Identifying Misconnection Hotspots Using Coliforms and Biofilm Communities

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Submitted to the University of Hertfordshire

in partial fulfillment of the requirements of the degree of

Doctor of Philosophy

September 2015

I would like to dedicate this thesis to my loving parents, Surv. Chike & Mrs Nkechi Nwanekezie

Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and Acknowledgements.

Chidinma Onyinyechi Chiejina

July 2016

Acknowledgements

I would like to express my gratitude to my supervisors, Dr. Veronica Edmonds-Brown (University of Hertfordshire) who provided me with adequate guidance and support, sharing with me her knowledge and expertise in the field, and Mr Richard Smith for his support during this study.

This project would also not have been possible without my parents, Surv. Chike & Mrs Nkechi Nwanekezie who provided the financial and moral support required for the study. I would also like to thank Lauren Billington at the Environment Agency for providing me with data on the River Lee and Pymmes Brook, Aiden Bygrave (University of Hertfordshire) for technical assistance with GIS, and also all the technical and laboratory staff at the University of Hertfordshire for their support and assistance.

My gratitude also goes to my brother Nnamdi Nwanekezie for driving me to sites and for his assistance with recording on-site measurements and my sister Uchenna Ojiako, for looking after my son on numerous occasions when I had to be away for laboratory and other research related work. I would also like to say thanks to my lovely son David who accompanied me on few occasions during field trips, Amaka Nwanekezie, Ikechukwu Nwanekezie and Onyedika Nwanekezie for all their support and encouragement.

Finally, I would specially like to thank my lovely Husband, Eric Chiejina who apart from his moral support, went out of his way to assist me with sample collection and with formatting my document using LaTeX.

Abstract

Sewage misconnections currently pose severe threats to water quality especially in urban areas in the UK. These misconnections lead to the discharge of untreated wastewater into receiving watercourses via surface water outfalls. Regular discharges from this source could lead to these watercourses failing to meet standards set by the Water Framework Directives. Despite the impacts resulting from this urban diffuse pollutant source, research into this area is limited. The study area is the River Lee, which is known to experience issues with water quality especially within the heavily urbanized lower Lee section. Misconnections are one of the major contributors to the poor water quality status of the River. In this study, the Lee was investigated using several parameters. The approach involved the monitoring of coliform bacteria to detect outfalls where misconnections were likely, then biofilm samples below selected outfalls were used for both clean and polluted sites to look for community types. Results showed severe pollution within some sections of the River particularly within the lower Lee. Pymmes Brook was used as a case study, both coliform bacteria and biofilm communities below outfalls were further investigated for clean and polluted sites. Results from the Pymmes Brook study also showed that this watercourse was experiencing severe issues with water quality, with elevated levels of coliform bacteria identified below polluted outfalls. Biofilm community data obtained during the pilot and experimental phases of study were analyzed using a range of multivariate techniques. Results of the analysis showed consistent

patterns in community structure within sites with similar water quality, with indicator species identified. Communities below polluted outfalls were composed mainly of species tolerant of organic pollution thus indicating a response of communities to misconnection discharges. Currently, the identification of misconnections are carried out through trackbacks, a process which is expensive and time consuming. During this study an inexpensive and rapid approach is proposed for assessing misconnections within a catchment. Using this method, misconnection hotspots are mapped out using coliform bacteria and biofilm communities. Using catchment data and predicted coliform bacteria from enumerated counts, misconnections within these hotspots are assessed. Having tested this approach on Pymmes Brook, a tributary of the River Lee, the results obtained were promising. Pending further verification, this system provides an economical and rapid tool for the assessment of misconnections within a catchment.

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List of abbreviations

ANOVA	Analysis of Variance
BMWP	Biological Monitoring Working Party
BOD	Biochemical Oxygen Demand
CAP	Community Analysis Package
CCA	Canonical Correspondence Analysis
CSO	Combined Sewage Overflows
D.O.	Dissolved Oxygen
DECORANA	Detrended Correspondence Analysis
DEFRA	Department for Environment, Food and Rural Affairs
DIY	Do It Yourself
EA	Environment Agency
ECOM	Ecological Community Analysis

EQR	Ecological Quality Ratios
GIS	Geographic Information Systems
IMS	Industrial Methylated Spirit
LA	Local Authority
PSWO	Polluted Surface Water Outfall
SLINC	Site of Local Importance for Nature Conservation
SSO	Storm Sewer Outfall
SSSI	Site of Special Scientific Interest
STW	Sewage Treatment Works
SUDS	Sustainable Urban Drainage Systems
TDI	Trophic Diatom Index
TWINSPAN	Two Way Indicator Species Analysis
UK	United Kingdom
WASc	Water and Sewerage Company
WFD	Water Framework Directives

WWTW Wastewater Treatment Works

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Chapter 1

Introduction

1.1 Water pollution

Water is a very important resource required by man for survival. However, the quantity of water available for use is limited. This is because a large percentage of the earth's water resources are contained in the oceans and ice caps, which make it difficult to access (Jones, 2014). Water pollution occurs when harmful and degrading substances or material are discharged into watercourses at a rate that could cause harm to organisms in a watercourse. Furthermore it can be said to include any man-made activity that increases the risk of degradation of the natural ecosystem of watercourses further reducing the water resources available for utilisation (Dugan, 2012; Moss, 2008). Several watercourses in the UK and around the globe are currently experiencing numerous water quality issues. In the UK for instance many rivers are presently faced with various pollution issues and between 2010 and 2013, less than one third of watercourses in England were recorded as having 'good ecological status' (Gov.UK, 2014; Halliday et al., 2014; Owen et al., 2012). This small percentage further emphasises the need to tackle the issue of poor water quality. Most of the issues leading to poor water quality are caused by various human activities, industrialisation, agriculture and urbanisation. A change in water quality due to all these factors reduces the

watercourses available for recreation, domestic, drinking, industrial and also agricultural purposes. Water pollutants from these sources could be physical, chemical or biological (Goel, 2006; Hawkins, 2015). The presence of pollutants in watercourses results in negative impacts on the environment, aquatic life and also people who rely on the affected watercourse for various uses (Snook and Whitehead, 2004)

Majority of the world's population are found in urban areas and thus there are several issues that arise as a result of urbanisation. Urbanisation comes with population increases and is associated with significant increases in the production and diversity of pollutants being discharged into watercourses. In addition, within urbanised areas, there is typically a 35 – 50% increase in impermeable surfaces (Reichard, 2010) such as car parks, buildings, roads, roofs and streets. All these impermeable surfaces prevent the absorption of surface water from rain and melting snow into the ground therefore increasing runoff. Approximately 55% of this surface water gets intercepted thus reducing ground water recharge (Ehlers et al., 2015; Pazwash, 2011). Furthermore, urbanisation alters the natural hydrological cycle, changing peak flow characteristics and the volume and quality of the runoff. This increased velocity and volume of the surface water runoff, causes a destruction of habitat and carries a variety of pollutants which end up being discharged into receiving watercourses (Wan et al., 2015). Some of the impacts of urbanisation on watercourses can be seen in Figure 1.1.

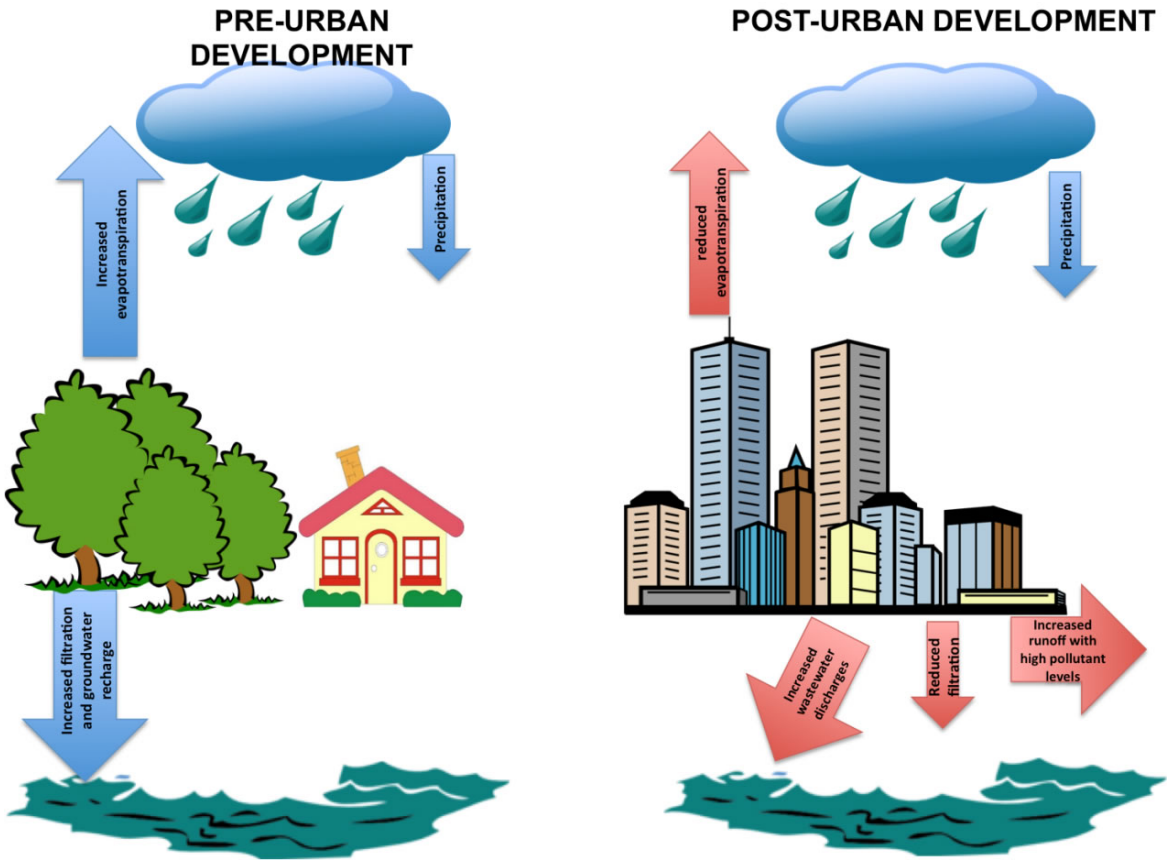


Figure 1.1: Diagram showing the impact of urbanisation on watercourses

1.2 Urban Runoff

The changes that occur due to urbanisation, industrialisation and constantly increasing population in urban areas (Sheng et al., 2013) cause a change in the natural hydrological cycle, therefore leading to changes the characteristics of peak flow as well as the quality and quantity of runoff produced (Scholes et al., 1998). Surface runoff usually occurs when the soil is over saturated and therefore has reached its maximum absorption capacity, or when the volume of water from rainfall or snow melt exceeds the soils ability to absorb it (Schaik et al., 2014). In highly urbanised areas, the runoff rates are usually high due to the increase in impervious surfaces such as roofs, car parks and roads. As previously mentioned, the increased surface runoff carries along a variety of pollutants, which are eventually discharged

into receiving watercourses. All these pollutants result from a diverse range of activities and sources therefore making it difficult for detection and management (European Environment Agency, 2014; Valtanen et al., 2014b). According to several research projects, one of the major threats to urban watercourses is the issue of diffuse pollution. Diffuse pollution occurs as a result of urban runoffs from houses and industries, agricultural runoffs and also surface runoffs (Kim et al., 2014; Osei-Twumasi et al., 2014). Individually, these non-point sources are not significant, however in combination, their resultant impacts to watercourses are usually severe (SEPA, 2013; Smith et al., 2015). Common sources and types of urban diffuse pollution, include pollution from vehicle emissions, oil and chemical spills from industries and road runoff, combustion products, emissions from transport and industries (Petrucci et al., 2014). Additional non-point sources of pollution and some associated pollutants also include pesticides and fertilisers from gardens (Venkateswarlu, 2014), faecal and pathogenic organisms from birds, pets, livestock, over capacitated sewage systems, sewage leaks and plumbing misconnections (Silva, 2013), heavy metals, chemicals from detergents, sediments, litter and debris (EPA, 2014; Rieuwerts, 2015). Factors that influence pollutant levels in watercourses include the frequency of storms, land use, catchment area and to a large extent the type of sewage system used (Scholes et al., 1998). Increased levels of these pollutants negatively impact on the affected watercourses by destroying natural ecosystem and habitat (Trathan et al., 2015). In addition, increased nutrient loads especially in streams with sufficient light availability could cause oxygen depletion, subsequently harming fish and other aquatic life (Roberts et al., 2014). Other negative impacts that could result include the excessive growth of algae from increased organic loading leading to reduced water clarity, blocked channels and thus the reduction of the aesthetic and recreational properties of such watercourses (Roberts et al., 2014). Residents of affected areas could also potentially be exposed to harmful toxins and pathogens which could lead to negative health impacts (Horwitz and Finlayson, 2011; Lofrano et al., 2015). Some urban diffuse pollution sources

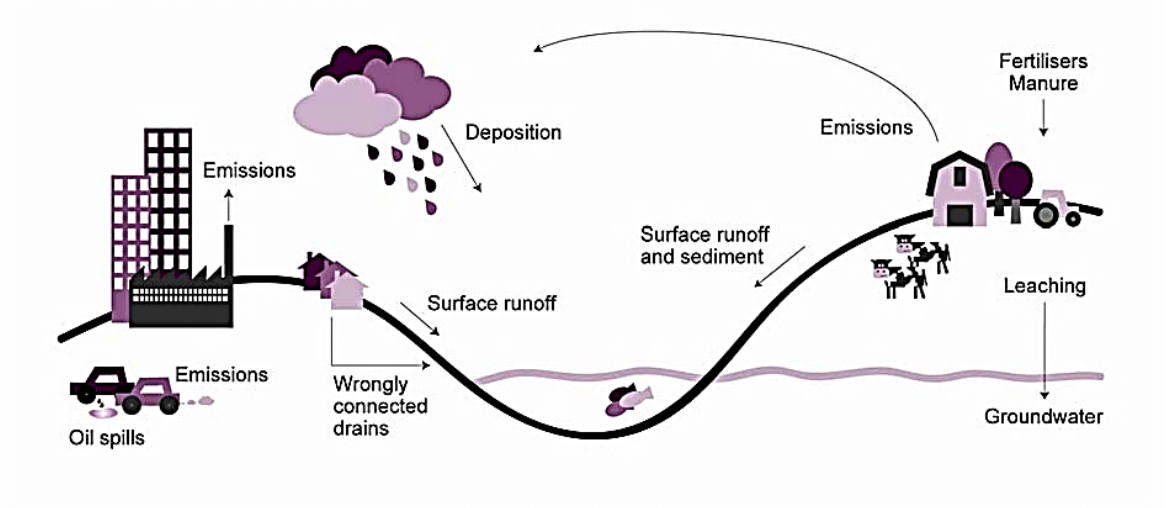


Figure 1.2: Common sources of urban diffuse pollution
Source: (Environment Agency, 2007)

mentioned previously are given in Figure 1.2. However, in the UK based on priority the key recognised sources apart from agriculture, include misconnections, pollution from industrial estates and urban runoff via road and other impervious surfaces as well as contaminated rivers (Gov.UK, 2014; Lundy and Wade, 2011; Yu, 2013). The impacts of urban runoff pollution are numerous and therefore to tackle them, all sources need to be assessed and managed at the catchment level. This is required in order to ensure that UK watercourses meet with the Water Framework Directives (WFD) requirements. It has been suggested that Sustainable Urban Drainage Systems (SUDS) such as filter strips, swales, constructed wetlands, be used to control and manage surface water runoff (Ellis et al., 2003). These are systems constructed to ensure a slower and more natural flow and discharge of surface water, providing a better chance of water absorption into the ground and the natural retention and breakdown of pollutants carried in surface runoff. Some of these systems have already been put in place to tackle surface water runoff especially in the London area (Environment Agency, 2007). However, despite all the measures being taken, poor water quality from urban runoff pollution still persists. This further emphasises the need for additional research to further understand and identify threats to

water quality in order to provide measures to manage and improve the quality of watercourses.

1.2.1 Coliform Bacteria

There is usually an abundance of microorganisms including pathogens that exist within urban rivers with pollution issues. Organisms could include bacteria, viruses, protozoa and so many others impacting negatively on the affected watercourses. Introduction of these organisms into watercourses are from a variety of sources that include sewage discharges as well as urban and agricultural runoff. Furthermore, some of these microorganisms can also be directly introduced into watercourses when birds and other animals defecate in them (Sanders et al., 2005). High levels of pathogenic organisms in watercourses which are introduced into watercourses via the above mentioned means, could lead to the spread of diseases such as typhoid, cholera and also hepatitis (Hamner et al., 2006). Poor ecological conditions could also result from these increased microbial loads, further emphasizing the need to improve the water quality status of affected watercourses. It is however usually difficult and expensive to test water samples for a wide range of pathogens and so watercourses are instead monitored for certain organisms that could indicate the presence of other pathogenic organisms. For several years, it has been a practice to detect recent faecal discharges by assessing the watercourses for the presence of these indicator organisms (Buckalew et al., 2015; Gleeson and Gray, 2002). One commonly used indicator is a group of bacteria known as coliform bacteria. These microorganisms are usually associated with human and animal faeces because they exist in high levels in the intestinal tract of humans and animals (Sivaraja and Nagarajan, 2014). Coliform bacteria are gram-negative bacteria belonging to the Enter-obacteriaceae family. They are lactose fermenters that possess $\beta -$ galactosidase and appear as yellow colonies on acid detection on membrane filters after incubation at two different temperatures, 30°C for 4 hours and then 37°C or 44°C for 14 hours (Burman, 1955; Dufour and Cabelli, 1975; Environment Agency, 2009a; Nollet and De Gelder, 2007; Service, 1980). When viewed

under the microscope, they appear as rod shaped bacilli. They are usually used as indicator organisms because although sometimes occurring in nature in soil and decaying vegetation, they exist in significantly high levels in faeces. Thus, their presence in high levels within water courses could be an indication of possible faecal contamination. With the exception of coliforms such as *E scherichia coli O157:H7* which could lead to internal bleeding, coliform bacteria are rarely pathogenic (Salvato et al., 2003). However, increased levels of these bacteria could indicate the presence of other organisms capable of causing diseases such as dysentery, cholera, typhoid and other waterborne diseases. Different species of coliform bacteria include *Escherichia coli, Citrobacter, Hafnia, Enterobacter, Yersinia, Serratia* and also *K lebsiella* (Environment Agency, 2009a; Meutia et al., 2014). The coliform bacteria group is usually classified into two categories, faecal coliforms and total coliforms. Faecal coliforms such as *Escherichia coli* and *Enterococci* are found exclusively in the gut and thus are more specific indicators of faecal pollution. On the other hand, total coliforms include all members of the coliform bacteria group including faecal coliforms and coliform bacteria found in the environment (Fiello et al., 2014). Due to their association with raw sewage increased levels of coliform bacteria within watercourses suggest major sources of faecal discharges in the UK such as plumbing misconnections, poorly treated or untreated discharges from Sewage Treatment Works (STW), urban runoff and agricultural runoff (Paruch et al., 2015; Thames 21, 2011). These increased bacterial levels impact severely on affected watercourses increasing the potential risk of residents contacting certain waterborne diseases due to other faecal pathogens that could be present. In addition, within such watercourses with excessively high levels of bacteria, there in an increase in oxygen demand, cloudy water and also bad odours usually associated with severely polluted water (EPA, 2012; Wan et al., 2014). There are several methods available for the assessment of sewage contamination of a watercourse and they include the measurement of biotic indices, Biochemical Oxygen Demand (BOD), Dissolved

Oxygen (DO), ammonia and suspended solids. However, coliform bacteria have for several years been used to indicate possible pollution issues within watercourses and when discovered in excessively high levels, further investigation of the watercourse is conducted (Dunk et al., 2008). Although coliform bacteria are widely used indicators of poor water quality, there are limitations to their utilisation.

These limitations being that several environmental variables affect their loadings and die-off rates. On introduction into watercourses, coliforms easily and quickly die-off with their survival rate dependent on factors such as temperature and D.O, sunlight, nutrient levels, protozoa grazing, and several other variables (An et al., 2002). Furthermore, although largely present in human faeces other sources of their discharge into watercourses could be due to pets, grazing animals and birds as well as from the environment (Wither et al., 2013).

The above mentioned limitations have in recent times led to several researchers questioning the reliability of using coliforms solely as an indicators of poor water quality (Ellis, 2004; Munn, 2011). Therefore in this research the study site was monitored for faecal contamination using coliform bacteria in combination with other parameters including biofilm communities.

1.2.2 Biofilms

The Water Framework Directive promotes the discovery and development of alternative biological techniques for the detection of changes in water quality due to pollution (Sabater et al., 2007). This research involves the use of coliform bacteria and the exploration of biofilm communities as tools for locating areas with faecal contamination from plumbing misconnections. Biofilms can be defined as a collection of microorganisms attached and living together as a community and interacting with each other. These communities are functional as a whole and the organisms that make up the biofilms contribute in different ways to the functioning of the general community as a whole. These organisms are usually attached to surfaces and embedded within a matrix of extracellular polymeric substances (Lee et al., 2014). Biofilms are usually

found growing on solid surfaces such as rocks and cobbles submerged in watercourses. These communities are usually composed of various microorganisms interacting and held together by a complex assemblage linked with polymers (Lee et al., 2014). Biofilm communities occur in various environments. However, in watercourses they are mainly found on plants submerged in the water, stones, sands and several other solid surfaces. They exist as an important part of river systems and play important roles towards the healthy functioning of ecological systems. Being at the bottom of the food supply chain in streams, they are very important energy sources for the aquatic food web (Ancion et al., 2013). Furthermore, biofilms in watercourses act as natural purifiers of watercourses similar to the trickle bed filters commonly used by Wastewater Treatment Works (WWTW) for the treatment of wastewater (Huang et al., 2014). This is due to their high removal capability of pollutants such as nitrates and phosphates as well as their ability to breakdown organic matter (Jin et al., 2012). In addition, being constantly exposed to the wide range of environmental conditions associated with watercourses, the changes and reaction of certain organisms within biofilm communities could assist towards the prediction of pollution issues. Several studies have established links between the existence of certain members within biological communities and the water quality status of rivers and streams. One of such studies was carried out on between 2005 and 2006 on the Llobregat River in Barcelona. Multivariate results obtained during the study showed that the presence of pesticides influenced the structure of diatom communities, with pesticide impacted sites dominated by species tolerant of eutrophic conditions such as *Gomphonema parvulum, Nitzschia umbonata, Navicula atomus var. permitis, Navicula subminuscula* and *Navicula veneta* (Ricart et al., 2010). Changes in environmental properties and parameters of streams could likely have a resultant effect on the structure of the communities within river biofilm. Therefore, these communities could potentially be used to indicate pollution within watercourses. Environmental changes could occur due to pollutant discharges

from sources such as plumbing misconnections and overcapacitated Combined Sewage Overflows (CSO's). Therefore if discovered to be effective in detecting faecal contamination, they could be used as a continuous monitoring tool because they could detect changes due to intermittent discharges (Fechner et al., 2012a).

1.2.3 Misconnections

In the United Kingdom, there are two types of sewage systems, the combined sewer system and the separate sewage system. Both systems are composed of the wastewater pipe and the surface water pipe. The foul water pipe transports untreated wastes from buildings to the WWTW for treatment prior to discharge while the surface water pipe takes rain water from parks, roofs and other impermeable surfaces and discharges it into watercourses in the area. In the combined sewage system, which was introduced in the Victorian era, the surface water pipe and the foul water pipe are constructed parallel to each other and interlinked at various points to enable dilution of the wastewater by the surface water. However, in the separate system, the sewage pipe and the surface water pipe are constructed separately without any links (Ellis and Butler, 2015; Faulkner et al., 2000). These two types of sewage systems both have implications for water quality and have been shown in previous studies to be two major routes through which pollutants are discharged into urban watercourses in the UK (Comber et al., 2013; Dunk et al., 2008; Faulkner et al., 2000). The issue with the combined sewer system results from the over capacitation of the sewage system due to increased waste production from the rapidly growing population within urban areas. These systems were originally constructed to discharge the diluted wastewater during peak storm periods, which only occurred approximately 2-3% of the time and as such were not leading to significant damage.

However, with increased wastewater production from a constantly increasing population within urban areas, the capacity of the sewage system is regularly exceeded resulting in frequent discharges from these CSO's (Faulkner et al., 2000). Several research projects have been carried out on urban diffuse pollution from CSO's. In addition, several remedial

works have also been conducted and future plans have been made to tackle pollution from CSO's. Unlike the CSO's which discharge when the system is overwhelmed, discharge from plumbing misconnections is continuous. Furthermore, even on identification and correction, several repeat misconnections still spring up (Dunk et al., 2008). This therefore indicates that further actions are required to deal with the issue and apart from remediation; a proactive approach needs to be applied. The issue of misconnections, despite being around for a long time, has not been widely studied. According to figures from several organizations and the few available studies, it is however a significant and widespread problem in UK. In 2013, it was estimated that at least 300,000 properties in the UK had a minimum of one misconnection. In addition, Thames Water estimates that one in ten properties is misconnected in the London area (Green, 2013). As a result, several watercourses in urban areas are severely polluted (Dunk et al., 2008). Findings from a previous study also suggest that there are a significantly large number of undetected misconnections currently existing in the sewage system (Ellis, 2013). Subsequently, Thames Water state that between 2013 and 2014, 1596 misconnected goods were identified in the Thames region (Thames Water, 2015). Although some are unverified, various estimates and figures have been provided for the levels of misconnections in the Thames region and the UK in general. However, results from a previous study carried out on 50,000 properties in the Thames region showed that although misconnection identification within the region varies, it generally falls within a range of less than 1% and 9%. This could be viewed as a small percentage; however the impacts on receiving watercourse have been significant. In addition to this, the research discovered that 3% of the identified misconnections are from toilets while a significant percentage of these misconnections were due to washing machines, hand basins and sinks. The distribution of the misconnection types found in this study is given in Figure 1.3.

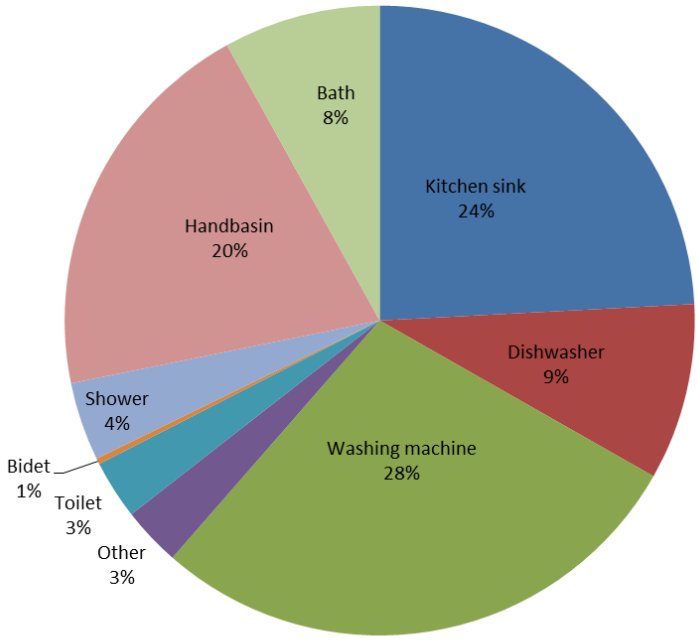


Figure 1.3: Distribution of misconnections in a 2008 study within the Thames region
Source: (Dunk et al., 2008)

A large percentage of plumbing misconnections arise during property extensions or constructions. These sewage misconnections occur in separate sewage systems, sometimes as a result of DIY plumbing. It also occurs sometimes when plumbers and builders, due to ignorance of the sewer system, illegally connect pipes carrying sewage material to the surface water pipes. In these cases, people connect pipes from washing machines, baths, dishwashers and other white goods direct to the surface water pipes (Edmonds-Brown and Faulkner, 1995). In addition, sometimes when people put in new toilets or extend their properties, the pipes are connected directly to the surface water pipes. As a result, grey water and untreated sewage are introduced into the surface water pipes thereby leading to their discharge into receiving watercourses. Another type of misconnection, although not very common, occurs when domestic surface water pipes are connected to the sewage pipes. During heavy rainfall events, the sewage pipes could become over capacitated, thereby leading to flooding into streets and watercourses (Edmonds-Brown and Faulkner, 1995).

Generally, it has been suggested that the most commonly occurring misconnections are from white goods and sinks. According to the Chartered Institute of Plumbing and Heating engineering, the most commonly occurring misconnections are dishwashers (10-15%), toilets (5%), sinks (10-15%), washing machines (35%) and the whole properties (5%) (Chartered Institute of Plumbing and Heating Engineering, 2014). All the above mentioned misconnections occur due to property owners, builders and plumbers having insufficient knowledge of the sewer system as well as the culture of DIY. Plumbing misconnections usually cause severe impacts on receiving watercourses mainly during low flow when there is a minimal chance of the pollutants being diluted (Edmonds-Brown and Faulkner, 1995). Figures provided previously in this thesis show how rampant this issue is in the UK. Therefore, this means that these misconnections are constantly discharging untreated wastewater into several watercourses. Impacts of this pollution include an increase in health risk of residents, bad odour and other aesthetic and physical impacts. Also common within impacted sites are the presence of sewage fungus, which is usually formed by long filamentous algae known as *Sphaerotilus* algae which subsequently forms a biofilm because several species of diatoms get attached to the filaments along with the polysaccharide matrix which binds the communities together. Most importantly the polluted wastewater from this source impact significantly on River and stream ecology and therefore leads to their failure to meet the aims of the Water Framework Directives and other water quality standards (Dunk et al., 2008). There are several potential reasons that could explain the lingering issue of diffuse pollution from plumbing misconnections. One of such is that misconnections are currently being tackled reactively. Investigations are mostly made when individual cases are suspected and reported by the residents. Most of the times these occur when discharges from misconnections have persisted for long enough for the impacts to be visible. Even then, identification of the misconnected property or appliance is usually through trackback surveys, which are usually expensive, difficult and time consuming (Dunk et al., 2008).

Secondly, there are various legislations available to tackle misconnections some of which are given in Table 1.1.

Table 1.1: Specific legislation available to tackle plumbing misconnections

Legislation	Relevance
The Water Industry Act 1991	Section 171 of this legislation provides sewerage undertakers with the right of entry to an area or property so as to determine whether a pollution offence has been committed and whether action should be taken or not (Legislation.Gov.UK, 2012). Furthermore, section 158, 159 and 168 provides an entry right to WaSCs to lay pipes and carry out investigations where required (Water UK Environment Agency, 2009). In addition, according to section 106(2)(b) of this legislation, in separate sewage systems, discharge of foul water or sewage into surface water pipes are prohibited while section 106(2)(c) banns individuals from directly linking drains or sewer to storm water overflow sewers (Legislation.Gov.UK, 2012). Section 109 of the Water Industry Act also states that it is an offence to cause a sewer or drain to communicate with a public sewer in violation of section 106. Part 2 of this section also provides sewerage undertakers with the right to disconnect drains and sewers that wrongly connected to public sewers. The undertakers also have the right to recover costs incurred from this work (Water UK Environment Agency, 2009). Finally, the Water Industry Act section 113 provides sewerage undertakers with the right to change or work on private drainage systems that they feel is objectionable or not conforming to the general sewage system in the area. However, costs incurred from such works cannot be reclaimed (Legislation.Gov.UK, 2012).
Water Resources Act 1991	The Water Resources Act 1991 section 161A provides the Environment Agency with the power to serve a notice to offending individuals or companies to conduct anti-pollution works within a specified time. This notice is served where it is discovered that a noxious, poisonous, polluting matter or solid waste material has been deposited or is likely to be deposited into any controlled waters. Section 85 of this legislation also states that it is an offence to either knowingly permit or cause noxious, poisonous or polluting material or solid waste matter to be introduced into controlled waters. Pollutant sources such as sewage misconnections to surface water pipes could lead to discharge of polluted material into controlled waters and therefore is said to be an absolute liability offence under this section of the Water Resources Act 1991 (Water UK Environment Agency, 2009)
The Building Act 1984	Section 59 of the Building Act 1984 empowers the Local Author-ity to take the necessary action if it feels that satisfactory drainage has not been provided for a building. In such cases, the Local Authority can serve the offenders with a notice requesting that the owner corrects the problem. However, if the offender does not comply with the notice, the Local Authority according to section 99 can undertake the work and reclaim costs incurred as a result (Water UK Environment Agency, 2009).

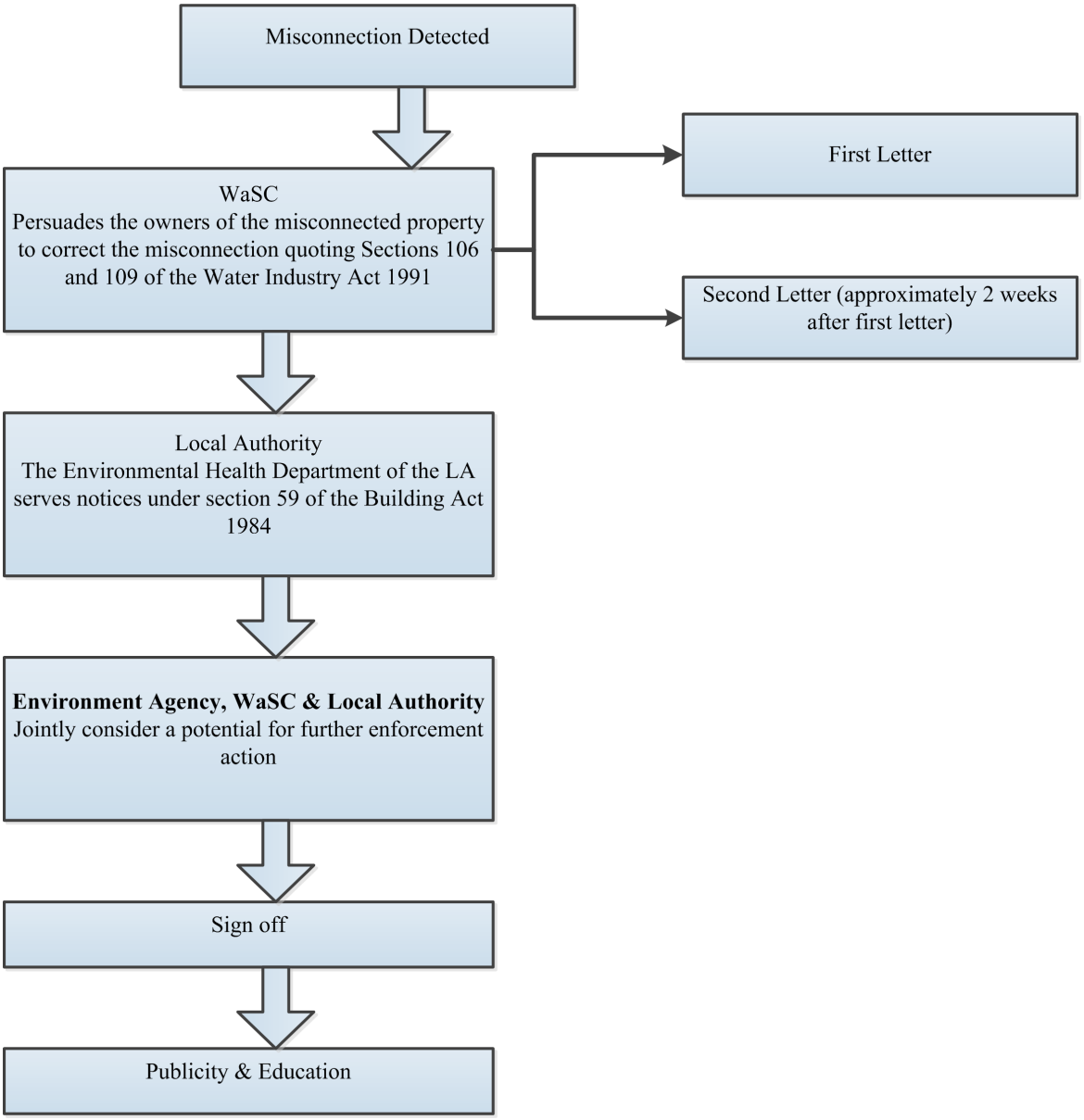


Figure 1.4: Diagram Showing the Process of Misconnection Investigation and Correction Adapted from: (Water UK Environment Agency, 2009)

Figure 1.4 also shows a procedure available for the rectification of plumbing misconnections in the UK. Generally, the law provides the Environment Agency and Local authorities with enforcement rights when dealing with misconnection offenders. Property owners are therefore responsible for the correction of misconnection issues and non-compliance could lead to prosecution based on the law (Water UK, 2010).

However, the lengthy procedure and communication required between the Water Companies, Local Authorities, the EA and the offenders makes it an intensive and time consuming process. Therefore the lengthy process could mean that even with identification of a mis-connected property it could take a while before it is corrected (Ellis, 2013). Implications of this, is the discharge of untreated wastes into watercourses including impacts such as oxygen depletion, a reduction in the ability of rivers to support aquatic life, an increase in human health risks, development of sewage fungus and other negative impacts (Bi et al., 2014; Environment Agency, 2013). All these further emphasize the urgent need for the development of various techniques and approaches to tackle this issue in order to prevent the constant discharges and resultant impacts from this pollution source.

1.3 Rationale, Aims and Objectives

It has been identified from the review of relevant literature that the River Lee is suffering from severe impacts mainly from urban diffuse pollution. One of the main sources has been attributed to the issue of plumbing misconnections (Petrucci et al., 2014; Snook and Whitehead, 2004). This misconnection issue causes the abundance of several pollutants within the river system and resultant impacts are severe. Current technique available for misconnection identification is misconnection trackbacks, a time consuming and expensive process.

1.3.1 Rationale for Study

As was discussed in the review of literature in this chapter, it is evident from previous studies and figures that misconnections occur rampantly in the UK, with misconnection discharges being one of the major contributors to poor water quality in several urban

Rivers including the River Lee, which is being investigated during this study. However, locating and tackling these misconnections is challenging because misconnection trackbacks are difficult and expensive. As discussed also in the literature review in this chapter, high coliform bacteria levels are usually found in human sewage and therefore untreated misconnection discharges are likely to contain these coliform bacteria in high levels. In addition, previous water quality studies have also shown the association of certain biofilms with sites of varying water quality status. This research is thus based on the hypotheses that there will be high numbers of coliform bacteria in the dirty sites and also that there will be differences between biofilm communities occurring in the clean sites and communities occurring in the dirty sites with misconnections. Based on this, the study aims were generated and certain objectives were set to assist towards the achievement of the aims. The study aims and objectives are provided below. If this research is successful, it will provide a new, easier and less expensive approach for locating misconnections and subsequently tackling them.

1.3.2 Aims

This research seeks to investigate the impacts of plumbing misconnections on the River Lee, using coliforms and biofilms to map out misconnection hotspots. Coliform numbers and biofilm communities will be used to assess sites with different water quality ("clean" and "dirty") status in order to assess the usefulness of this approach in locating areas with misconnection issues.

1.3.3 Objectives

i. To investigate the water quality status of the River Lee, water samples for coliform testing will be collected from 45 sites from both rural and urban reaches located on the

river. This initial sampling program will assist towards the decision on which areas of the River to focus on. These samples will be tested for coliform bacteria levels. Additional sites will be selected for an experiment, five on Pymmes Brook and one control site in an artificial stream at Bayfordbury. Water samples from these sites will be collected repeatedly over several months and tested for both coliform bacteria numbers as well as a range of physicochemical parameters.

ii. Stones collected from the river benthos during the biofilm pilot stage, from ten sites on the Lee (all identified using results from the coliform pilot) for the identification of biofilm communities. Furthermore, during next stage, which is the biofilm experiment, benthic biofilm occurring within the same six sample sites used during the coliform experiment will also be assessed. This will involve repeated collection of biofilm samples over a longer period (four months) to identify communities and possible patterns in the structure of communities within clean and dirty sites.

iii. Landscape and Land use data will also be used to calculate runoff coefficients for the study area.

iv. In addition, census data will be collected for the study area and used in conjunction with coliform bacteria, land use and landscape data to locate potential hotspots.

v. Several multivariate analysis techniques will be used to test how effective the coliform and biofilm approach is as a tool for assessing misconnection occurrence.

1.4 Thesis Structure

This thesis has been laid out to include seven chapters as well as the reference and appendices sections. Chapter 3, 4, 5 and 6, are the chapters in which data collected during the study have been analysed. These chapters have been structured to include the introduction, methodology, results and discussion sections. This structure is similar to that of scientific publications and therefore it will assist towards the use of these chapters for writing up scientific papers in future. A summary of the content of each of the seven chapters is given below.

Chapter 1, the introduction chapter provides some background information and literature review of the study. It explains urban runoff pollution with a particular focus on pollution from misconnections. It also provides a description of the major parameters used during the study to assess the quality of the study catchment and sub catchment. The study aim and objectives are also provided within this chapter.

Chapter 2 is the site description section and it provides a description of the River Lee catchment and the sites used during the coliform and biofilm pilot studies of the river. It also provides a description of the River Lee sub catchment (Pymmes Brook) and the sites used during the coliform and biofilm experiment within this sub catchment of the Lee.

In chapter 3, the sites selected on the River Lee for the pilot stage of the research were assessed for their water quality status using coliform bacteria concentrations at sites. The chapter also provides information on the coliform bacteria levels identified for Pymmes brook during the experimental study phase.

Chapter 4 provides information on the biofilm pilot stage of the research. The chapter presents results of an initial study into the composition of species/ families found within certain sites with different pollution statuses. Results of the initial multivariate analysis of communities found within the two groups, clean and dirty sites are also provided.

Chapter 5 contains further and long-term investigation of biofilm communities within the two groups of sites. Several multivariate techniques were also used to analyse these communities and detect patterns in their structure as well as their relationship to some environmental factors.

In chapter 6, runoff coefficients are estimated for Pymmes Brook, the Lee subcatchment used for the experimental study. These coefficients are then used to further investigate misconnection hotspots within the Pymmes Brook area.

The last chapter, chapter 7 provides a summary and discussion of the research findings.

Chapter 2

Site Description

2.1 The River Lee Catchment

The River Lee is a large river system with several tributaries that discharge into it at various locations within the southeastern part of England. The rivers and its tributaries include several navigation channels and urban water bodies. Many of these tributaries are chalk streams, which are unique and fragile environments of great ecological and economical value (The Wild Life Trusts, 2015). Similar to several UK rivers and chalk streams, the Lee and its tributaries have been exposed to extensive channel modification, reduced flow from excessive water abstraction, excessive clearing of vegetation in addition to significant discharges of several pollutants from urban and agricultural runoff over the years. All these have detrimental effects on such watercourses especially the chalk streams. These negative effects include significant impacts such as increase in fish mortality, loss of fish production, alterations in community structure and diversity resulting in the dominance of species tolerant of pollution and excessive growth of algae which could be unpleasant to look at. All these impacts contribute towards the overall poor water quality status of these streams (Old et al., 2014).

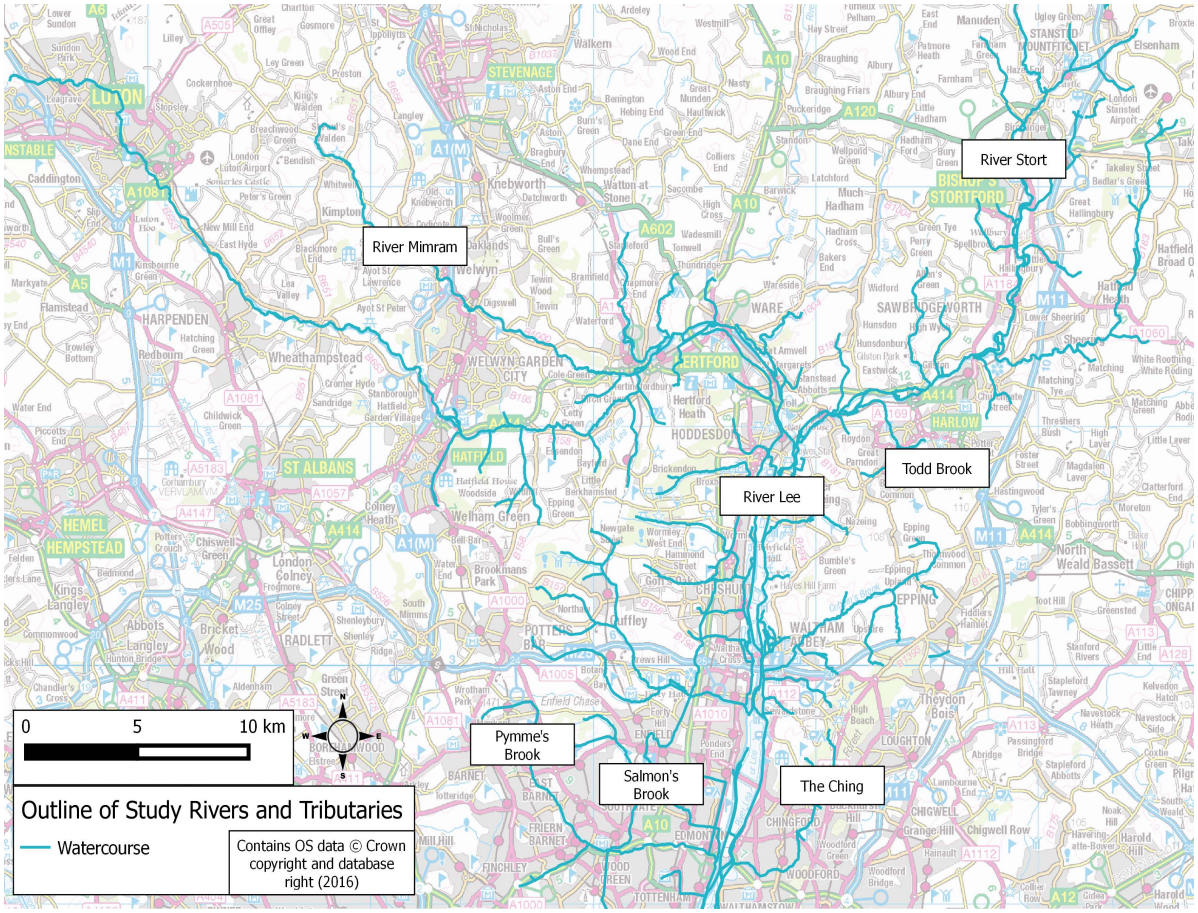


Figure 2.1: Map of the River Lee and tributaries used during the study

The River Lee a major tributary of the River Thames is a large lowland river system. It flows through a catchment area of approximately 1420km^2 with its source located at a spring in Marsh Farm Bedfordshire (Snook and Whitehead, 2004). From the source, it flows south-east through Hertfordshire and several towns in Greater London down to its confluence with the River Thames (Copp and Roche, 2003). Figure 2.1 shows a map of the River Lee and the six tributaries studied. There are approximately two million people living within the River Lee catchment area. The geology of the Lee catchment is mainly chalk, tertiary deposits and London clay (Snook and Whitehead, 2004). The Upper Lee section starts from the source of the river in Leegrave, Luton. Most of this section from its source to Fields Weir in Hoddesdon, Hertfordshire is predominantly rural. Although there has been a marked increase in housing since the 1950's, consequently leading to an increase in urbanisation within this upper section of approximately 15%. Geology of this northern part of the river is

composed mainly of cretaceous chalk. In addition, land use is for the most part agricultural, with large stretches of arable farmland located in the area. Several tributaries of the river are located within the upper Lee catchment. These include the River Mimram and the River Beane, both located in Hertford. These two tributaries feed into the River Lee at Horn's Mill and Hartham Common respectively. Other tributaries include the Rib, which also flows into the Lee at Hertford, the River Ash, which has its confluence with the Lee at Stanstead Abbots and also the River Stort, which joins the Lee at Hoddesdon (Segond et al., 2007). All these tributaries are Chalk Rivers.

The Lower Lee catchment on the other hand is predominantly urbanised. Geology in this section of the Lee is made up mainly of London clay and tertiary deposits. There are also several tributaries located within this section of the River Lee. Some of the tributaries include the Ching, Cobbins Brook, Salmons Brook, Rags Brook, Small River Lee, Turkey Brook, Dagenham Brook, Pymmes Brook, Mosselle Brook (Environment Agency, 2015) and several other watercourses, all of which are predominantly on clay. The high level of urbanisation in some of these areas has a negative impact on the watercourse. This urbanisation leads to increasing human impacts from activities such as water abstraction, navigation and modification of water channels. There is also an increased discharge of pollutants from runoff from the increase in impermeable surfaces such as car parks and roofs. Plumbing misconnections and combined sewage overflows also contribute to the increased pollution loads. The River Lee has been subjected to significant pressures from a lot of these processes for several decades thus contributing to a continuous decline in water quality. In addition, some areas within the lower Lee section have been split into channels known as the Lee Navigation to allow canal boat transportation. These date back to the period of invasions where a shallower channel was designed to beach longboats coming up the river from the Thames from Leamouth (Hadfield, 1981). In later years, it also served as a source of water for London. Within the lower Lee catchment, to prevent excessive flooding

in areas prone to flooding, some side channels were also constructed to provide flood relief during flood incidents. However, the locks constructed to prevent flood within these channels cause reduced flow and thus deposition and accumulation silt of in these areas (Snook and Whitehead, 2004). There are several drainage trenches, small tributaries and culverts that discharge into the Lower Lee. Some of the sections of the above mentioned tributaries are found in open channels while others are concealed within culverts and pipes below the ground surface. This concealment has led to several sewage misconnections being left undiscovered subsequently leading to increased pollution of the River from sewage discharges (Environment Agency, 2012).

There are also discharges of treated wastewater from various Wastewater Treatment Works into the River Lee. For instance within the upper Lee, excessive water abstraction has led to the drying out of springs from which the river is fed. This is made worse during dry weather periods and therefore approximately 80% of the water within this section is from discharges from the East Hyde Wastewater Treatment Works in Luton (Copp and Roche, 2003; River Lee Catchment Patnership, 2015). Other Sewage Treatment Works (STW) that discharge treated wastewater into the river in the upper Lee section include the Hatfield Mill Green, Rye Meads and also Harpenden STW. Moreover, within the southern section of the river, the major effluent discharge is from the Deephams STW in Edmonton which discharges into Pymmes Brook via Salmon Brook. This STW handles a wastewater effluent of approximately 460,000 m^3 on a daily basis (Ellis, 2006). The River is also a main source of water supply and therefore is subjected to excessive water abstraction (Snook and Whitehead, 2004). This has been ongoing for several centuries and therefore has led to both hydrological and ecological issues especially during low flow periods. During such periods when there is a significant reduction in rainfall, resident's water usage exceeds the rate of replenishment. Subsequently, this leads to the drying up or

reduction of such rivers and could lead to the loss of aquatic life (Ecologist, 2010). The low flows also lead to dilution issues and thus poor water quality especially in locations such as the flood relief gate area in the lower Lee (Environment Agency, 2012).

Generally, the River Lee has had a long history of pollution from several man made activities, some of which have been discussed above. However a key source of this pollution is currently through urban runoff mainly from misconnections and Combined Sewage Overflows (CSO's) (Snook and Whitehead, 2004). This is particularly an issue within the Lower Lee catchment area because of the increased population and urbanization associated with these areas. The numerous roads, highways and other impermeable surfaces located along the river, particularly in the lower Lee lead to increased and constant road and urban runoff from these sources as well. These discharges and inputs are usually worse during first flush events, which occur when heavy rain falls after a dry antecedent period. The first flush events lead to the flushing of leaves, debris, oil, road grit and other significant pollutants into the River Lee via catchpits. There is an airport in the Lee catchment in Luton and therefore pollutants are introduced within this section both from this source as well as runoffs from residential and industrial properties.

The Lee has suffered from excessive build-up of sediments especially in the lower Lee area (Snook and Whitehead, 2004). Essentially, high temperatures with high levels of organic matter see increased bacterial activity to break down the organics, this depletes the available oxygen and during a first flush event see deoxygenated water flushed from catchpits into the river. There are in addition high levels of siltation from roads, construction etc. Siltation of the bed of the river creates a homogenous substrate, which apart from being unsuitable for higher invertebrate life also contains heavy metals, which stick to the colloids of the fines (Blettler et al., 2015). The excessive build of sediments between Tottenham Lock and Lea Bridge became a source of concern for the Environment Agency. Therefore in 2009 to improve the water quality within this section prior to the London Olympic, arrangements were

made for the removal of accumulated sediments from the affected area. On removal, sediments had to be sent to landfill because they were composed of both toxic and non-toxic material (Environment Agency, 2012).

Additional discharges are made into the River Lee from a couple of farms. For instance, the Westmill Trout Farm discharges an effluent above 4000m^3 daily into the Rib. An effluent of similar volume is also discharged daily into the Ash (Snook and Whitehead, 2004).

There have been some water quality researches carried out on the River Lee previously, and results from these studies indicate that the River is experiencing severe impacts from human activities and also has poor water quality (Patroncini et al., 2014; Snook and Whitehead, 2004). A study on the River Lee carried out by Snook and Whitehead discovered the highest levels of metals, nutrients and microorganics within the lower Lee catchment and the Lee navigation, both highly urbanised sections of the Lee. It was also discovered that microbiological levels significantly exceeded standards stipulated by the European Bathing Water Directives. The poor water quality especially within the lower Lee section was also discovered to be impacting on aquatic life. This was evident from the poor aquatic life observed within this area of the River. Further assessments of the macrobiological community also showed a very poor quality within this area (Snook and Whitehead, 2004). Another study carried out on the Lee Navigation discovered that concentrations of dissolved oxygen were significantly low within the Lee Navigation area. This was potentially attributed to the high levels of bacteria and polar compounds identified during the study (Patroncini et al., 2014). Furthermore, results obtained from a snapshot water quality testing commissioned by Thames21 in 2011 showed that several tributaries of the River Lee were suffering from severe water quality issues. During the study, it was discovered that all the tributaries investigated had levels of reactive phosphates above 1mg/l. Some of the sites within Dagenham brook, Moselle brook, Pymmes brook and Salmons brook had reactive phosphate levels above 12mg/l Thames21,

2011). Sewage and chemical contamination from residential properties were suggested as possible causes of the increased phosphate levels (Thames21, 2012). The study also identified extremely low levels of dissolved oxygen within some of the sites with the lowest percentage saturation of D.O. (ranging between 10 – 30 %) identified in Salmons Brook, Pymmes Brook and Moselle Brook (Thames21, 2011). The later also had a lot of sewage fungus on the riverbed. During the investigation, the presence of raw sewage in the Ching was also noted (Thames21, 2012). Generally all these studies indicate that the River Lee suffers from anthropogenic impacts resulting in poor water quality. These poor water quality issues need to be addressed to ensure that the River meets the requirements of the Water Framework Directive.

2.2 Sample sites and criteria for selection of sites

The sample sites used in this research have been split between three phases of the study, the sites used for coliform bacteria survey of the Lee, sites used for the biofilm pilot study and the sites used for the biofilm experimental study. The strategy of this research provided a framework for the design of a systematic study to effectively achieve the study goals. Figure 2.2 shows a flow chart of the criteria for sample site selection. Sites were sampled during the River Lee coliform pilot and the results obtained were used as a basis for the selection of sites for the biofilm pilot and the experimental phase of the study. Sites selected for the biofilm pilot study and the final experimental (coliform and biofilm) study were classified under clean and dirty category based on the results of the coliform pilot study. For the biofilm pilot, ten sites were selected from the 45 coliform pilot sites, five of these were from sites with coliform counts less than 1×10^4 cfu/ 100ml (clean sites), while the remaining five (dirty sites) were selected from sites with coliform bacteria numbers greater than 1×10^4 cfu/100ml.

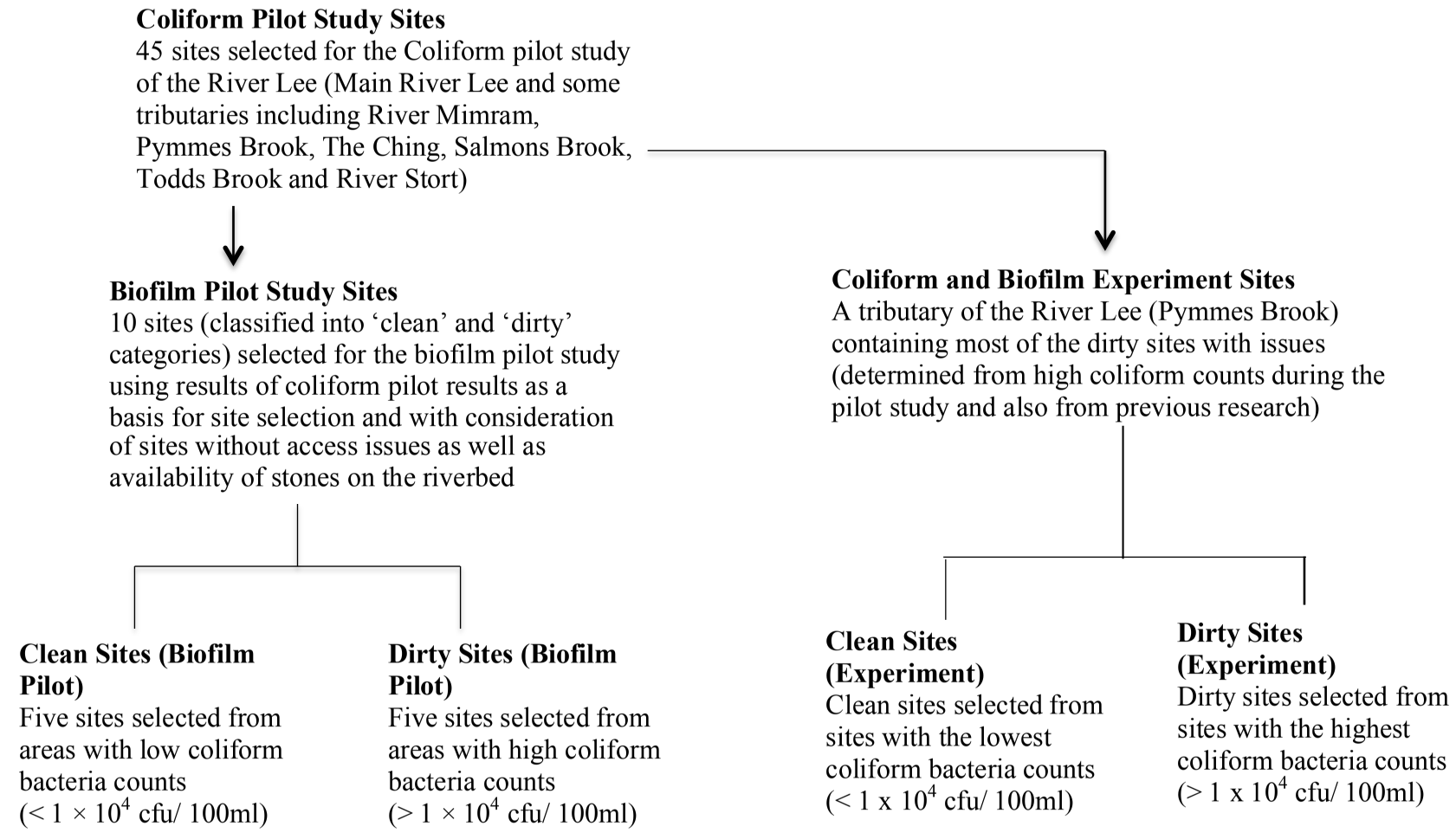


Figure 2.2: Flow chart showing the criteria used for sample sites selection

Similarly, sites selected for the experiment were also a subset of the 45 coliform pilot sites and clean and dirty sites were also selected based on the coliform results obtained during the coliform pilot. In addition, Pymmes Brook was considered because according to previous research, the brook has been known to experience severe diffuse pollution issues (from misconnections and CSO's). However, it was also evident from previous research that overflows from CSO's have reduced significantly (Faulkner et al., 2001). In addition, sites selected on the brook were not located around any agricultural land or inputs from Wastewater Treatment Plants (WwWTP).

2.2.1 River Lee coliform pilot sample sites

The River Lee coliform pilot was carried out in 2013 along the main river and six of its tributaries, Pymmes Brook, The Ching, River Stort, Salmons Brook, River Todd and the Mimram. A list of sites with pollution issues was provided by the Environment Agency. Using this list and a map of the area, an on-ground investigation was conducted to identity accessible areas with outfalls. 45 sites were studied during the coliform survey. Impacts of urbanisation on coliform bacteria and other pollutant levels within watercourses have been widely noted in previous researches (Azzam et al., 2014; Khatri and Tyagi, 2015; Ouattara et al., 2014; Schoonover and Lockaby, 2006; Snook and Whitehead, 2004). Therefore, sites selected included both rural and urban to determine if the coliform bacteria levels were concentrated within the urbanised areas and also to pre-determine potential problem sites to focus on later in the study. Table 2.1 shows a list of the 45 sample sites and the map showing the site locations are given in Figure 2.3. Furthermore, some of the Lower Lee sites in Figure 2.3 were extremely clustered, therefore another map was provided in Figure 2.4 to show a zoomed in section of the River with a clearer view of these sites.

Table 2.1: A list of sites sampled during the coliform survey

Site name	Site number	Easting	Northing
Source of the Lee (Five Springs) Bancroft	1	506100	224800
Road (Birdsfoot Lane) Kingsdown Avenue	2	507586	224050
Brooks Street	3	508874	224043
Recreation Ground Manor Road	4	508663	221967
Cold Harbour Lane Harpenden	5	509716	220926
Leaside Court (a)	6	514101	215585
Leaside Court (b)	7	514414	215300
Leasey Bridge (Cherry Lane)	8	514564	215341
Wheathhampstead Carpark, Meadow Lane	9	516171	214421
Rye House Gatehouse	10	517889	214071
Fisherman Way (a)	11	538397	210009
Fisherman Way (b)	12	538654	209431
Above Ware lock	13	538847	209752
Mimram Road	14	535891	214008
Bessemer Road	15	531807	212326
Stanborough Park	16	526107	214157
Pymmes Park	17	522907	211053
Montagu Road	18	533709	192407
Church Street	19	535159	193329
Littlebury	20	532898	194629
Opposite Cunard Crescent	21	532991	194443
Park Ridings	22	532604	195100
The Chines	23	531998	195437
Slades Hill	24	531877	195302
Jacks Lake	25	531229	196864
Baring Road	26	527218	197251
Cathill 1	27	526859	196532
Cathill 2	28	527219	195426
Oakhill Park	29	527283	195041
Parkside Gardens	30	527751	194832
Eastwalk/ Westwalk	31	528229	194635
Waterfall walk	32	528329	194370
Vincent	33	529095	192967
Winchester	34	538926	191671
Studley Road	35	538383	191160
Beech Hall Crescent	36	538665	191503
Hale End	37	538616	191351
Falmouth (The Charles Road)	38	538670	191664
Waterhall Avenue	39	539026	192049
South Mills	40	539118	192861
Piglane (Twyford Lock)	41	549551	220055
Tendering Road (Todd Brook)	42	549378	219246
Tripton Road (Todd Brook)	43	543922	208917
Howard Way (Todd Brook)	44	544500	209045
	45	544886	209431

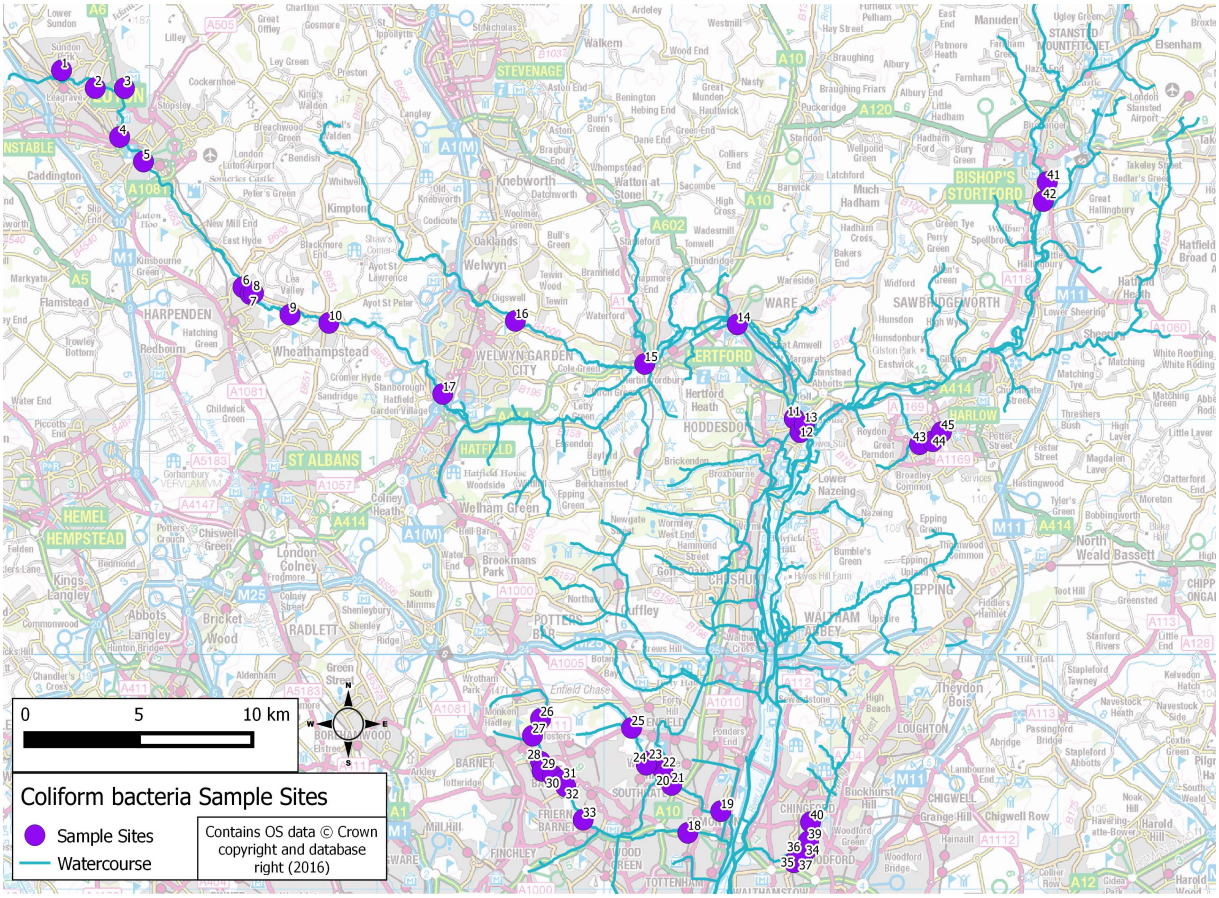


Figure 2.3: Map of the River Lee indicating the 45 coliform bacteria sample sites

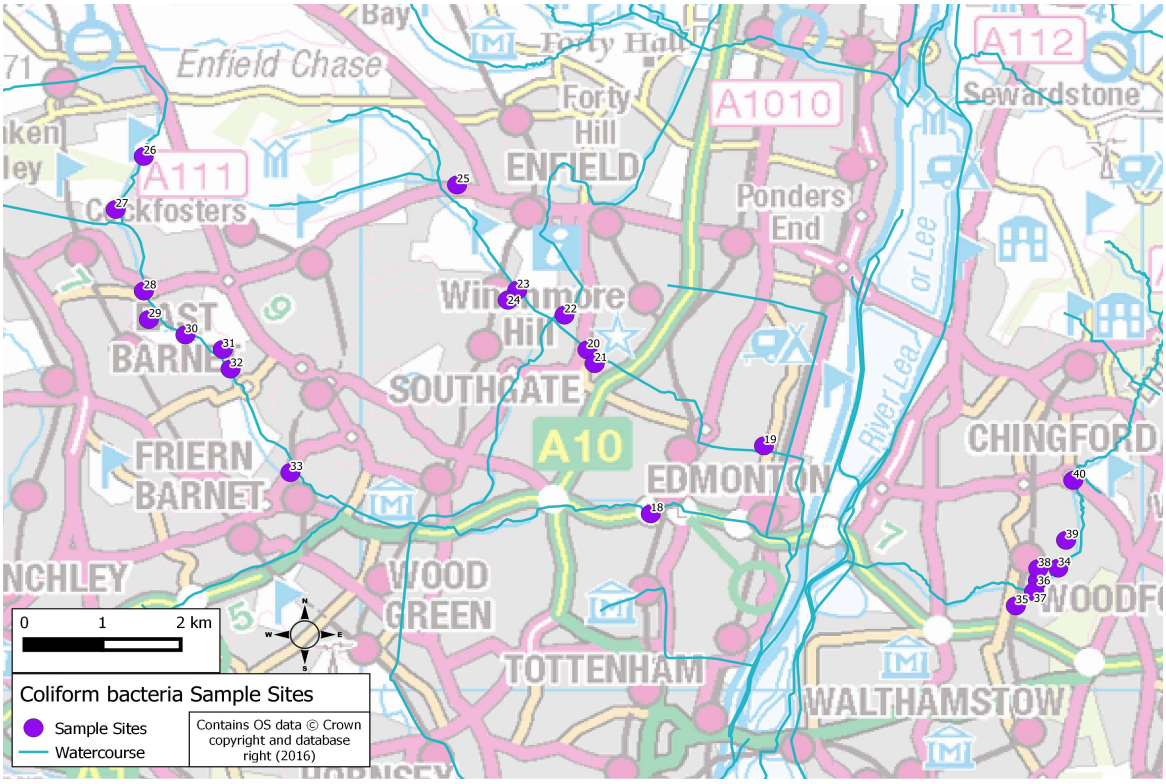


Figure 2.4: Zoomed in section of the map in Figure 2.3

2.2.1.1 Source of the Lee (Five Springs)

The first sample site shown in Figure 2.5 is located at the source of the River Lee in Five Springs Luton. Here the water flows from a gated outfall below a housing estate on the outskirts of the town. The sample was collected downstream of the outfall below the car park of a fifteen story high-rise building. The channel at this site was quite narrow with a stream width between 0.45 – 0.75 metres while the depth at the time of sampling was approximately 4cm. Furthermore, the substratum within this section was made up of a few large cobbles gravels and sand. The river banks were almost bare with short grasses on both sides of the watercourse.



Figure 2.5: Sample site in Five Springs, Luton

2.2.1.2 Brooks Street sample site

Flowing beneath a bridge in Brooks Street, Luton, the River Lee can be seen passing alongside the A6 road and bordered on the opposite side by the Training depot day nursery. This Brooks street site shown in Figure 2.6 is located within a highly urbanised section of Luton and several residential buildings are located here. Grasses dominated the riparian vegetation which was present on just one side of the river as the left bank is an artificial bank made of bricks. Also present are a few trees and some watercress *Rorippa nasturtium-aquaticum*. Channel width and depth are approximately 2.5 metres and 10cm respectively. Water flow at this section of the river was low and the substratum was made up of a combination of silt and cobbles.



Figure 2.6: Brooks Street site

2.2.1.3 Bessemer Road site

At this site, the River Mimram a tributary of the River Lee flows underneath Bessemer Road. The site is located within a relatively rural area with only a few residential properties. There is a pumping station, the Digswell pumping station here, located upstream of area where the water sample was collected. In January and February 2013, this pump was said to have overflowed leading to five sewage overflow incidents from this station, therefore causing a lot of concern for the locals and sampling here was from a request from local community groups. The watercourse within this section of the Mimram looked relatively clear and showed a riverbed mainly made up of gravels. Furthermore, the river at this site is relatively shallow and at the time of sampling had a depth of between 12cm to 15cm. Channel width varied between 3-4 metres. Emergent vegetation was present in some sections of the watercourse and was composed mainly of watercress *(Nasturtium officinale)*. Vegetation on the riverbank was made up of several trees, which include hornbeams and a few shrubs and grasses. Figure 2.7 shows the Bessemer Road sample site.



Figure 2.7: River Mimram at Bessemer Road

2.2.1.4 Southmill Road sample site

This sample site is located on the River Stort, another one of the River Lee's tributaries with its confluence with the Lee in Hertford. The area is also urbanised with several residential and a few industrial properties. At the site, both sides of the riverbank were covered by very dense vegetation, which include trees and shrubs. Some of the species identified include common Nettle *(Urtica dioica)*, Himalayan balsam *(Impatiens glandulifera)* and Alder *(Alnus glutinosa)*. The water in this section of the river appeared clear, flowing over a substratum composed mainly of silt and cobbles. Channel width varied between 3 and 4 metres while the water depth was between 3 - 8cm. Water sample was collected from below an outfall found in this site. Figure 2.8 shows the Southmill Road sample site.



Figure 2.8: The Southmill Road site on River Stort

2.2.1.5 Tripton Road sample site

The Tripton Road site is located on Todd brook, a tributary to the Stort. This section of the catchment is located within a relatively built up area made up mainly of residential buildings. Vegetation on the riverbanks was dense and shaded the watercourse in some sections therefore reducing the amount of light penetration into the brook. The grasses approximately 0.5 metres away from the riverbank on the right hand side of the brook appeared to have been recently cut and that portion of the bank was almost bare. Plant species identified within this section of the brook include Sycamore *(Acer pseudoplatanus)*, Holly *(Ilex aquifolium)*, Ivy *(Hedera helix)* and false brome *(Brachypodium sylvaticum)*. Channel width varied between 1 - 1.5 metres wide and the depth was between 2 – 5cm. River substratum was of London clay and a few small cobbles. Figure 2.9 shows a picture of the sample site and a second picture in Appendix A shows the outfall located in the site.

Figure 2.9: The Tripton Road sample site

2.2.1.6 Montagu Road site

The Montagu road site was one of the sites sampled on Salmons Brook, a tributary of the River Lee in London. The Brook has its source at Enfield Chase adjacent to the previous Enfield Old Park a former nature reserve located in Enfield. At this site, the brook flows alongside the Salmons Walk pathway and is flanked by two cemeteries, the Tottenham Park cemetery and the Edmonton Federation Jewish cemetery. The watercourse also flows underneath a road in the area. When sample was collected, construction work under the Environment Agency's flood alleviation scheme had already commenced. This area is prone to flooding and therefore the scheme aims to reduce flooding by increasing the height of the banks within this section of the river, creating a culvert beneath Montagu Road and to increase the earth mounds around the recreation ground to improve floodwater containment and temporal storage. Located within a high-density area, the brook can be seen behind several residential buildings in the area. There are also several industrial properties located within this area of the watercourse. As at the time of sampling, riparian vegetation seemed to have been cleared. As result, the riverbanks were almost bare and composed mainly of London clay soil. Large amount of debris could also be seen both on the banks and the riverbed within this section of the brook. Channel width was approximately 2 meters and the substratum was made of clay, silt and cobbles. In addition, the depth of the water at this time varied between 8cm to 10cm. Water sample was collected from below an outfall located in this site. A picture of the Montagu Road sample site is given in Figure 2.10.



Figure 2.10: The Montagu road sample site in London

2.2.1.7 Pymmes Park site

The Pymmes Park site in Edmonton is located in a densely urbanised area with several residential houses. The Park is classified as a site of Archaeological Importance, a Site of Local Importance for Nature Conservation (SLINC) and also a Metropolitan Open Land (London Borough of Enfield, 2014). There is an outfall into a lake in this site and this can be seen in Figure 2.11. It has been suggested that this lake suffers from severe pollution impacts. Between November 2012 and May 2013, four different pollution events were recorded leading to sewage contamination and severe reduction in dissolved oxygen levels of the lake (Thames21, 2013). Previous reports also suggest that several misconnections exist in the area leading to the discharge of untreated faecal waste into

the Lake. On visiting the site, it was immediately discernable that the lake environs had an extremely bad odour, a smell often associated with polluted watercourses. Vegetation within this section is predominantly made up of grasses. There are also some shrubs and trees which include species such as the weeping willow *(Salix babylonica)*, plane trees *(Platanus)*, parkland oak *(Quercus robur)* and horse chestnut *(Aesculus hippocastanum)* trees. The water at this site was very turbid, making it difficult to see the riverbed.



Figure 2.11: The Pymmes Park Lake sample site

2.2.2 Sites sampled during the River Lee biofilm pilot study

Table 2.2 shows the ten sites selected for the biofilm pilot study. Locations of the ten sites are also given in the map in Figure 2.12. Descriptions of these sites can also be seen below with the exception of most of the Pymmes Brook sites including Jacks Lake, Cathill, Parkside Gardens and Eastwalk/Westwalk sites all located within the Pymmes Brook subcatchment. These sites mentioned were used in the biofilm experiment and therefore will be discussed in the biofilm experiment site description section.

Table 2.2: Sites sampled during the biofilm pilot study

Clean Sites		Dirty Sites	
Site name	Site number	Site name	Site number
Bancroft/ Birdfoot lane	1	Vincent	6
Kingsdown Avenue	2	Hale End	7
Leaside Court (a)	3	Cathill	8
Tewin Bury	4	Parkside Gardens	9
Jacks Lake	5	Eastwalk/Westwalk	10

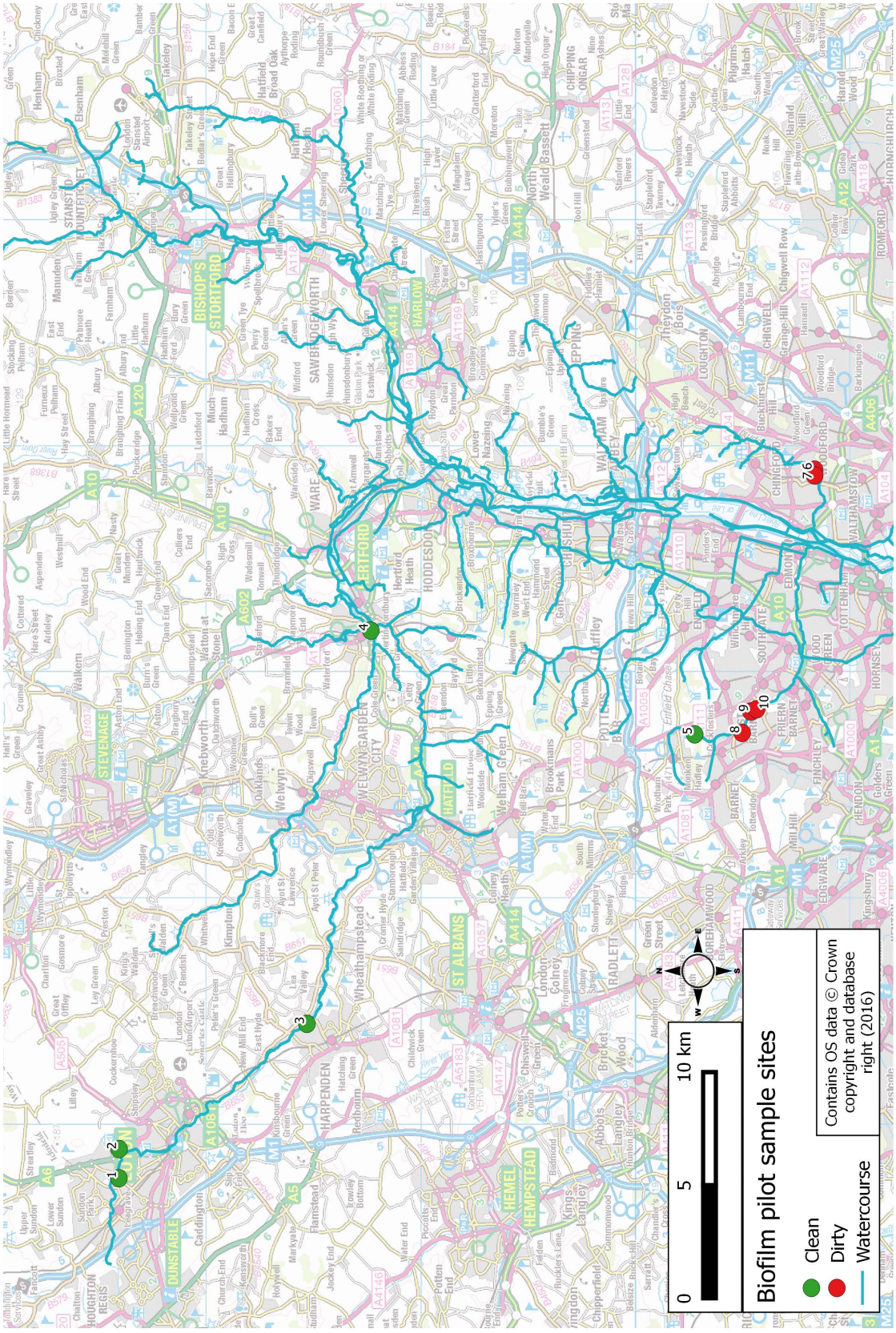


Figure 2.12: Biofilm pilot sample sites

2.2.2.1 Bancroft/ Birdfoot lane site

This sample site shown in Figure 2.13 is located opposite the Icknield Primary School in Luton, Bedfordshire. Flowing through a residential area, the river passes through a concrete culvert under a road in the area. The stream is bordered at the right by a concrete pathway and mowed lawn approximately 1.5 metres away from the banks and at the left by a large mowed park. Riparian vegetation at this site is mainly short grasses, some watercress and a few scattered trees. In-stream vegetation was also present and consisted of a few patches of short grasses. Substratum at the site is mainly fine sand, cobbles and gravels. Furthermore the water depth was approximately 15cm and the width was approximately 3 metres. The height from the water level to the bank varied between 10cm to 25cm at this sample site.



Figure 2.13: The Bancroft/ Birdsfoot Lane site

2.2.2.2 Kingsdown Avenue sample site

The Kingsdown Avenue site, which can be seen in Figure 2.14, is located in an urbanised and highly residential area of Luton. Within this section of the catchment, stream channels were quite narrow at approximately 0.4 metres wide. The depth varied between approximately 2cm and 3cm. Bank height at this site was also relatively low at approximately 10-15cm high. Substratum was predominantly clay and a few small cobbles. Riparian vegetation was mainly made up short grasses, a few tall grasses, a few broad- leaved docks *(Rumex obtusifolius)* and watercress *(Nasturtium officinale)*. Two outfalls adjacent to a narrow concrete pathway were identified behind a housing estate located in the area.



Figure 2.14: The Kingsdown Avenue site

2.2.2.3 Leaside Court (a)

The Leaside Court (a) site shown in Figure 2.15 is located behind the Waterside Office Complex along Lower Luton Road in Harpenden. Land use is mainly residential with few industrial properties. Several residential developments, mainly new build can be seen close to the watercourse at this site. In addition, the water, which passes under a road with a bridge located at this site, is fast flowing over a substratum made of gravels and sand. This section of the River Lee is relatively wide with a width of approximately 4 - 6 metres and a depth of approximately 10cm. River bank height also varied between approximately 10cm to 35cm. Vegetation on one bank of the River was almost exclusively made up of amenity grassland and three weeping willow trees. There is also a concrete road located over 10 metres away from the water's edge. Riparian vegetation on the other bank is made up of a very dense cluster of trees and some shrubs. There were two outfalls located at this section of the River.



Figure 2.15: The Leaside Court (a) site and outfall

2.2.2.4 Tewin Bury (Mimram)

The Tewin Bury sample site is located on a section of the River Mimram, a very rich chalk stream in Hertfordshire. At this place, the river passes through a farm hotel in a designated Site of Special Scientific Interest (SSSI). The area supports a wide range of habitats including reed beds and wet meadows therefore making it attractive to several birds, water voles, insects and other species. Several reed species can be found growing in stream along the channels and they include species such as reed sweet grass *(Glyceria maxima)*, bulrush *(Typha spp.)* and common reed *(Phragmites australis)*. Riparian vegetation also includes species such as townhall clock *(Adoxa moschatellina)*, horsetail *(Equisetum spp.)*, butterbur *(Petasites)* and greater tussock sedge *(Carex paniculata)*. Channel width and depth at this site were approximately 5 - 6 m and 35cm respectively.

2.2.2.5 The Vincent and Hale end sites

The Vincent and Hale end sites are located within a short distance of each other with similar characteristics and as such will be discussed together. These two sites shown in Figures 2.16 and 2.17 respectively are located on the Ching, a watercourse that originates in Epping Forest. The Ching, a tributary of the River Lee is located in a highly urbanised area of London. It is reported that there are several misconnections impacting severely on certain sections of river (Thames Water, 2009). Land use is mainly residential with several 1930's built houses located around the river at these sites. There is a lawn tennis court located here as well as the Oakhill Primary School. This section of the River flows through very dense vegetation dominated mainly by brambles *(Rubus fruticosus agg.)* and some trees. This vegetation limited the amount of light penetration into the water. Stream channel width varied between 2 to 3 metres and the depth was approximately 6cm. There were scanty sections of short grasses scattered all over the riverbank, which had a height of approximately 20cm from the surface of the water. Above

the banks, the River is bordered on one side by a concrete pathway and on the other side by a mown grass lawn located adjacent to the water just before a school playground in the area. Water in this section was slow flowing over a substratum made up of a few stones and silt. Two outfalls were located here and samples were collected from below these outfalls.



Figure 2.16: The Vincent sample site



Figure 2.17: An outfall located in the Hale end sample site

2.2.3 Sample Sites for Biofilm Experiment

The pilot as well as previous work (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000) provided an insight into problem areas to focus on and so sample sites for the biofilm experiment were selected based on the quality and also physical accessibility of sites. Six sites were monitored during the study, one control site and five sites on Pymmes Brook, a tributary of the Lee, located in North London. Figure 2.18 shows a map of the densely urbanized Pymmes Brook catchment. This catchment has an area of approximately 42.6 km^2 and is located above Edmonton Silver Street. The watercourse flows from its source in Monken Hadley Common, and passes through several areas some of which include New Barnet, East Barnet, Friern Barnet, Palmers Green and Edmonton. The Brook then meets up Salmons Brook downstream of Edmonton's Silver Street above the Banbury Reservoir and then flows south of Tottenham Marshes where it has its confluence with the River Lee. Land use within the Pymmes brook catchment is mainly residential and over 80% of the catchment area through which the watercourse passes is urbanized (Centre for Ecology and Hydrology, 2014). Figure 2.19 shows a map of the catchment area with the built up and wooded sections. Historic data obtained from previous research shows that this Brook has been severely polluted from urban diffuse sources of which CSO's and misconnections constitute the major routes of pollutants into the watercourse (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000). Coliform bacteria levels identified during the coliform survey also confirmed this poor water quality status. The sites monitored during the biofilm experiment are discussed below.

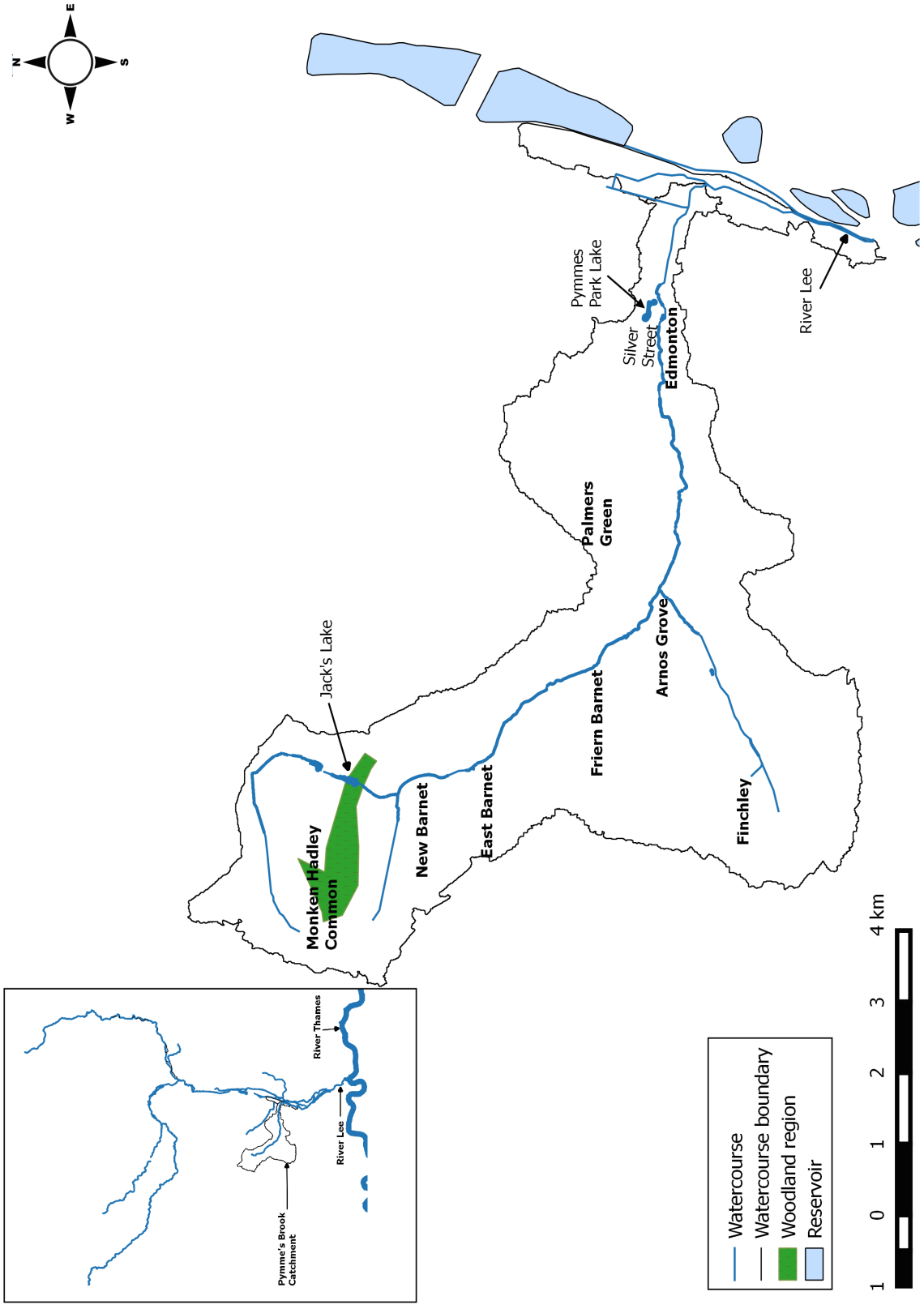


Figure 2.18: A map of the Pymmes Brook catchment

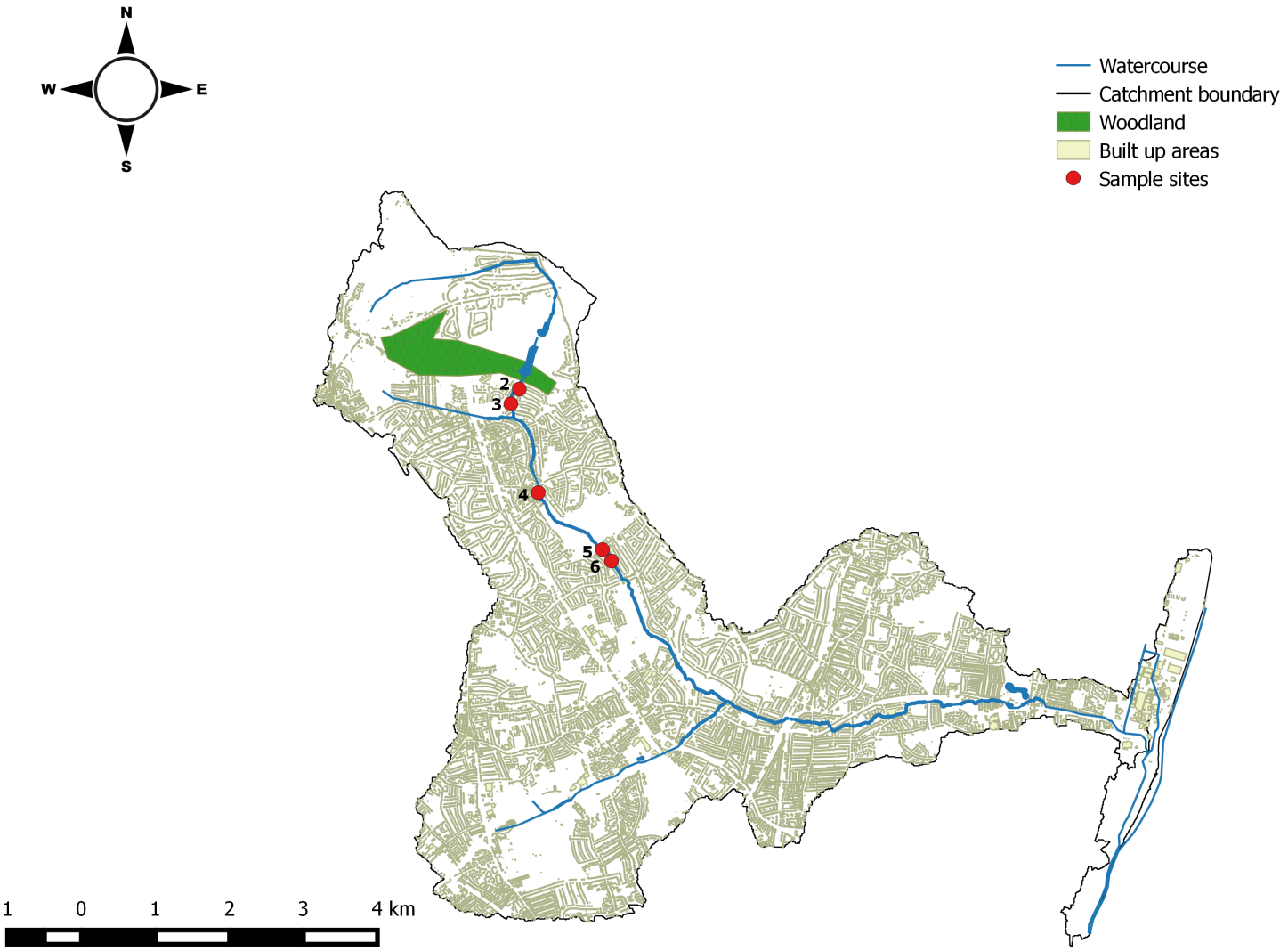


Figure 2.19: Map of Pymmes Brook with sample sites used for the experiment

2.2.3.1 Bayfordbury (Control site)

Site 1, the Bayfordbury sample site was located in an artificial stream in Bayfordbury. This model stream is located within a glasshouse in the University of Hertfordshire's Bayfordbury field station. Pymmes Brook the study watercourse has a geology of clay stream overlying chalk. The Bayfordbury site was therefore selected as the control site because it has similar geology, however unlike Pymmes Brook, it is an isolated system, known to be relatively clean with limited pollutant inputs.

2.2.3.2 Jacks Lake

The Jacks Lake sample site is located at the brooks headwaters in Monken Hadley Common. This site is located within a relatively rural section of the Pymmes Brook catchment. Jacks Lake previously served as a boating lake in the Victorian era, however it is currently being used for fishing (Hadley Angling and Preservation Society, 2005). Riverbed and banks at this site are composed of London clay with alluvial deposits and gravels. Water depth varied between approximately 3cm and 15cm. Furthermore, vegetation in this section of the catchment is mainly woodland vegetation composed of several trees, few grasses and some shrubs, which include species such as the midland hawthorn and common hazel *(Corylus avellana)*. Figure 2.20 shows the Jacks Lake sample site.



Figure 2.20: The Jacks Lake sample site

2.2.3.3 Baring Road site

The Baring Road site (site 3) which is located close to Livingstone primary and nursery school, is the next site situated downstream of Jacks Lake. The Jewish Community Secondary School, a recently constructed school founded in 2010, is also situated further west of the sample site. Here, the watercourse can be seen flowing underneath a bridge and alongside several residential buildings. Riparian vegetation was very dense at the Baring Road site with species such as the field maple tree and common hazel *(Corylus avellana)* seen growing on the banks and heavily shading the watercourse in some sections. Other vegetation seen here include species such as stinging nettles *(Urtica dioica)*, polypody fern *(Polypodium spp.)*, common brambles, cow parsley, dandelions and the dock leaf *(Rumex obtusifolius)*. The bank is made up of London clay soil type while riverbed substratum is composed of clay and gravels. Bank height from the level of the watercourse ranged between approximately 1.5 to 2 metres high while channel width and depth were between 3 - 4 metres and 3 - 8 cm respectively. The 2013 pilot study as well as a previous study on this site (Nwanekezie, 2009) identified it as a relatively clean section of the brook. Water sample was collected from below an outfall located in the site. On two occasions during sample collection, this outfall was discovered to be discharging foamy water into the watercourse. Figure 2.21 shows the Baring Road sample site.



Figure 2.21: The Baring Road site

2.2.3.4 The Cathill sample site

The Cathill site (site 4) which can be seen in Figure 2.22, is the third study site selected on Pymmes Brook. Previous studies carried out have shown that this section is a major problem area in the Brook. For several years, this particular area of the catchment has been known to experience severe water quality issues (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000; Nwanekezie, 2009). In one of the studies carried out, it was discovered that apart from an extremely poor ecology, there was a constant occurrence of bad odour and growth of sewage fungus downstream of the outfall located in the site (Faulkner et al., 2000). This sample site is situated in a densely urbanised area of the catchment. Several residential buildings, a car park, offices and roads are located in this area. Riparian vegetation consists of some timothy grass *(Phleum pratense)*, false brome *(Brachypodium sylvaticum)* brambles *(Rubus fruticosus agg.)*, some stinging nettles *(Urtica dioica)*, curled dock weed *(Rumex crispus)*, polypody fern *(Polypodium spp.)*, broad leaf dock and also some field maple trees. Furthermore, at this sample site, there is a mowed lawn bordering the watercourse on the left side next to a concrete pathway, a block of residential buildings and a road. On the right side of the watercourse, there is a car park and several residential buildings. Channel width was approximately 2 - 3 metres wide while the depth varied between 3 to 15cm. Bank height from the water level varied and was between 0.5 - 1.5 metres high. The previously reported bad odour was also discovered during sampling. Water in this section of the Brook could be seen flowing over a substratum composed of clay soil type, gravels and large cobbles.



Figure 2.22: The Cathill sample site

2.2.3.5 Parkside Gardens

Site 5, the Parkside Gardens site shown in Figure 2.23 is the fourth sample site located downstream of the brook. Running alongside long stretches of amenity grass, the brook can be seen owing underneath a bridge in the area. This section of the catchment is also highly urbanised with several residential buildings located here. Some trees including weeping willows *(Salix babylonica)* species can be seen growing both on the mowed lawn bordering the watercourse as well as on the riverbanks. Riparian vegetation include species such as stinging nettle *(Urtica dioica)*, broad leaf dock *(Rumex obtusifolius)*, a few dandelions and also cow parsley. The watercourse at this site can be seen owing over a substratum mainly composed of clay and gravels. The depth and channel width are approximately 8cm and 2.5 metres respectively. While the bank height was between 30 - 35cm.



Figure 2.23: Parkside Gardens sample site

2.2.3.6 Eastwalk/ Westwalk site

The last sample site selected on Pymmes brook was the Eastwalk/Westwalk site (site 6). A picture of this site can be seen in Figure 2.24. Also located within a densely urbanised section of the catchment, the watercourse can be seen flowing beneath a bridge in the area. At the site, the brook is sandwiched between long stretches of amenity grass. There are also concrete pathways some metres away from the watercourse on both sides. Land use within the area is mainly residential with blocks of residential properties seen on both sides of the watercourse. There were no shading from large trees or shrubs and as a result the watercourse had unlimited light penetration. Riparian vegetation seen at the Eastwalk/Westwalk site includes species such as the stinging nettle *(Urtica dioica)*, cow parsley and broad leaf dock *(Rumex obtusifolius)*. On physical observation, the flow of the river seemed quite slow with a mass of debris causing blockage in a section downstream of the bridge. The presence of bad odour was also noted on all sample days and dense collection of sewage fungus made it difficult to see the riverbed in most parts of the site. However, directly underneath the bridge, there was an area that was slightly visible and it showed a riverbed with some debris and a substratum made up of London clay soil type and large cobbles. Furthermore, during sampling in August, a large quantity of foam was discovered in this section of the watercourse possibly indicating washing machine activity via a misconnection. Other characteristics of the Eastwalk/Westwalk site include a channel width of approximately 2 - 2.5 metres wide and a depth of approximately 24cm. Bank height from the water level was also approximately 40cm high.



Figure 2.24: The Eastwalk/ Westwalk sample site

Chapter 3

Assessment of the Impacts of Coliforms on Water quality of the River Lee

3.1 Introduction

The discharge of untreated wastewater into rivers in urban areas could lead to the abundance of several pathogenic organisms including bacteria, protozoa and viruses (Staley et al., 2012). Such polluted watercourses pose threats to the environment and severe risks to public health (Teklehaimanot et al., 2014). Therefore amongst residents living around such watercourses, the occurrence of skin irritation, gastrointestinal illnesses and other waterborne illnesses could potentially increase. In the UK, there have been several recorded cases of disease outbreaks resulting from contact with polluted water. For instance in 2012, there was a gastrointestinal outbreak that occurred amongst participants of a swimming event on the River Thames (Public Health England, 2013). Similarly, there was also a norovirus outbreak that occurred in Strathclyde loch among swimmers in 2012 (Fewtrell and Kay, 2015).

Monitoring the coliform bacteria levels within watercourses could assist towards the assessment of its quality and associated risks. Levels identified are usually compared against standard levels stipulated by the relevant legislation. Currently in the United Kingdom, there

are no guidelines or regulations provided for the levels of coliform bacteria in urban rivers and streams. However, the EU Bathing Water Directives (76/160/EEC) (EEC, 1976) stipulate guidance and imperative (threshold) levels for certain indicator organisms in designated bathing waters. The UK is however transitioning into the new Bathing Water Directive (2006/7/EC) (EC, 2006) which when implemented, will be much stricter than the current directive (Huang et al., 2015). According to the current directive (76/160/EEC), guidance and imperative levels for faecal coliforms are 100 cfu per 100ml and 2,000 cfu per 100ml respectively while for total coliforms, guidance and imperative levels are 500 cfu per 100ml and 10,000 cfu per 100ml respectively. There is no mandatory level for faecal *Streptococci*, however guidance level provided is 100 cfu per 100ml.

As mentioned previously, multiple parameters will be applied to assist towards the identification of misconnection hotspots on the study catchment. Misconnections introduce untreated faecal waste into watercourses therefore increasing pathogen levels and the potential for the occurrence of related illnesses within misconnection hotspot areas. The River Lee catchment cannot be monitored for all the organisms usually associated with faecal waste, therefore coliform bacteria was selected as the bacterial indicator. Coliform bacteria a re a group of Gram negative, non-spore forming bacteria that ferment lactose and appear as rod shaped bacilli under the microscope (Kumawat and Sharma, 2015). These bacteria grow at a pH range of 4.4 to 9 and temperature ranging between 10°C and 45°C (Prescott et al., 1990). Optimal temperature and pH for the growth of these organisms are 37°C (Nelson et al., 1996) and 6-7 respectively (Prescott et al., 1990). Although rarely pathogenic with few exceptions, they are usually associated with faeces and have been commonly used as indicators of contamination from sewage (Pal, 2014).

The use of coliform bacteria as indicators of human faecal contamination has some limitations. This is because their occurrence in watercourses could be due to a variety of sources such as wild animals, birds, runoff from farmlands and industrial processes

(Dombek et al., 2000; Ferguson and Signoretto, 2011; Hachich et al., 2012; Leclerc et al., 2001). However, sewage discharges from sources such as failed septic systems and misconnections have also been shown to be major contributors of coliform bacteria levels in watercourses (Ahmed et al., 2005; Griffin et al., 2001). As a result, despite their limitations, they are widely used in water quality assessments (Mounjid et al., 2014; Noble et al., 2003; Schreiber et al., 2015). Their detection in significantly high concentrations could provide an early indication of an increase in microbial loadings from sewage contamination. Therefore in this current study, bearing in mind their potential limitations, they were used in combination with the monitoring of biofilm communities for the location of hotspots within the study catchments. The monitoring of coliform bacteria was carried out in two phases. The first being a pilot study of the River Lee and some of its tributaries, and the second focused on Pymmes Brook one of the tributaries as a case study. The River Lee coliform pilot was conducted to provide information on likely problem areas within the river and therefore determine sections or tributaries to focus on for the next stages of the research.

3.2 Methodology

3.2.1 Coliform sample site selection

The coliform pilot study was conducted along the River Lee and some of its tributaries between March and July 2013. Several steps were taken to predetermine sample sites to be used during the pilot. Firstly, the Environmental Agency's water quality data summary for UK Rivers was studied to provide an idea of potential problem areas (Environment Agency, 2009b). An on-ground investigation was also conducted to identify accessible areas with proximity to outfalls. Using all these information obtained, 45 sample sites were selected along the stretch of the River Lee for the coliform bacteria pilot. Sites were selected both upstream and downstream of the River. In some sites with minor accessibility issues

water samples were collected using a bucket and rope. The second coliform monitoring phase was carried out within the biofilm experimental study between April and August 2014. Six sites were selected for the study, five located on Pymmes Brook and a control site in Bayfordbury artificial lake. These sites were classified into clean and dirty site categories.

3.2.2 Field methods

A risk assessment was conducted before the collection of all samples. Water samples were collected using clean, 100ml plastic bottles with screw tops. A total of 45 water samples were collected during the pilot, one sample (100ml) per study site. However, for the experiment, five replicate 100ml water samples were collected from each of the selected six sites. Representative samples were collected mid-channels with proper consideration of outfall locations and accessibility. Since the issue being investigated was misconnections, most of the samples were taken at low flow to further maximise the chance of identifying coliform bacteria from recent faecal discharges (Faulkner et al., 2000). The plastic bottles were filled with river water and closed with the screw tops. Bottles were then labelled with site location, date and time. All samples were then stored at approximately 4°C and taken to the laboratory for analysis within 4 hours. All these were done based on standard methods for the examination of waters and associated materials (Environment Agency, 2000, 2009a).

3.2.3 Laboratory methods

Water analysis for coliform bacteria was started immediately on arrival at the laboratory. This was done generally within six hours of sample collection. The membrane filtration technique was used to assess the water samples for coliform bacteria levels. Four sub-samples using different sample volumes (0.01ml, 0.1ml, 1ml and 10ml) were filtered per sample. This was done to be able to obtain the best plate for counting, the recommended range usually between 20 – 80 colonies where possible.

Each sub-sample was filtered through a membrane filter with a diameter of 47mm and pore sizes of 0.45 μm. For sites for which sample volume filtered was less than 10ml, approximately 20 ml of Ringers solution was first added to the funnel before adding the water sample to aid the distribution of the bacteria on the surface of the filter paper (Environment Agency, 2000, 2009a). To culture the coliform bacteria, membrane filters were then placed on previously prepared sterile absorbent pads (soaked through with lauryl sulphate broth) and then incubated at two different temperatures, 30°C for a 4 hour duration and then 44°C for 14 hours. When cultured using lauryl sulphate broth, coliform bacteria ferment the lactose in the broth and produce acid during the fermentation process. On detection of acidification, the pH indicator (phenol red) causes a colour change to yellow, therefore all yellow colonies formed were regarded as coliform bacteria (Environment Agency, 2009a). These colonies were counted and subsequently, the number of coliform bacteria per 100ml of the water sample was calculated and recorded as coliform forming units (cfu) per 100mls.

3.3 Data analysis methods

3.3.1 GIS mapping

The coliform data obtained during the River Lee survey and the experiment on Pymmes Brook were processed using the Geographic Information Systems (GIS) Software QGIS version 2.8.2. Firstly, layers were obtained from the Edina digimap website and then these were uploaded on QGIS software and the results overlayed on the maps. Maps generated indicated concentrations and distributions of coliform bacteria within the study catchment.

3.3.2 Statistical analysis

Statistical analysis was conducted on the coliform data obtained for the Pymmes Brook experiment using IBM SPSS base package version 22. This software was used to calculate basic descriptive statistics including means and standard errors for the coliform dataset.

3.4 Results

3.4.1 River Lee coliform survey

A map was produced to show the coliform bacteria results obtained during the River Lee coliform survey. This map is given in Figure 3.1 below. The results obtained for the coliform bacteria survey of the River Lee showed that the worst levels seemed to occur mainly in the tributaries located within the densely urbanised reaches of the lower Lee. Apart from the River Stort and Todd Brook sites, coliform concentrations identified within the Upper Lee were relatively low compared to the levels identified within majority of the sites located in the lower Lee tributaries. For instance, of the fifteen sites located between the source of the river in Luton and the Fisherman Way (a) and (b) sites in Hoddesdon, thirteen sites fell between the 1×10^2 cfu and 5×10^3 cfu per 100ml range. Coliform concentrations within the remaining two sites, the Leaside Court (a) and Leaside Court (b) sites in Harpenden were 5.2×10^3 cfu per 100ml and 6.1×10^3 cfu per 100mls respectively. On the River Mimram, coliform levels were relatively low, falling also within the 1×10^2 cfu and 5×10^3 cfu per 100ml range as shown on the map. The two Mimram sites, Mimram Road and Bessemer Road had relatively low concentrations with both having coliform bacteria levels of 3×10^2 cfu per 100ml. Coliform levels within Southmills and Piglane, the two sites located on the Stort exceeded the EU Bathing Water Directive threshold at 1.3×10^4 cfu per 100ml and 1.5×10^4 cfu per 100ml respectively. The Todd Brook sites also exceeded the threshold levels. Levels within Tendering Road and Tripton Road were within the 1×10^4 to 5×10^4 cfu per 100ml range and Howard way exceeded this range at 9.7×10^4 cfu per 100ml.

Within the lower Lee tributaries, coliform levels exceeded the maximum accepted levels in most of the sites. On the Ching for instance, coliform levels were high across all the sites sampled. Three of these sites, Beech Hall Crescent, Falmouth and Waterhall Avenue were within the range of 5×10^4 to 1×10^5 cfu per 100ml, while coliform levels

within the rest of the sites exceeded 1×10^5 cfu per 100ml. In addition within Pymmes Brook, coliform concentrations detected in three sites, Cathill 1, Cathill 2 and Parkside Gardens were over 1×10^5 cfu per 100ml. The site with the highest coliform bacteria counts was the Cathill 2 site and levels within this site exceeded 3×10^5 cfu per 100ml. Only two sites, Jacks Lake and Baring Road had relatively low coliform bacteria concentrations falling between 1×10^2 cfu per 100ml and 5×10^3 cfu per 100ml. The remaining sites on the Pymmes Brook subcatchment fell within the range of 1×10^4 to 5×10^4 cfu per 100ml. The third tributary, Salmons Brook also had high coliform bacteria levels identified in most of the sites sampled. Only one site within this Brook had coliform levels below 5×10^3 cfu per 100ml. Levels within two of the Salmons Brook sites were over 1×10^5 cfu per 100ml with Park Ridings having 1.5×10^5 cfu per 100ml and Slades Hill having 1.2×10^5 cfu per 100ml. The rest of the sites within Salmons Brook were within the range of 1×10^4 to 5×10^4 cfu per 100ml.

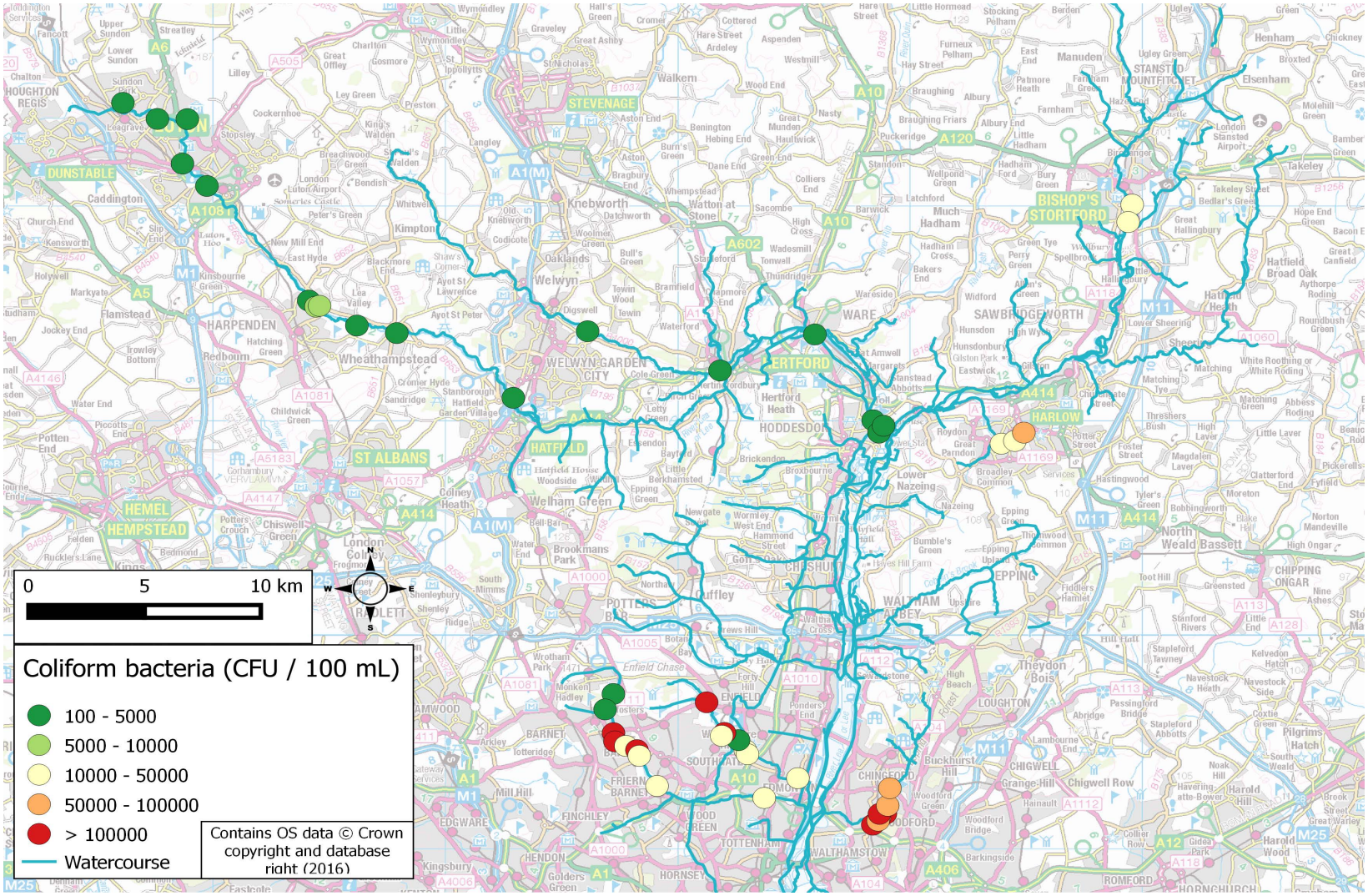


Figure 3.1: River Lee Coliform survey concentration and distribution (site names and numbers are provided in Table 2.1 and Figure 2.3

3.4.2 Pymmes Brook experimental study coliform results

The coliform results obtained for the Pymmes Brook catchment during the experimental study also showed poor coliform bacteria water quality in several sites. Sites with evidence of this contamination include Baring Road, Cathill, Parkside Gardens and Eastwalk/Westwalk. The map in Figure 3.2 shows mean coliform bacteria counts for the Pymmes Brook 2014 study. Figure 3.3 also shows the mean coliform bacteria levels for all the sites with the error bars in the graph indicating standard error of the mean. Results of the study showed that levels were low in the Bayfordbury and rural Jacks Lake sites. Generally within the Brook, Figures 3.2 and 3.3 show a clear pattern of coliform bacteria increase from the rural to the urban areas of the catchment. To test for variations in coliform levels between the sites over the study period, a one-way ANOVA test was conducted. The calculated F value ($F = 3.054$) was larger that the critical F value ($F(5,24) = 2.63$), this difference is considered significant at ($p = 0.028$). The lowest coliform bacteria concentrations were identified in Jacks Lake, while Cathill appeared to be experiencing the worst quality with coliform levels exceeding 2.5×10^5 cfu per 100ml as shown on the map in Figure 3.2. The graph also indicates that coliform bacteria levels were low in the control site at Bayfordbury and in rural Jacks Lake. After Jacks Lake, there was a sharp increase in coliform bacteria levels at Baring Road and levels continued to be high up to the Eastwalk/Westwalk site, the last sample site downstream of the Brook. Levels within the last four sites downstream were exceptionally high with the highest level identified in Cathill. Coliform concentrations within the Cathill site were around 2.8×10^5 cfu per 100ml. Figures 3.4 – 3.9 below also show monthly coliform bacteria concentrations for each site. The graphs show that variations in coliform bacteria levels did not follow a consistent trend in the control site. On the other hand, results obtained for Pymmes Brook showed that for all the sample sites, July and August had

the highest coliform bacteria concentrations. While the results provided above indicate coliform concentrations recorded for the sites during the study period, there are however several variables that affect coliform bacteria, leading to their increased levels or die-off rates within watercourses. These variables include rainfall, temperature, solar radiation and several other variables. Two major parameters, rainfall and solar radiation were identified from the literature and further investigated. It has been shown in previous studies that rainfall leads to the flushing and subsequent discharge of bacteria and other pollutants into watercourses (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000; Scholes et al., 2007; Tornevi et al., 2014), whilst solar radiation has been noted as the single most influential parameter affecting coliform bacteria mortality in watercourses (Auer and Niehaus, 1993; Rozen and Belkin, 2001; Šolić and Krstulović, 1992; Whitman et al., 2004). To investigate the effects of these parameters on coliform bacteria levels recorded during this current study, rainfall and solar radiation data were obtained for the sample period. The rainfall data was collected by the Bayfordbury weather station and the data on solar radiation was obtained from Kings College, London.

The rainfall data given in Figure 3.10 shows the total rainfall that occurred one week prior to sampling. From the rainfall graph, it can be seen that no rainfall event occurred the week before sampling in April and May while rainfall in June was limited with a total rainfall of 0.4mm recorded the week prior to sample collection. On the other hand, July and August had the highest occurrence of rainfall the week before sampling. Rainfall recorded for these two months were 15.7 mm and 38.5 mm respectively.

Furthermore, the solar radiation records obtained from Kings College is given in Appendix D while the radiation records on the days sampled in each study month are provided in Figure 3.11. These records show that for the sampling days, May and June had the highest solar radiation records (516W/m^2) while the lowest record (216 W/m^2) during the study was in August.

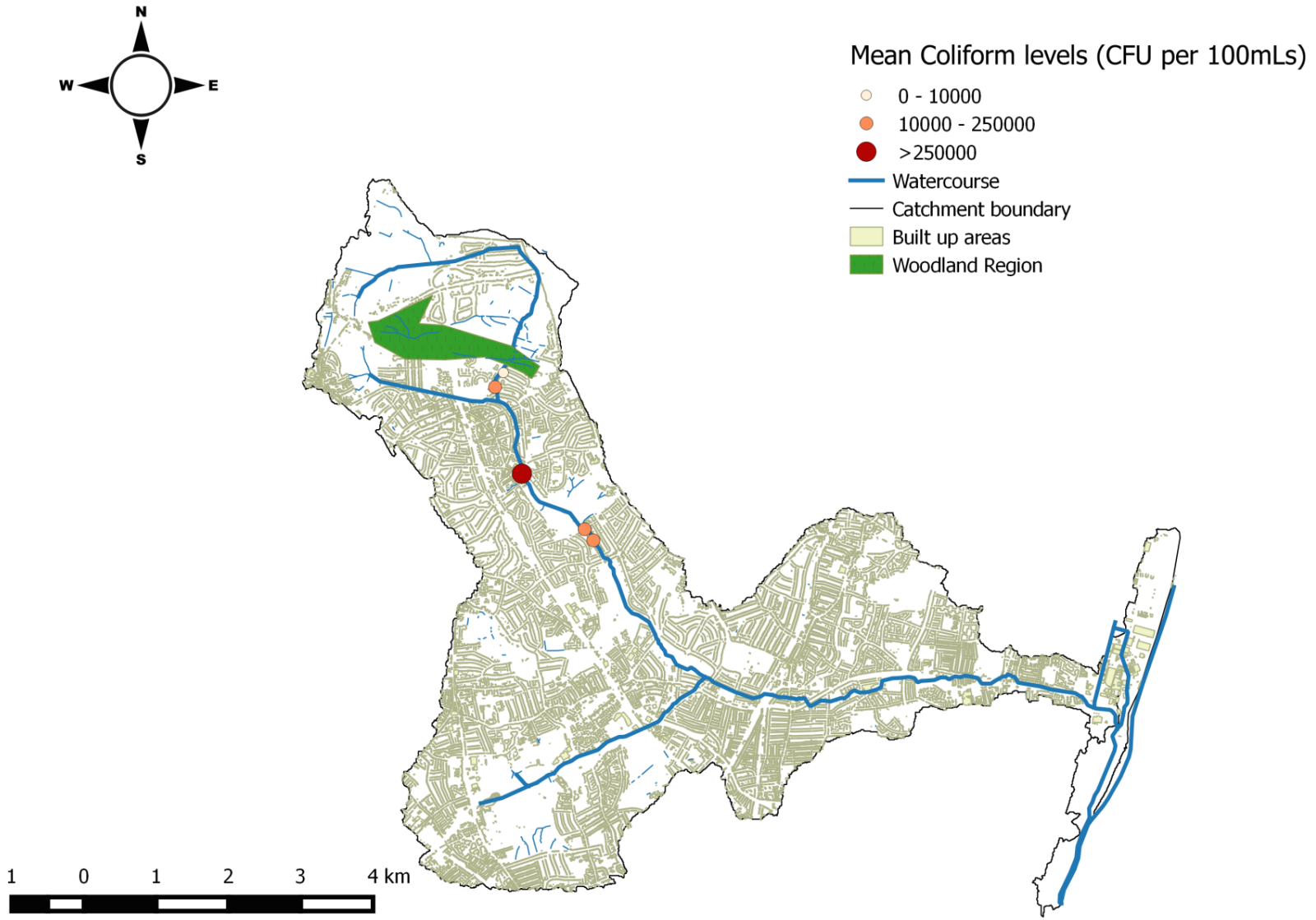


Figure 3.2: Pymmes Brook map with 2014 mean coliform bacteria counts

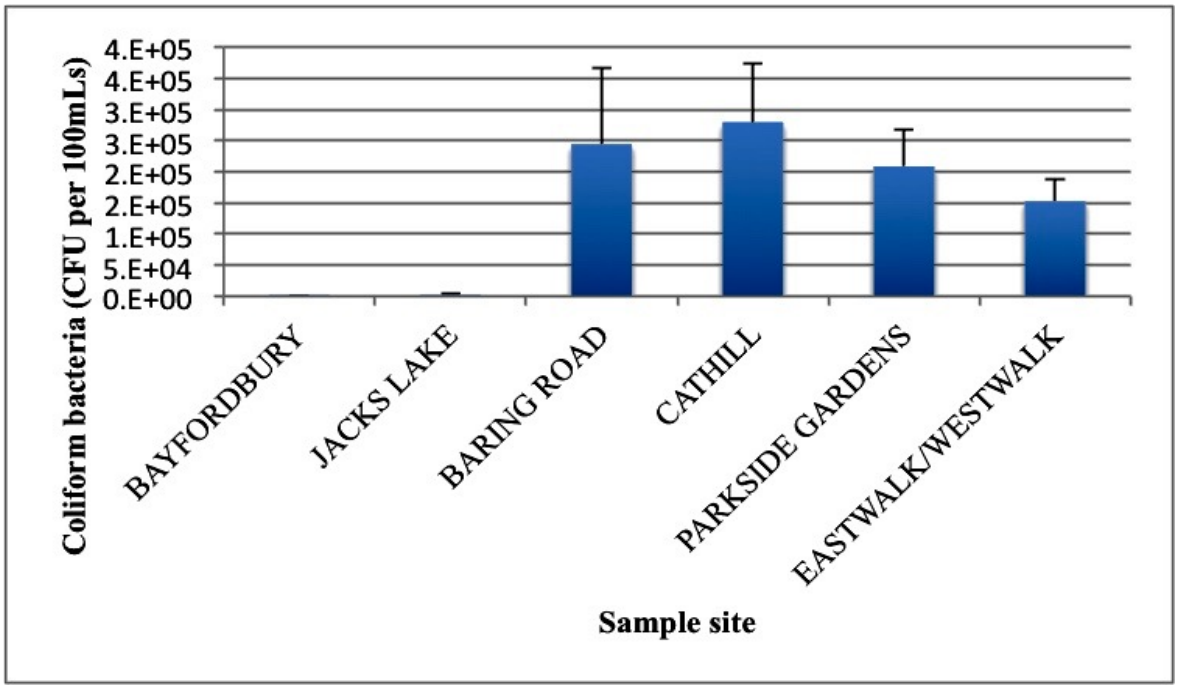


Figure 3.3: Mean coliform levels (Pymmes Brook 2014)

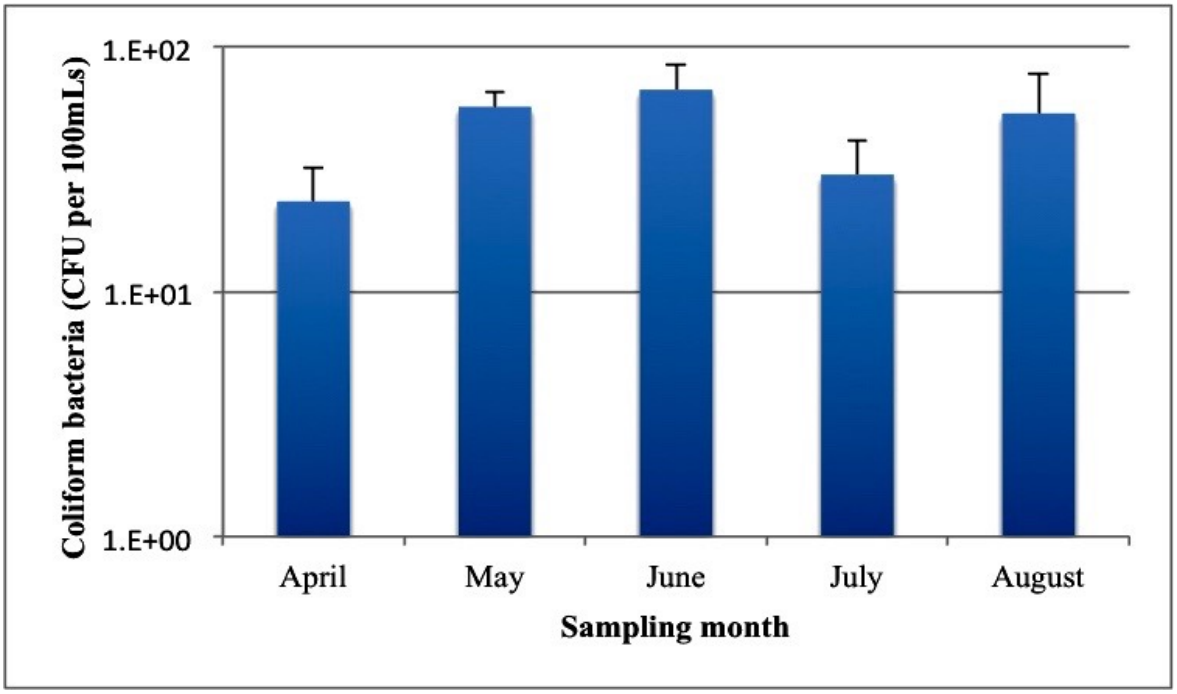


Figure 3.4: Monthly mean coliform counts for Bayfordbury

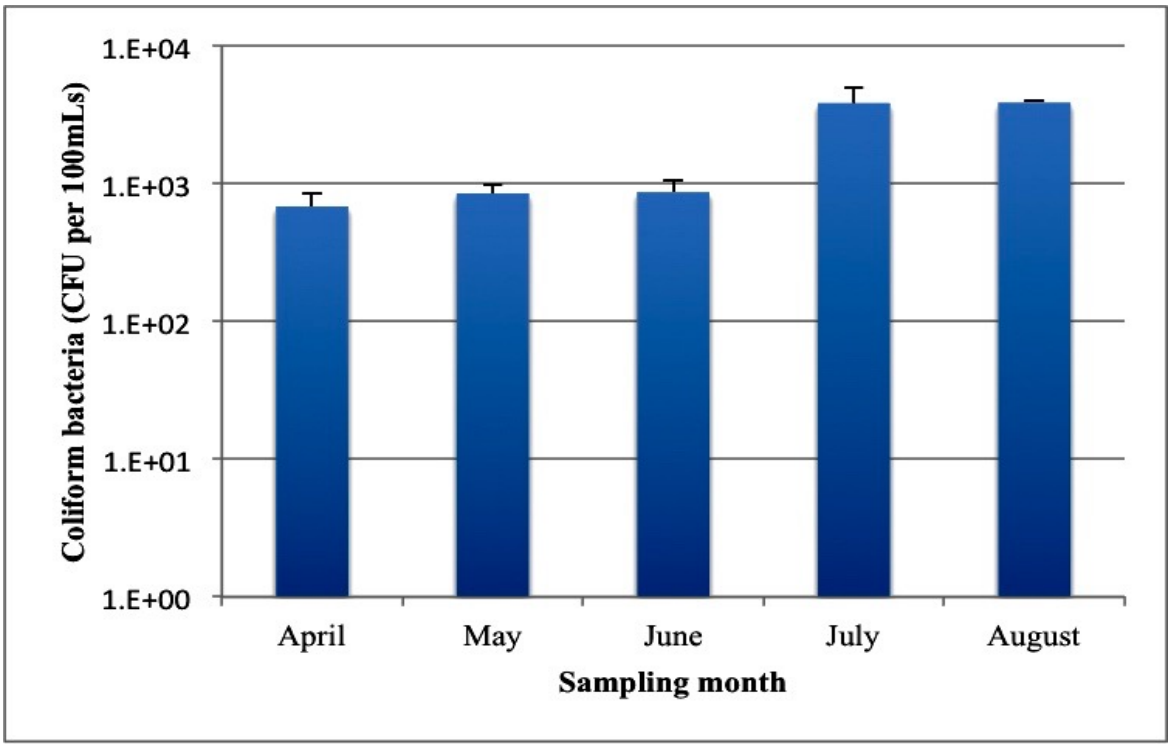


Figure 3.5: Monthly mean coliform counts for Jacks Lake

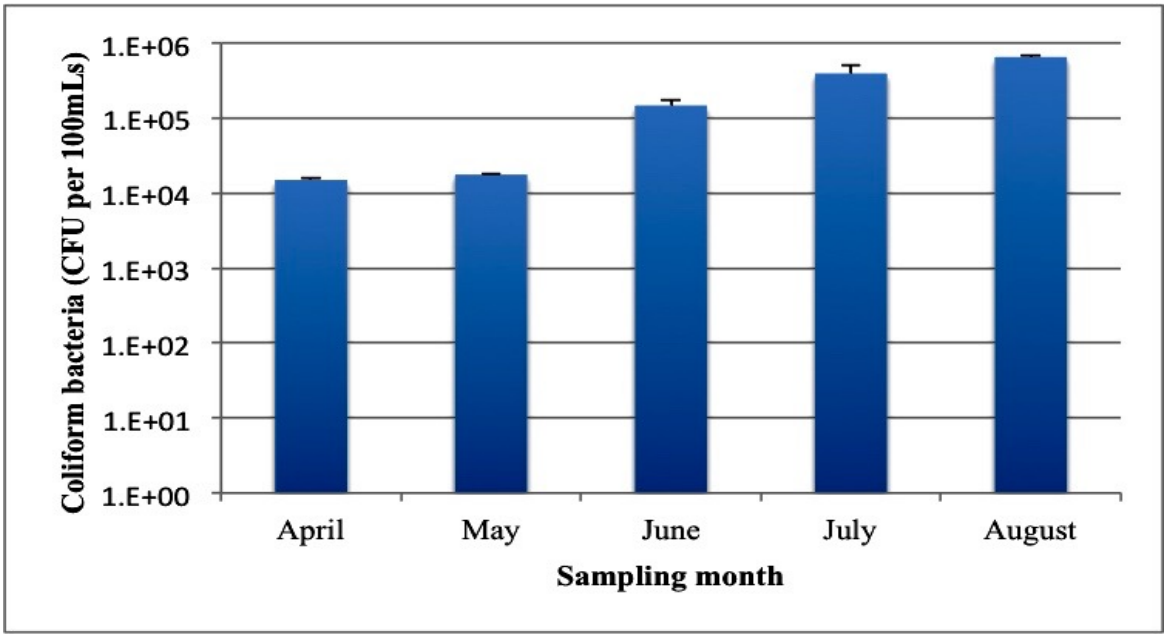


Figure 3.6: Monthly mean coliform counts for Baring Road

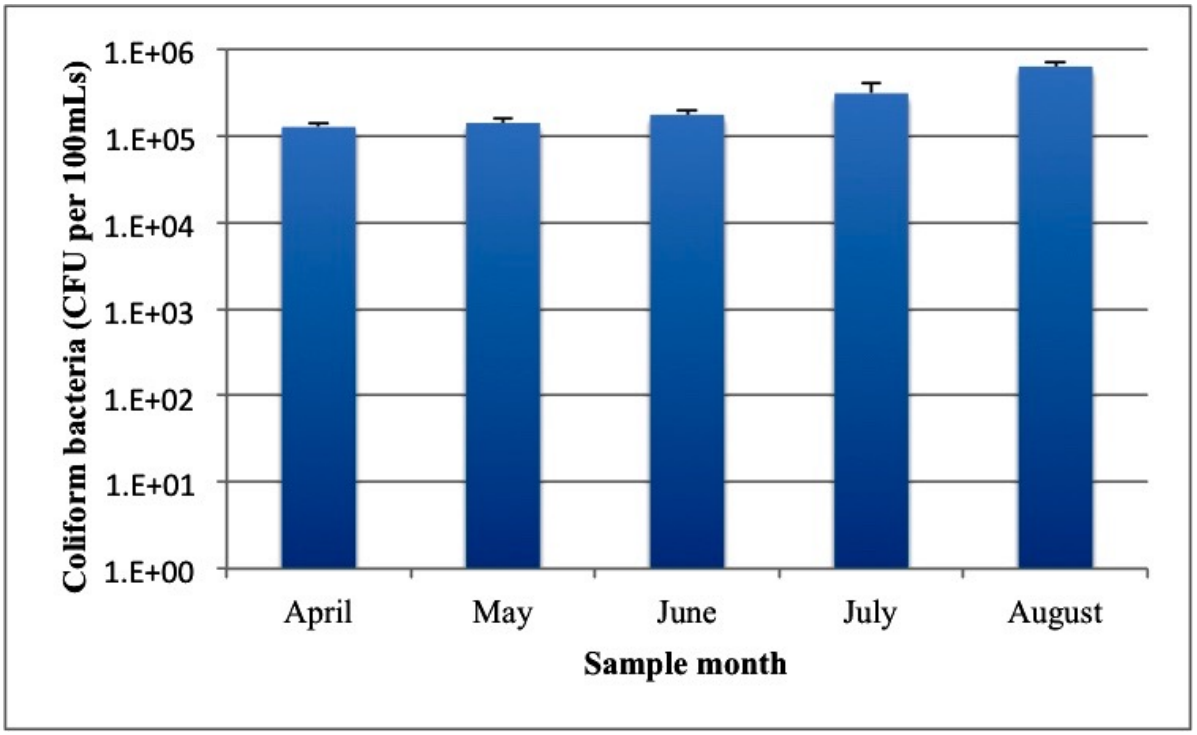


Figure 3.7: Monthly mean coliform counts for the Cathill site

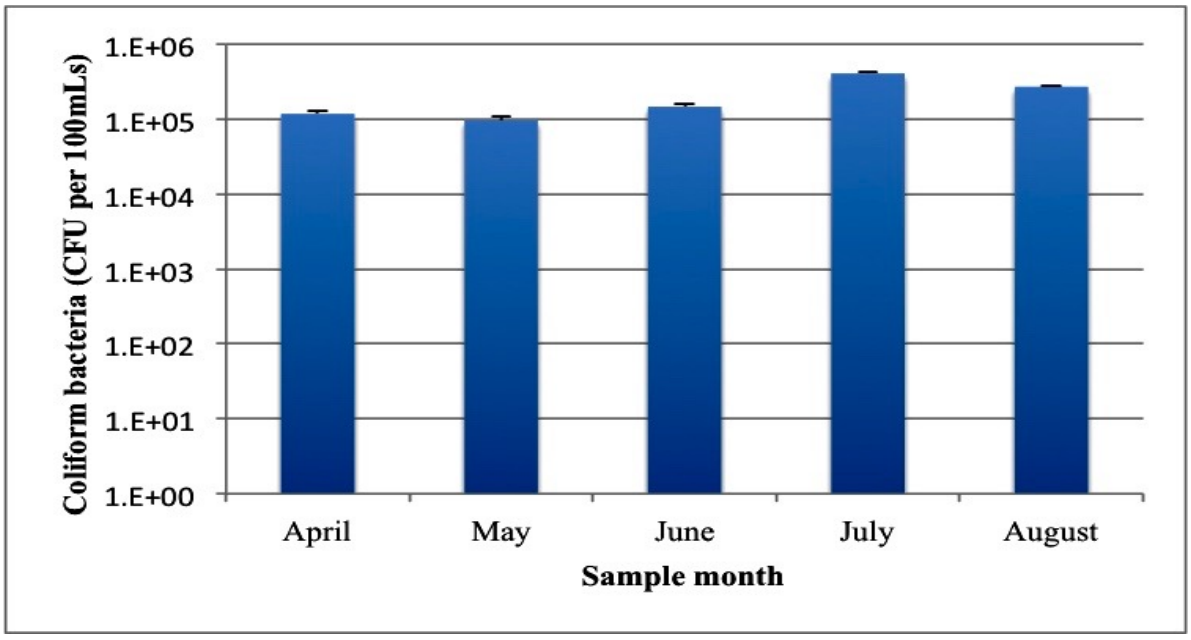


Figure 3.8: Monthly mean coliform counts for Parkside Gardens

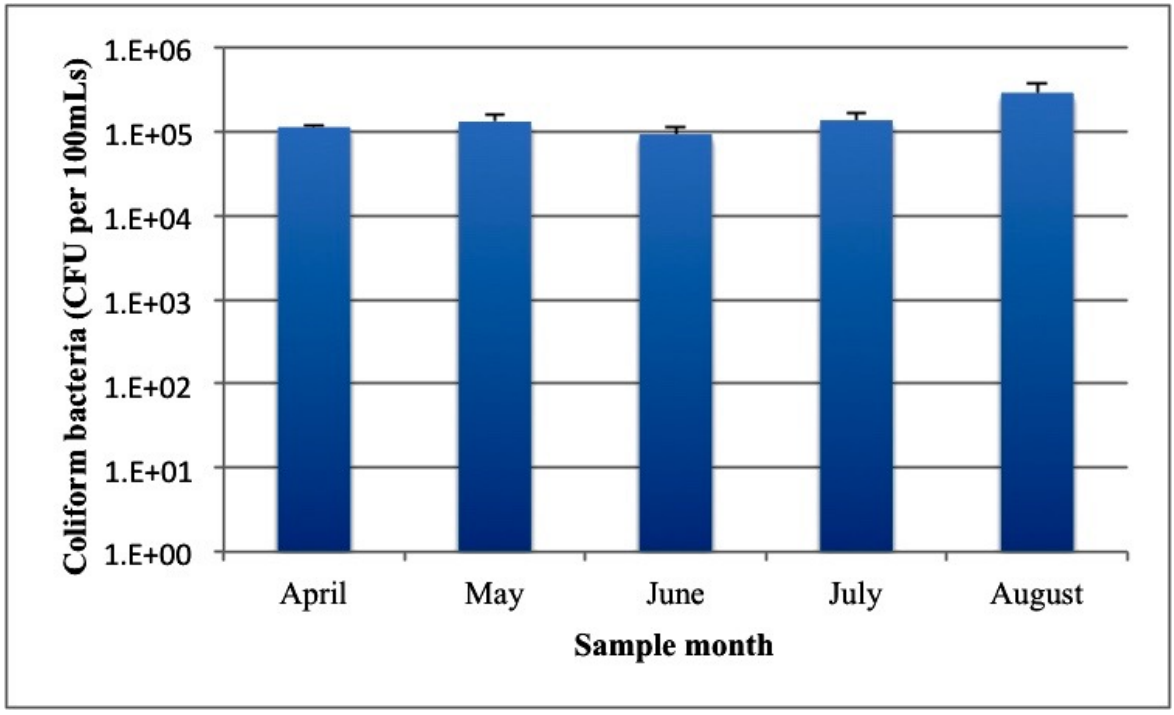


Figure 3.9: Monthly mean coliform counts for the Eastwalk/Westwalk site

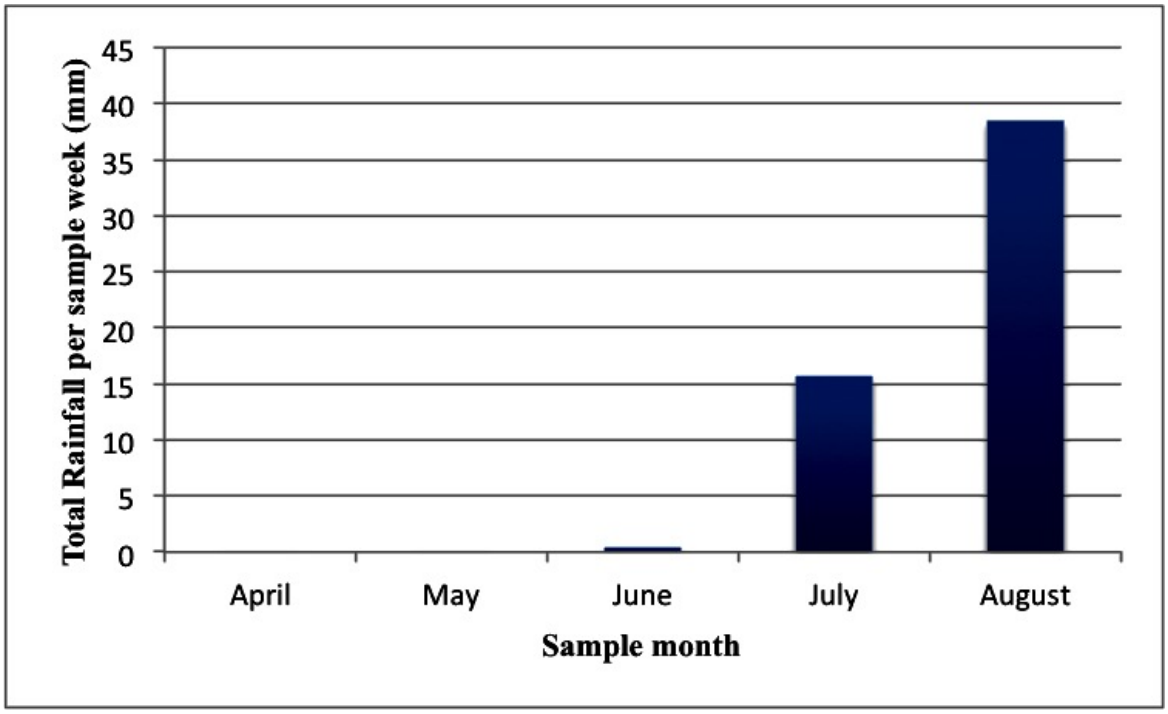


Figure 3.10: Total rainfall data for sample weeks the study period

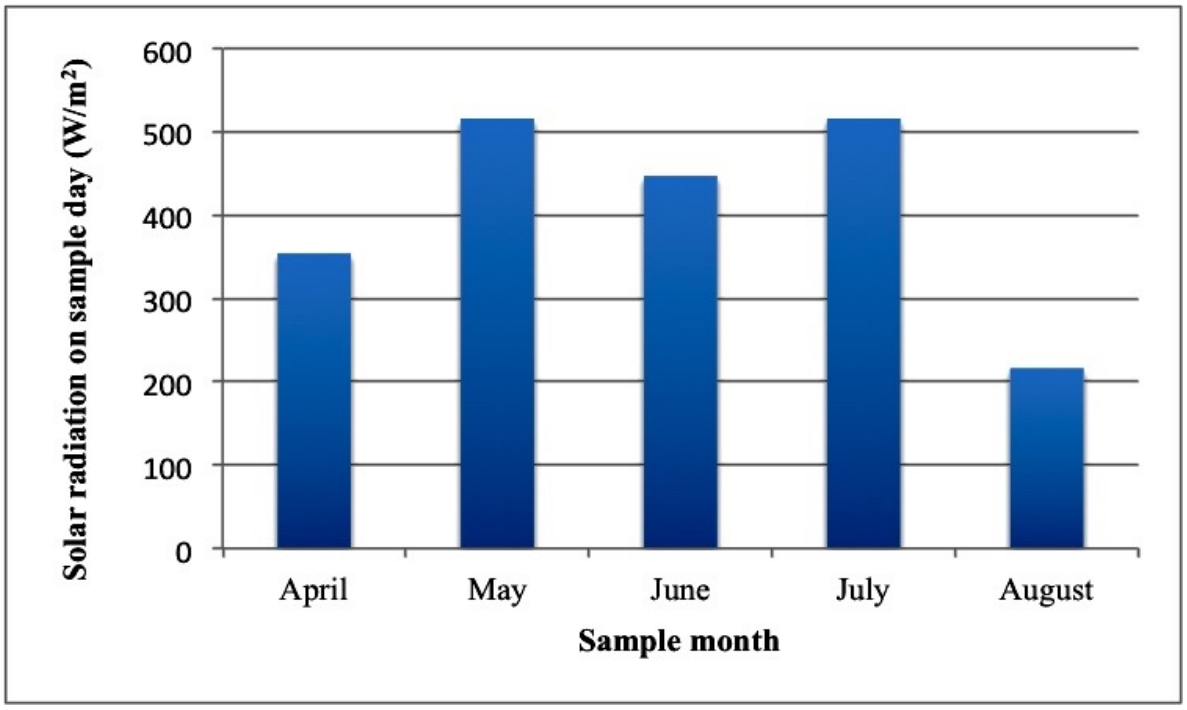


Figure 3.11: Solar radiation record for the sampling period

3.5 Discussion

Results obtained during the coliform bacteria survey of the River Lee and the tributaries studied suggested that coliform bacteria levels within most of upper Lee sites were relatively low compared to the lower Lee tributaries. The results of the survey also showed that within the Upper Lee section, sites located on the main river from Luton down to Hoddesdon and the River Mimram had the highest quality in terms of parameters analysed. Levels within the River Stort and Todd Brook were however high with all the sites located within the two watercourses exceeding 1×10^4 cfu per 100ml which suggests the presence of poorly treated or untreated wastewater (Water UK/Environment Agency, 2009). The tributaries within the Lower Lee however appeared to have the worst coliform bacteria water quality with majority of the sites exceeding 1×10^4 cfu per 100ml. In the Ching, all sites sampled exceeded this level with some sites exhibiting coliform bacteria levels above 1×10^5 cfu per

100ml. Excessively high coliform bacteria counts were also identified within all sites located on Pymmes Brook, the only exceptions being the Jacks Lake and Baring Road sites. Water quality also appeared to be poor in Salmons Brook with all sites except the site opposite Cunard Crescent exhibiting levels above 1×10^4 cfu per 100 ml. Generally, results obtained indicated that within majority of the sites in the lower tributaries coliform bacteria levels detected far exceeded the mandatory limits provided by the EU Bathing Water Directives. In urban areas, levels such as those identified in some sites within these watercourses suggest inputs of inadequately treated wastewater or raw untreated wastewater from Sewage Treatment Works and urban diffuse sources such as CSO's and plumbing misconnections. However, given that some of the sample sites in these sub-catchments of the Lee are not located downstream of sewage treatment works, the possible explanation for the grossly polluted status is likely to be due to CSO's and plumbing misconnections.

3.5.1 Bacteriological (Coliform bacteria) quality of Pymmes Brook

Mean coliform results obtained for the experimental study showed excessively high coliform bacteria levels in all the urban downstream sites. According to the guide provided in the UK for the assessment of Polluted Surface Outfalls, coliform counts ranging between 1×10^4 and $>1 \times 10^5$ cfu per 100ml suggest contamination from inadequately treated or untreated faecal waste (Water UK/Environment Agency, 2009). Based on this, it is clear that the only Pymmes Brook site that seems unaffected is the Jacks Lake site, which had low coliform bacteria counts during the entire sample period. As mentioned previously, this site is located in a rural section of the Brook and as such it is expected that there will be relatively low inputs of urban diffuse pollutants in the watercourse within this section. Mean coliform bacteria levels in Baring Road, Cathill, Parkside Gardens and Eastwalk/Westwalk ranged between 1.5×10^5 and 2.8×10^5 cfu per 100 ml. The Cathill site had the worst levels with mean coliform counts of approximately 2.8×10^5

cfu per 100ml. These results and the data collected for the Brook during the River Lee coliform survey indicate that the Brook is experiencing severe water quality issues. This also agrees with findings of previous research projects that Pymmes Brook is suffering severely from urban diffuse pollution (Edmonds-Brown and Faulkner, 1995; Faulkner et al, 2000). However, from the results obtained in this current study, coliform levels appear to have reduced dramatically in this catchment. This is because coliform bacteria counts obtained during some of these previous studies were recorded to be as high as 2 to >5 million cfu per 100ml in some sites especially Cathill which was suggested to be the main problem area of the Brook. Sources of these excessive bacteria levels were mainly attributed to CSO's and sewage misconnections (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000).

Coliform bacteria obtained during the experimental study in 2014 showed high levels at the four downstream sites during the entire study period. The only exception to this was the Baring Road site, which had relatively low levels in April and May 2014. Apart from this period, the pollution of the Brook was evident across all the affected sites regardless of the sample month and during periods of low flow and rainfall events. While CSO's impact on watercourses during periods of high flow, increased coliform bacteria counts from plumbing misconnections occur both during periods of low flow and high flow. This therefore suggests that the major source of the increased coliform concentrations in the Brook is likely due to misconnections. Confirmation of this suspicion is only possible through trackbacks to the misconnected property. Although, misconnection trackbacks were not conducted in this current study because such trackbacks require access to the sewage system. However, previous trackbacks conducted on the Cathill Storm Sewer Outfall (SSO) discovered misconnections in the catchment with a severe misconnection problem found particularly around Northumberland Road (Edmonds-Brown and Faulkner, 1995). During the River Lee coliform survey and also during previous studies (Faulkner et al., 2000; Nwanekezie, 2009) the Baring Road site had relatively low coliform

bacterial levels. Coliform levels were also low at this site in April and May during the 2014 experimental study period, and then started to climb in the month of June. From June, which had levels above 1×10^5 cfu per 100ml, the Baring Road site started to experience coliform concentrations similar to the other downstream sites. The possible explanation behind these increased concentrations is the discharge of untreated sewage from additional misconnections in this area.

The graphs in Figures 3.5 – 3.9 show increased coliform bacteria levels across all the Pymmes Brook sites in July and levels continued to be high in August. Although compared to the other sites, which experienced a massive increase (43.20% to 77.4%) in coliform counts in July, the Eastwalk/Westwalk site just had a smaller increase from 9.3×10^4 cfu per 100ml in June to 1.4×10^5 cfu per 100ml in July. No significance tests can be performed on just the two values, however this constitutes a 32.1% difference. There are several factors that have been known to influence coliform bacteria survival and subsequently concentrations within watercourses. Two major parameters, rainfall and solar radiation were investigated during this study. The results obtained from the Pymmes Brook experiment showed the highest coliform bacteria concentrations in July and August, the months that had the highest rainfall during the study period. This is in line with the findings of previous studies where it was discovered that an increase in rainfall leads to the flushing of bacteria and other pollutants into watercourses. According to these studies, the effect of this flushing on the affected watercourse has also been shown to linger for several days after the high rainfall event (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000; Scholes et al., 2007; Tornevi et al., 2014).

In terms of the solar radiation, Figure 3.11 shows that on the days sampled, the lowest solar radiation (216 W/m2) was recorded for August and the highest coliform bacteria counts were obtained for all the Pymmes Brook sites during this month whilst counts recorded on the sample days in April, May, June and July had solar radiation records ranging between 354 W/m2 and 516W/m2. Furthermore, the coliform bacteria records obtained during the

study indicated that all the Pymmes Brook sites experienced an increase in coliform bacteria levels in July. However, the increased coliform levels in the Eastwalk/WestWalk site was not as elevated as in the other four Pymmes Brook sites (Jacks Lake, Baring Road, Cathill and Parkside Gardens). This site (Eastwalk/Westwalk) had the highest sunlight penetration, given the complete absence of tall vegetation on the riverbanks unlike the other sample sites therefore suggesting that coliform bacteria levels may have been affected by the sunlight during this month. This conforms to available literature which suggests that solar radiation causes a reduction in coliform bacteria concentration within watercourses and therefore an increase in solar radiation could lead to increased coliform die-off rates (Fulton et al., 2004; Patricia, 2012; Whitman et al., 2004). Solar radiation is harmful to these bacteria because on penetration through the bacterial cells, it could cause a rearrangement and thus damage of the cell's DNA. This may subsequently result in the death of the bacteria (Meador et al., 2014; Rozen and Belkin, 2001; Sinha and Häder, 2002) leading to a decline in their numbers within watercourses.

The solar radiation and rainfall data examined during the experiment suggested that these factors caused some levels of variation in the coliform bacteria levels of the watercourse, however, it was also clear from the coliform results that coliform bacteria levels were still significantly higher in the dirty sites than in the clean sites.

Chapter 4

Pilot Study Investigating the Impacts of Misconnections on Biofilm Communities in the River Lee

4.1 Introduction

As mentioned in Chapter 1, the Water Framework Directive promotes the discovery of alternative biological techniques for assessing pollution of watercourses. Although the use of diatoms is included for WFD biological assessment, the time taken to identify to species level and issues of seasonality pose limitations on their use. The Environment Agency uses software such as DARLQ2 to calculate the Trophic Diatom Index (TDI). TD14 and TD13 scores are provided for river samples and the program is used to calculate various metrics and EQR status (Kelly et al., 2008a). Biofilms have been used extensively for the monitoring of watercourses due to their ability to provide a means for the early detection of effects resulting from pollutant discharges of toxicants, nutrients and organic matter. This is because they are sensitive to water quality changes resulting from the discharges of these pollutants (Burns and Ryder, 2001; Sabater et al., 2007). This response could be evident either as

long-term changes to the biofilm community structure or temporary physiological changes (Fore and Grafe, 2002; Navarro et al., 2002; Roni et al., 2005). The ability to react rapidly to water quality changes suggests that existing communities during sample collection are indicative of the water quality conditions in that area. Another advantage of using biofilms as water quality indicators is that biofilm sampling can also be carried out easily and relatively quickly. Moreover, because of the presence of different groups of organisms within the biofilm structure, with only a small sample, one could have the advantage of monitoring both autotrophic organisms as well as heterotrophic organisms (Burns and Ryder, 2001).

All these qualities make them a useful and cost efficient tool in the measurement of impacts over a long time and impacts due to intermittent discharges from sources such as plumbing misconnections. Changes in the community structure could thus help in the prediction of river and stream quality. Evidence of the use of these organisms in pollution monitoring can be found in several studies in Europe (Brümmer et al., 2000; Fechner et al., 2012a; Rimet, 2012; Rimet et al., 2009; Villeneuve et al., 2013).

Biofilm communities are a complex group of organisms attached to surfaces and joined together by exopolymeric substances (Lee et al., 2014). In watercourses they are usually formed on the surfaces of stones, plants, and other solid surfaces found in and around the watercourse (Fechner et al., 2012). The biofilm community structure typically comprises of autotrophic organisms such as green algae, blue-green algae and diatoms as well as heterotrophic organisms such as protozoa, bacteria and fungi (Romaní et al., 2013). In addition, due to their trophic interactions with the autotrophs and heterotrophs, metazoans often become part of the community and are therefore regarded as part of the biofilm (Ackermann et al., 2011).

Biofilms are very useful for the proper functioning of the aquatic system. They play an important role in the storage and cycling of carbon in rivers (Romani et al., 2004). In addition, levels of extracellular enzymes are usually high within river biofilms and these enzymes assist

towards the breakdown of organic matter (Lear and Lewis, 2012). Invertebrates, fish and some other aquatic organisms also graze on river biofilms therefore making them important energy sources for the aquatic food web, supplying nutrients from the bottom up (Ancion et al., 2013). Furthermore, some members of the community require nutrients for cell growth and development and based on this, biofilms are used for purification processes in Wastewater Treatment Works (WWTW). Similar to this, biofilms in river systems also play a major role in the uptake of nutrients and the breakdown of organic matter therefore assisting towards the natural purification of watercourses (de Beer and Schramm, 1999; Huang et al., 2014). Evidence of this natural purification ability has been well documented in previous studies. Some of these studies have shown the reduction of pollutants such as phosphate, organic matter and ammonium downstream of WWTW and point inputs of raw sewage (Aristi et al., 2015; Elósegui et al., 1995; Marti et al., 2004; Merseburger et al., 2005; Von Schiller et al., 2008). As mentioned previously, biofilm communities are usually made up of a variety of organisms, however the dominant groups typically found within river biofilms are bacteria, algae and protozoa. Having been known to respond to changes in water quality, pollutant inputs would possibly determine the type of communities that occur within the biofilm structure. Therefore biofilms in watercourses receiving untreated sewage discharges from sources such as misconnections are likely to consist of communities that are tolerant to sewage pollution.

One of the major groups that make up river biofilms is the algae group consisting mainly of green algae (Chlorophyta), Diatoms (Bacilliarophyta) and Blue-green algae (Cyanobacteria). Amongst the above-mentioned algae groups, the most studied are the diatoms. Diatoms are widely used for water quality monitoring because of their sensitivity to water quality changes due to increased nutrients, dissolved organic matter, pollution from acidification, pesticides and heavy metals and many other pollutants (Kelly et al., 1995; Lavoie et al., 2014; Morin et al., 2010; Potapova and Charles, 2007; Smol and Stoermer, 2010). Diatoms

are very common and are found in almost all aquatic ecosystems (Schowe and Harding, 2014) and are very useful tools for monitoring because of their high rate of reproduction, easy colonisation of substrates, and their rapid reaction to pollutants (Cox, 1991; Venkatachalapathy and Karthikeyan, 2013a). Certain algae have been known to occur in abundance within sewage impacted watercourses containing high levels of organic matter and nutrients. Algae communities common within such heavily impacted sites are dominated by species such as *Nitzchia palea, Cyclotella meneghiniana* (Kwandrans et al., 1998; Silva-Benavides, 1996; Venkatachalapathy and Karthikeyan, 2013b), *Gomphonema parvulum* (Juliara Stahl, 2013; Venkatachalapathy and Karthikeyan, 2013a), *Synedra ulna* (Kshirsagar et al., 2012). The diatom, *Navicula viridula* has also been associated with sites heavily impacted by sewage (Butcher, 1932; Kshirsagar et al., 2012). Pictures of some of these diatoms are provided in Figure 4.1

Protozoa are another major group of organisms that are abundant in river biofilms and the biological wastewater treatment systems (Pauli et al., 2001). The trickle bed system, one of the biological treatment processes employs the use of biofilms for sewage treatment. Natural river biofilms work in a manner similar to the trickle bed process of wastewater purification (de Beer and Schramm, 1999; Huang et al., 2014). WWTP effluents usually contain high concentrations of organic matter and nutrients (Rawat et al., 2011). The trickle bed filter process involves the use of biofilms, mainly bacteria and protozoa which breakdown the organic matter present in the wastewater (Rehman et al., 2012). In this process, the untreated wastewater goes through different treatment stages which result in the removal of organic matter and reduction of nutrients therefore yielding an effluent that could be discharged into watercourses (Grady Jr et al., 2011; Peters, 1930). Firstly, the sewage is passed through settling tanks to remove the heavy particles and then the liquid overlying the settled solids is then gradually sprayed over a stacked bed of gravels. The wastewater then undergoes gradual purification during its passage through the gravel beds and perforated tiles below and is finally

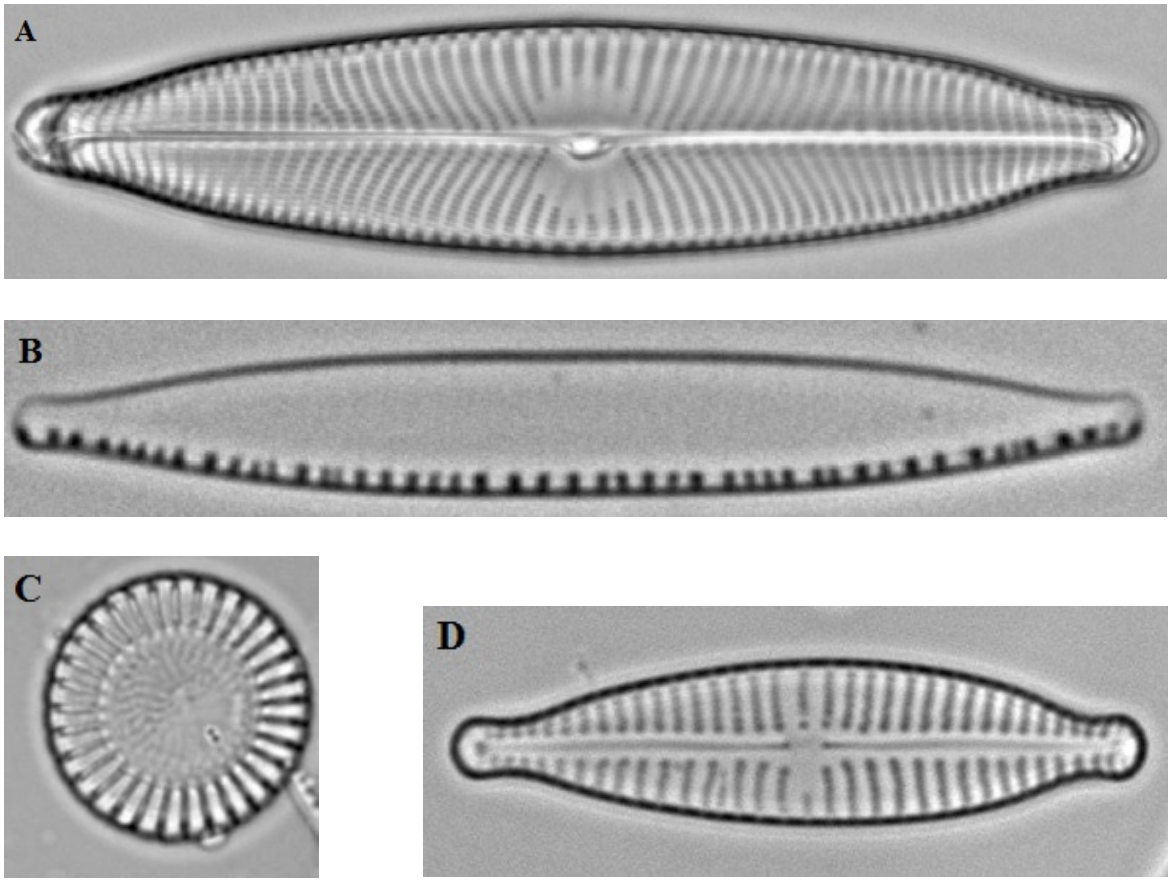


Figure 4.1: Some common diatoms found in polluted watercourses, A: *Navicula viridula*; B: *Nitzchia palea*; C: *Cyclotella meneghiniana*; D: *Gomphonema parvulum*; Adapted from: (NCL, 2015).

collected at the base as treated effluents. Biofilms are seeded and develop on the trickle bed surface utilising the high levels of nutrients contained in the sewage, and the supply of oxygen at the top (Dhokpande et al., 2014; Grady Jr et al., 2011; Rehman et al., 2012). Although some metazoans and other organisms occur, communities within these biofilms are mainly bacteria and protozoa. The major organisms involved in the sewage treatment process are the bacteria. However, protozoans form a principal part of the biofilm purification process especially in modern treatment systems, which receive lower concentrations of nutrients and organic matter (Madoni, 2011; Pauli et al., 2001). Protozoa are classified into three major groups, which include the flagellates, the ciliates and the amoebae (Patterson, 1996; Thorp and Rogers, 2014). However, ciliates contribute the highest number of species to the

protozoan communities found within trickle bed filter biofilms (Pauli et al., 2001). Several protozoan organisms that are associated with the sewage treatment process in WWTW are also found in polluted watercourses receiving high organic and nutrient loads from untreated wastewater discharges (Curds, 1982). Common examples of protozoa found in the trickling filter processes include *Vorticella*, *Cinetochilum* and *Aspidisca* (Madoni, 2011; Pauli et al., 2001). Other key protozoan and metazoan organisms that have also been detected within sewage treatment processes in the UK include *Spirostomum*, *Mastigamoeba*, *Paramecium caudatum*, *Centropyxis*, *Colpidium*, *Philodina* and *Aelosoma* (Peters, 1930). Figure 4.2 shows some of the organisms mentioned.

The association of certain biofilm organisms with untreated sewage as discussed above imply that biofilm communities have a potential for use as indicators of sewage discharges or other high nutrient discharges into watercourses from sources such as misconnections. This use of biofilms was tested in this current study to explore whether community assemblages found at misconnection hotspots could be used as indicators of discharges from misconnections.

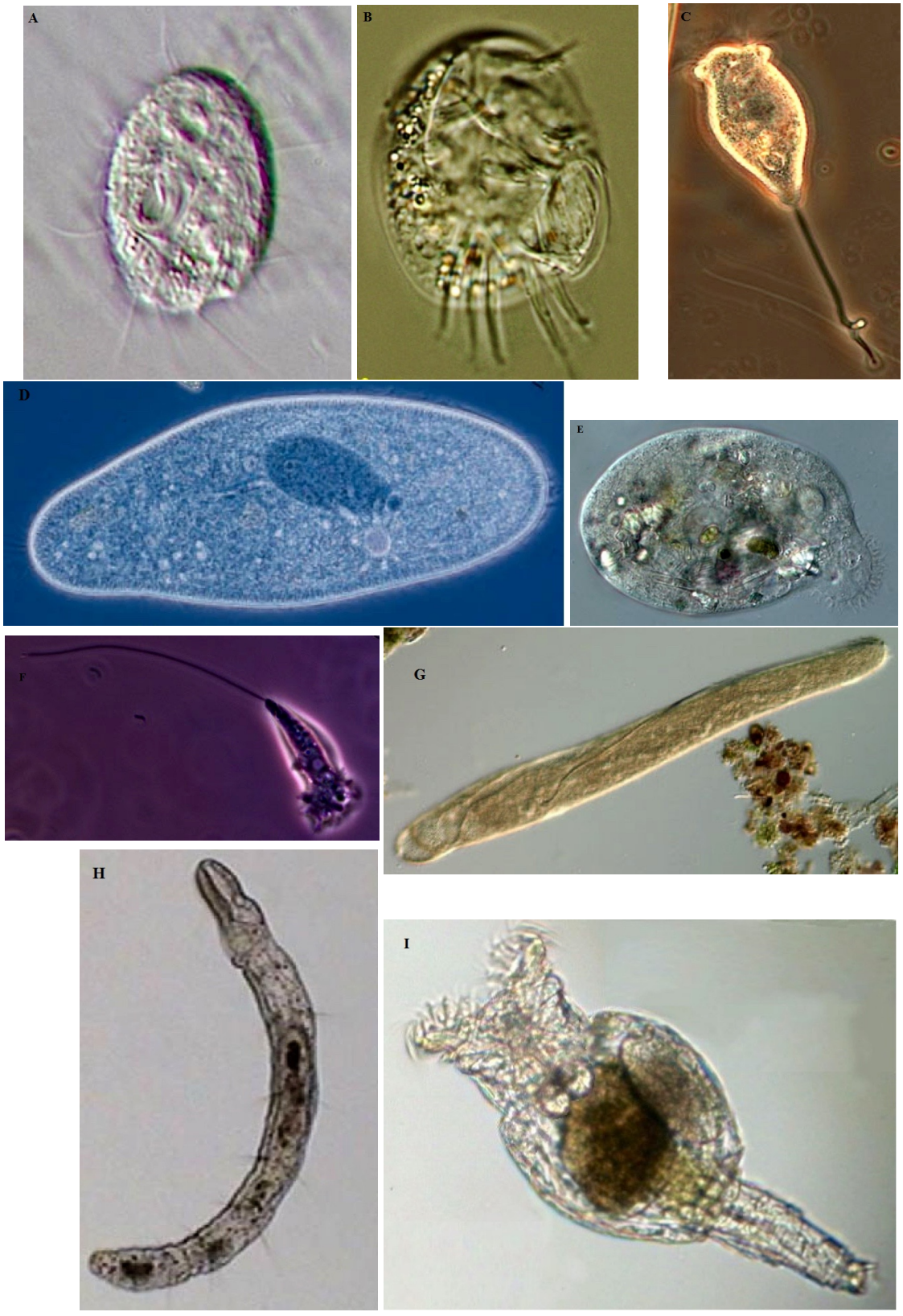


Figure 4.2: Some protozoa and metazoa encountered in sewage treatment processes, **A**:*Cinetochilum*; **B**:*Aspidisca*; **C**:*Vorticella*; **D**:*Paramecium caudatum*; **E**:*Pelomyxa*; **F**:*Mastigamoeba*; **G**:*Spirostomum*; **H**: *Aelosoma*; **I**: *Philodina*; Adapted from: (MBL, 2015; Microscopy UK, 2015).

4.2 Methodology

4.2.1 Biofilm pilot sample site selection

The biofilm pilot was carried out in August 2013, on the main River Lee and some of its tributaries including the Mimram, the Ching, Salmons Brook and Pymmes Brook. Ten sample sites were selected from the 45 sites investigated during the coliform pilot study. A list and map of these sites are given in the site description chapter (Chapter 2). Selection of sites was done based on proper consideration of site accessibility and availability of stones on the riverbed. Selected sites were classified into two groups, clean sites and dirty sites. Sample collection was based on guidelines provided by the European Union standard methods EN 13946: 2003 (CEN, 2003; Kelly, 2013; Kelly et al., 1998).

4.2.2 River Lee benthic biofilm communities

Epilithic biofilms were sampled during the pilot study period. Ten stones were collected at random from the natural riverbed substratum at each of the selected sites. Stones were collected from all the sites on 1st of August 2013. This was done to prevent as much as possible potential variations in biofilm structure due to different environmental conditions. Collected stones were briefly rinsed in river water to remove debris and other contamination loosely adhered to the stone's surface. After collection, these stones were placed in labelled plastic bags half filled with river water, stored in a cool and dark condition and taken to the laboratory for preparation within approximately six hours.

In the laboratory a toothbrush rinsed in sterilised water and rubbed on a clean surface, was used to scrub the biofilm off a defined surface of $3cm^2$ (Hayashi et al., 2011). This sample was then preserved using Industrial Methylated Spirit (IMS) (Kelly et al., 1998; Suthers and Rissik, 2009) pending identification of communities. During the identification

process, communities were counted under a under a bright field microscope (GX L1500) using the Sedgwick Rafter Counting chamber method (Francoeur et al., 2013; LeGresley and McDermott, 2010) and identified with the aid of a number of taxonomic keys (Fitter and Manuel, 1986; Kelly et al., 2005; Patterson, 1996; York and John, 2005). For relatively small cells four horizontal strips were chosen at random and the cells within counted. However all the large organisms within the chamber were counted (Karlson et al., 2010; Peters, 1930).

4.3 Data analysis

4.3.1 Data management

A site by species matrix was created on an excel spreadsheet from the data obtained from the counting and identification of communities.

4.3.2 Diversity calculation

The diversity measure used to analyse species diversity in the sample sites was the Simpsons Index of Diversity, which is suggested to be one of the most robust and meaningful diversity measures available. The diversity index, introduced in 1949 by Edward Simpson, to describe the probability that a second individual randomly picked from a community should be of the same specie as the first. The equation used for the Simpsons Index calculation is given in is given in Box 4.1 below.

Box 4.1: Equation for the Simpsons Index of Diversity

$$\mathbf{D} = \sum \frac{n[n-1]}{N[N-1]} \tag{4.1}$$

Where:

n the total number of organisms of a particular specie

N the total number of organisms of all species

The reciprocal (1/D) of the index is often used therefore as the index increases; diversity increases (Magurran, 2009).

4.3.3 Measure of similarity

A test of similarity between the ten River Lee sites was conducted using the Sorenson's similarity index (Sørensen, 1948). This examines the occurrence of species within sites, identifying the species that each site has in common and assigning a score between 0, meaning complete species dissimilarity and 1 meaning complete species similarity. The equation for the Sorenson's index is given in Equation 4.2 in Box 4.2 below.

Box 4.2: Equation for the Sorenson's similarity index

$$S = \frac{2c}{a + c} \quad (4.2)$$

Where:

S The Sorensons coefficient of similarity

a The number of species found in site A

b The number of species found in site B

c The number of species that site A and site B have in common

4.3.4 Multivariate analysis

A number of multivariate analyses were carried out on the biofilm community data using the Community Analysis Package (CAP) program using Pisces Environmental software. The use of multivariate techniques to assess the response of biological communities is increasingly used in research (ter Braak and Verdonschot, 1995). These techniques are able to provide a clear illustration of structural changes in biological communities along a pollution gradient (Cao et al.,1996). The biofilm pilot data was analysed using classification and ordination multivariate techniques. Techniques used were the Two Way Indicator Species Analysis (TWINSPAN) and Detrended Correspondence Analysis (DECORANA) techniques. TWINSPAN is a commonly used classification technique (Cao et al., 1996) that works by grouping communities/samples according to their hierarchical relationship. In this technique, samples are arranged by a series of correspondence analysis ordinations and as a result are split into groups. The samples are first divided into two groups, the negative group and the positive group with indicator species for each of the groups. Subsequently, each group is then divided into smaller sub-groups based on how similar the species composition are (Hill, 1979b). On the other hand, DECORANA looks for site and specie groupings in ordinal space. To decide whether to use a unimodal or linear ordination method, DECORANA was first calculated for the data set and the length of the first axis was greater than four Standard Deviation (S.D) units, meaning that the dataset was heterogeneous. Unimodal methods such as DECORANA are more suitable for heterogeneous

datasets and therefore DECORANA was selected for the analysis. In this technique the data is rearranged in low dimensional space such that dissimilar samples are furthest apart while similar samples are clustered together (Hill, 1979a). The multivariate analysis was carried out using totals calculated from the addition of numbers of individuals identified within the ten replicate samples obtained per sample site.

4.4 Results

4.4.1 Biofilm community diversity and distribution

During the River Lee biofilm pilot study in 2013, a total of 159 taxa was recorded, 83 of which belonged to the algae group. Within this group, phylum Bacillariophyta had the largest number of individuals with a record of 57 taxa. Genus *Navicula* contributed the highest number of species (7) within this phylum. Other major contributors to the phylum Bacillariophyta include *Nitzschia* (6), *Cymbella* (6) and *Gomphonema*, which had four species recorded during the study.

The protozoan group contributed 55 taxa to the total number of species in the study and was made up mostly of the ciliates (phylum Ciliophora) while the metazoan group had 21 taxa. A list of the individuals recorded during the study is given in Table 4.1. It should however be noted that the list of communities recorded and presented in this thesis may not completely reflect the biofilm communities in their natural environment (the study sites) as the effect of preservation would likely have led to the loss of certain organisms. Therefore, the biofilm communities recorded would be slightly different from the original biofilm community in the sample sites. Furthermore, Table 4.2 provides the taxon richness and diversity index figures for identified communities. The Diversity index measure of the communities did not show a clear and consistent pattern between the clean sites (Sites 1, 2, 3, 4 and 5) and the dirty sites (6, 7, 8, 9, and 10). However, the sites with the highest diversity were Site 1 (Bancroft/ Birdfoot lane) and Site 4 (Tewin bury), which had index scores of 17.527 and 15.477 respectively.

Table 4.1: List of individuals identified during the River Lee biofilm pilot

Algae	**Protozoa**	**Metazoa**
Achnanthes inflata	*Acanthamoeba*	*Aelosoma*
Achnanthes granulata	*Actinophrys*	*Anuraeopsis navicula*
Amphora	*Actinosphaerium*	*Asellus aquatica*
Ankistrodesmus	*Amoeba*	*Baetidae*
Anthophysa	*Arcella*	*Chaetogaster*
Asterionella	*Aspidisca*	*Chironomid larvae*
Aulacosera	*Astasia*	*Dugesia polychroa*
Cocconeis placentula	*Bursaria*	*Erpobdellidae*
Caloneis bacillum	*Calyptotricha*	*Flatworm*
Caloneis silicula	*Centropyxis*	*Gammaridae*
Campylodiscus noricus	*Cercomonas*	*Gastotrich*
Carteria	*Chaenia*	*Glossiphoniidae*
Chamaesiphon	*Chlamydodon*	*Helodidae larva*
Chlamydomonas	*Cinetochilum*	*Hydroporinae larvae*
Chlorella	*Cochliopodium*	*Nematode*
Chlorococcum	*Coleps*	*Oligochaete*
Chlorogonium	*Colpidium*	*Pericoma larvae*
Chroococcus	*Cryptodifflugia*	*Rotaria*
Cladophora	*Didinium*	*Rotifer (Philodina)*
Closterium	*Difflugia*	*Rotifer Bdelloidea*
Cocconeis	*Dileptus*	*Tubifex*
Coscinodiscus lacustris	*Epiclintes*	
Cosmocladium	*Euglena*	
Cyclotella	*Euglypha*	
Cyclotella meneghiniana	*Euplotes*	
Cylindrocapsa	*Glaucoma*	
Cymatopleura elliptica	*Halteria*	
Cymatopleura solea	*Heterophrys*	
Cymbella cistula	*Lacrymaria*	
Cymbella ehrenbergii	*Litonotus*	
Cymbella lanceolata	*Mallomonas*	
Cymbella naviculiformis	*Mastigamoeba*	
Cymbella prostrata	*Mastigella*	
Cymbella ventricosa	*Mayorella*	
Diatoma	*Menoidium*	
Euastrum	*Nuclearia*	
Eunotia	*Ochromonas*	
Fragilaria	*Opercularia*	
Gomphonema angur	*Paramecium caudatum*	
Gomphonema gracili	*Pelomyxa*	
Gomphonema olivaceum	*Phacus longicauda*	
Gomphonema parvulum	*Phacus pyrum*	

Table 4.1: List of individuals identified during the River Lee biofilm pilot (continued)

Algae	Protozoa	Metazoa
Gyrosigma acuminatum	*Phialina*	
Gyrosigma attenuatum	*Prorodon*	
Hantzchia amphioxys	*Rhynchomonas spp*	
Melosira	*Spathidium*	
Microspora	*Spirostomum*	
Navicula cryptocephala	*Stentor*	
Navicula cuspidata	*Stichotricha*	
Navicula gracilis	*Stylonchia*	
Navicula minima	*Tetrahymena*	
Navicula oblonga	*Urceolus*	
Navicula radiosa	*Uroglena*	
Navicula reinhardtii	*Urotricha*	
Navicula viridula	*Vorticella*	
Nitzschia acicularis		
Nitzschia amphibia		
Nitzschia palea		
Nitzschia recta		
Nitzschia sigmoidea		
Nitzschia sublinearis		
Oedogonium		
Oscillatoria		
Pediastrum		
Phormidium		
Pinnularia		
Rhoicosphenia abrevata		
Rhipidodendron		
Synedra acus		
Rhoicosphenia curvata		
Scenedesmus		
Spirogyra		
Staurastrum		
Stauroneis phoenicenteron		
Stephanodiscus		
Stigeoclonium		
Suirella ovata		
Suirella splendida		
Synedra ulna		
Tribonema		
Ulothrix spp.		
Vaucheria		
Volvox		

Table 4.2: Taxon richness and diversity parameters of communities for the River Lee biofilm pilot study in 2013

Site	Taxon Richness	Simpsons Index
Site 1	70	17.527
Site 2	54	14.951
Site 3	42	7.545
Site 4	32	15.477
Site 5	53	9.295
Site 6	42	12.838
Site 7	38	8.103
Site 8	25	10.442
Site 9	36	12.653
Site 10	22	10.469

4.4.2 Biofilm community similarity patterns

Results of the similarity test between the ten River Lee sites are provided in Table 4.3. As mentioned previously in the methodology section of this chapter, the index used for the measure of similarity between sites was the Sorenson's Index of similarity. Sites with the highest similarities (figures greater than 0.5 which approximates to 50% similarity) are highlighted in the table. From the analysis it is clear that the dirty sites had the highest similarity during the study.

Table 4.3: Similarity (Sorensons) patterns between the River Lee sites. Green highlights sites with 50 - 59% similarity, yellow represents sites with 60 - 69% similarity, blue highlights sites with 70 -79% similarity and red represents sites with >80% similarity

	Site1	Site2	Site3	Site4	Site5	Site6	Site7	Site8	Site9	Site10
Site1										
Site2	0.571429									
Site3	0.495868	0.538462								
Site4	0.311927	0.347826	0.325							
Site5	0.458015	0.491228	0.588235	0.444444						
Site6	0.319328	0.27451	0.355556	0.128205	0.3					
Site7	0.295652	0.244898	0.302326	0.135135	0.229167	0.666667				
Site8	0.271845	0.232558	0.297297	0.193548	0.238095	0.694444	0.794118			
Site9	0.283186	0.208333	0.333333	0.138889	0.297872	0.757576	0.692308	0.853659		
Site10	0.222222	0.195122	0.314286	0.172414	0.35	0.647059	0.59375	0.692308	0.741935	

4.4.3 River Lee biofilm community structure (biofilm pilot)

A dendogram showing the TWINSPAN classification of sites is provided in Figure 4.3. Site classification was only taken to the second level. At the first level, sites separate out with Sites 1, 2, 3, 4 and 5 (Bancroft/Birdfoot lane, Kingdown avenue, Leaside Court (a), Tewin bury and Jacks Lake) positioned on the left and sites 6, 7, 8, 9, 10 (Vincent, Hale end, Cathill, Parkside gardens and Eastwalk/Westwalk) on the right side. At the second division, the clean sites are further split into two groups with Sites 1, 2, 3 on one side and Sites 4 and 5 on the other side. The dirty sites also split at the second division with sites 6 and 7 on the left and Sites 8, 9 and 10 on the right.

Figure 4.4 shows the DECORANA plot of the River Lee biofilm pilot sites with Axis 1 accounting for 67% of the variation. The graph shows a separation between the dirty sites (Sites 6, 7, 8, 9 and 10), which are circled and the clean sites (Sites 1, 2, 3, 4 and 5). The dirty sites have similar communities and are positioned together in ordinal space between 0 and 70 on the first Axis and 120 and 160 on the second Axis. The dirty sites are grouped clearly and taxa common within these sites include *Nitzschia acicularis, Hantzschia amphioxys, Melosira, Suirella ovata, Gomphonema parvulum, Navicula minima, Navicula viridula, Nitzschia palea Nitzschia amphibia, Nitzschia sigmoidea, Mastigamoeba, Cinetochilium and Pelomyxa*.

The clean sites did not group as closely together as the dirty sites. However, some species found in high abundance within the clean sites include *Amphora ovalis, Nitzschia sublinearis, Achnanthes granulata and Achnanthes inflata*.

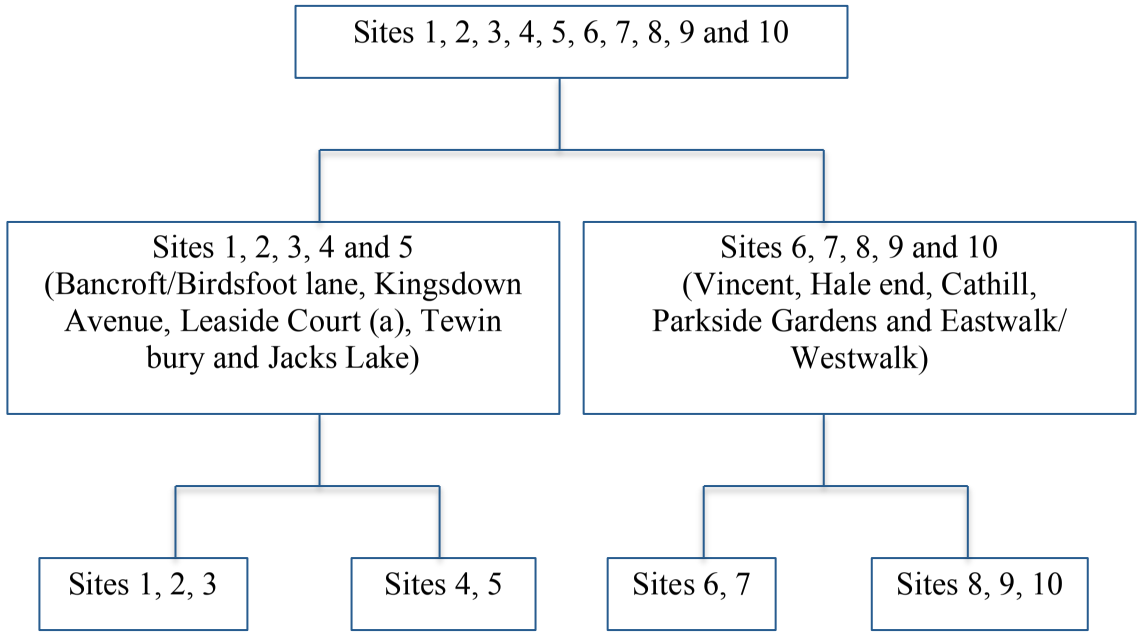


Figure 4.3: TWINSPAN classification of the ten biofilm pilot sites, sites 1,2,3,4 and 5 (are clean sites and sites 6,7,8,9 and 10 are dirty sites.

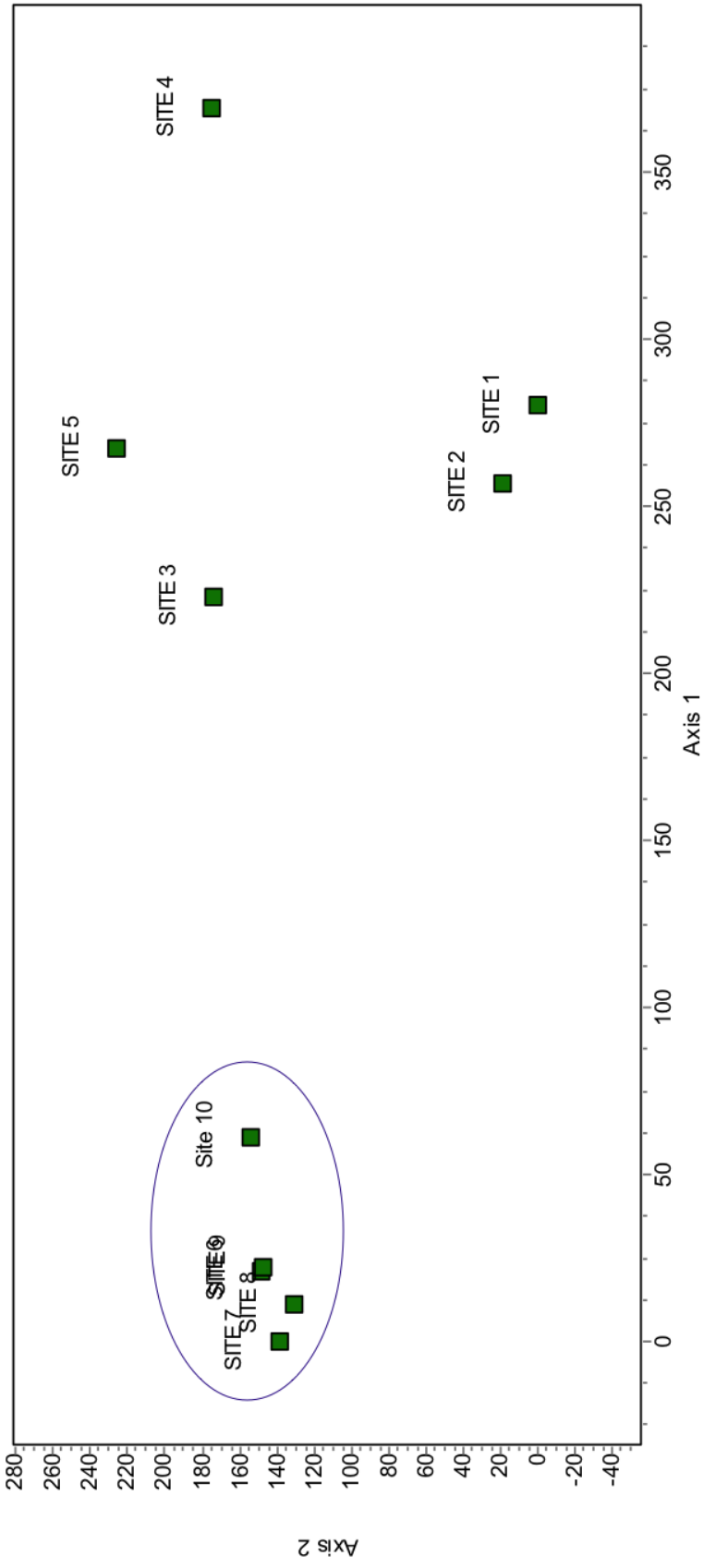


Figure 4.4: DECORANA ordination plot for the River Lee biofilm pilot. Clean and dirty sites separate out (dirty sites circled). Axis 1 accounts for 67% of the variation

4.5 Discussion

Communities from clean sites were selected and communities within these sites were compared to communities within the selected dirty sites. The dirty sites were located on two tributaries of the River Lee, the Ching and Pymmes Brook noted in previous studies as suffering impacts of misconnections. For instance misconnection trackbacks conducted previously on a $1km^2$ residential catchment area of the Ching identified 347 misconnections. Investigation of coliform bacteria levels and Biochemical Oxygen Demand (BOD) below the Polluted Surface Water Outfall (PSWO) identified coliform bacteria levels as high as 2.42×10^4 MPN/100ml and BOD levels of 275mg/l (Ellis and Butler, 2015). Misconnections have also been detected within the Pymmes Brook catchment and previous studies have identified coliform bacteria levels exceeding 5 million cfu/100 ml, levels indicative of raw sewage below a PSWO in the catchment (Edmonds-Brown and Faulkner, 1995). Coliform levels identified for sites within these two catchments (The Ching and Pymmes Brook) during this current study have also been excessively high (Chapter 3).

Biofilm communities have several characteristics that make them a potential tool for monitoring misconnection impacts and thus locating misconnection hotspots within a catchment. They occur within most watercourses (Lawrence et al., 2015) and therefore are constantly exposed to pollutant inputs from misconnections. Furthermore, biofilm assemblages are capable of responding quickly to changing environmental conditions due to their short life cycle. Their ability to respond rapidly to these changes makes them useful for monitoring intermittent discharges from misconnections (Fore and Grafe, 2002). Misconnections discharge untreated wastewater into watercourses therefore causing an increase in organic (Alves et al., 2012) and nutrient loadings (Comber et al., 2013) and biofilms react to the presence of such inputs because they have different levels of tolerance to pollutant loadings (ter Braak et al., 2004). This results to shifts in the community structure from organisms that

are completely sensitive to the increased loadings and those that are tolerant of mild background levels of such pollutants to organisms that are tolerant of high levels of such pollutants.

Diversity measures such as the Simpsons Index of diversity can provide information on trends in the occurrence of communities (Magurran et al., 2010). However the Simpsons diversity index used to assess community diversity during this pilot did not show a clear trend between the dirty and clean sites. This could possibly be due to the fact that biofilm communities were only sampled once and long-term data sets provide more information on the variety and abundance of species at various locations and at selected time intervals (Magurran et al., 2010).

Similarity indices provide a summary of the species composition within sites and the Sorenson's similarity index is one of the most widely used similarity index in ecological studies (Xu et al., 2005). The index scores (Table 4 .3) for the sites showed that the highest scores ranging between 0.59375 to 0.853659 were recorded for the dirty sites therefore indicating that the species composition within the dirty sites were similar. This conforms to a similar study, which recorded the highest similarity scores for sites with the most similar water quality (Maznah and Mansor, 2002). The possible reason being that the higher levels of increased organic and nutrient loading contained in misconnection effluents are more likely to support increased numbers of these communities especially those species particularly tolerant to such levels.

With regards to the multivariate analysis, the TWINSPAN classification showed a separation between clean and dirty sites. In addition, the DECORANA plot showed the dirty sites (Vincent, Hale end, Cathill, Parkside gardens and Eastwalk/Westwalk sites) clustered together and clearly separated from the clean sites (Bancroft/Birdfoot lane, Kingsdown avenue, Leaside Court (a), Tewin bury and Jacks Lake sites) in ordinal space. Species found in high abundance within the clean sites include species which have been known to occur in fairly clean water (Kalyoncu et al., 2009; Kelly et al., 2005;

Kwandrans et al., 1998; Soininen, 2002; Szulc, 2007). While communities present and abundant within the dirty sites include several *Nitzschia* and *Navicula* species as well as other algae and protozoan organisms mentioned in the results section. The most tolerant species could be considered as indicators for polluted watercourses (Lange-Bertalot, 1979). The communities such as *Gomphonema parvulum, Nitzschia acicularis, Hantzschia amphioxys, Mastigamoeba* and other organisms common within the dirty sites during this pilot have been known to tolerate high levels of organic matter and nutrients (Butcher, 1932; Kelly et al., 1995; Patterson, 1996; Silva-Benavides, 1996; Venkatachalapathy and Karthikeyan, 2013a; Yallop, 2008). All the species mentioned also occurred consistently across all the dirty sites, therefore they could be potential indicators of misconnection discharges.

This pilot study, provided information based on a short-term investigation. As the results of the pilot were promising, to further investigate biofilm communities within misconnected areas and thus locate hotspots, the next stage involved conducting a biofilm experiment in more details. Details of this are presented in Chapter 5.

Chapter 5

Experimental study of the impact of sewage misconnections on River Lee biofilm communities (Pymmes Brook case study)

5.1 Introduction

Biofilm communities are composed of a wide range of organisms including bacteria, algae, and protozoa embedded in exo-polymeric substances (Ancion et al., 2013). They are usually made up of several species that react in different ways to environmental changes in water quality. Therefore species reaction to such changes usually results in changes to the biofilm and species composition and thus leads to the occurrence of species that are tolerant to those particular environmental conditions. As a result, biofilm communities may provide a means for assessing the ecological health status of watercourses (Broady, 2003).

Water pollution has become a serious cause for concern in many urban areas in the UK. The current study area, the River Lee has long been known to experience issues with poor water quality. One of the major sources of pollution into the river is from diffuse sources such as CSO's and misconnections (Snook and Whitehead, 2004). This is particularly an issue in the lower Lee section of the watercourse. Untreated wastewater gets discharged into the river from these sources resulting in severe impacts on the watercourse such as reduced D.O, eutrophication and loss of biodiversity (Snook and Whitehead, 2004). Coliform results obtained during the pilot stage of this study suggest that the watercourse is still experiencing issues with poor water quality in some areas.

Misconnections usually discharge untreated wastewater into watercourses thus leading to increased nutrient loads and organic matter in affected rivers. Because biofilm communities are usually sensitive to environmental changes resulting from pollutant loadings, they are one of the major parameters being used in this study in an attempt to locate misconnection hotspots. A sub catchment of the Lee, Pymmes brook was selected for further investigation of biofilm communities along a pollution gradient. For several years, the Pymmes brook watercourse has been experiencing severe water quality issues from diffuse sources, which have been suggested to be mainly due to CSO's and plumbing misconnections (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000). The coliform results obtained during the pilot illustrate that the brook is still experiencing water quality issues. Although an initial pilot study was carried out on several sections of the River Lee, the Pymmes Brook biofilm study was conducted to investigate biofilm communities over a longer period of time, to observe seasonal changes over time. Algal compositions require sufficient light availability and therefore the study covered between spring and end of summer. Rivers are dynamic systems subjected to a wide range of environmental conditions from relatively clean to extremely polluted waters. Biofilm communities are sensitive to environmental variables such as light, D.O. levels as well as nutrient and organic loadings. Therefore biofilm species

composition is determined by these environment variables in watercourses (Duong et al., 2007). In the initial biofilm pilot, details of which are presented in chapter 4, epilithic biofilms were sampled from the natural riverbed substrata. These are the recommended substrata type for monitoring biofilms, two reasons being that they are readily available on riverbeds, easier to sample and less likely to be tampered with (Kelly, 1998). However, for the main experiment, to monitor the change in community over time, a uniform substrate where equal sized samples could be taken was considered the best option for making comparisons and reducing substrate variability.

A previous study has also suggested that the reaction to changes in water quality conditions is more rapid in recently developing communities on artificial substrates than in communities long established on the natural riverbed substrata (Iserentant and Blancke, 1986).

5.2 Methodology

5.2.1 Biofilm experiment sample sites

The biofilm experiment was carried out between May and August 2014 and the six sites selected for the biofilm experiment were the same sites used during the Pymmes Brook coliform study (Chapter 3). As with the biofilm pilot sites, these six sites were grouped into potentially clean sites and dirty sites. A list of the biofilm experiment sites and a map showing their locations are given in Table 5.1 and Figure 5.1.

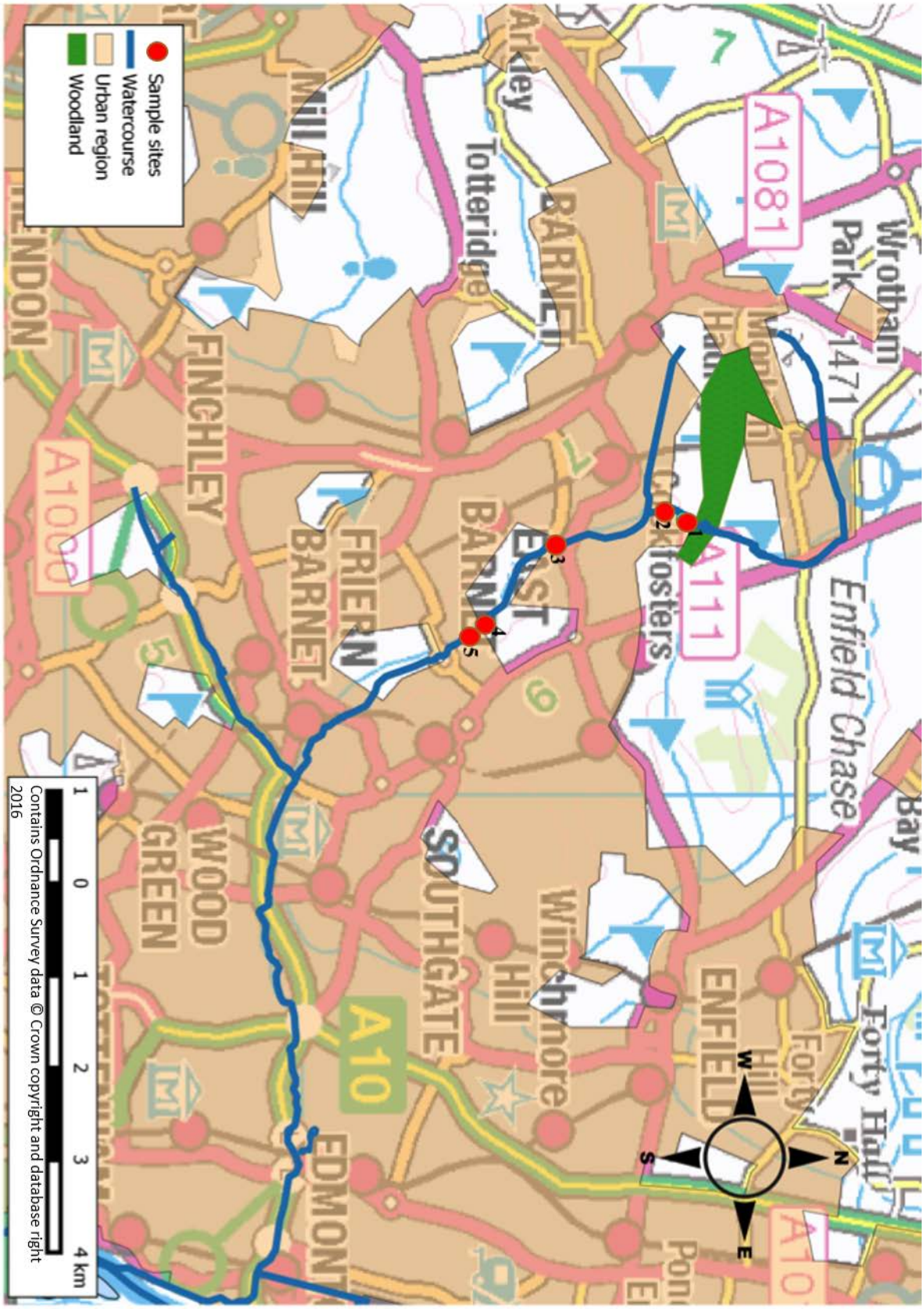


Figure 5.1: Map showing the Pymmes Brook biofilm experiment sites

Table 5.1: Sites sampled during the biofilm experiment study

Site name	Site number
Bayfordbury	1
Jacks Lake	2
Baring Road	3
Cathill	4
Parkside Gardens	5
Eastwalk/Westwalk	6

5.2.2 Physico-chemical parameters

During biofilm sample collection, physiochemical parameters of sites were also assessed. Physicochemical data such as Dissolved Oxygen (D.O.), flow rate (cumecs), conductivity (μS), pH and temperature (°C) were measured on site using portable meter probes. Measurement of conductivity and pH were carried out using a multi-probe meter (Hanna Combo HI-98129) while D.O. and temperature were measured using a D.O meter probe (Extech Instruments, Model 407510a). Furthermore, water samples were also collected for the analysis of nitrates, phosphates and ammonia levels (HACH kit method). Further details on physicochemical methods are provided in Appendix C.

5.2.3 Sampling and identification of biofilms

Epilithic biofilms were also sampled during the biofilm experiment period. Artificial substrates (unglazed clay building tiles) were attached with twines to tent pegs, which were then hammered into the riverbed at all the selected sites. These artificial substrates measuring approximately 25cm by 150cm were submerged at random one tile for each month of sampling below outfalls in the selected sites. Pictures showing submerged tiles are given in Figure 5.2. When submersing the substrates, heavily shaded areas were avoided as much as possible so there was sufficient light availability (Kelly, 1998). Where possible, hidden areas, unlikely to be accessed by the public, were preferred for submerging the tiles as urban areas are often subject to vandalism. The artificial substrates were placed on the riverbed

in April 2014 and then sampled randomly at monthly intervals from each of the selected sites between May and August 2014. On collection, substrates were lightly rinsed in river water to dislodge unwanted contaminants as well as debris and then temporarily placed in transparent plastic bags, which were then filled halfway with river water. Samples were kept in a cool dark place and taken to the laboratory within four to six hours. In the laboratory, biofilms within defined sections (2.54 cm^2) were scrapped from tiles using a toothbrush. Four replicates were randomly obtained from the collected tiles. Similar to the biofilm pilot, samples were preserved using Industrial Methylated Spirit 70% (IMS) pending community identification. Communities present were also counted and identified using the Sedgwick Rafter Counting Cell method details of which were provided in the biofilm pilot methodology section in Chapter 4.



Figure 5.2: Pictures of submerged tile substrates

5.3 Data analysis

5.3.1 Statistical analysis

Firstly, a site by species matrix was created on an Excel spreadsheet from the community identification and counts. Means and standard errors were also generated for sample sites per month of sample collection using the IBM SPSS base package version 22.

5.3.2 Diversity calculation

Similar to the biofilm pilot, the diversity measure used to measure communities identified in the biofilm experiment was the Simpson's Index of Diversity. Details of this diversity measure and the equation used for its calculation are provided in Chapter 4.

5.3.3 Multivariate analysis

Multivariate techniques were also used for the analysis of communities identified during the experimental period. The Community Analysis Package (CAP) and Ecological Community Analysis (ECOM) programs (Pisces Conservation LTD) were used for the multivariate analysis. Analyses carried out include Two Way Indicator Specie Analysis (TWINSPAN), Detrended Correspondence Analysis (DECORANA) as described in the previous chapter (chapter 4) and Canonical Correspondence Analysis (CCA), which is an improvement on ordination techniques such as DECORANA. This technique not only performs ordination of species and sites simultaneously, it also incorporates the analysis of environmental variables in the process. The technique illustrates the strength of a physicochemical parameter on the species composition. By plotting the species, sites and environmental variables together on low dimensional space, habitat preferences of species are clearly illustrated (Henderson and Seaby, 2008; ter Braak and Verdonschot, 1995). For the CCA analysis, an additional data

matrix containing the environmental data is added to the site by specie matrix used for the other multivariate analysis. A Pearsons correlation analysis was also carried out to look for associations between the environmental parameters and the axes. The multivariate analysis was carried out using totals calculated from the addition of numbers of individuals identified within the four replicate samples obtained per sample site.

5.4 Results

5.4.1 Physicochemical parameters (Pymmes Brook)

Results of the physicochemical parameters measured during the study period are given in bar charts in Figures 5.3 to 5.10 below. There was little variation in pH levels and conductivity across all the sample sites as expected. Conductivity levels ranged between 424 – 944 µS/cm and were within the 50 – 1500µS/cm range normally recorded for most streams (Haddis et al., 2014), while pH levels ranged between 7.66 and 8.1. Dissolved Oxygen (D.O.) was relatively high in Bayfordbury and Jacks Lake sites (the clean sites) while for most of the study period there was a downstream decline in D.O. from the Baring Road site. During the study, the lowest D.O. levels ranging between 0.8 to 5.9 mg/l were recorded for the last four downstream sites. Exceptions when D.O. was relatively high occurred during April and May for Baring Road and August for all the last four sites. Records for all the sites also showed flow rates ranging between 0.01 and 0.57 ms^{-1}. The highest flow records during the sample period were also obtained in July and August for all the sample sites the only exception being the Bayfordbury site. Levels during these two months ranged between 0.08ms^{-1} recorded in July for the Eastwalk/Westwalk site and 0.57ms^{-1} recorded in August for the Cathill site. Nitrate levels were lowest in the Bayfordbury and Jacks lake sites during the study while the highest concentrations (5 – 10mg/l) were recorded in the last four sites. Ammonia and phosphates also had a similar trend with the lowest levels recorded for the Bayfordbury and Jack Lake sites and the highest levels recorded in the Baring Road, Cathill, Parkside Gardens and Eastwalk/Westwalk sites. Ammonia and

phosphate levels within these four sites mostly ranged between 1 – 4mg/l and 0.5 – 5mg/l respectively. Water temperature between sites varied little compared to most of the other physicochemical parameters measured during the study and ranged between 13.7 and 22.5°C.

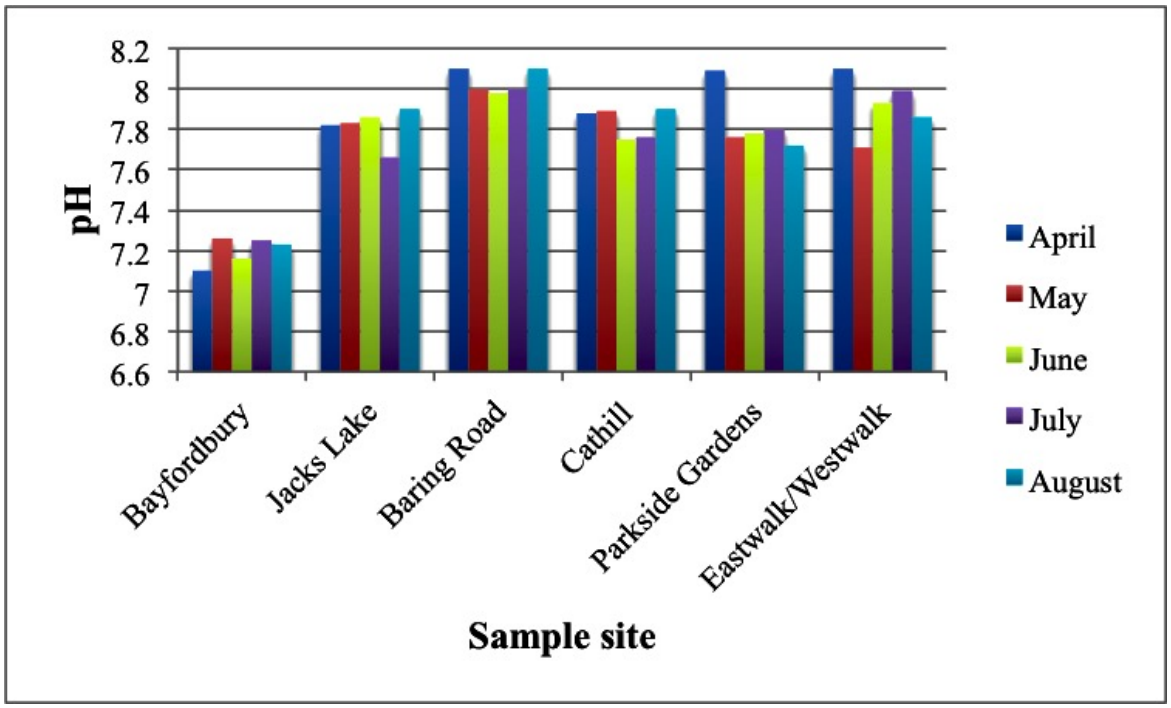


Figure 5.3: pH records for the sample sites between April and August 2014

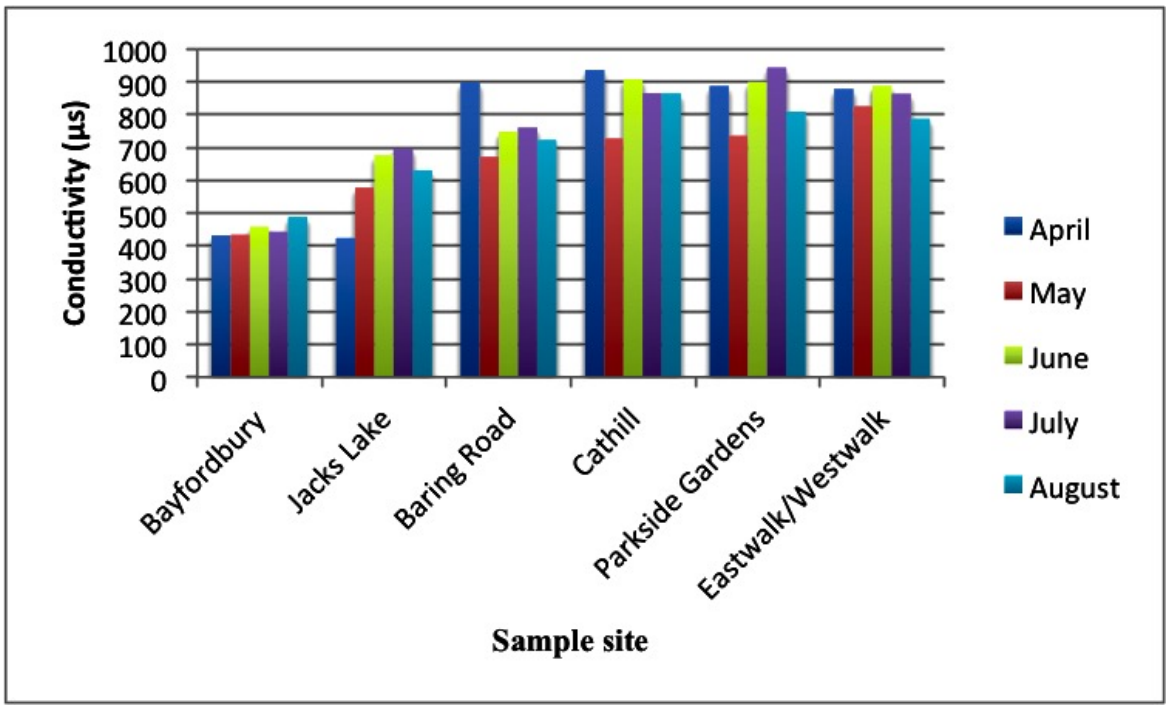


Figure 5.4: Conductivity levels within the sample sites between April and August 2014

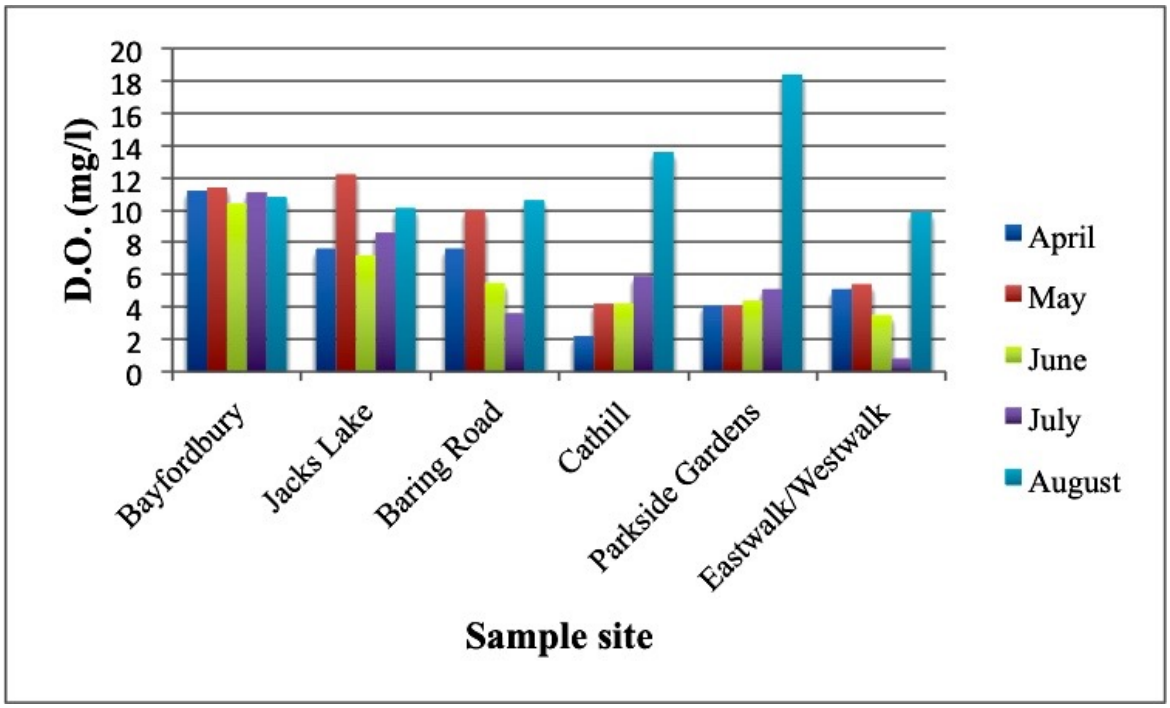


Figure 5.5: D.O levels within the sample sites for the 2014 study

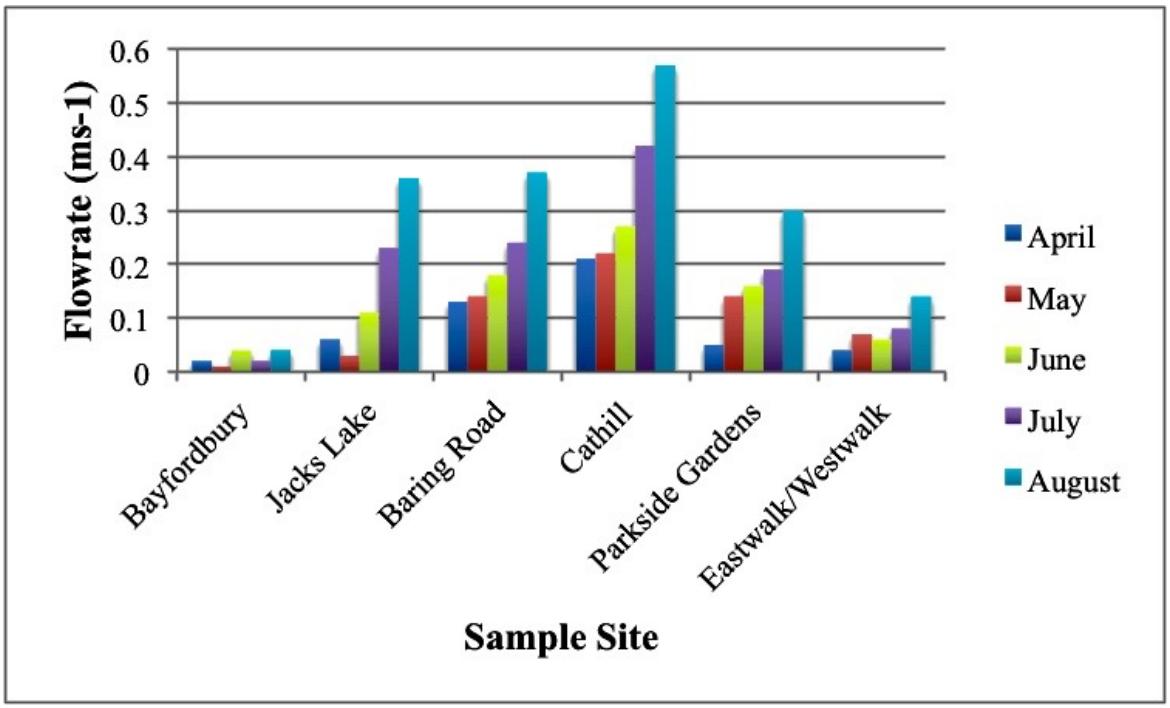


Figure 5.6: Flow records for the sample sites between April and August 2014

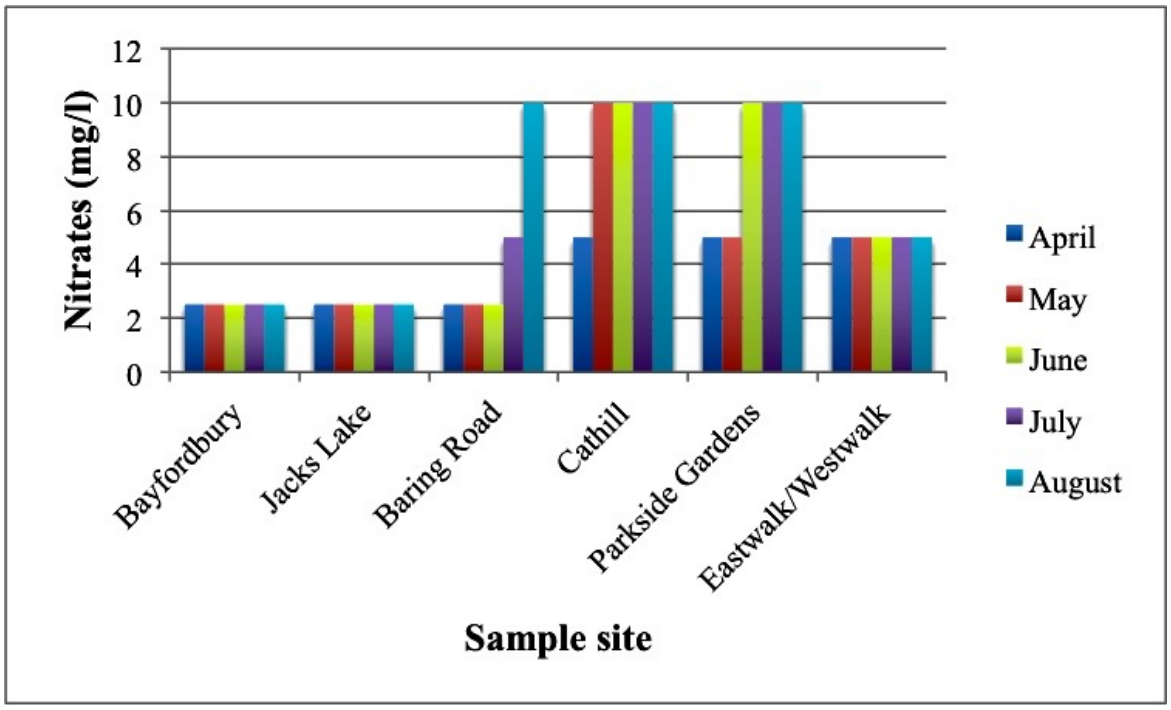


Figure 5.7: Nitrate concentrations within sites in the 2014 study

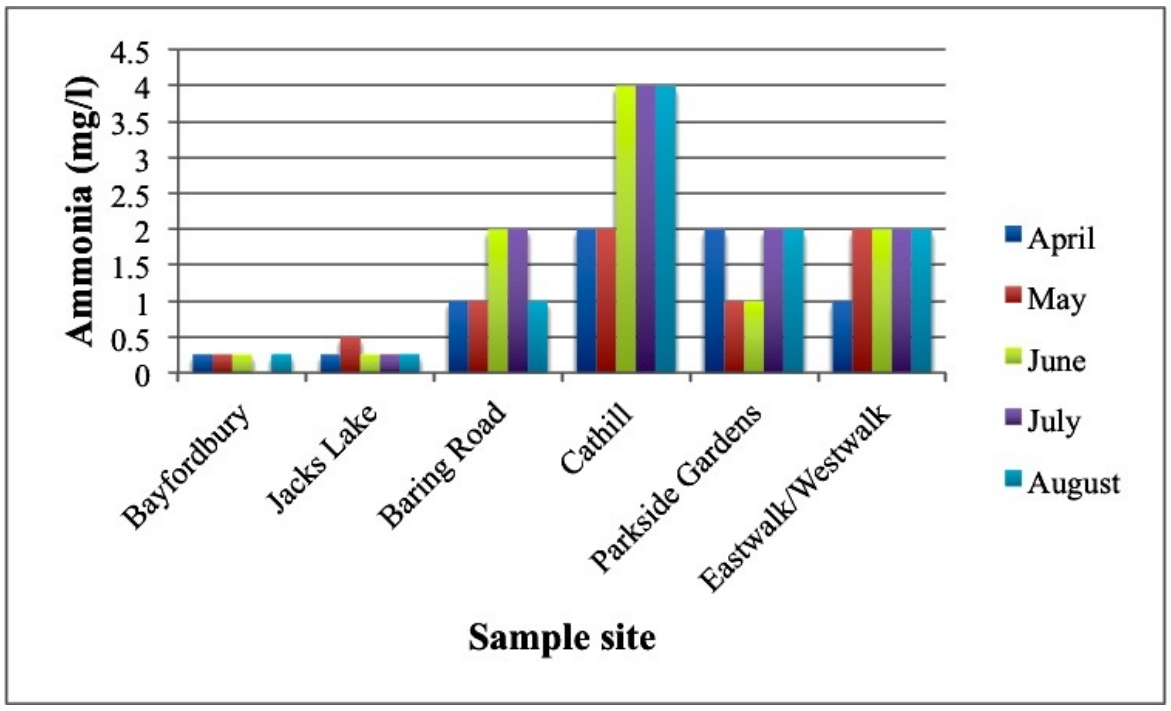


Figure 5.8: Ammonia concentrations within sites in the 2014 study

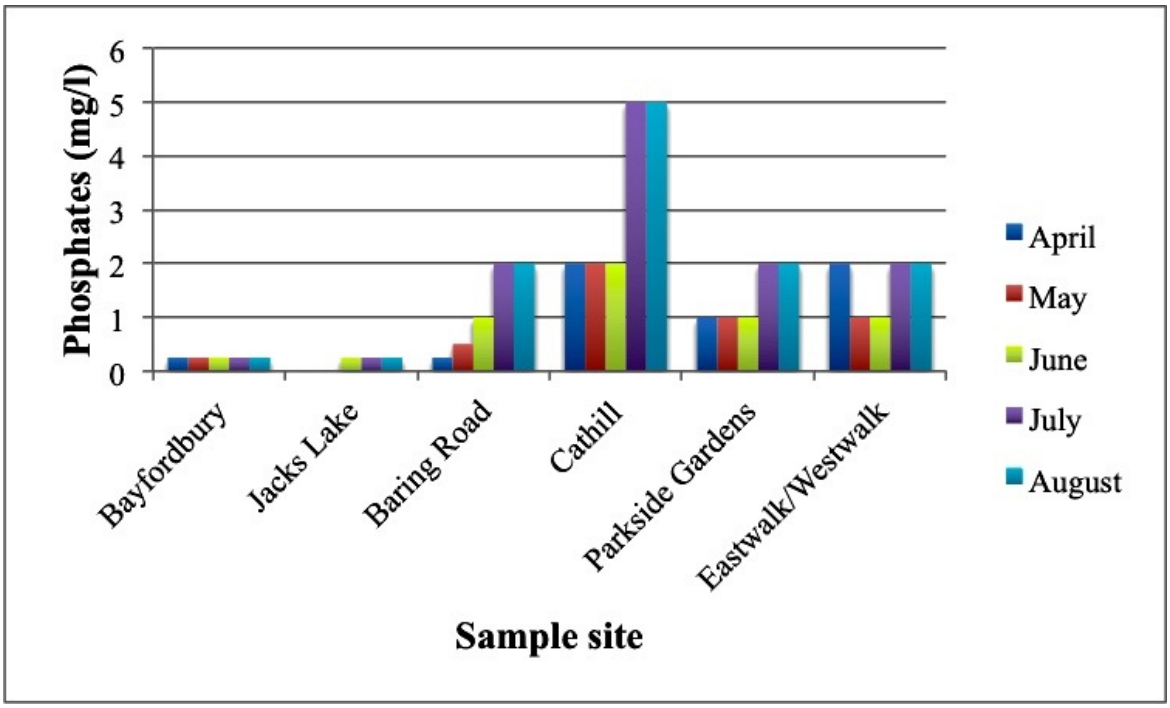


Figure 5.9: Phosphate concentrations within sites between April and August 2014

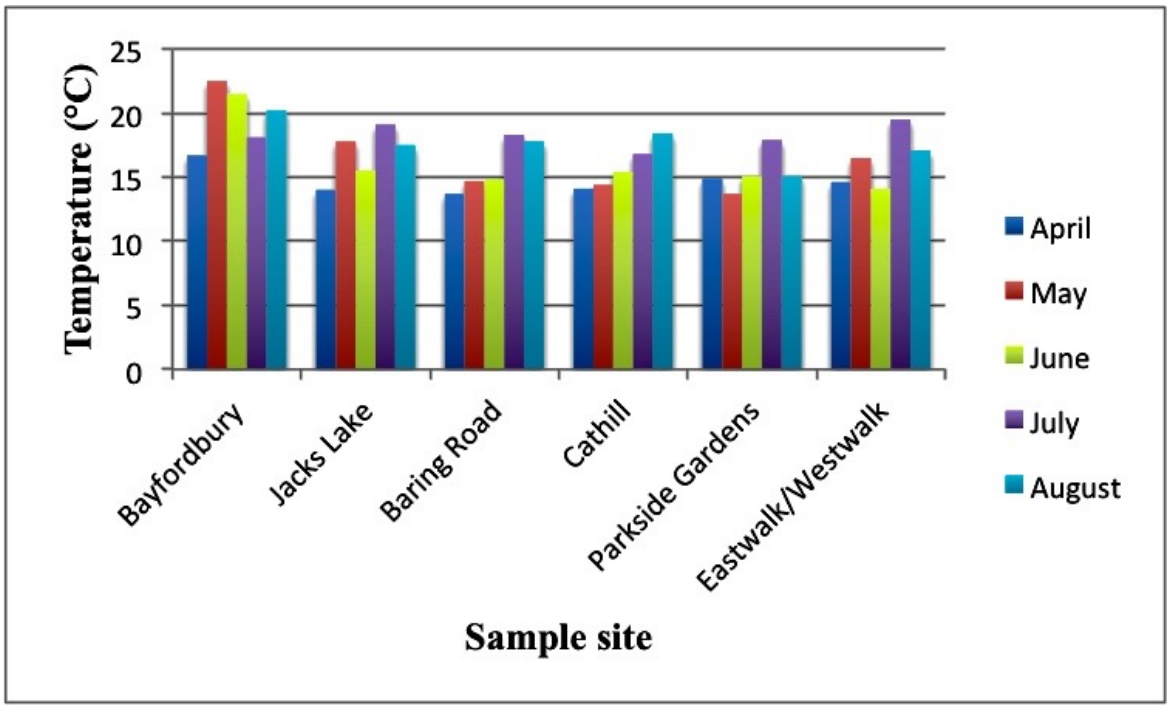


Figure 5.10: Water temperature records for sampling sites in the 2014 study

5.4.2 Diversity and abundance of communities identified during the biofilm experiment

A total of 152 taxa were identified and these communities were collated into three groups, algae, protozoa and metazoa. Amongst the algae group 58 genera under different phyla were recorded. The most common was phylum Bacillariophyta (diatoms). The genera with the largest taxa were, *Nitzschia* (9 taxa), *Navicula* (8 taxa), *Cymbella* (6 taxa), *Suirella* (5 taxa) *Gomphonema* (4) and *Achnanthes* (4). There were 38 protozoan groups recorded, the largest of which was the Ciliophora (ciliates) with 22 taxa. The metazoans had only 17 taxa recorded. Table 5.2 shows a list of these individuals while Table 5.3 shows the taxon richness and diversity index scores for the sites (through May – August 2014). From Table 5.2 it can be seen the highest taxon richness was recorded for the dirty sites (Sites 4, 5 and 6) and Site 3. Taxon richness within these sites ranged between 50 - 62 taxa throughout the entire study period. Based on the Simpson's index scores, during the four months study, the highest diversity occurred in the dirty sites (4, 5, 6) and in site 3 (Simpsons Index scores ranging between 12.2 and 26.36) the only exception being site 3, which in July had a score of 8.2.

Table 5.2: A list of individuals recorded during the biofilm experiment study.

Algae	**Protozoa**	**Metazoa**
Amphora ovalis	*Amoeba*	*Aelosoma*
Amphora pediculus	*Mastigamoeba*	*Tubifex*
Eunotia bilunaris	*Pelomyxa*	*Erpobdella octoculata*
Aulacosera ambigua	*Arcella*	*Glossiphonia complanata*
Aulacosera granulate	*Centropyxis*	*Dugesia polychroa*
Caloneis bacillum	*Cryptodifflugia*	*Phagocata vitta*
Caloneis silicula	*Difflugia*	*Anuraeopsis navicula*
Navicula cryptocephala	*Mayorella*	*Philodina*
Navicula cuspidata	*Actinophrys*	*Rotaria rotatoria*
Navicula gracilis	*Astasia*	*Rotifer (unidentified)*
Navicula oblonga	*Phacus longicauda*	*Physidae*
Navicula radiosa	*Phacus pyrum*	*Nematode*
Navicula reinhardtii	*Euglena viridis*	*Asellus aquatic*
Navicula viridula	*Menoidium*	*Gammarus pulex*
Navicula minima	*Rhipidodendron*	*Chironomid larvae*
Gyrosigma acuminatum	*Aspidisca*	*Simuliidae larvae*
Gyrosigma attenuatum	*Euplotes*	*Gastrotrich*
Pinnularia viridis	*Coleps*	
Stauroneis phoenicenteron	*Halteria*	
Nitzschia acicularis	*Stentor*	
Nitzschia amphibia	*Opercularia*	
Nitzschia dissipata	*Vorticella*	
Nitzschia frustulum	*Spathidium*	
Nitzschia hungarica	*Spirostomum*	
Nitzschia palea	*Paramecium putrinium*	
Nitzschia recta	*Paramecium caudatum*	
Nitzschia sigmoidea	*Calyptotricha*	
Nitzschia sublinearis	*Cinetochilum*	
Hantzchia amphioxys	*Colpidium*	
Diatoma vulgare	*Tetrahymena*	
Synedra acus	*Chaenea*	
Synedra ulna	*Lacrymaria*	
Fragilaria spp.	*Didinium*	
Meridion circulare	*Dileptus*	

Table 5.2: A list of individuals recorded during the biofilm experiment study (continued)

Algae	**Protozoa**	**Metazoa**
Martyana martyi	*Litonotus*	
Raphoneis amphicerous	*Epiclintes*	
Tabellaria	*Plagiopyla*	
Cyclotella comta	*Prorodon*	
Cyclotella meneghiniana		
Stephanodiscus hantzchii		
Stephanodiscus astrae		
Coscinodiscus lacustris		
Melosira varians		
Chrysococcus rufescens		
Dinobryon		
Mallomonas caudata		
Vaucheria		
Stigeoclonium tenue		
Ankistrodesmus		
Bulbochaete mirabilis		
Oedogonium		
Chlamydomonas reinhardtii		
Chlamydomonas spp		
Pandorina morum		
Phacotus lenticularis		
Volvox		
Chlorococcum		
Scenedesmus quadricauda		
Microspora		
Chlorella spp		
Chlorella pyrenoidosa		
Stichococcum bacillaris		
Cladophora		
Rhizoclonium hierogyphicum		
Cylindrocapsa		
Sphaerotilus		
Ulothrix		
Closterium		
Euastrum oblongum		
Netrium		
Spirogyra spp.		
Chamaesiphon		
Oscillatoria		
Phormidium		

Table 5.3: Taxon richness and diversity parameters of communities for the biofilm experim-ental study (May – August 2014).
Letters refer to the month sampled and numbers to sites.

Site	Taxon richness	Simpsons Index
M1	22	7.98
J1	23	6.924
JY1	13	6.756
A1	16	6.73
M2	27	6.421
J2	22	10.412
JY2	20	6.347
A2	29	13.806
M3	53	18.919
J3	50	12.253
JY3	40	8.22
A3	36	13.575
M4	56	18.204
J4	53	23.897
JY4	50	14.282
A4	52	25.832
M5	53	21.656
J5	53	20.755
JY5	55	18.75
A5	53	16.613
M6	58	12
J6	58	26.358
JY6	62	19.241
A6	53	19.098

The complete results of identified communities and the numbers recorded are presented in the appendix section of this thesis. The results recorded during the study showed a minor difference in the identified communities between May, June, July and August. The difference between months is likely due to seasonal succession. However, clear differences in communities were noted been clean and dirty sites. The mean distribution of communities for each of the study sites are given in the bar charts in Figures 5.11 to 5.34. From examining the recorded taxa for the entire study period, (May - August), it was noted that some organisms always occurred in a particular site category especially the dirty sites. In addition, there were also some individuals that occurred abundantly in these sites especially the dirty sites. Table 5.4 shows a list of the dominant taxa as well as taxa that always occurred only in clean sites and dirty sites.

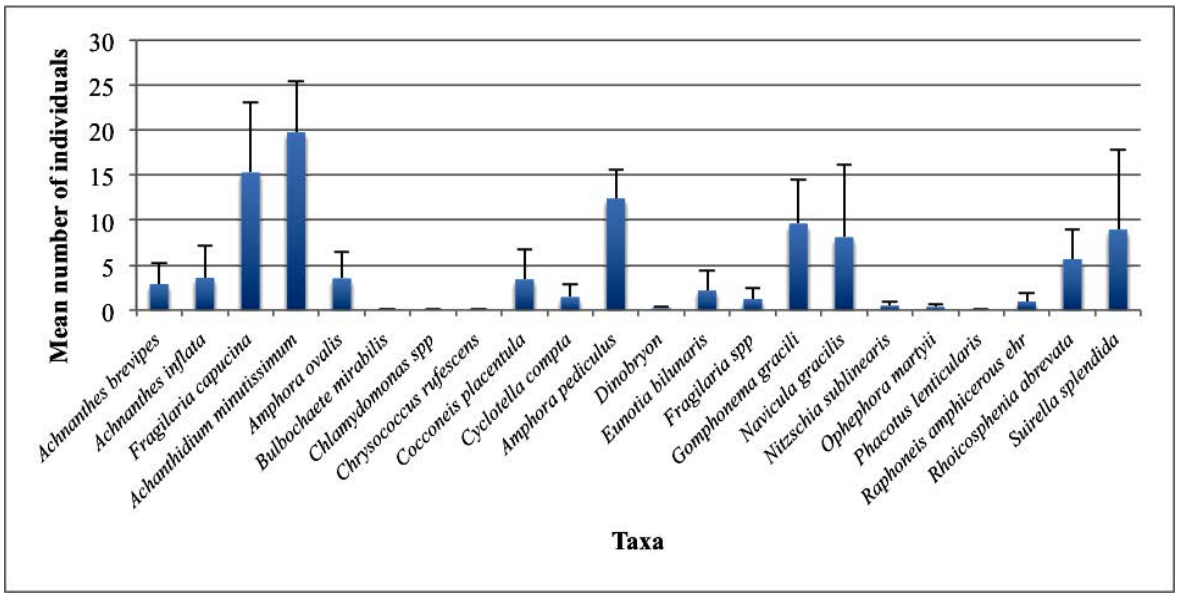


Figure 5.11: Mean and standard error for individuals present at site 1 across all replicates in May 2014

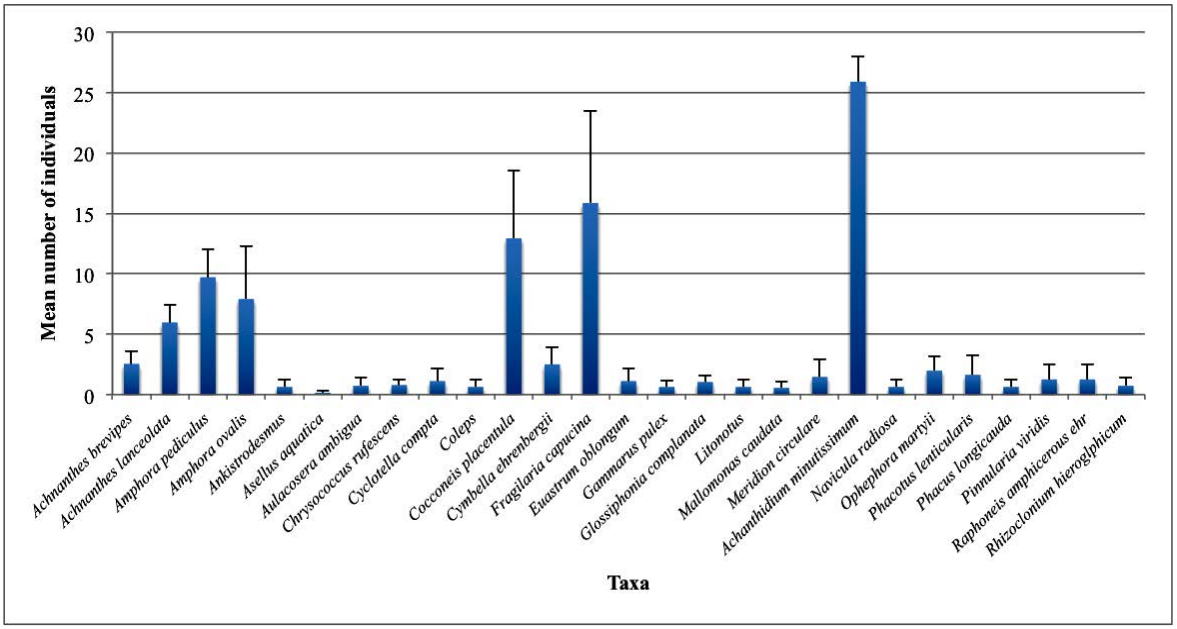


Figure 5.12: Mean and standard error for individuals present at site 2 for all replicates in May 2014

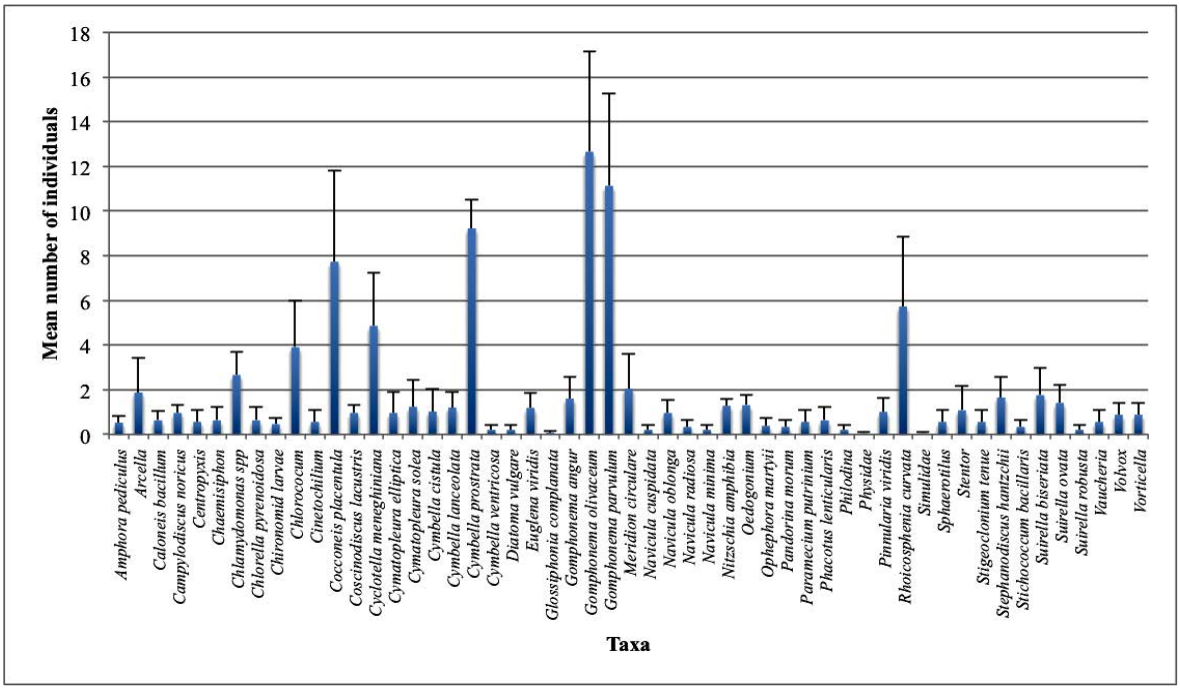


Figure 5.13: Mean and standard error for individuals present at site 3 for all replicates in May 2014

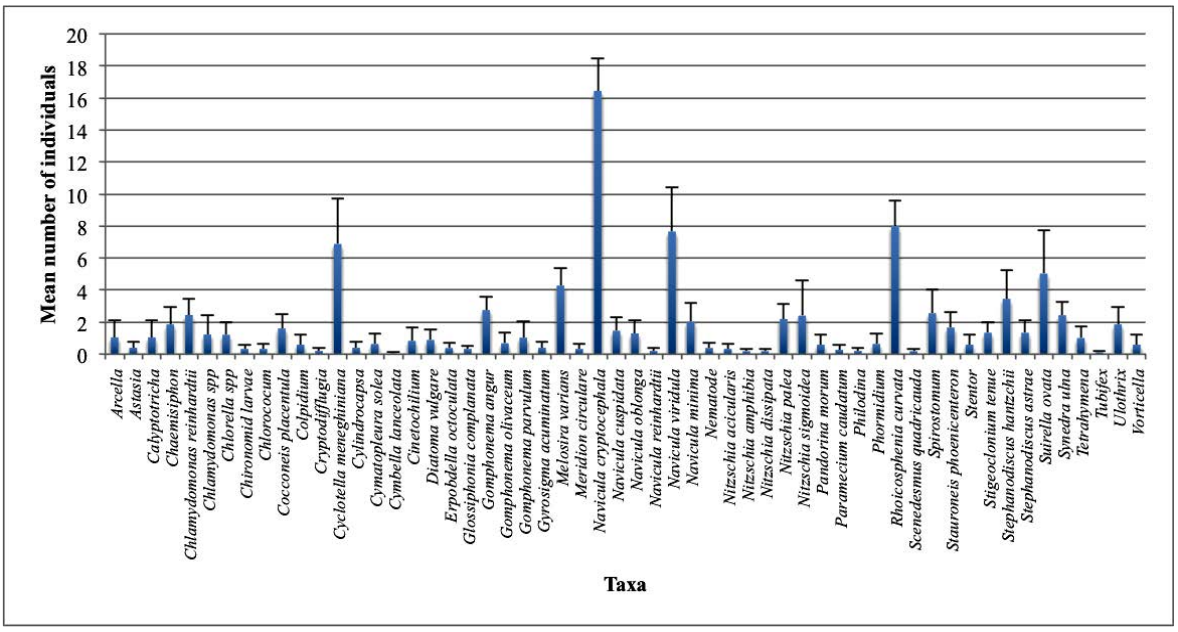


Figure 5.14: Mean and standard error for individuals present at site 4 for all replicates in May 2014

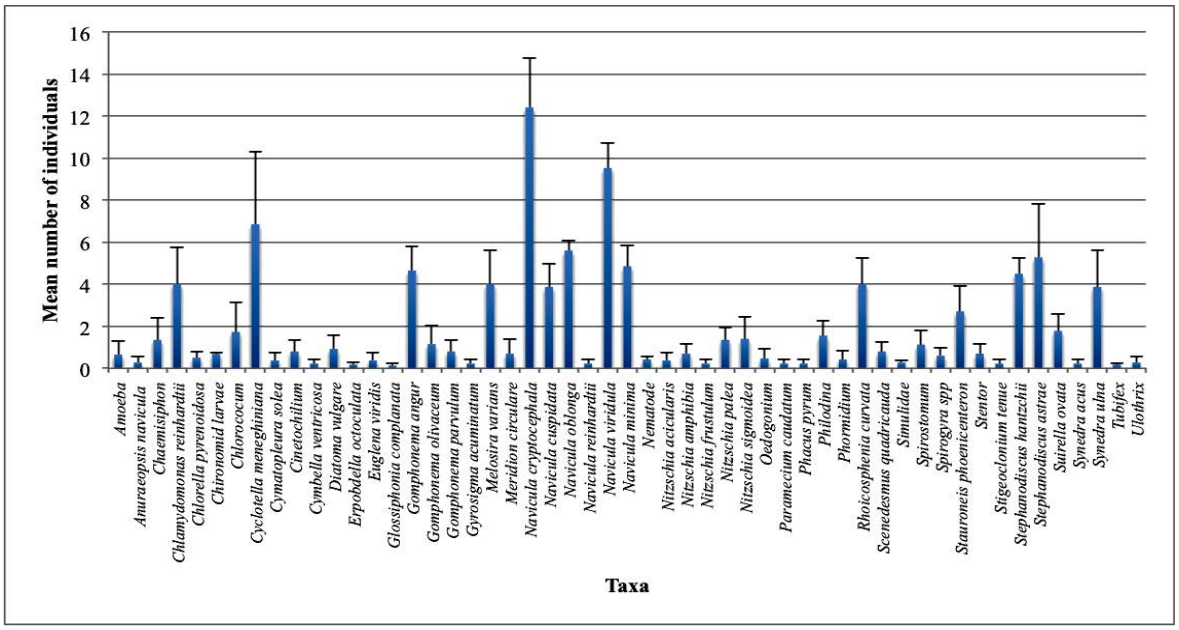


Figure 5.15: Mean and standard error for individuals present at site 5 for all replicates in May 2014

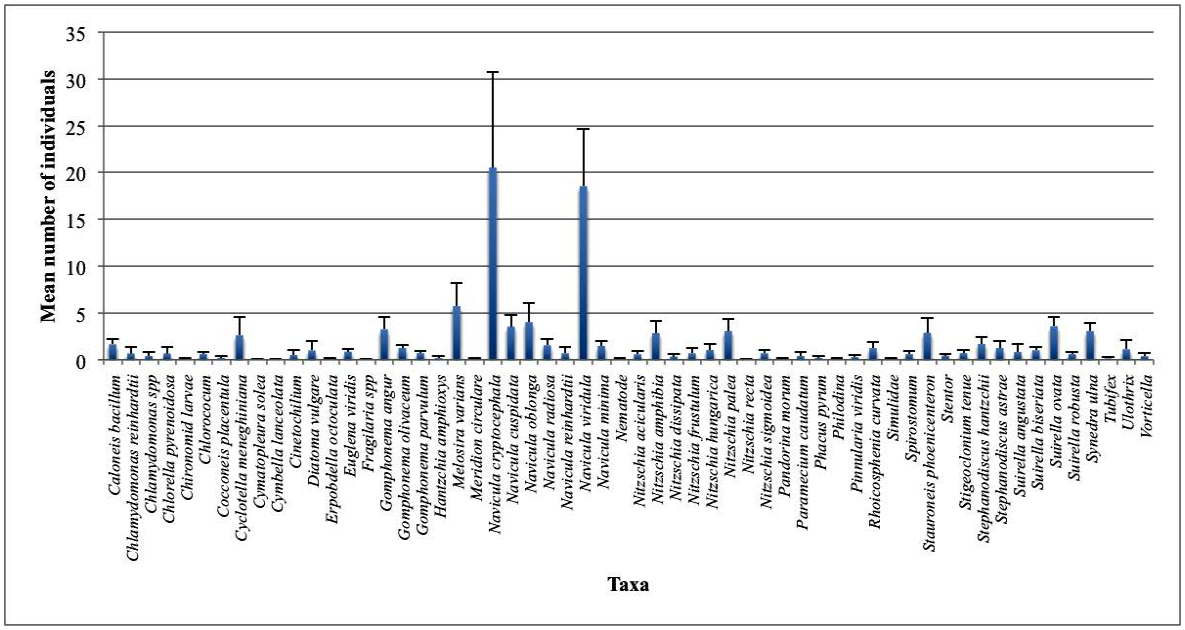


Figure 5.16: Mean and standard error for individuals present at site 6 for all replicates in May 2014

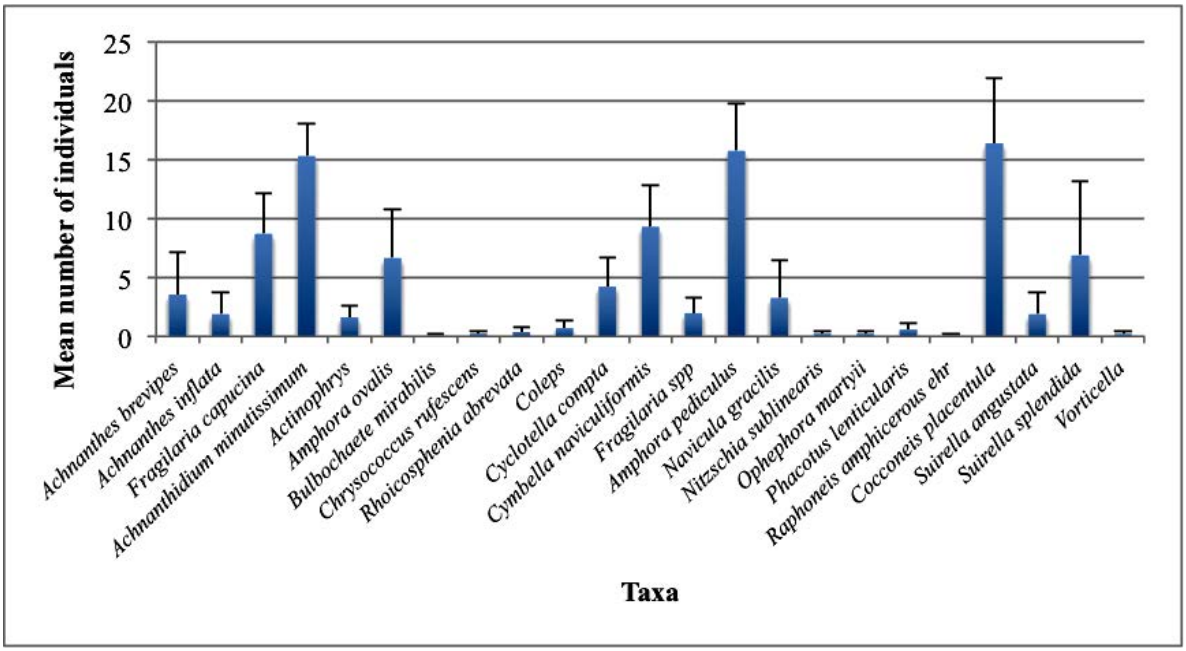


Figure 5.17: Mean and standard error for individuals present at site 1 for all replicates in June 2014

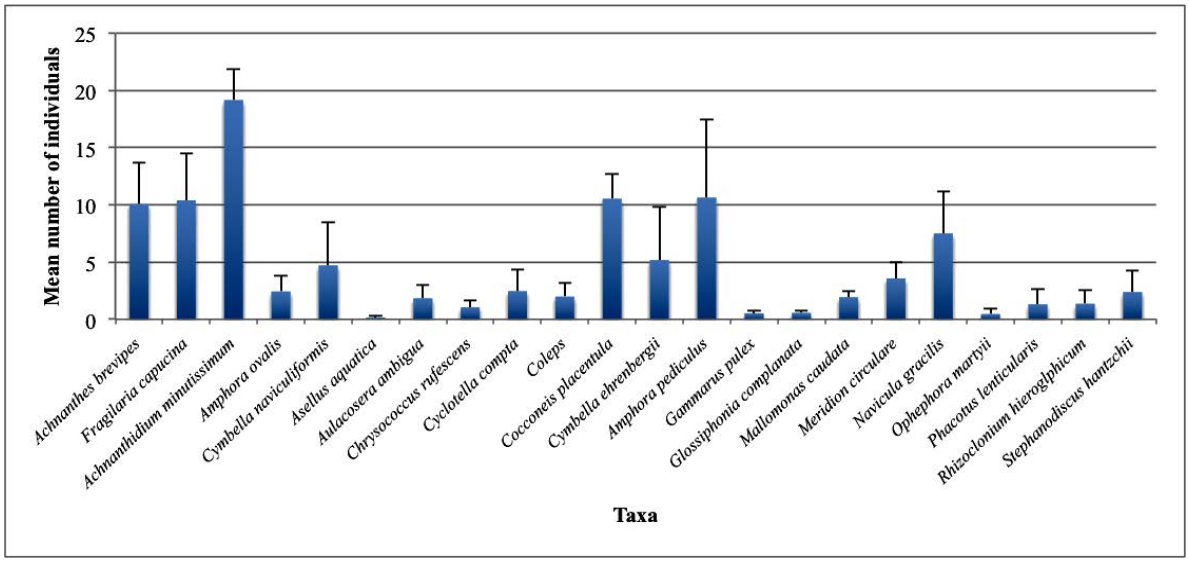


Figure 5.18: Mean and standard error for individuals present at site 2 for all replicates in June 2014

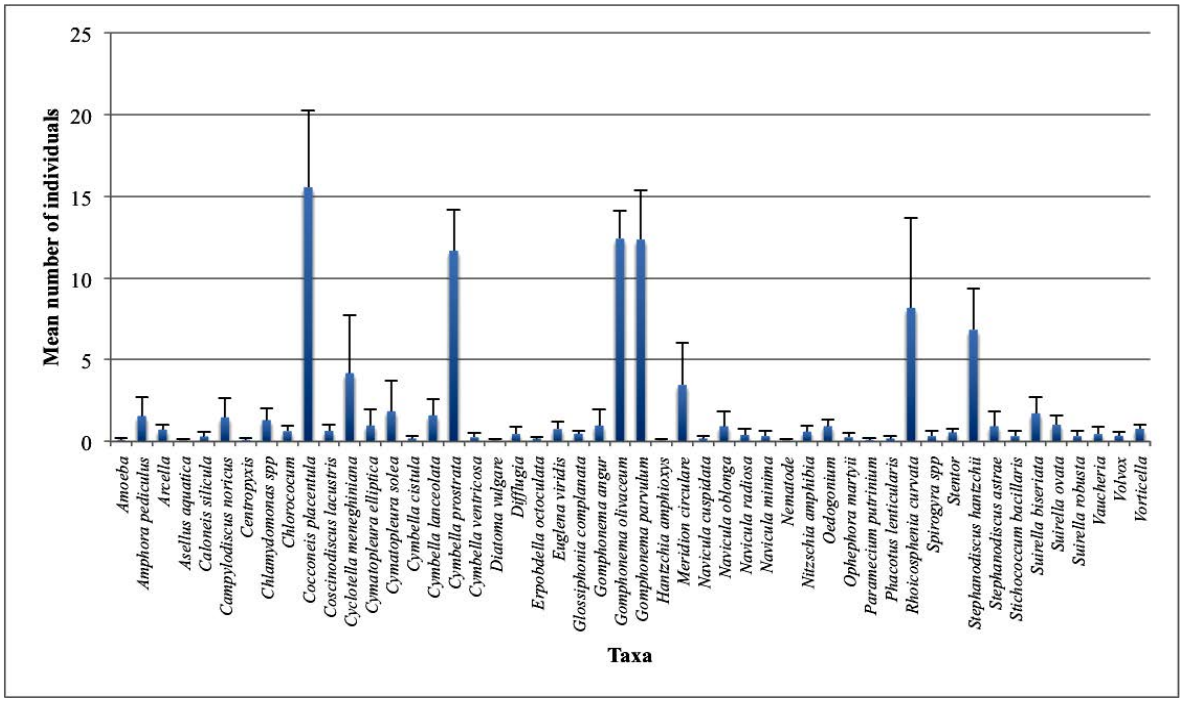


Figure 5.19: Mean and standard error for individuals present at site 3 for all replicates in June 2014

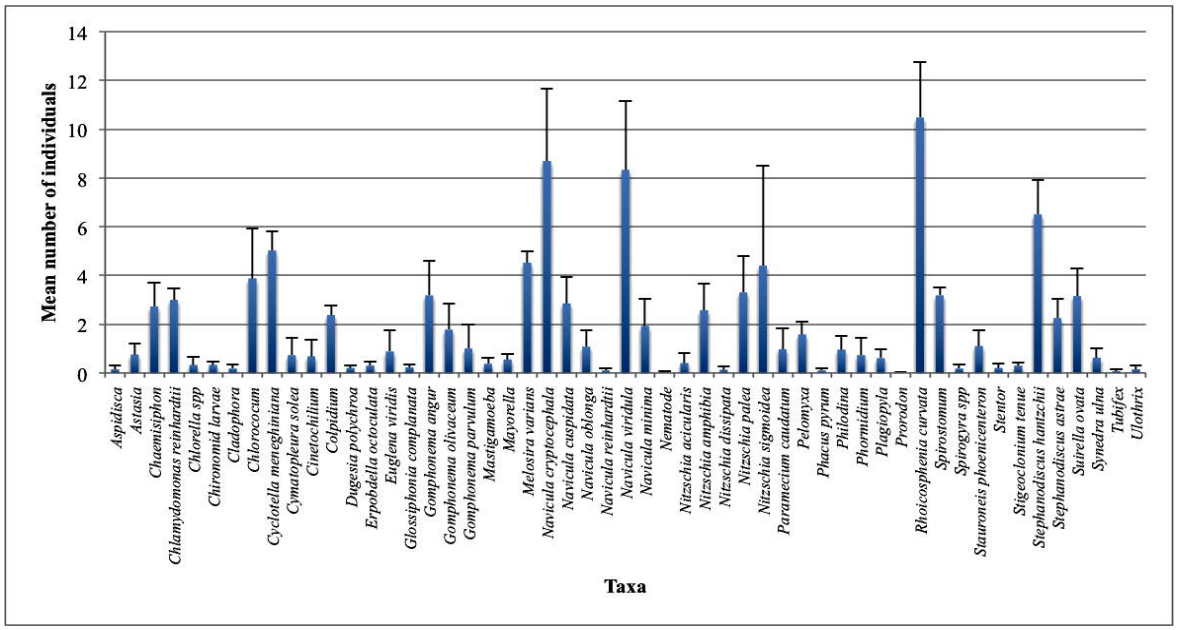


Figure 5.20: Mean and standard error for individuals present at site 4 for all replicates in June 2014

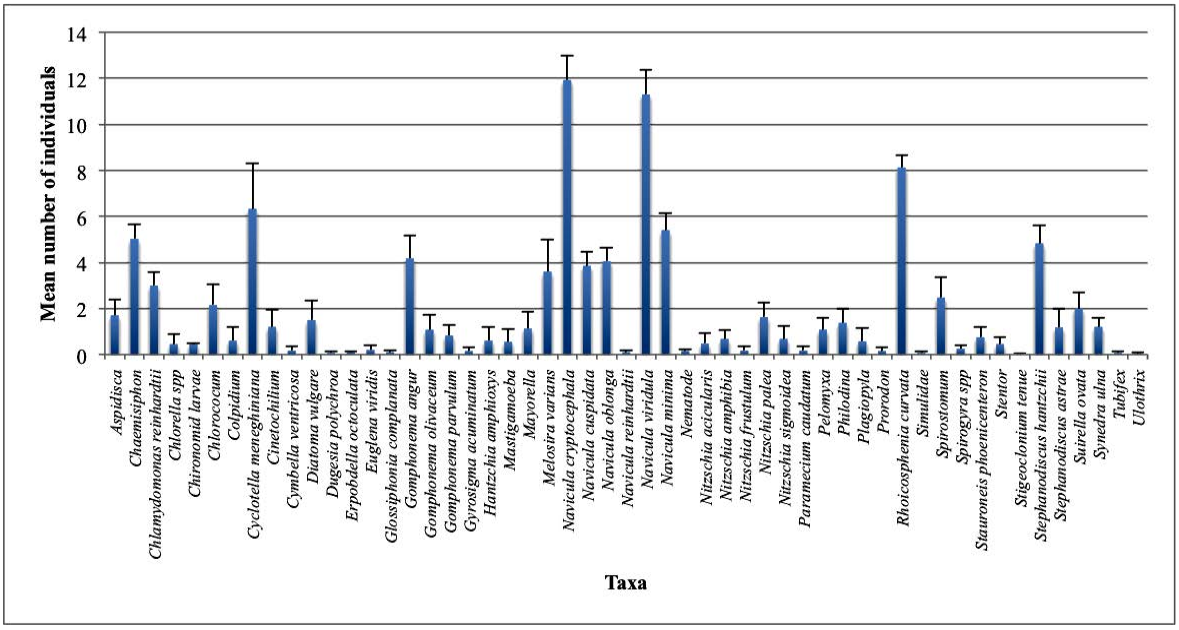


Figure 5.21: Mean and standard error for individuals present at site 5 for all replicates in June 2014

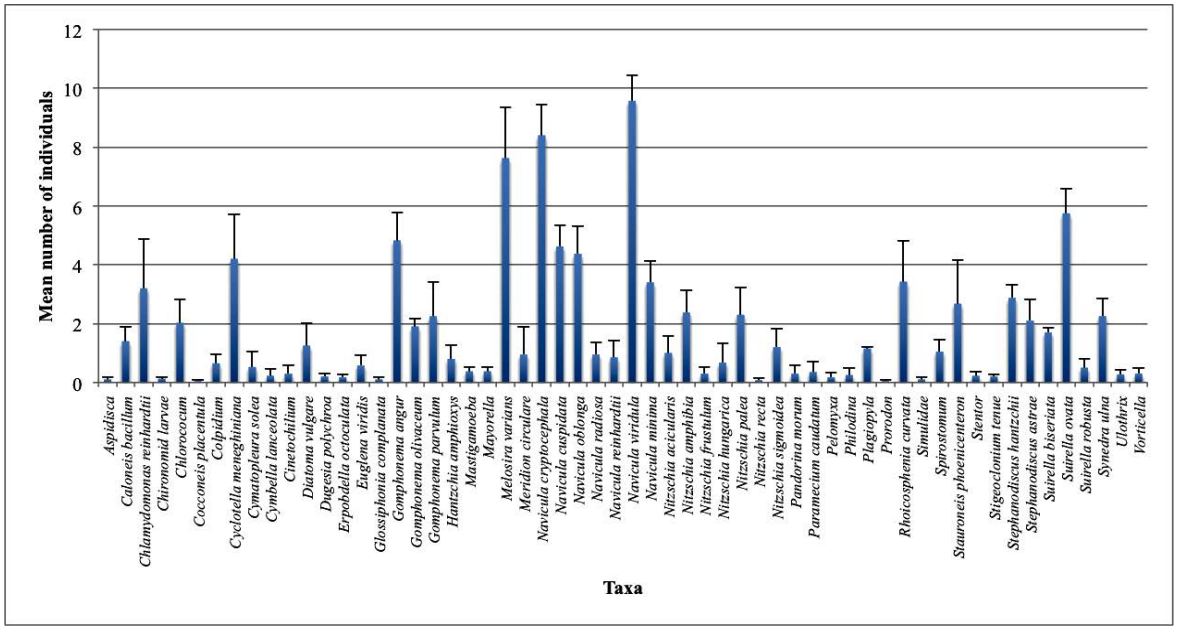


Figure 5.22: Mean and standard error for individuals present at site 6 for all replicates in June 2014

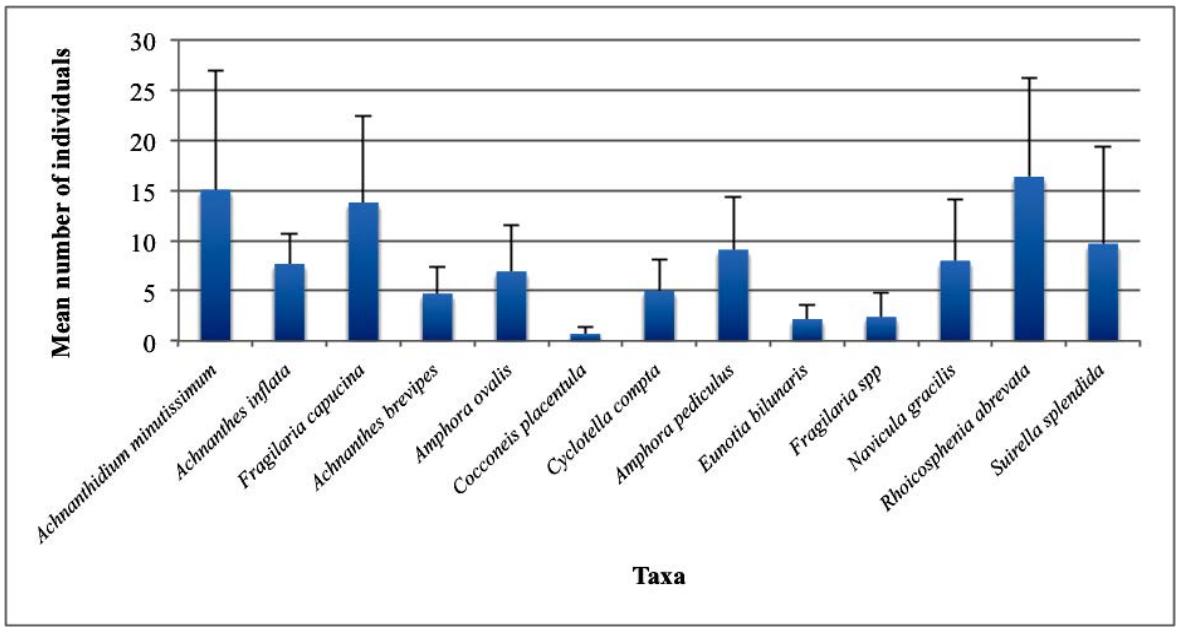


Figure 5.23: Mean and standard error for individuals present at site 1 for all replicates in July 2014

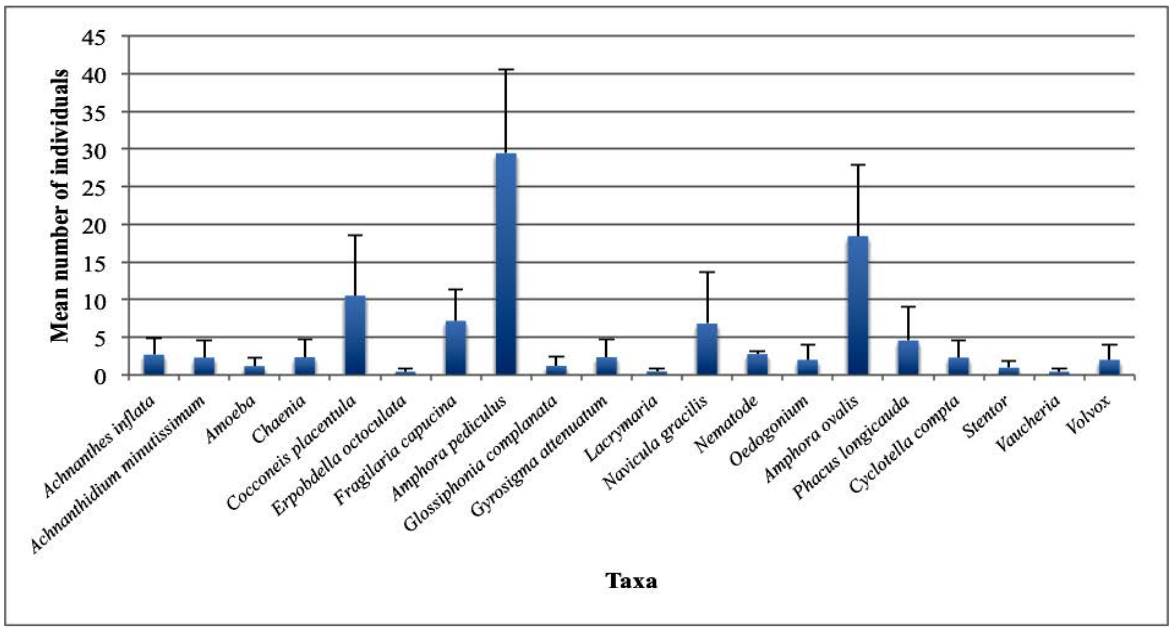


Figure 5.24: Mean and standard error for individuals present at site 2 for all replicates in July 2014

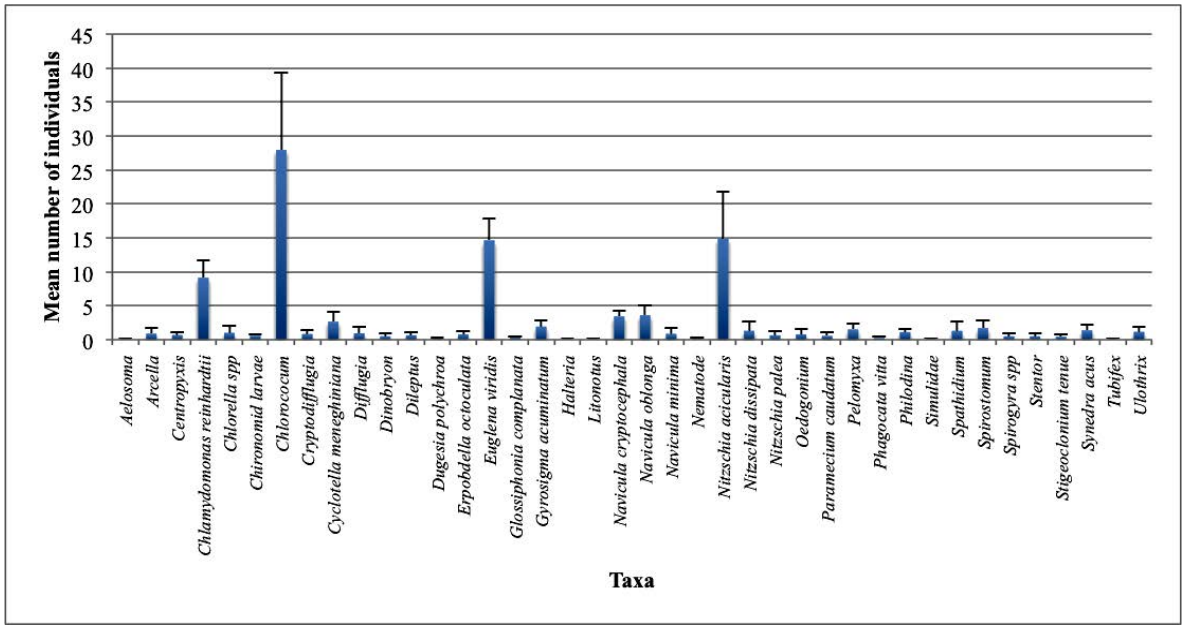


Figure 5.25: Mean and standard error for individuals present at site 3 for all replicates in July 2014

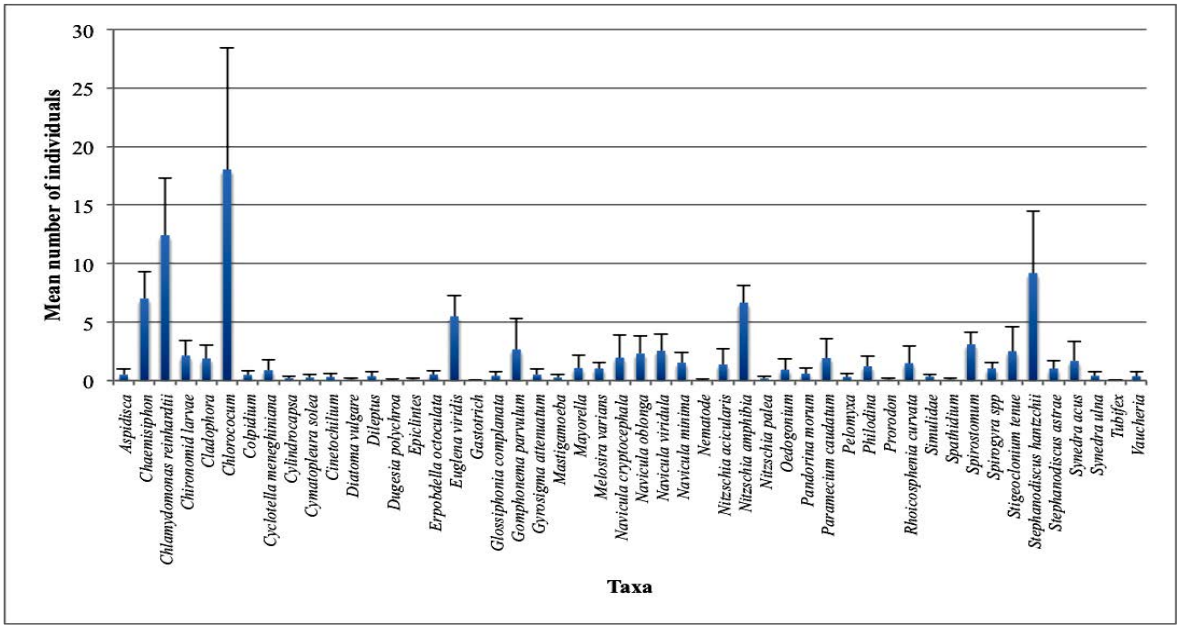


Figure 5.26: Mean and standard error for individuals present at site 4 for all replicates in July 2014

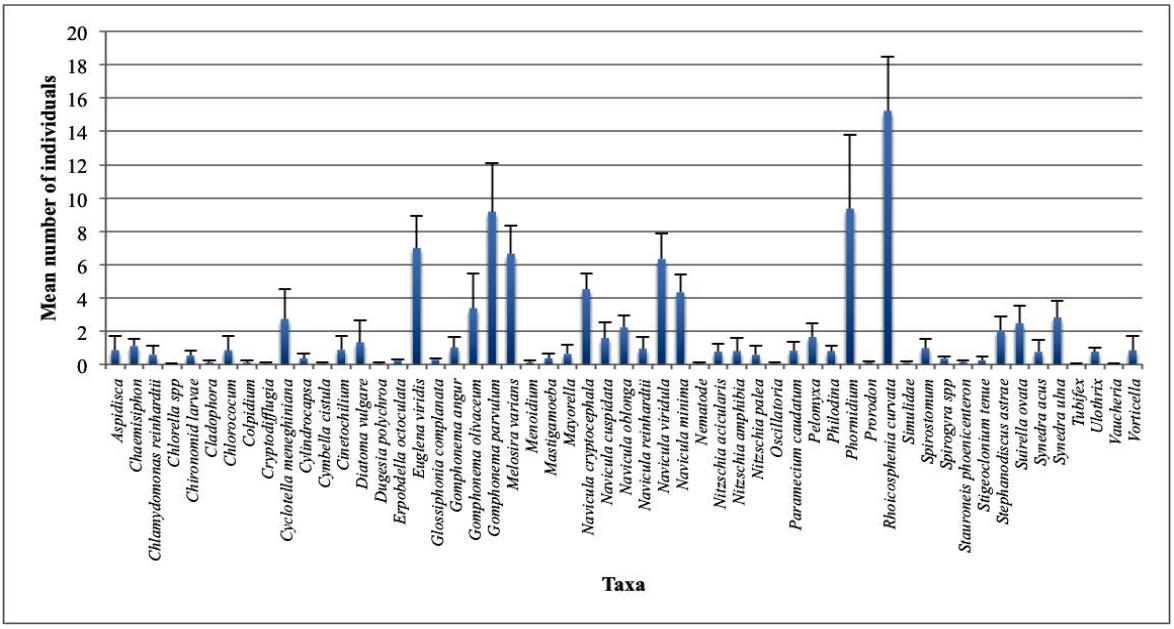


Figure 5.27: Mean and standard error for individuals present at site 5 for all replicates in July 2014

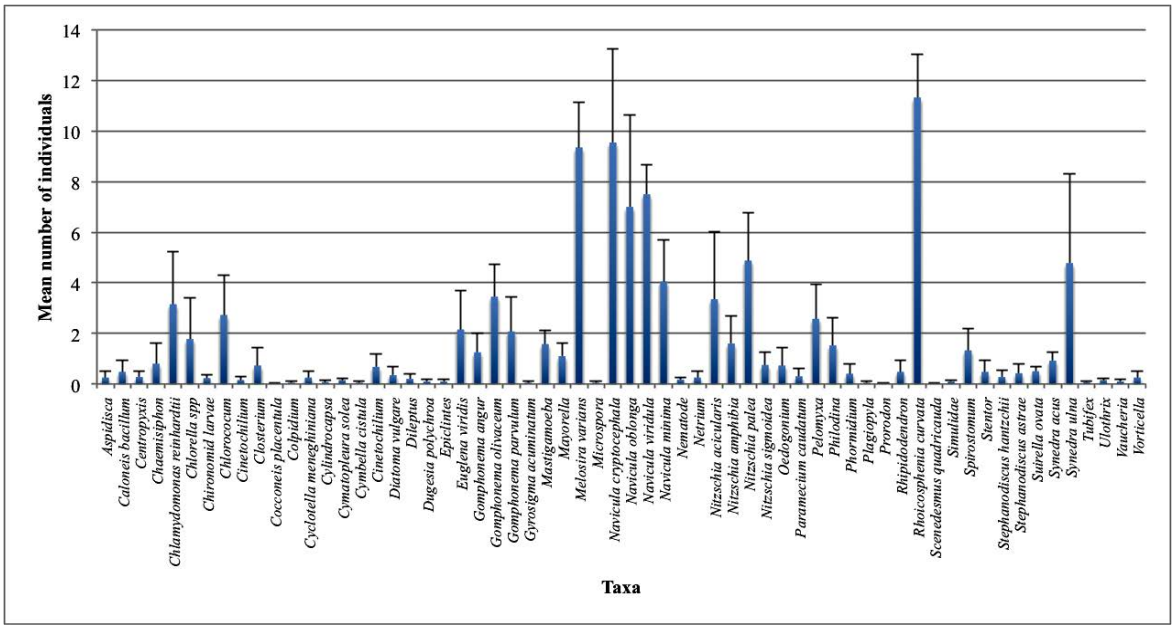


Figure 5.28: Mean and standard error for individuals present at site 6 for all replicates in July 2014

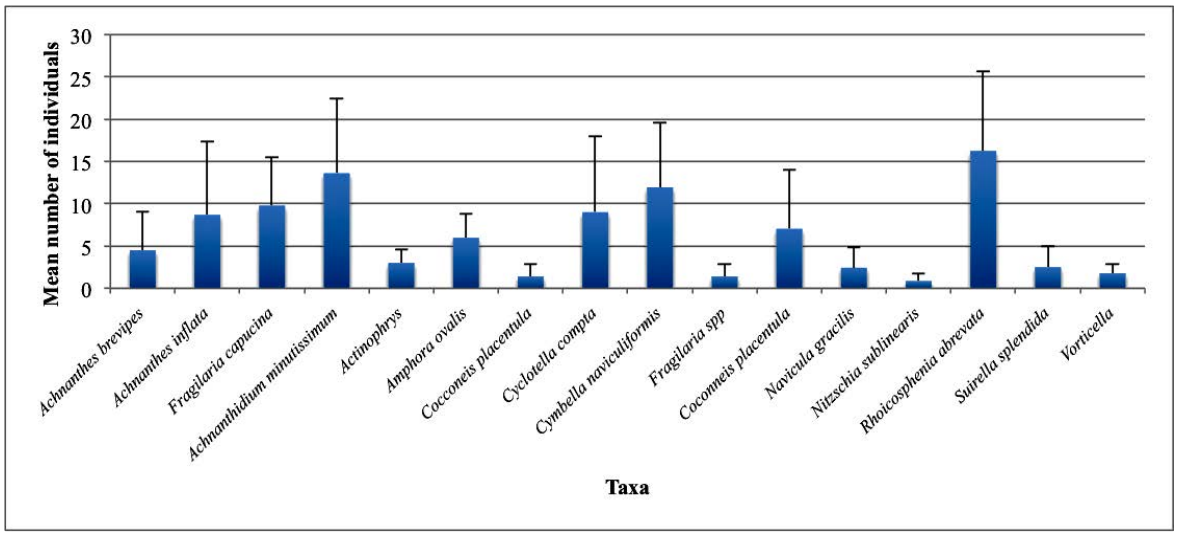


Figure 5.29: Mean and standard error for individuals present at site 1 for all replicates in August 2014

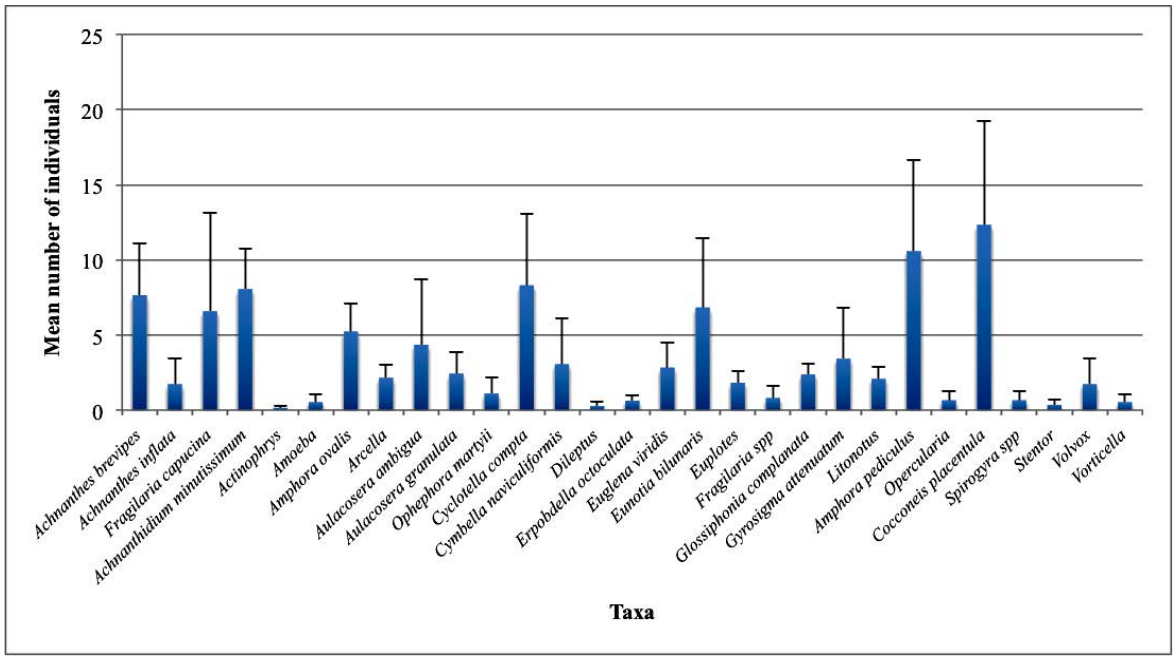


Figure 5.30: Mean and standard error for individuals present at site 2 for all replicates in August 2014

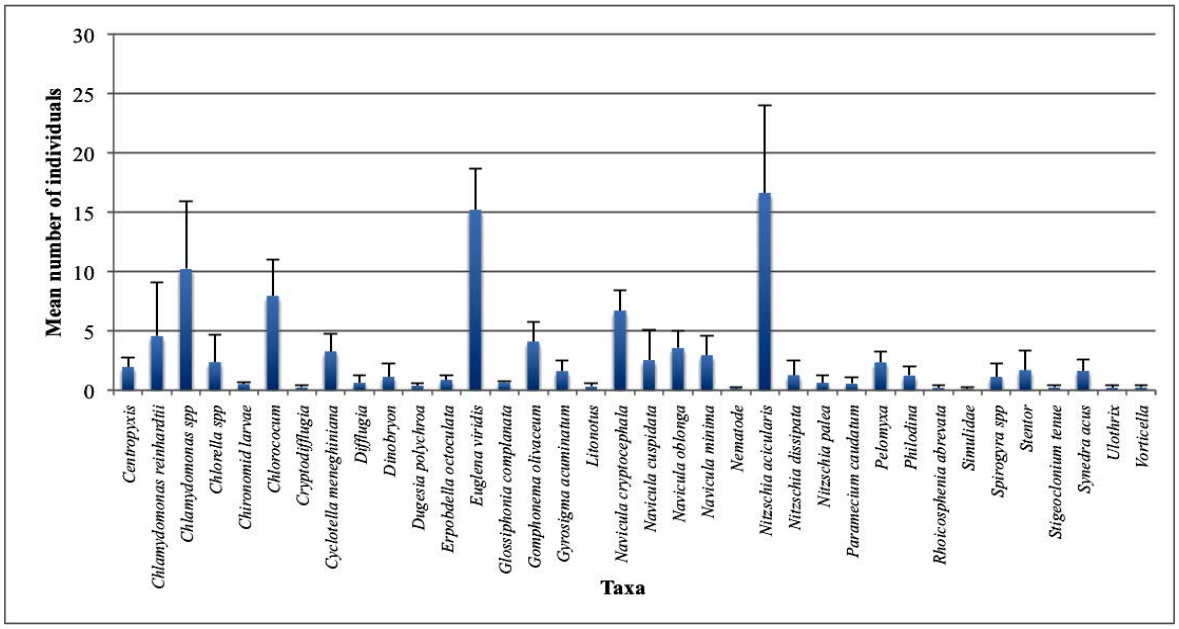


Figure 5.31: Mean and standard error for individuals present at site 3 for all replicates in August 2014

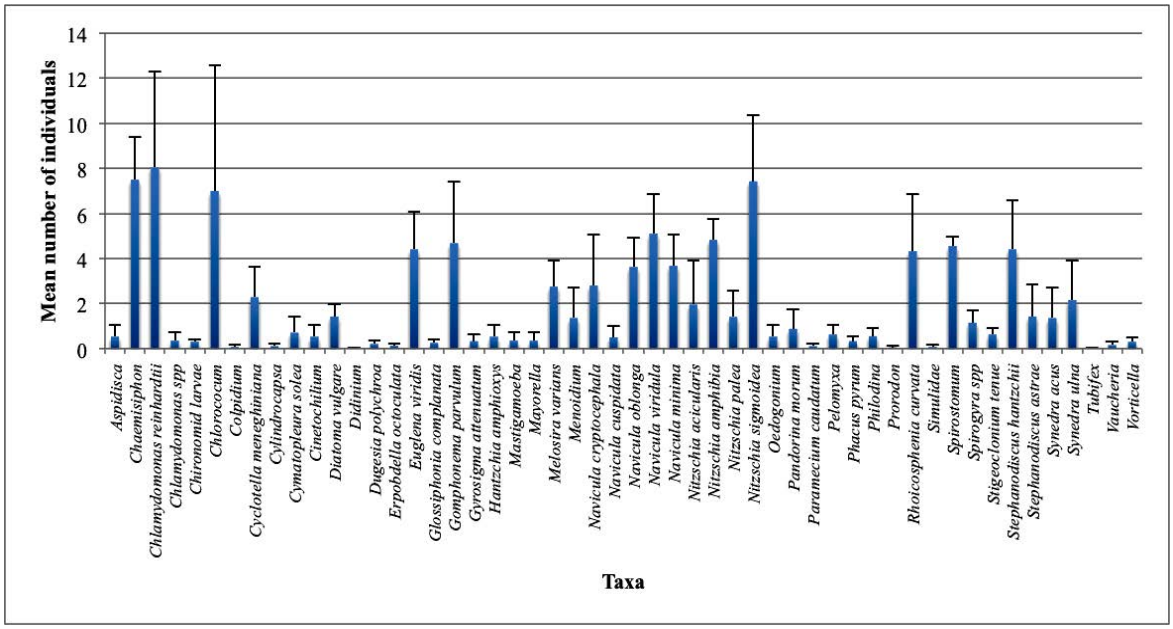


Figure 5.32: Mean and standard error for individuals present at site 4 for all replicates in August 2014

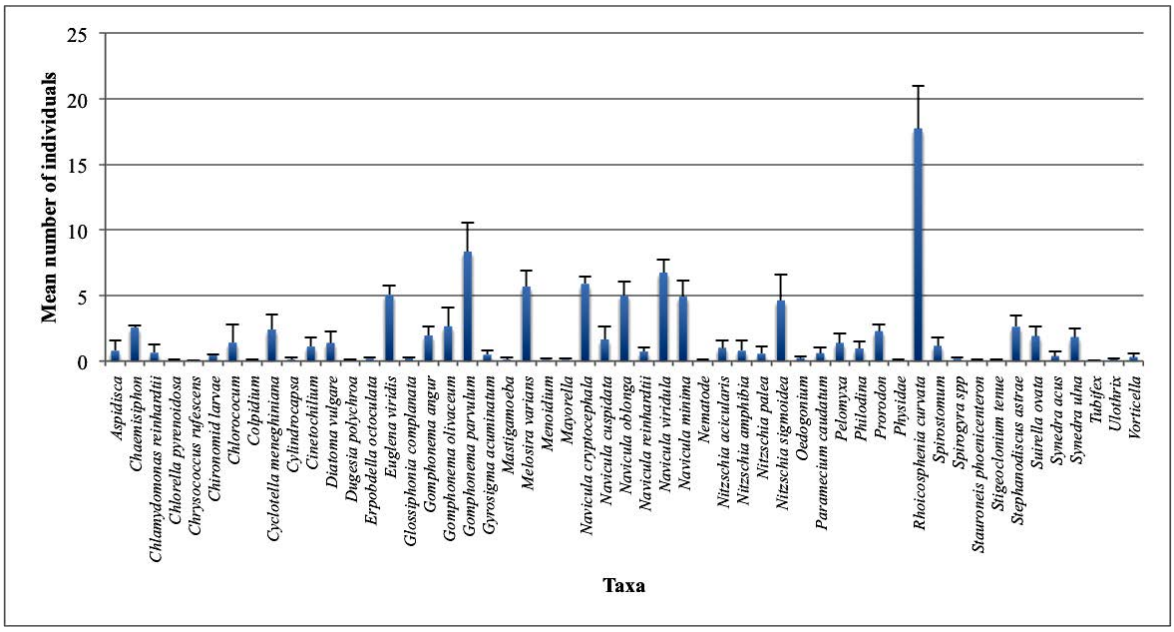


Figure 5.33: Mean and standard error for individuals present at site 5 for all replicates in August 2014

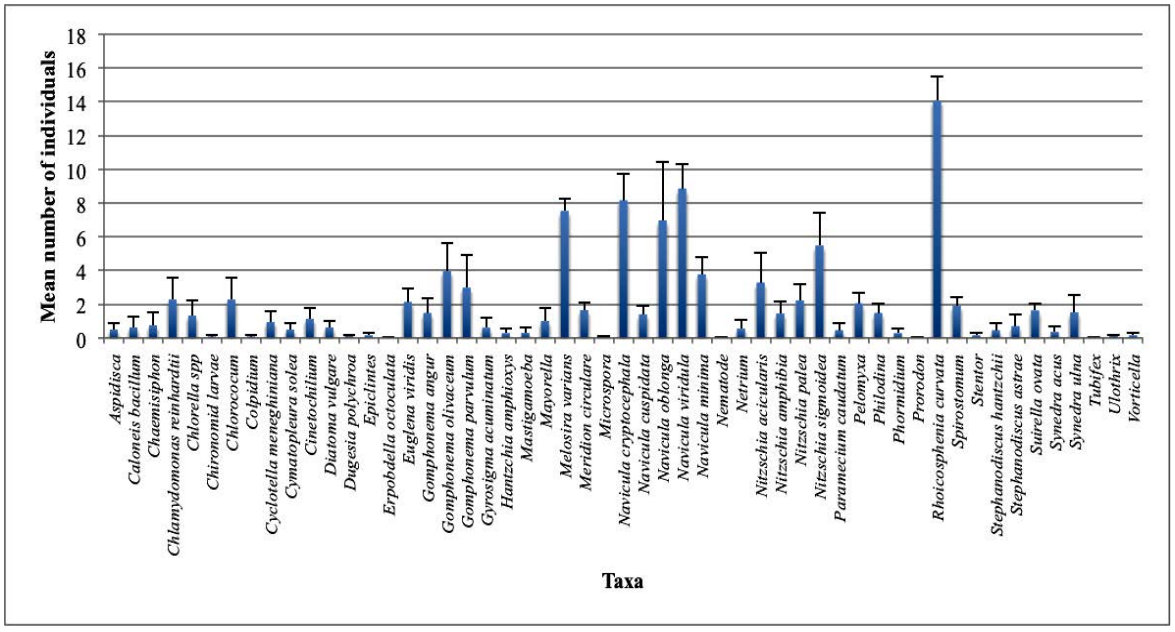


Figure 5.34: Mean and standard error for individuals present at site 6 for all replicates in August 2014

Table 5.4: Dominant taxa and taxa constantly occuring in particular site categories (clean or dirty) during the study (May to August).

Site 1 (Clean site)	Site 2 (Clean site)	Site 3 (Appears to be transitioning from clean to dirty)	Site 4 (Dirty site)	Site 5 (Dirty site)	Site 6 (Dirty site)
Fragilaria capucina	*Achnanthidium minutissimum*	*Gomphonema olivaceum*	*Navicula cryptocephala*	*Navicula cryptocephala*	*Navicula cryptocephala*
Achnanthidium minutissimum	*Fragilaria capucina*	*Gomphonema parvulum*	*Rhoicosphenia curvata*	*Navicula viridula*	*Navicula viridula*
Amphora pediculus	*Cocconeis placentula*	*Cocconeis placentula*	*Navicula viridula*	*Cyclotella meneghiniana*	*Melosira varians*
Amphora ovalis	*Amphora ovalis*	*Cymbella prostrata*	*Cyclotella meneghiniana*	*Chironomid larvae*	*Chironomid larvae*
Cyclotella compta	*Cyclotella compta*	*Euglena viridis*	*Chironomid larvae*	*Chlamydomonas reinhardtii*	*Chlamydomonas reinhardtii*
Cocconeis placentula	*Amphora pediculus*	*Nitzschia acicularis*	*Chlamydomonas reinhardtii*	*Chlorococcum*	*Chlorococcum*
Rhoicosphenia abrevata	*Navicula gracilis*	*Chlorococcum*	*Chlorococcum*	*Cinetochilium*	*Cinetochilium*
Cymbella naviculiformis		*Chironomid larvae*	*Cinetochilium*	*Gomphonema parvulum*	*Gomphonema parvulum*
		Chlamydomonas reinhardtii	*Gomphonema parvulum*	*Melosira varians*	*Navicula minima*
		Navicula cryptocephala	*Melosira varians*	*Navicula minima*	*Navicula oblonga*
		Navicula minima	*Navicula minima*	*Navicula oblonga*	*Nitzschia acicularis*
		Navicula oblonga	*Navicula oblonga*	*Nitzschia acicularis*	*Nitzschia amphibia*
		Nitzschia palea	*Nitzschia acicularis*	*Nitzschia amphibia*	*Nitzschia palea*
		Paramecium caudatum	*Nitzschia amphibia*	*Nitzschia palea*	*Paramecium caudatum*
		Philodina	*Nitzschia palea*	*Paramecium caudatum*	*Philodina*
		Chlamydomonas spp.	*Paramecium caudatum*	*Philodina*	*Spirostomum*
			Philodina	*Spirostomum*	*Synedra ulna*
			Spirostomum	*Synedra ulna*	*Mastigamoeba*
			Synedra ulna	*Rhoicosphenia curvata*	*Mayorella*
			Colpidium	*Mastigamoeba*	*Aspidisca*
			Mastigamoeba	*Mayorella*	*Pelomyxa*
			Mayorella	*Aspidisca*	*Colpidium*
			Aspidisca	*Pelomyxa*	*Prorodon*
			Pelomyxa	*Colpidium*	*Rhoicosphenia curvata*
			Prorodon	*Prorodon*	
			Stephanodiscus hantzschii	*Phormidium*	
			Chlamydomonas reinhardtii		
			Chaemisiphon		

5.5 Biofilm community structure

Results for the TWINSPAN classification analysis are given as a dendogram in Figure 5.35. The dendogram showing the results was only taken to the second level identifying three groups (A, B and C). The first division shows a seperation of sites into two groups. The seperation showed site 1 (Bayfordbury) and site 2 (Jacks Lake) on the left side of the division while the right side showed site 3 (Baring Road), site 4 (Cathill), site 5 (Parkside Gardens) and site 6 (Eastwalk/Westwalk). The group of sites on the right further seperated into two groups with the May and June samples (M3 and J3) for Baring Road on the left side and the rest of the sample sites on the right side. The DECORANA plot for sites between May and August 2014 are given in Figure 5.36 below and show a separation of sites into three groups A, B and C which relate to the groupings in the TWINSPAN dendogram. Axis 1 accounts for 94% of the variation while axis 2 accounts for 17% of the variation. The clean and dirty sites separate out with the clean sites positioned on the right side of the graph and the dirty sites positioned on the left side. The dirty sites 4, 5, 6 and an addition of site 3 in July and August clustered at the top left part of the graph and had similar communities during the entire study period (May – August). Generally the results of the examination and analysis of communities identified during the study showed clear differences in communities between clean and dirty sites. It was clear especially within the dirty site category, that there were certain communities characteristic of the dirty sites (site 4, 5 and 6). These communities were always present or abundant in the dirty sites (sites 4, 5 and 6) all through the experiment. They include protozoan organisms such as *Cinetochilium, Paramecium caudatum, Spirostomum, Colpidium, Mastigamoeba, Mayorella, Aspidisca, Pelomyxa, Prorodon, Chlamydomonas reinhardtii,* algae such as *Navicula cryptocephala, Navicula viridula, Navicula minima, Navicula oblonga, Rhoicosphenia*

Rhoicosphenia curvata, Cyclotella meneghiniana, *Chlorococcum*, *Gomphonema parvulum*, *Melosira varians*, *Nitzschia acicularis*, *Nitzschia amphibia*, *Nitzschia palea*, *Synedra ulna*, *Chaemisiphon*, *Stephanodiscus hantzschii*, *Phormidium* as well as two metazoans, *Philodina* and early instar *Chironomid* larvae. Species common within the clean sites (sites 1 and 2) include *Achnantidium minutissimum*, *Fragilaria capucina*, *Cocconeis placentula*, *Amphora ovalis* and *Amphora pediculus*.

Examples of the monthly DECORANA plots, using the May and August biofilm data, are given in Figure 5.37 and Figure 5.38. The monthly plots show sites 1 and 2 always positioned on the right side of the graph and sites 3, 4, 5 and 6 on the left side of the graph. May and August correlation data are shown as examples in Table 5.5 and 5.6 as the same general pattern is repeated across all months. In May, Axis 1 relates most strongly with conductivity, phosphates and coliforms while Axis 2 correlated positively with pH and in August, Axis 1 related most strongly to conductivity, phosphates, ammonia and coliforms while Axis 2 relates positively to temperature. Significant correlations are highlighted in red and most of the parameters are negative correlations.

The Canonical Correspondence Analysis (CCA) was conducted using eight environmental parameters (conductivity, phosphates, ammonia, D.O., coliforms, temperature, pH and flow). The CCA ordination plots for a combination of species and sites and a plot of the sites alone are given in Figures 5.39 and 5.40 respectively. It is possible to create rank plots from CCA plots to show a clearer association with individual environmental parameters (Henderson and Seaby, 2008). Untreated domestic wastewater discharges from misconnections usually contain high levels of bacteria and organic matter (Wright, 2004) and there are certain organisms that can tolerate poor water quality resulting from such sewage discharges. The coliform bacteria variable was one of the major

variables strongly associated with Axis 1. Therefore a sample rank plot using this variable is provided in Figure 5.41 to show the association of the biofilm communities with the coliform variable. Organisms associated with the dirty sites are closer to the coliform line while those located further away from the line are less tolerant of the dirty sites. The large number of species in the rank plot made it difficult to view all the species therefore a few representatives were selected and labeled. *Mastigamoeba, Navicula viridula* and *Nitzschia palea* are closely associated with the high coliform levels. The graph is interpreted based on the direction of the green arrow (Henderson and Seaby, 2008) therefore according to the coliform rank plot, *Mastigamoeba, Navicula viridula* and *Nitzschia palea* are more tolerant of poor water quality conditions associated with high coliform bacteria levels than *Amphora pediculus* and *Fragilaria capucina*.

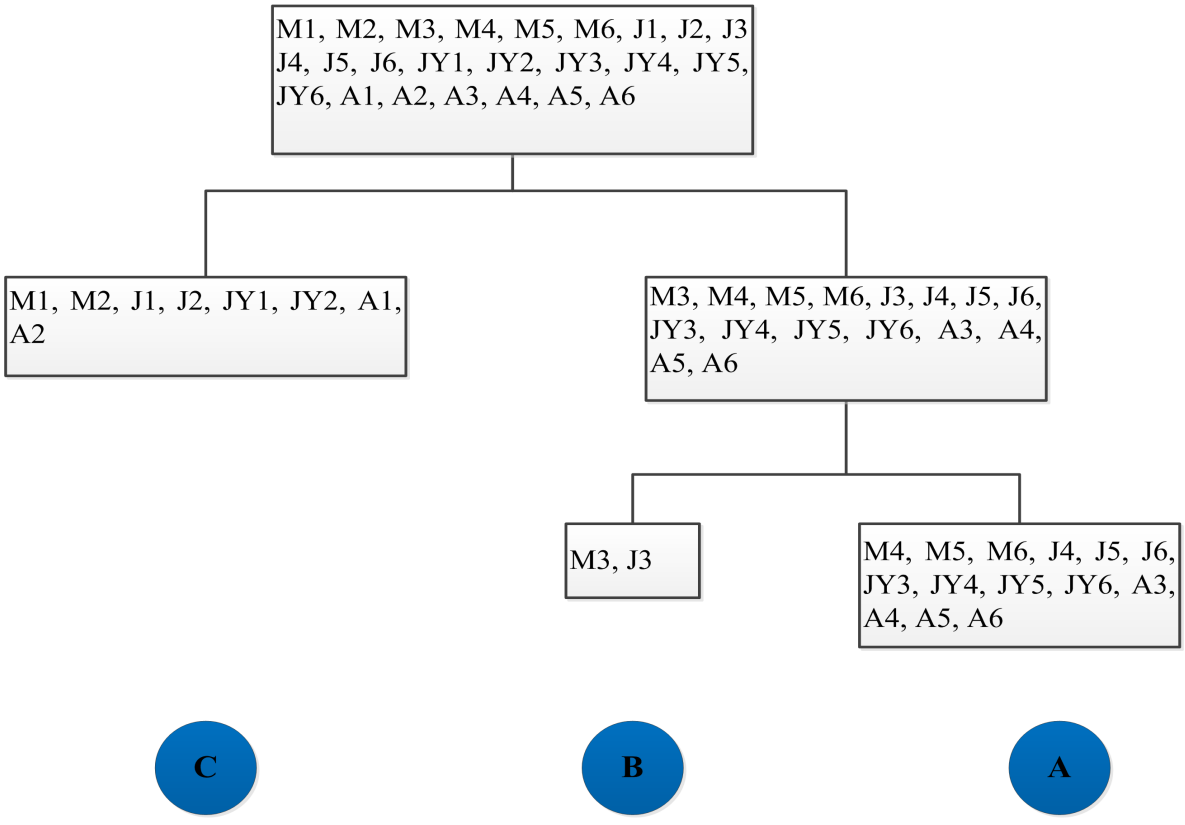


Figure 5.35: TWINSPAN dendrogram showing a separation of sites one to six into groups A, B and C for M (May), J (June), JY (July) and A (August) 2014

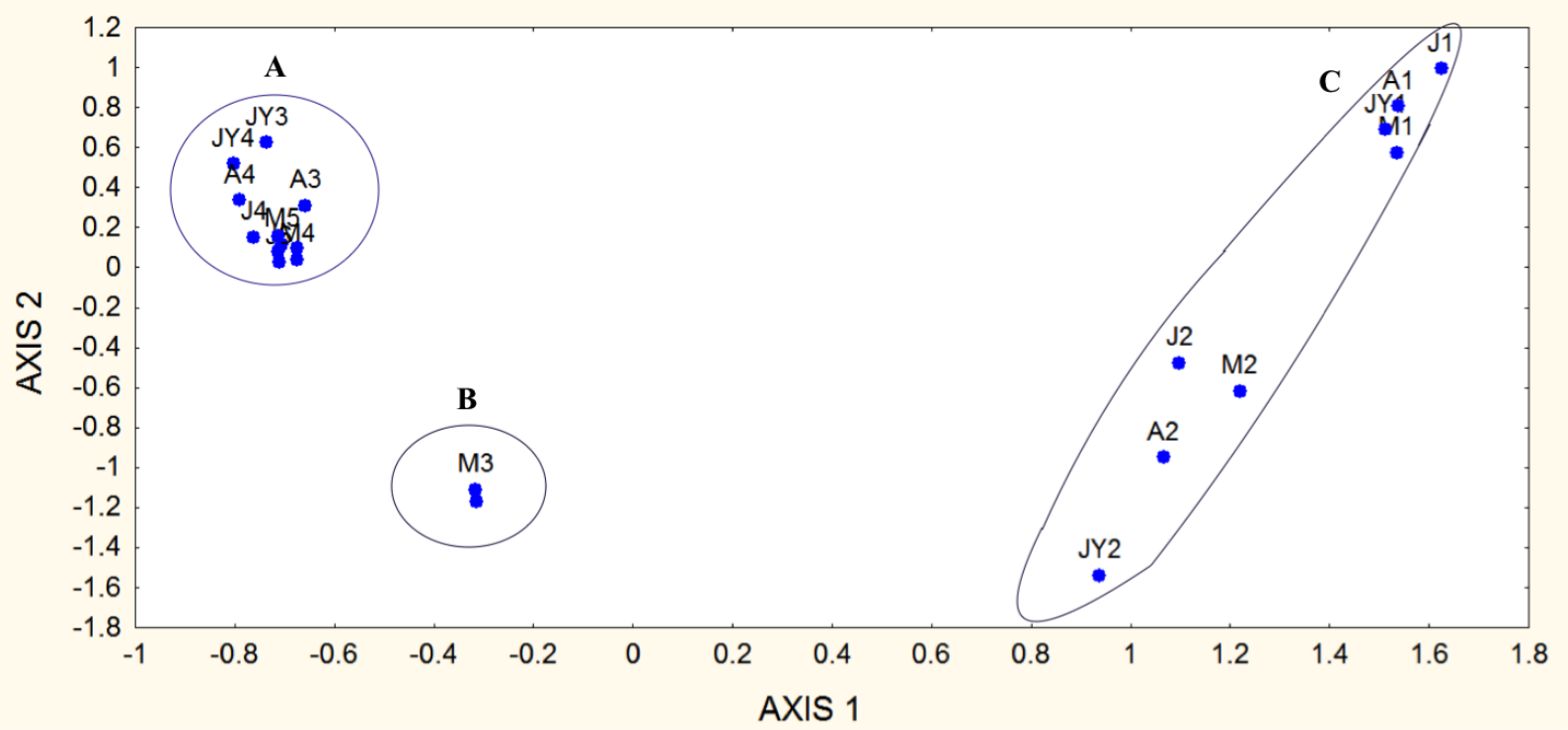


Figure 5.36: Sites ordination using DECORANA for the months of M (May), J (June), JY (July) and A (August) 2014. Sites separated into three groups, A, B and C, which correspond to the TWINSPAN groups.

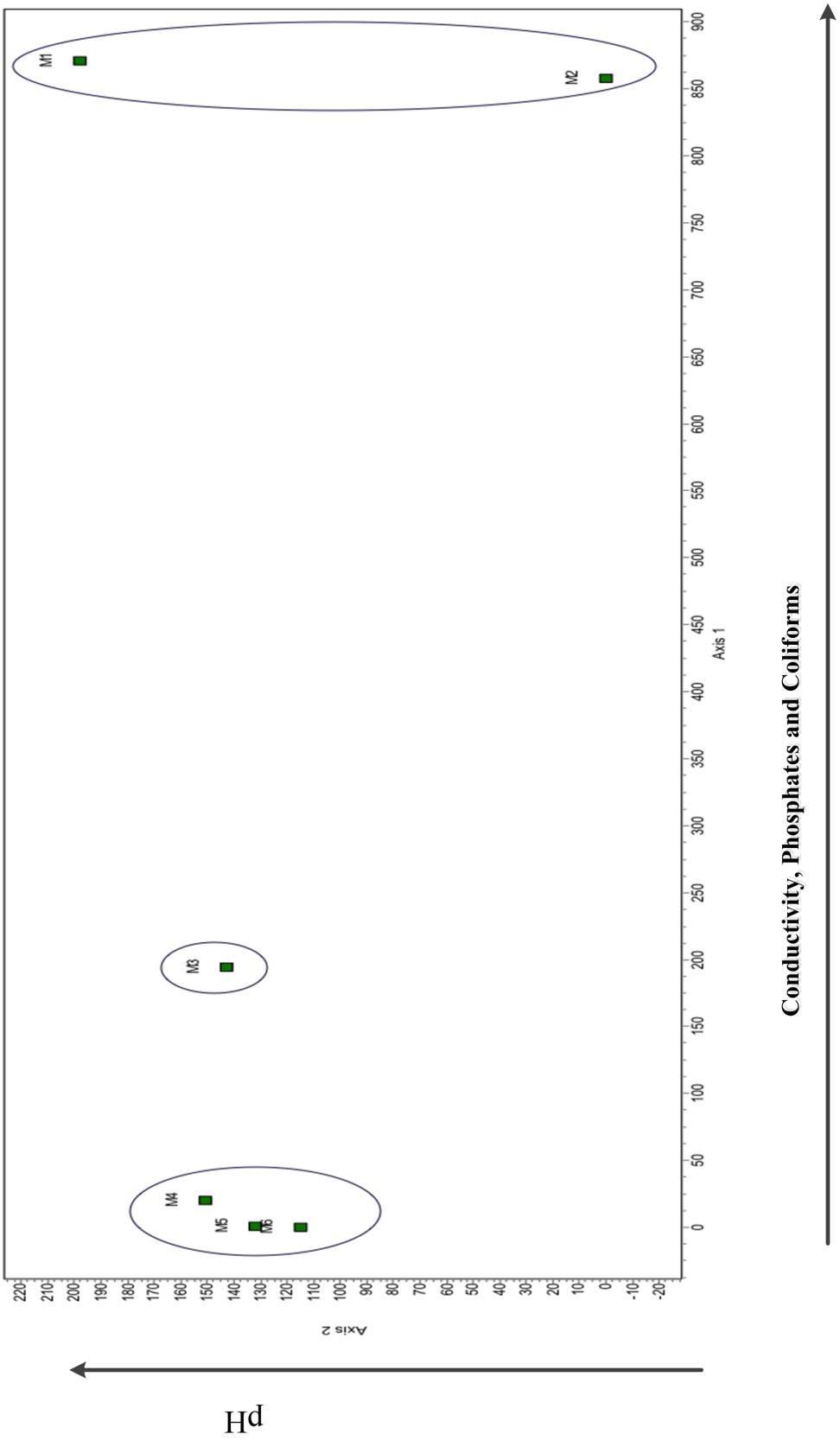


Figure 5.37: Sites ordination using DECORANA for the month of May 2014

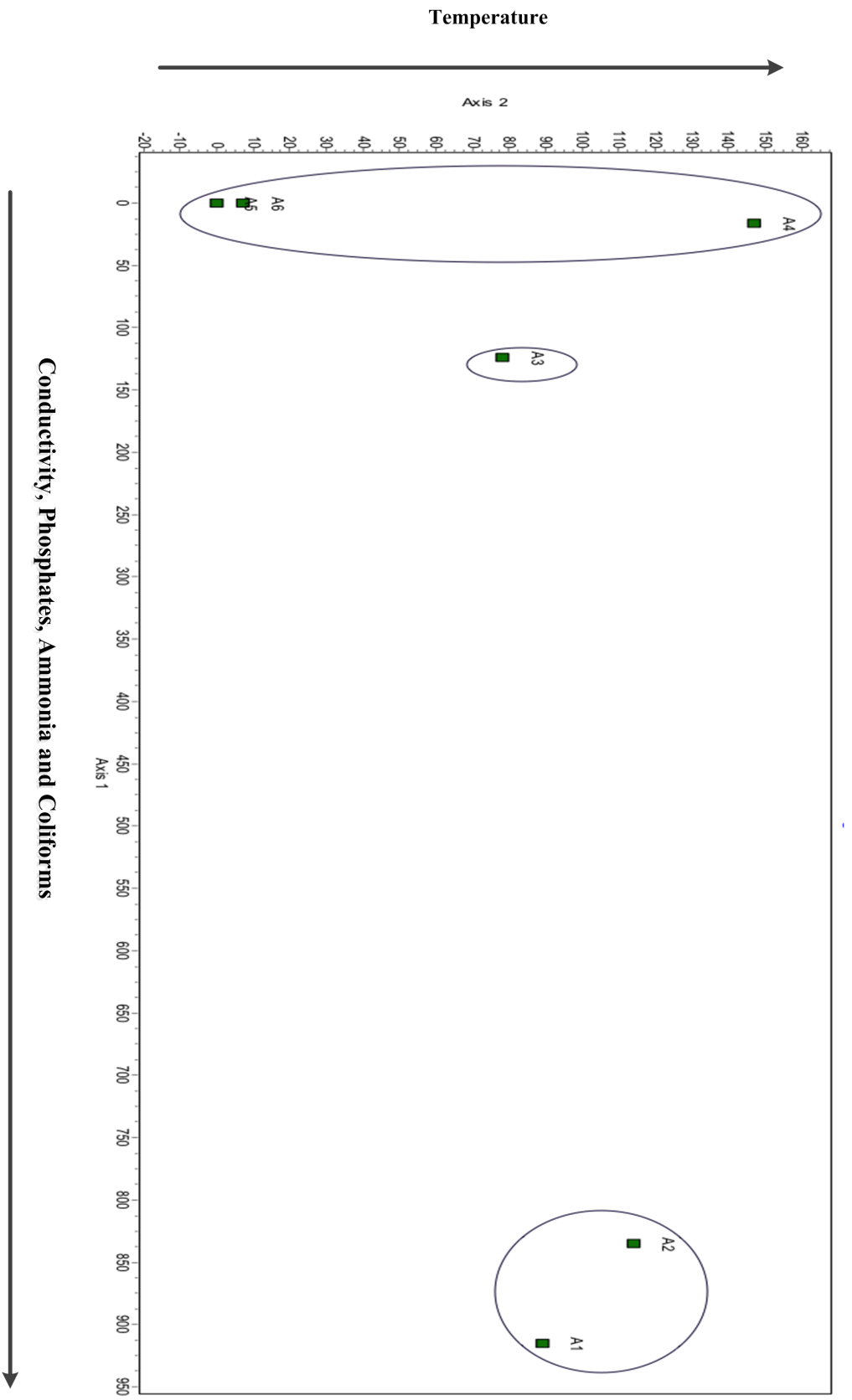


Figure 5.38: Sites ordination using DECORANA for the month of August 2014

Table 5.5: Pearsons correlations of the Eigenvalues of the first two axes and the environmental variables in May (The strongest correlations are marked in red and they are significant at p< .05000)

	Flow	**Cond.**	**pH**	**D.O**	**Temp.**	**Phos.**	**Ammo.**	**Coli**
Axis 1	-0.70	-0.90	-0.54	0.45	0.69	-0.96	-0.64	-0.92
Axis 2	0.21	-0.14	-0.56	0.21	0.49	0.15	0.25	0.31

Table 5.6: Pearsons correlations of the Eigenvalues of the first two axes and the environmental variables in August . (The strongest correlations are marked in red and they are significant at p< .05000)

	Flow	**Cond.**	**pH**	**D.O**	**Temp.**	**Phos.**	**Ammo.**	**Coli**
Axis 1	-0.46	-0.92	-0.58	0.54	0.60	-0.91	-0.85	-0.78
Axis 2	0.45	-0.19	0.04	0.31	0.64	-0.42	0.41	0.15

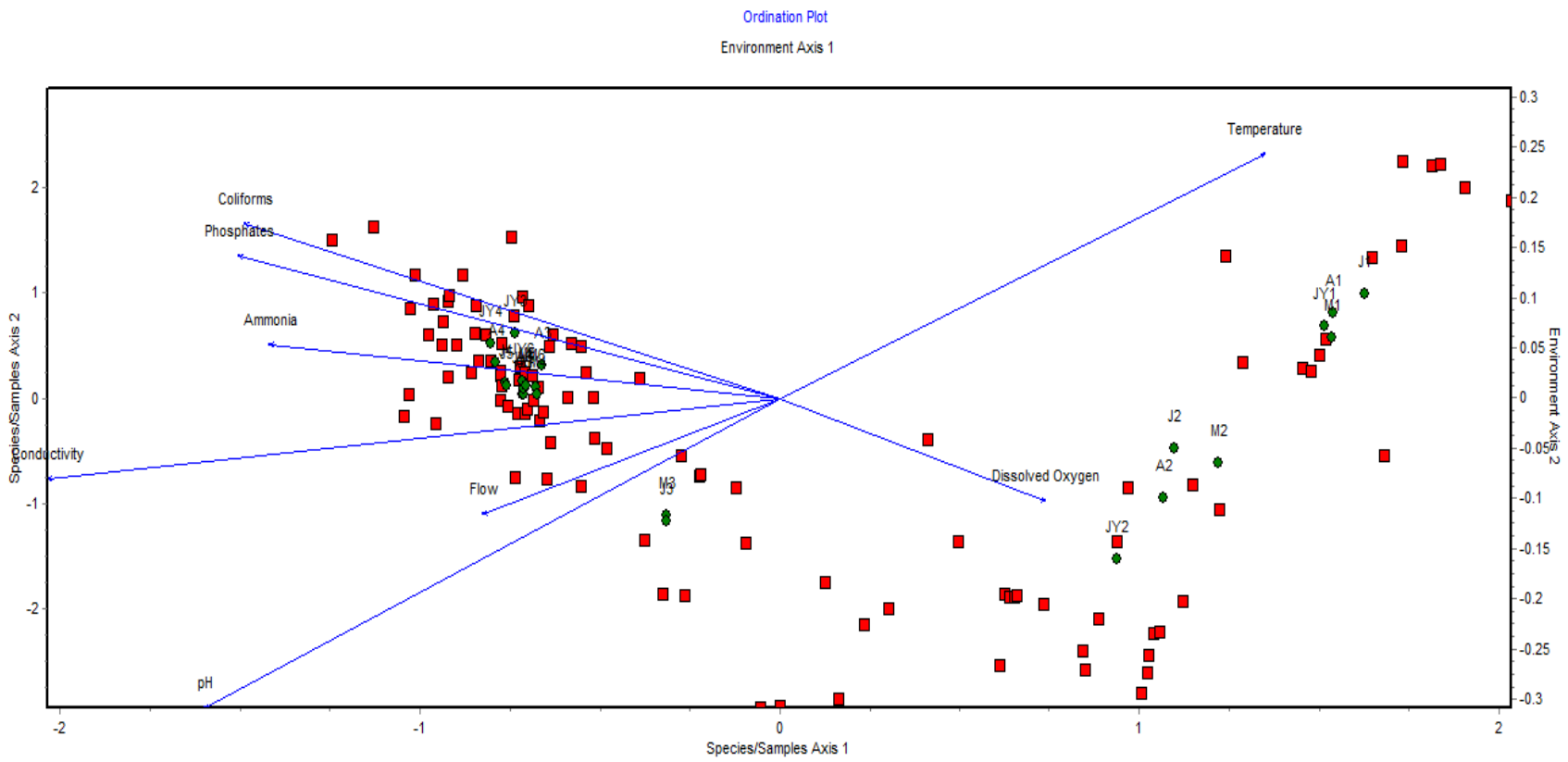


Figure 5.39: CCA plots for the months of M (May), J (June), JY (July) and A (August) 2014 for sites one to six. Sites are indicated by the green dots, species by the red squares and the environmental variables by the blue arrows in the plot. The longest arrow generally has the greatest influence on the community and here it is temperature followed by conductivity and pH. There is little variation amongst sites for these variables, however, coliforms and phosphates also have long arrows relating to increasing levels of pollutants and these are where the 'dirty' sites are found in ordinal space. Dissolved oxygen has a positive relationship with the cleaner sites to the right of the figure, which for Axis 1 have eigenvalues of +0.5-2 for sites 1 and 2. For Axis 2, eigenvalues range from - 0.3 to 0 for site 2 and from 0 to + 0.25 for site 1 - which is the clean, control site.

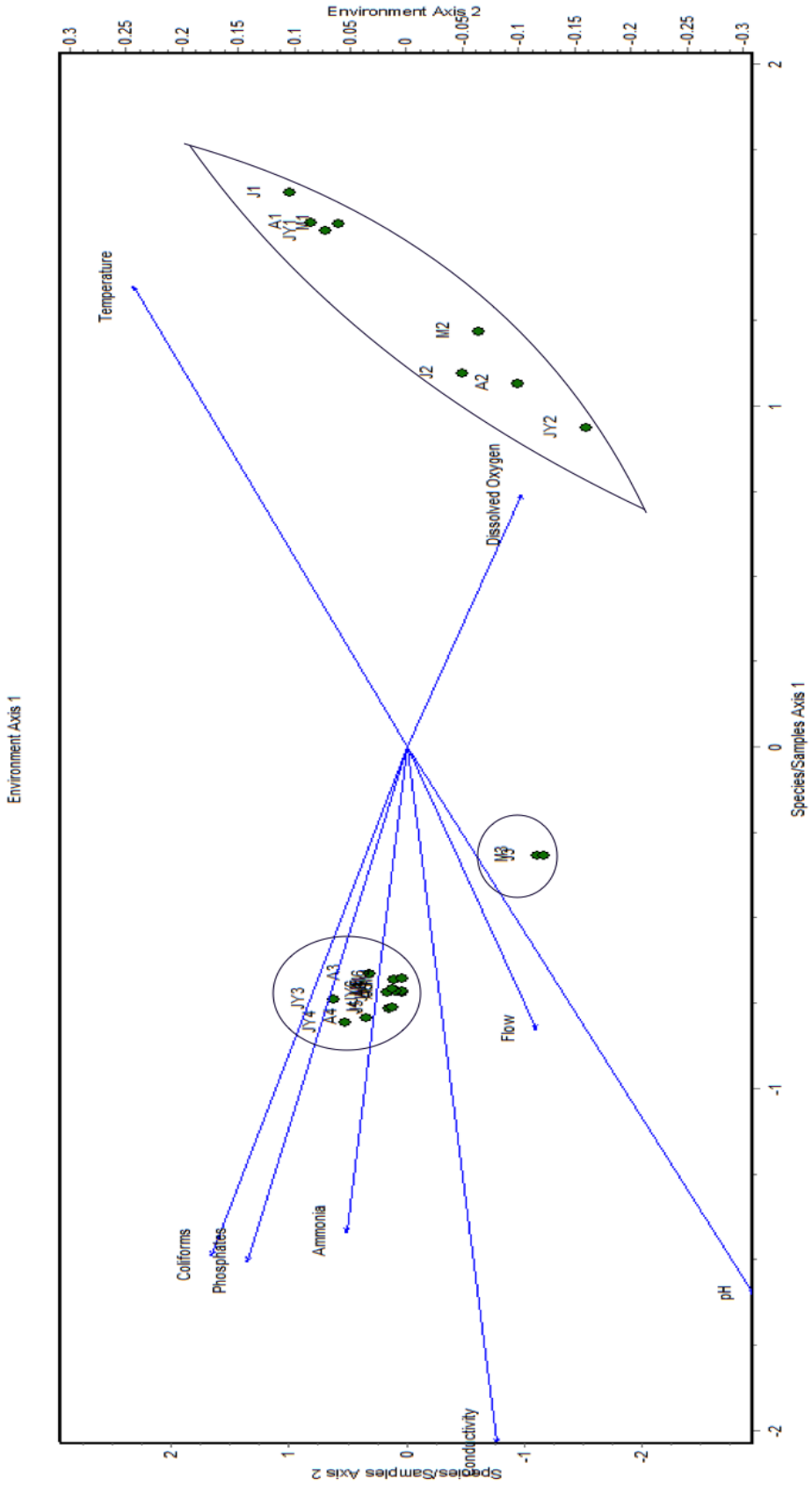


Figure 5.40: CCA Site plots for the months of M (May), J (June), JY (July) and A (August) 2014. Sites are indicated by the green dots the environmental variables by the blue arrows in the plot. Ellipses manually drawn to show clusters in the dataset

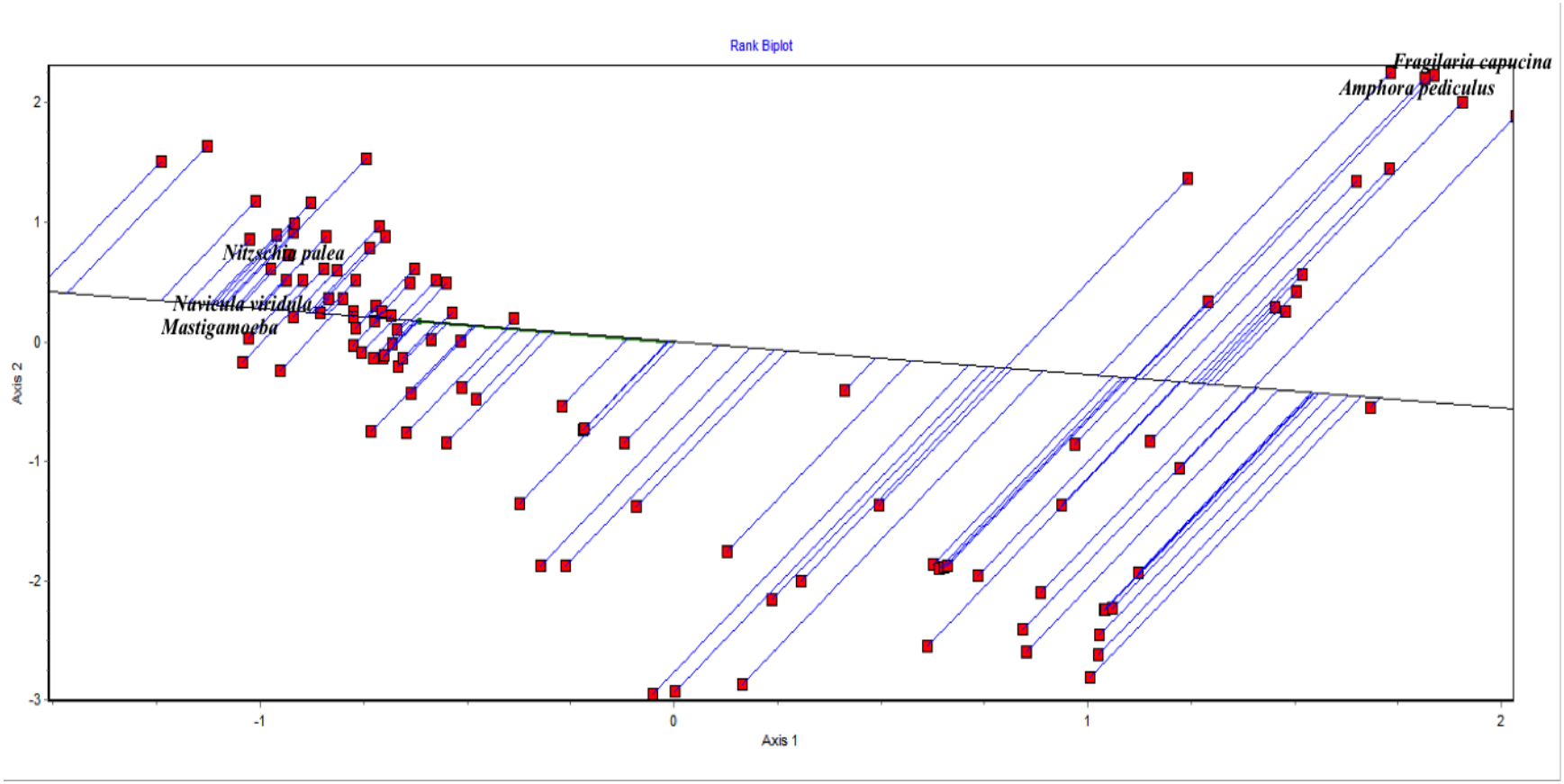


Figure 5.41: CCA rank plot for one major variable (coliform). Red squares indicate the species with a few named examples. Species close to the arrow on the left are species highlighted previously

5.6 Discussion

The biofilm experiment carried out in this current study investigated the impact of misconnection discharges on biofilm communities within the Pymmes Brook catchment, a sub catchment of the River Lee. The structure of communities below outfalls in the dirty sites Cathill (site 4), Parkside Gardens (site 5) and Eastwalk/Westwalk (site 6) were compared with the selected clean sites Bayfordbury (Site 1), Jacks Lake (Site 2) and Baring Road (Site 3). During the study, differences between the community structures within the two categories of sites were noted. Species composition within the dirty and clean sites was consistent throughout the entire study period with the exception of Baring Road. Although this site was initially selected as a clean site, it was however noted during the study that it had more species in common with sites 4, 5 and 6 than it had with sites 1 and 2 and appears to be a transition site. There were only a few species strictly characteristic of sites 1 and 2 found in Baring Road in May and June.

Diversity indices such as species richness and diversity are commonly used in ecological studies to measure communities and how they relate with their environment (Avidano et al., 2005). Although most ecological studies have shown that species diversity reduce in polluted sites, however, in this study the Simpsons Index showed that diversity was greater in the dirty sites than in the clean sites. This is in line with previous studies, which have shown that diversity measures are not always able to accurately provide indications of pollution impacts (Archibald, 1972; Bellinger et al., 2006; Beyene et al., 2009; Pratt et al., 1981; Stevenson, 1984). Findings from a study which investigated benthic diatoms located in an area of the Sandusky River experiencing poor water quality from sewage effluent discharges, suggests that it is possible for polluted sites to have higher species diversity than less pollutant-impacted sites (Stevenson, 1984). Sewage discharges contain increased loads of organic matter, nutrients and bacteria that biofilm communities feed on. Sites enriched with these

pollutants usually encourage the growth of benthic biofilms therefore increasing the number of individuals in the biofilm, although communities within such sites are likely to be mainly composed of taxonomically related species tolerant to such poor water quality conditions (Corcoll et al., 2014; De-la Ossa-Carretero et al., 2012; Patterson, 1996; Pinedo et al., 2015). This has similarities with the way sewage filter beds are designed to operate. In terms of the multivariate analysis, a clear separation occurred between the clean and dirty sites. The TWINSPAN groupings separated sites with Bayfordbury and Jacks Lake on one side and Cathill, Parkside Gardens, Eastwalk/Westwalk and Baring Road on the other side. At the second division, Baring Road (May and June) moved away (in ordinal space) from the rest of the dirty sites/samples. The same pattern was observed in the DECORANA plot which also separated into three groups with Cathill, Parkside Gardens, Eastwalk/Westwalk and July and August samples for Baring Road making up the first group (A) while group C was composed of the Bayfordbury and Jacks Lake sites. The last group (group B) was made up of the Baring Road site/sample for May and June. Species occur within a characteristic restricted habitat range and therefore tend to have increased abundance when environmental conditions are within their optimum. Based on this, changes in community structure occur along an environmental gradient. Direct gradient analyses such as CCA therefore make it possible for specie abundance along an environmental gradient to be inferred. With the CCA plot, the distribution of communities along an environmental axis can be visualized (ter Braak et al., 2004). The CCA analysis of communities within this study showed that coliforms, ammonia, conductivity and phosphates were the parameters that mainly explained the communities and the sites that linked most with these parameters were Cathill, Parkside Gardens, Eastwalk/Westwalk and Baring Road in July and August. Biofilm communities within these sites were mainly characterised by certain organisms, details of which are provided in Table 5.4 and section 5.5. Algae with several members of the *Navicula* and *Nitzschia* genera, *Melosira varians, Gomphonema parvulum, Cyclotella meneghinana, Stigeoclonium tenue, Synedra ulna*. Most of these species especially *Nitzschia palea*,

Gomphonema parvulum and members of the *Navicula* genera have been noted in previous studies as pollution tolerant organisms (Alakananda et al., 2013; Bellinger and Sigee, 2015; Brown and Olive, 1995; Butcher, 1932; Cairns et al., 1973; Joir and Saxena, 1992; Kalyoncu et al., 2009; Kelly, 1998; Kelly et al., 2005, 2008b; Kumar, 2002; Lange-Bertalot, 1979; Palmer, 1969; Silva-Benavides, 1996; Venkatachalapathy and Karthikeyan, 2013a).

In addition, most of the protozoan organisms found in this study to be common within the dirty sites have been previously noted to occur in a number of WWTW as well as a number of sewage polluted watercourses. These protozoa which are mainly bacteria feeders and commonly occur in areas of high organic and bacteria loadings include *Mastigamoeba*, *Mayorella*, *Aspidisca*, *Chlamydomonas reinhardtii*, *Colpidium*, *Cinetochilium*, *Paramecium caudatum*, *Pelomyxa*, *Prorodon* and *Spirostomum* (Đatkauskienë, 2012; Lackey, 1938; Patterson, 1996; Sawyer, 1990; Xu et al., 2005).

Furthermore, *Chironomids* and *Philodina* have been previously noted as pollution tolerant metazoans capable of surviving in areas with high levels of organic matter and reduced oxygen levels (Đatkauskienë, 2012; Beneberu et al., 2014; Halpern and Senderovich, 2014).

Misconnections discharge untreated sewage into watercourses thus leading to the abundance of various pollutants including organic matter, nutrients and heavy metals (Dunk et al., 2008; Ellis and Butler, 2015; Faulkner et al., 2000). The presence and abundance of these pollutants in affected watercourses causes a decline and loss of species such as those that occurred only in the clean sites during this study and the abundance of communities that can tolerate such conditions (Hughes and Dunham, 2014; ter Braak et al., 2004). Investigation of biological communities are therefore founded on the presumption that individual species differ in their tolerance to pollutants and therefore increased levels of those pollutants potentially lead to the abundance of the pollution tolerant organisms and the reduction or elimination of pollution sensitive ones.

The investigation of communities located below Polluted Surface Water Outfalls (PSWO) in the study area showed that some communities were always present while some occurred

abundantly in certain site categories (clean and dirty sites). This was particularly evident in the dirty site category. For instance, *Achnanthidium minutissimum* was always present in site 1 and 2 (clean sites) for most of the study period. On the other hand, some organisms such as *Navicula viridula*, *Nitzschia palea* and *Mastigamoeba* were always present in sites 4, 5 and 6 (dirty sites). These organisms as well as all others shown in the dirty site category in Table 5.4 and section 5.5 could therefore form a basis for indicator species for the detection of misconnection hotspots. Although no misconnection trackbacks were conducted during this study, as it would have required access to the sewer system. However, apart from previous trackbacks, which have identified some misconnections in these areas, the dirty sites, especially Cathill, are located within an area of the Pymmes brook catchment shown in previous studies to be suffering severe pollution impacts and known misconnections. In addition, communities within these sites have previously been linked with increased organic loadings and nutrients from untreated wastewater discharges therefore suggesting that pollutant discharges from misconnections in the area are likely to lead to the structural changes in the biofilm communities in the dirty sites with the abundance of these pollutant tolerant species. As seen from the results, the community patterns for the three groups are remarkably consistent.

In conclusion, although it should be noted that the biofilm results obtained during the pilot and experiment phases of the research would be slightly different from the actual communities in the sites. This is because processes such as sample preservation using IMS as well as scraping and washing samples off the stones and tiles would likely have led to the loss of certain organisms therefore skewing the results. However despite these, results obtained during the experiment carried out on Pymmes Brook and the pilot in Chapter 4 still identified clear differences between biofilm communities in the clean sites and communities in the dirty sites with certain potential indicators identified. Therefore, the dirty site indicators identified during this part of the study could potentially be used as indicators of misconnection issues on similar catchments.

Chapter 6

Misconnection hotspots in the Pymmes Brook Catchment

6.1 Introduction

In separate sewage systems there are two types of pipes, the wastewater pipe and the surface water pipe. The wastewater pipe carries untreated wastewater from dishwashers, sinks, washing machines, toilet and other domestic wastewater and sends them to the Wastewater Treatment Works (WWTW) for treatment before being discharged into receiving water-courses. The surface water drain on the other hand takes surface water from roads, roofs or driveways and discharges them straight into receiving watercourses. The issue that is usually experienced in separate sewage systems is the issue of sewage misconnections. These mainly occur during DIY plumbing and property extensions when householders and some-times builders and plumbers either illegally or unknowingly connect the wastewater pipe to sewage pipe (Dunk et al., 2008; Edmonds-Brown and Faulkner, 1995; Ellis and Butler, 2015; Faulkner et al., 2000). Figure 6.1 shows a picture of a correctly connected property and a wrongly connected property. The house on the left side has all the foul water pipes from a toilet, washing machine and bathtub rightly connected to the main foul water sewer, which

transports untreated waste from these sources to the WWTW. On the other hand, the house on the right side has a toilet wrongly connected to the surface water sewer subsequently leading to the discharge of untreated waste along with surface water from the roof into the receiving watercourse.

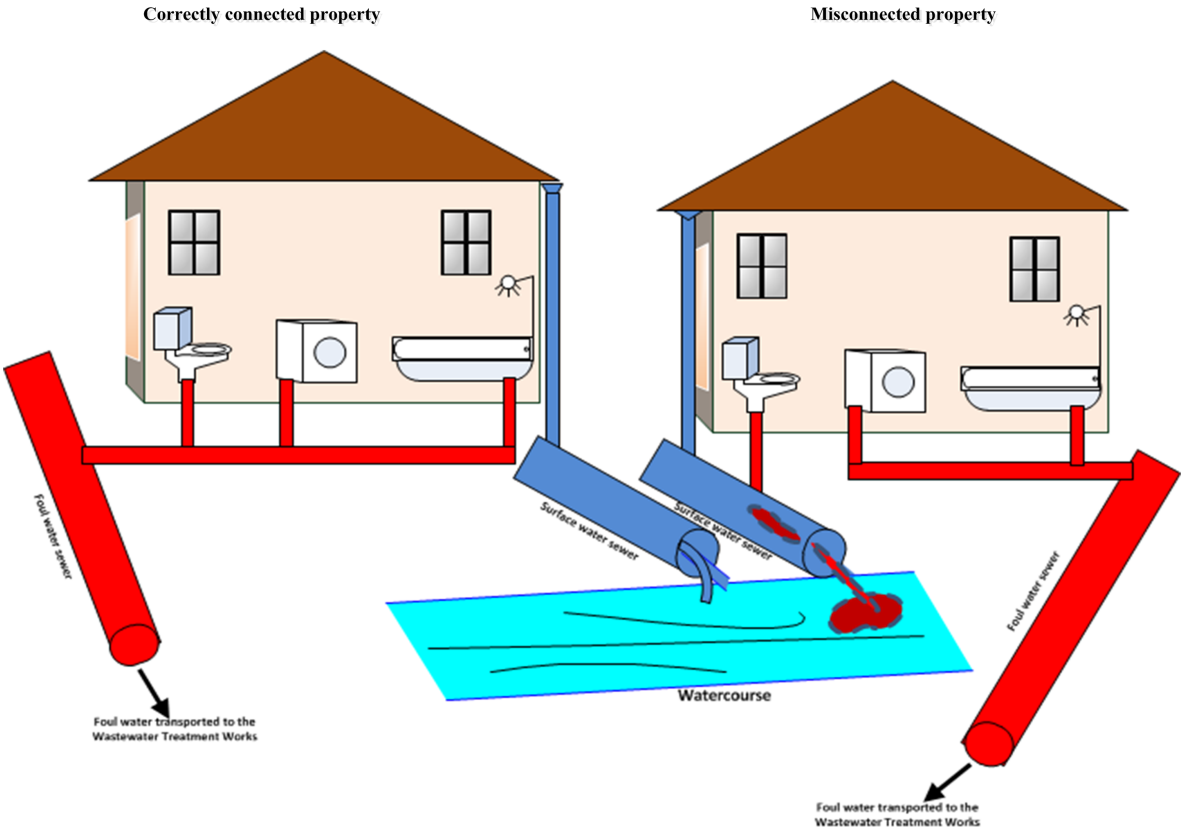


Figure 6.1: Picture showing a properly connected property (house on the left side) and a misconnected property (house on the right side)

A network of surface water pipes usually feeds outfalls through which surface water is discharged into receiving watercourses. Misconnection hotspots are those particular outfalls within a hydrological boundary fed by surface water pipes that are experiencing these misconnections and discharging untreated sewage into the watercourse, leading to high pollutant in-stream concentrations and impacts. Unlike CSO's which discharge during heavy rainfall events, the discharges of sewage from misconnections happen intermittently

and the most severe impacts can occur during low flow periods when there is a reduced chance of the dilution of pollutants discharged (Edmonds-Brown and Faulkner, 1995).

Pymmes Brook, the watercourse investigated during this part of the study has had a long history of poor water quality from diffuse pollution. Although a heavily urbanised catchment with a large area of impervious surfaces, contributions to pollutant loadings from road, car parks and other impermeable surfaces are expected. However, according to previous studies CSO's and misconnections appeared to be the major diffuse sources of pollutants into the brook (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000). Previously within this catchment, discharges from CSO's were noted to occur regularly during heavy rainfall events. This progressively worsened as urbanisation increased therefore resulting in severe water quality deterioration on the brook (Faulkner et al., 2000). The poor state of the brook became a source of concern and as a result, remedial construction work was carried out to address the pollution issues. The remedial work which was termed the East Barnet Low Level intersecting foul water sewerage scheme aimed to address the poor water quality by increasing the holding capacity of the sewage system. The diameter of a large proportion of the main sewers had to be increased as a result. One of the projects carried out within the scheme was the removal of seven problematic CSO's in order to prevent discharges from these sources into the watercourse (Green, 2000). Although some CSO's remained operational within this catchment, discharges via these sources did not occur as frequently as they previously used to. Apart from the removal of problematic CSO's and the increase in the capacity of sewer pipes, larger 'Storm King' tanks were built to increase the storage capacity of the whole system. This was done to aid the storage of water and prevent flooding especially during heavy storm events after which it can then be discharged gradually into the watercourse. These tanks were built in Victoria Park located upstream of the brook, in the Oakhill Park area and in Brunswick Park further downstream. The construction work however only addressed the catchments

over capacitated CSO's issue. A study conducted after the completion of the projects within the construction scheme showed some improvement in the brooks water quality. However this improvement was only minor and therefore could be attributed to the flushing of the system within the Pymmes Brook catchment (Green, 2000).

Several areas within the Pymmes Brook catchment have been known to experience issues with misconnections (Edmonds-Brown and Faulkner, 1995; Green, 2000). While the locations of some of these misconnections stayed undiscovered, some others were found and rectified. For instance, a survey conducted on streets in the south-eastern part of Oakhill park resulted in the discovery of approximately twenty misconnections, which were subsequently rectified (Green, 2000). Misconnections are major contributors to high bacteria loads within watercourses. Apart from bacteria loads, they also contribute to the increased levels of other pollutants within rivers and streams such as organic material and heavy metals. The presence of high concentrations of these pollutants is detrimental to the ecological health of receiving watercourses and as such misconnection issues need to be addressed. Using coliform bacteria and biofilm communities, potential hotspots have been located in the Pymmes Brook catchment. In this part of the study, coliform bacteria levels will be predicted from enumerated coliform counts (Chapter 3) and used together with catchment and population data to assess misconnections within these hotspots. This would provide a rapid and inexpensive approach for assessing misconnections within a catchment. Identified hotspots could then be targeted at the sub catchment with the aim of reducing the occurrence of misconnections.

6.2 Methodology

6.2.1 Desk Survey

A desk survey was carried out to assign runoff coefficients for the study sub catchments. Subsequently, bacterial loadings into the brook were predicted and used along with census data to assess misconnection rates within the sub catchments. Details of the methods used are provided below.

6.2.2 Runoff coefficients and bacterial loadings for the study sub catchments

The method used to calculate runoff coefficients in the Pymmes brook catchment study was the Rationale Method. This technique has been around since the 1980's (Dhakal et al., 2011) and since its introduction it has been the most commonly used method for sizing sewers for flood and pollution control (O'Loughlin et al., 1996; Watts and Hawke, 2003). Despite its simplicity, it is proven to be a very effective and accurate tool for runoff estimation (Grimaldi and Petroselli, 2015). Using the catchment characteristics, the rational method tries to predict or model what would occur in a catchment during rainfall events. In the model, catchment characteristics are used to assign runoff coefficients for the analysis of rainfall-runoff relationships. Runoff coefficient (C) is a dimensionless coefficient that relates to the amount of precipitation that remains as runoff after infiltration, interception and water storage in depressions such as ditches and puddles. Therefore the runoff coefficient of an area increases as impermeable areas increase and decreases as more permeable areas such as forest and parks increase (Dhakal et al., 2011).

Catchment characteristics were determined using a map of the Pymmes brook catchment which was obtained from the Edina Digimap website. The ordinance survey map, which is

given in Figure 6.2, was used to determine the drainage area of the two study sub catchments within the larger Pymmes Brook catchment area. Land use categories were then identified using the map and the area of each category measured and recorded.

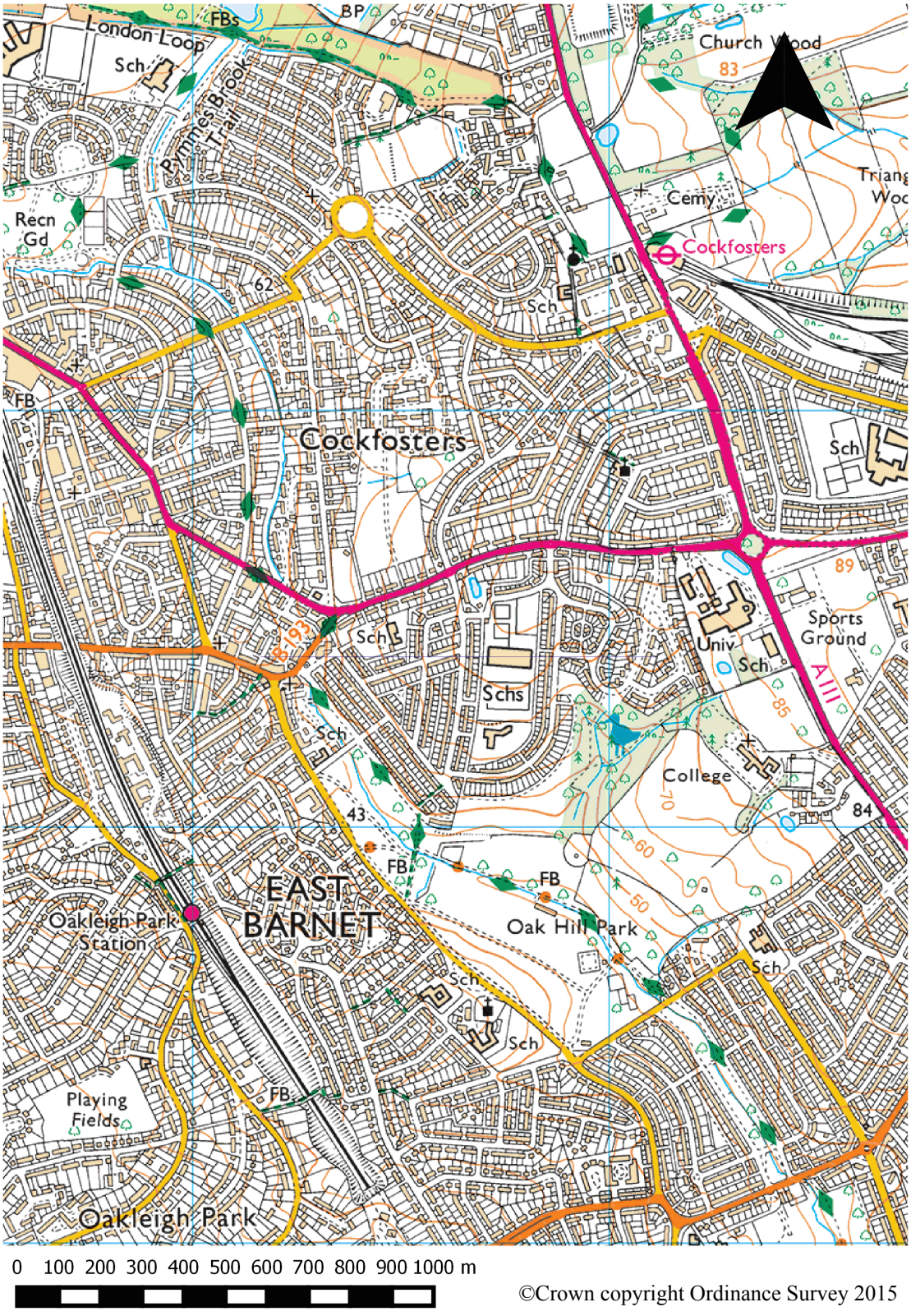


Figure 6.2: The map used to estimate land use/ land cover categories for the study area

There are coefficients values available for the estimation of different land use/land cover categories. These tables have been compiled over several decades and used to speed up the process of calculation (Cleveland et al., 2011). The table used for assigning coefficients for this part of the study is given in Appendix E. Runoff coefficients for individual land use sections identified in the two Pymmes brook sub catchments were assigned using the runoff coefficient table. The Area weighted coefficient (C) was then calculated using Equation 6.1 (Akan and Houghtalen, 2003) below:

$$\textbf{Area weighted runoff coefficient(C)} = \frac{Total\ Area \times Runoff\ coefficient}{Total\ Area} \qquad (6.1)$$

Using the runoff coefficient calculated for the study sub catchments, the annual bacteria loading to the Brook was estimated using the 'Simple Method'. This method requires a minimal amount of information including drainage area, runoff coefficient of study area, pollutant concentration and annual rainfall to calculate pollutant loads within surface runoff. In this method, chemical pollutant loads are estimated as a product of annual runoff volume and concentration of the pollutant [L(lbs)= 0.226 × R × C × A]. Furthermore, to estimate bacteria loads, the chemical loads equation is modified to account for differences in unit (Pitt, 1998; Scheuler, 1999; Stormwater Centre, 2015). The equation for bacteria is given in Equation 6.2 below.

Box 6.1: Simples Equation for estimating bacteria load in annual runoff.

$$\mathbf{L} = 1.03 \times 10^{-3} \times \mathbf{R} \times \mathbf{C} \times \mathbf{A} \quad (6.2)$$

Where:

L = Annual load (Billion Colonies)

R = Annual runoff (inches) which is calculated with the equation

C = Bacteria concentration (Number/100ml)

A = Area (acres)

1.03 × 10−3 = Unit conversion factor

To calculate the bacteria loads using the above equation, annual runoff (R) was first calculated using equation 6.4 (Stormwater Centre, 2015).

Box 6.2: Equation for estimating annual runoff

$$\mathbf{R} = \mathbf{P} \times \mathbf{P}_j \times \mathbf{R}_v \quad (6.3)$$

Where:

R = Annual runoff (inches)

P = Annual rainfall (inches)

P_j = Fraction of rainfall that produces runoff (usually 0.9)

R_V = Runoff coefficient

6.2.3 Estimation of Misconnections in the study area

The bacteria loadings calculated for the study area was then utilised along with census data within equation 6.4 (Rhew, 2009) to calculate percentage misconnections in the area.

Box 6.3: Equation for estimating percentage misconnections.

$$\mathbf{L = 37.85 \times N \times Q \times C \times F} \quad (6.4)$$

Where:

L = Daily bacteria load

37.85 = Value for standardization

N = Number of households in the area

Q = Discharge rate for each household (obtained by multiplying the average household size by the per capita wastewater production rate per day)

C = Concentration of domestic wastewater

F = Misconnection rate (%)

6.3 Results

6.3.1 Catchment data of the study sub catchments (Area & Runoff coefficient)

There were six land use/ land cover categories identified for the study sub catchments. These categories and their areas are shown in Table 6.1 and Figure 6.3. In the study sub catchments, residential land use occupied the highest area with 837.63 acres followed by parks, which occupied an area of 112.4 acres. Concrete areas within the catchment occupied 47.09 acres,

Table 6.1: Land use/land cover categories assigned for Jacks Lake and Cathill sub catchments

Land use/landcover	Area (Acres)	Runoff coefficient	Individual areas × Runoff coefficient
Residential	837.63	0.75	628.22
Concrete	47.09	0.85	40.03
Asphalt	31.54	0.95	29.96
Forest	31.98	0.25	8.0
Park	112.40	0.20	22.48
Unimproved	18.47	0.30	5.54

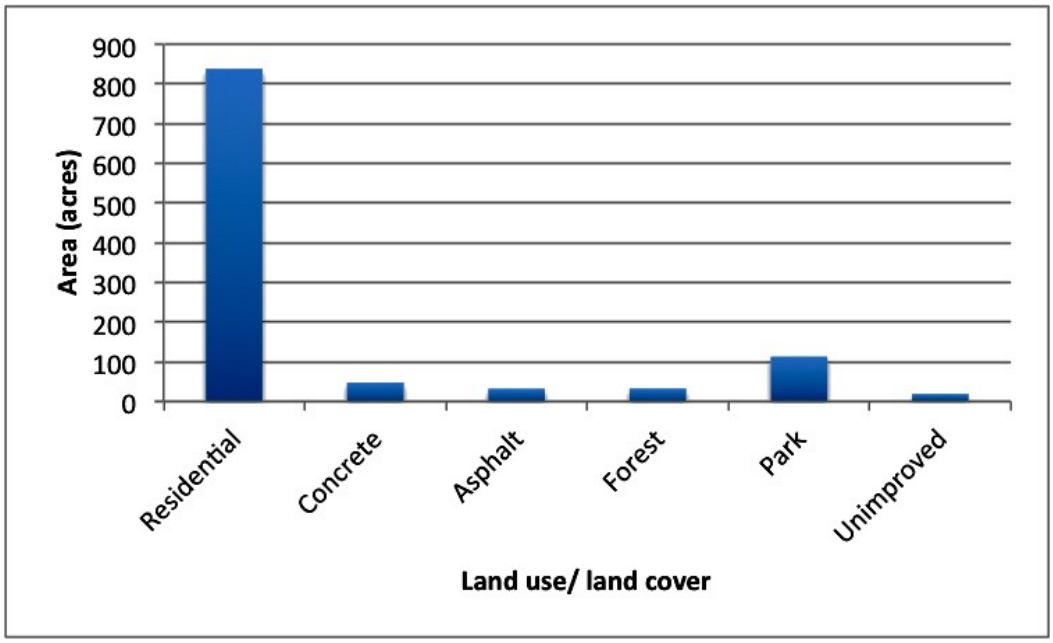


Figure 6.3: Bar chart showing different land use land cover categories and their areas

while forests had an area of 31.98 acres. The last two categories, asphalt and unimproved occupied the smallest areas in the catchment with 31.54 acres and 18.47 acres respectively.

Using the equation:

Area weighted runoff coefficient (C) $\frac{Total\ Area \times Runoff\ coefficient}{Total\ \ Area}$ (Equation 6.1)

Where:

The total area is 1079.11 acres. Based on Equation 6.1, the Area weighted coefficient (C) for the two Pymmes brook sub catchments is 0.68.

6.3.2 Annual bacteria loading to Pymmes Brook

The results obtained from the calculation of annual runoff and predicted bacteria loads contained runoff in the study sub catchments are given below.

Using the equation:

$$\mathbf{R} = \mathbf{P} \times \mathbf{P}_j \times \mathbf{R}_v \qquad \text{(Equation 6.3)}$$

Where:

Annual rainfall (P) obtained for 2014 was 51.18 inches (MetOffice, 2015), the fraction of rainfall that produces runoff ($\mathbf{P}_j$) is 0.9 and the calculated runoff coefficient ($\mathbf{R}_v$) was 0.68.

Based on Equation 6.3, annual runoff of the study sub catchments is 31.32 inches.

Results of the calculation of bacteria load in annual runoff are also shown below. Using Equation 6.2:

$$\mathbf{L} = 1.03 \times 10^{-3} \times \mathbf{R} \times \mathbf{C} \times \mathbf{A} \qquad \text{(Equation 6.2)}$$

Where:

Annual runoff (**R**) is 31.32 inches, bacteria concentration/100ml (**C**) is 177,409/100ml and the Area (**A**) is 1079.11 acres. Based on (Equation 6.2), Annual load (**L**) is 6,175,901 billion colonies.

6.3.3 Misconnections in the Study Area

Results of the estimated misconnections in the study sub catchments are provided below.

Using equation 6.4 below:

$$\mathbf{L} = 37.85 \times \mathbf{N} \times \mathbf{Q} \times \mathbf{C} \times \mathbf{F} \qquad \text{(Equation 6.4)}$$

Where:

The daily bacteria load (L) is 1.692×10^{13} counts/day. The study area is located within the East Barnet ward and according to the most recent UK census, the number of households within the East Barnet ward is 6531 and the average number of residents within households is 2.5 (Barnet London Borough, 2015*a*). Therefore, the number of households using the sewer system (N) is 6531. The discharge rate from each household (Q) was obtained by multiplying the average number of residents within households (2.5) by 38 gallons which is the UK's per capita wastewater production (Friedler et al., 2013). Furthermore, the coliform bacteria concentration in domestic water (C) is 1×10^7 counts/100ml (Clark and Boutin, 2001; Kadlec and Wallace, 2008; Kathy, 2011; Liu and Liptak, 1999; Sullivan and Krieger, 2001) and the value required is the percentage misconnections (F). Based on Equation 6.4, the percentage misconnection estimated for the study area was 7.21%.

6.4 Discussion

In this phase of the current study, catchment characteristics and population data were used to investigate misconnection hotspots in the Pymmes Brook catchment. Impervious surfaces in an area are usually used to assess the degree of urbanisation. Urbanisation results in an increase in impermeable areas and has thus been known to impact negatively on hydrological systems (Valtanen et al., 2014*a*). Furthermore, an increase in impervious surfaces within a catchment has been shown to increase the volume of surface water runoff therefore increasing flood potential and pollutant inputs into receiving watercourses (Page et al., 2015). The data obtained showed that the study area is highly urbanized and occupies a total area of 1079.11 acres. According to the land use proportions; the major land use within the area is residential which occupies approximately 78% of the total area. Runoff coefficients have been shown in previous studies to increase as catchment imperviousness increases (Dietz and Clausen, 2008; Pauleit et al., 2005). The high runoff coefficient 0.68 and resultant

increase in annual runoff is expected because the study area is located within a densely built up area made up mainly of impervious surfaces. Catchment data obtained during this part of the study suggest that large volumes of surface runoff are introduced into Pymmes Brook annually. Increased storm water runoff washes several pollutants including coliform bacteria off surfaces and discharges them into receiving watercourses. It has been previously suggested that birds and dogs represent two major contributors to increased bacteria loadings within surface runoff from impermeable surfaces (Ellis, 2004).

Although faecal inputs from birds have been suggested to greatly influence coliform levels within watercourses, a previous study showed that the rate of contamination from this source is to a large extent dependent on the abundance of the birds within the area (Meerburg et al., 2011). However, bird population in the Pymmes Brook area is not particularly high and the ones present usually roost away from the stream. In 2014, birds seen around the area include rooks, seagulls, mallard ducks, herons, blue tits and blackbirds. However most of these bird sightings were occasional. The major bird population according to the records were the rooks of which a maximum of 60 birds were recorded and seagulls which were only seen in bad weather. Most of these birds were however seen in the Oakhill park area of the brook, not in the Baring Road, Cathill, Parkside Gardens and Eastwalk/Westwalk areas of the brook, areas in which high coliform bacteria levels were identified during this study. Dog fouling has also been suggested to be a major contributor of bacteria levels within urban streams. However results from a previous study showed that although dogs are an important coliform source to watercourses, this source could be controlled. The study showed a significant decrease in coliform levels after proper education on disposal of waste was provided to people residing in the area (Ervin et al., 2014). In the UK there are several management approaches available for the control of dog fouling. The Clean Neighbourhoods and Environment Act 2005 which repeals Dogs (Fouling of Land) Act 1996, provides local councils with the power to create byelaws regarding dog mess. Under this legislation,

most of the local authorities require that dog owners carry poop scoopers and disposable bags to clean up after their pets. In several parks and public places, dog bins are provided for the disposal of the collected dog poop. The legislation also provides local authorities with the power to issue fixed penalty orders and in cases whereby there is no local rate, the fixed penalty rate of £75 applies. Magistrates can also issue a maximum penalty of £1000 in severe cases (UK Environmental Law Association, 2014). In London Borough of Barnet, the issue of dog fouling is particularly taken seriously and there is an online service on the councils website where members of the public could report issues relating to dog fouling (Barnet London Borough, 2015b). The predicted value for bacteria levels within the annual runoff in the study area was approximately 6.18×10^{15} colonies. Whilst it is expected that there will be contributions to these bacteria levels from dogs, birds and other sources, these contributions are likely to be minimal. The water quality issues in the brook dates back to several years as is evident from previous studies within this catchment. As mentioned previously in the introductory section of this chapter, the brook has been experiencing diffuse pollution issues which were initially suggested to be mainly due to CSO's and plumbing misconnections.

Within some of the studies carried out on the brook, the poor water quality was evident from high coliform bacteria levels ($>5 \times 10^6$), bad odours and sewage fungus. Below outfalls with suspected misconnections, Biological Monitoring Working Party (BMWP) scores were also discovered to be low (Edmonds-Brown and Faulkner, 1995; Faulkner et al., 2000). The engineering works carried out in the area in 1995 was said to have significantly improved the quality of the brook resulting in a significant drop in coliform bacteria concentrations especially in the upper urban areas of the catchment (Ellis, 2004). The attenuation tanks also constructed as part of the engineering construction works reduced the occurrence of raw sewage discharges into the brook from over capacitated sewer systems therefore contributing towards this improved water quality (Faulkner et al., 2001). The engineering works however only addressed the issue of CSO's within the catchment. The poor water quality of the brook

still remained especially downstream of Cathill with the Cathill PSWO still discharging untreated wastewater into the watercourse (Ellis, 2004). This poor water quality was also evident during this current study. Since the issue of misconnections within the catchment was not addressed, it is therefore suggested to be the most likely contributor to the bacteria levels within storm water runoff in the catchment especially in areas of high coliform bacteria concentrations.

There are several visual and non-visual methods through which discharges from which misconnections can be identified. Visual investigation usually involves looking for aesthetic signs of misconnection discharges such as sewage fungus, scum, oil, faeces, bad odour, grey water, used toilet paper and foam (Dunk et al., 2008; Ellis and Butler, 2015; Water UK/Environment Agency, 2009). On the other hand, non-visual methods require testing water samples for coliform bacteria levels, chemicals as well as the measurement of biotic indices (Water UK/Environment Agency, 2009). However, the only way to identify the particular misconnection responsible for the discharges is through trackbacks to the source (DEFRA, 2009; Edmonds-Brown and Faulkner, 1995; Ellis and Butler, 2015; Water UK/Environment Agency, 2009). These misconnection trackbacks are usually expensive, labour intensive and time consuming. Therefore most times, misconnections are only investigated and detected when discovery of misconnection discharges or impacts are made and reported by members of the public (Water UK/Environment Agency, 2009). However misconnection discharges are intermittent thus limiting this chance discovery (DEFRA, 2009). Therefore in the majority of cases, misconnections are discovered and reported when discharges have gone on for long enough to produce visual aesthetic impacts. Considering all these, it is necessary to deal with the issue of misconnections proactively. The method suggested in this study is the mapping and targeting approach, whereby misconnection hotspots are detected and the areas within these hotspots are targeted for the reduction of misconnections. The bacteria prediction made during this study was used to estimate the percentage misconnections within the study sub

catchment. Results of this current study suggest that approximately 7.21% of the households within the area are misconnected meaning that approximately 467 properties out of the 6531 properties are misconnected. Although no trackbacks were conducted, this estimate is in line with a previous study carried out within the Thames region which suggested that property misconnections were within the range of < 1 – 9% (Dunk et al., 2008). Similar results have also been obtained from actual investigations into 6000 properties located around Muswell Stream, a watercourse which discharges into Pymmes Brook in Palmers Green, Enfield. During this investigation, which was conducted by Thames Water between 2009 and 2014, it was discovered that 7% of the properties were misconnected. 975 household appliances including 26 toilets within 448 properties were discovered to be discharging untreated wastewater into Muswell Stream (Thames Water, 2015). Substantial amounts of pollutants and impacts could potentially result from a small proportion of misconnections in a catchment area (de Haan et al., 2011; Ellis, 2013). It is evident from the high coliform bacteria counts obtained for the brook during this study (Chapter 3), that these misconnections appear to be having severe impacts on the watercourse. These impacts could be aesthetic in nature, resulting in unsightly discharges as well as bad and unpleasant odours as observed within some of the sites investigated in this study. Impacts could also be severe because increased activity from high bacteria concentrations could lead to the depletion of D.O. within watercourses and could thus affect biodiversity and the ecological health of receiving waters. Toxic inputs such as pharmaceutical compounds could also result from these misconnections therefore contributing to negative impacts on water quality (Ellis, 2006).

Presently in the UK there is a procedure available for investigating and tackling misconnections. When misconnection discharges are discovered or reported, the Water and Sewerage Company (WaSC) can only notify the owners of the misconnected property because they are not provided with the power to enforce misconnection rectification. On non-compliance after a given period, the WaSC informs the Local Authority requesting that they serve an

enforcement notice under section 59 of the Building Act 1984 (Water UK/Environment Agency, 2009). However, because issues relating to sewer and effluent discharges are the responsibility of the Environmental Agency and WaSC, there is little motivation for the LA's to take action. In addition, the whole investigation and rectification procedure has to be conducted within six months otherwise the data is considered out-dated and irrelevant (Ellis and Butler, 2015). Apart from being lengthy, the process involves several linkages and transfers between the relevant organisations. These links and transfers are prone to breakdowns, which could eventually result in the information becoming out-dated therefore significantly increasing processing costs and subsequently the total cost of misconnection rectification.

The legislative issues discussed above make it difficult to tackle misconnection issues. Apart from this, even if misconnections are tracked and identified, the legislative approach may not be able to prevent future reoccurrence. Issues with misconnections could thus be a continuous one because people keep moving and carrying out building extensions, which could potentially lead to the introduction of additional misconnections. This is because a lot of people do not know the difference between the pipes therefore it is easy to get the connections wrong. This therefore emphasises on the need for education and continuous public awareness. The identification of misconnection hotspots would thus be useful because identified hotspots could be dealt with at the sub catchment level by targeting homeowners and builders within such areas and applying the education and campaign approach.

Chapter 7

Discussion and conclusion

7.1 Introduction

Sewage misconnections occur when sewage pipes carrying untreated wastewater from a number of domestic appliances in mainly residential properties are wrongly or illegally connected to the surface water pipes carrying relatively clean water from roofs, driveways and discharging them into watercourses. These misconnections lead to the discharge of untreated wastewater into receiving watercourses especially in urban areas. The wastewater instead of being routed to the WWTW for treatment is discharged into streams and rivers therefore causing significant ecological and water quality impacts on receiving watercourses (Edmonds-Brown and Faulkner, 1995). Substantial amounts of organic matter, nutrients and bacteria are present within these misconnection discharges. During the breakdown process of these materials, available oxygen within the watercourse is used up and therefore regular discharge of untreated sewage causes a significant reduction in D.O. as well as the abundance of sewage fungus on the river bed and impacts on ecological life (Bi et al., 2014). The impacts of misconnections could be severe even at low misconnection percentages in a catchment (Ellis, 2013). Although the true national scale of the misconnection issue is not completely known, regular discharges from misconnections could potentially lead to

several UK watercourses failing to meet the water quality standards of the Water Framework Directives and also a reduction in the ecological status of affected watercourses (Ellis and Butler, 2015). As a result, misconnections are currently considered as one of the top priority urban diffuse pollutant sources in the UK (Gov.UK, 2015).

It has long been established that biological indicators could provide information on water quality status of rivers and streams (Sabater et al., 2007). These indicators could be used as relatively fast and economical tools for monitoring watercourses. In the Pymmes Brook catchment for instance, a previous study recommended the use of invertebrates as a relatively economical tool for monitoring misconnection effluents. During the study, macro-invertebrate scores were found to decline significantly below Cathill PSWO, a particularly problematic outfall in the catchment (Faulkner et al., 2000). Therefore for this current study, coliform bacteria and biofilm communities were explored as tools for locating potential misconnection hotspots.

7.2 Overview and implication of findings

7.2.1 The impacts of misconnection discharges on coliform bacteria

During the coliform survey and experimental study phases (Chapter 3), coliform concentrations recorded for some of the study sites were excessively high. Results obtained for the River Lee sites during the survey indicated that coliform levels were the highest within most of the Lower Lee sites. Records obtained during the Pymmes Brook coliform bacteria experiment also suggested that Pymmes Brook was still experiencing poor water quality. Coliform concentrations within some sites especially Cathill, far exceeded 100,000 cfu per 100ml suggesting faecal discharges into the Brook. Environmental conditions such as solar radiation (increased UV rays from sunlight) and rainfall create many opposing variables therefore affecting coliform bacteria concentrations in

watercourses (Patricia, 2012). Results obtained during this study show that while levels could increase due to rainfall, Ultraviolet rays from exposure to sunlight could potentially lead to reduced concentrations in watercourses. Essentially, concentrations within watercourses are not always easy to predict. Although not a robust tool for river monitoring, coliform bacteria monitoring could be useful tools highlighting areas or sites that require further investigation.

7.2.2 Impacts of misconnections on biofilm communities (diversity and structure)

Biofilm communities (algae, protozoa and metazoa) were investigated as potential indicators of misconnection discharges during this study. It was assumed that the high levels of organic matter within untreated wastewater discharges from misconnections would impact on biofilm communities, thus causing a change in the structure of communities below misconnected outfall. Communities were analysed using diversity indices as well as several multivariate techniques.

Significant differences in diversity were not observed between clean and dirty sites during the biofilm pilot (Chapter 4). With regards to the biofilm experiment (Chapter 5), it was noted that species diversity was higher in the dirty sites than in the clean sites. These observations suggest that diversity indices are not always robust measures of trends in the occurrence of biofilm communities. While some studies have found the use of diversity indices as effective measures of communities (Johnston and Roberts, 2009; Ju et al., 2014), others have found that they were not accurate measures of biological communities (Bellinger et al., 2006; Beyene et al., 2009; Staley et al., 2012; Stevenson, 1984), as appears to be the case here.

Unlike the diversity measures applied during this study, multivariate techniques were more effective measures of the structure of communities along a gradient and are therefore suggested to be more useful tools for the investigation of biofilm community's reaction to pollution in watercourses. During the pilot (Chapter 4) and the main biofilm experiment

(Chapter 5), biofilm community structure was found to change below outfalls in the dirty sites. Results obtained showed evidence of the occurrence of particular communities within clean and dirty sites. Certain species were found to occur abundantly within communities below outfalls in the dirty sites. These species were also consistent across all the dirty sites and from available literature it is noted that these organisms are common indicators of increased levels of nutrients and organic material, which are often major components of misconnection discharges (Lundy et al., 2012). These particular species could be the basis of a group of indicators for detecting misconnection hotspots. In addition, diffuse pollution in the Pymmes Brook catchment has been on-going for over 30 years (Edmonds-Brown and Faulkner, 1995) now and since the discharges from CSO's are no longer common within the catchment (Faulkner et al., 2001), the possible explanation behind the changes in biofilm structure in the dirty sites is misconnection discharges.

7.3 Tackling misconnections (hotspots)

Results obtained during this study highlights the potential threats of misconnection discharges to the quality of watercourses located in catchments with separate sewer systems (Green, 2013). The issue of misconnections has been a longstanding issue within the Pymmes Brook catchment. Misconnections are also common within several urbanised catchments in the UK.

The annual bacteria loading into the brook was predicted to be approximately 6.18×10^{15} colonies. In addition, the misconnection hotspot calculations for the Pymmes Brook sub catchment suggested that approximately 7.21% of the households within the area are misconnected. While this estimated proportion of misconnections could be said to be relatively low, the high coliform bacteria counts identified during the study suggest that these misconnections are impacting severely on Pymmes Brook. This lays further emphasis on the severe impacts that could result from a small proportion of misconnections in a catchment. Although trackbacks were not conducted to identify actual misconnections within the study

area, the estimated 7.21% misconnections was similar to the 7% obtained for the Muswell Stream catchment, a watercourse discharging into Pymmes Brook in Enfield. These figures also corresponded well with the percentage misconnection range of <1 – 9% range suggested for catchments in the Thames region (Dunk et al., 2008).

Various misconnection estimates have been provided for the Thames region and catchments within different areas of the UK. Although some of the estimates have not been verified, these estimates as well as figures from actual trackback investigations suggest that misconnection issues are rampant in the UK. According to the most recent UKWIR report, dealing with misconnections is an expensive operation. It is suggested that costs for the identification of suspected misconnections in the UK could run up to approximately £190,000,000 (UKWIR, 2013).

Currently, tackling misconnection issues relies heavily on the chance discovery of misconnection discharges and then using trackback surveys which are very expensive and time consuming, the misconnections are ideally identified and dealt with. The whole process is however lengthy and involves a great expenditure. Aesthetic signs are not always present for misconnection discharges to be discovered and reported. For instance, although large amounts of sewage fungus, foam and bad odour were noted in Cathill and Eastwalk/Westwalk, these signs were completely absent in Parkside Gardens throughout the study period. In addition it was only on one sampling occasion that foam was noted in the Baring Road site. Therefore in cases whereby aesthetic signs are absent, misconnection discharges could go on for an extended period without being discovered. Even when misconnections are identified and rectified, it does not prevent the reoccurrence of additional misconnections in the catchment. This is because people constantly move houses and in doing so, property extensions are sometimes carried out therefore increasing the chances of misconnection reoccurrence. Evidence of repeat misconnections has been observed in some areas in London. For instance, a pollution tracking study carried out on properties in the Milk Street

catchment area in London identified 102 misconnected appliances including toilets, baths, dishwashers and kitchen sinks that were polluting the watercourse. These misconnections were rectified, however within two years, it was discovered that five misconnected properties were discharging untreated wastewater via the same outfall again. Therefore tackling the issue of misconnections could only be fully effective when it is done alongside stakeholder education (Dunk et al., 2008). The mapping and targeting approach proposed in this current study could provide an easy and inexpensive way of dealing with the sewage misconnection issues. In this approach, misconnection hotspot areas with high coliform bacteria loadings and the abundance of pollution tolerant biofilms could be identified. These hotspots could then be investigated and the proportion of misconnections within the sub catchment assessed. As shown in chapter 6, estimating the impacts of misconnection hotspots would only require catchment information, population data as well as coliform bacteria data for the assessment of misconnections within the catchment. The identification of misconnection hotspots makes it possible for identified hotspots to be targeted at the sub catchment level. This could go a long way towards reducing the occurrence or reoccurrence of misconnections within targeted sub catchments therefore preventing the severe ecological impacts that could result from misconnection discharges. The legislative process for the correction of misconnections does need to be reviewed in order to make it less difficult to rectify misconnections when they are identified. Currently the misconnection identification and correction process is lengthy and requires several linkages and transfers between the Water and Sewerage Companies (WaSC), the Local Authorities and the Environment Agency. In addition, if correction is not carried out within six months of the identification of the misconnection, the information is considered as out-dated. It is, however, difficult to ensure that correction is carried out within this time-frame. For instance, the Local Authorities (LA) are not constitutionally required to correct misconnections. Although on a property owner's refusal to correct misconnections, the WaSC needs to request that the Local Authorities serve enforcement notice since they are

the only ones that have the power to enforce corrections. There is however little motivation for the LA to do so. An option that could be considered in this case would be to provide the WaSC with the power to enforce corrections on property owners instead of having to go through the long process of requesting LA enforcement.

Tackling misconnections through public engagement and education may present a more effective solution to misconnections than the use of legislation. A MORI poll carried out in 2009 with focus groups in London, the Midlands and Torquay showed that 1 in 3 property owners are ignorant of whether they have a combined or separate drainage system. Results of the poll indicated that 8 in 10 people automatically assumed that their pipes were correctly connected since professionals carried out their plumbing installations. In addition, 7 out of 10 people did not think that their misconnections would be discovered (Myers, 2014). Essentially, it was noted that people had different perceptions on whose responsibility it was for misconnection correction while some others are put off by the potential costs of correction (Ellis and Butler, 2015).

Campaign and education programs raise public awareness, which will help the stake-holders value water and yield broader societal benefits (Myers, 2014). Such campaigns and public awareness programs will target residents and the relevant building trade professionals, providing them with information regarding misconnections including responsibility for correction. There are several initiatives currently available in the UK for creating awareness about misconnections. For instance, Thames Water creates awareness on misconnections via their website (http://www.thameswater.co.uk/help-and-advice/8203.htm). In addition, the Environment Agency, WaSC, Local Authorities and several other organisations are currently working in partnership as the National Misconnections Strategy Group (NMSG) to provide information via the Connectright campaign (Ellis and Butler, 2015). The Connectright website (http://www.connectright.org.uk/) provides information about water pollution and advice to residents on how to check their properties for misconnections, all aimed towards

preventing misconnections. Through the connectright website, residents are also provided with information on drainage systems and responsibilities, including how their actions could be affecting local watercourses and how they could face legal action which could lead to fines of up to £50,000 or imprisonment (Connect Right, 2014). Apart from providing misconnection information, residents within misconnection hotspots could be motivated to identify and report misconnections in their properties. Certain discounts for correction could be provided to residents who identify and report misconnections in their property. The WaSC could even offer free correction works to residents who report their misconnections within a specified time. Subsequently, costs for misconnection corrections might be counterbalanced by the reduced cost of investigation (JBA Consulting, 2014). In addition, the WaSc are required to provide the public with access to sewer maps via the Local Authorities. However, the public are only granted access to these maps through a computer in the council offices. Providing these maps through the web may encourage residents and building professionals to access them and obtain information on whether they are dealing with a combined or separate sewage system as well as provide a better understanding of where the sewage and surface water pipes run (JBA Consulting, 2014).

7.4 Conclusion and future work

In this current study, the River Lee and some of its tributaries were investigated with the aim of locating misconnection hotspots. The project proposed a relatively economical mapping and targeting hotspot approach for tackling misconnection issues. This approach involved the use of multiple parameters, coliform bacteria and biofilm communities for the location of hotspots. Although coliform bacteria have several issues relating to variability in watercourses, when deciding sites that require further investigation, they could be used as a tool to reveal sites with potential misconnection issues. The results of the biofilm investigation also suggest that they could be used as important tools for locating hotspots. It

was also shown during this project that coliform bacteria levels predicted from enumerated counts in water samples could also be used along with catchment and census data to assess misconnection hotspots in a catchment. Identification of hotspots makes it easier to target such areas and provide residents and tradesmen (plumbers, builders etc) with the required education, information and campaigns in order to reduce the issue of misconnections. The misconnection hotspot mapping and targeting approach would therefore provide a fast and cheap way to tackle misconnections issues at the sub catchment level.

Additional research especially within the academic sector, would be valuable to help discover inexpensive and rapid approaches that could be used in the industry to monitor misconnections and other sources of pollution within watercourses. This is especially important in the case of misconnections, as research in this area is limited. Furthermore, the approach using predicted coliform bacteria concentrations from enumerated counts was only tested on the Pymmes Brook catchment. Pymmes Brook is a good template for other urban rivers, especially in the London area and it would be interesting to see it explored in other urban catchments using longer-term coliform bacteria data.

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Appendix A

Additional sample sites pictures



Figure A.1: Brook Street site



Figure A.2: Recreation ground, Manor Road (off gypsy hill)(A)



Figure A.3: Recreation ground, Manor Road (off gypsy hill)(B)



Figure A.4: Cold harbour Lane, Harpendon



Figure A.5: Leaside Court sample site



Figure A.6: Leasey Bridge, Cherry Lane



Figure A.7: Leasey Bridge, Cherry Lane(B)



Figure A.8: Wheathampstead car park, Meadow Lane



Figure A.9: Rye house Gatehouse site(A)



Figure A.10: Rye house Gatehouse site(B)



Figure A.11: Fisherman way (a)



Figure A.12: Above Ware lock at Priory Street



Figure A.13: A section of the River Mimram



Figure A.14: Stanborough Park site



Figure A.15: Outfall at Montague Road (Salmons Brook)



Figure A.16: Church street sample site



Figure A.17: Sample site at Littlebury



Figure A.18: Sample site opposite Cunard crescent (Salmons Brook)



Figure A.19: Piglane, Twyford Lock (River Stort)



Figure A.20: Outfall at Tendering Road (Todd Brook)



Figure A.21: Howard way (Todd Brook)



Figure A.23: Outfall at the Cathill sample site



Figure A.22: Tripton Road sample site (Todd Brook)



Figure A.24: The Cathill sample site

Appendix B

Preparation of media and reagent

B.1 Membrane Lauryl sulphate broth

Peptone	40g
Yeast extract	6g
Lactose	30g
Phenol red (0.4 % m/v aqueous solution)	50 ml
Sodium lauryl sulphate - specially pure	1g
Water	1 litre

The required ingredients are measured and poured into the water in a flask. The content of the flask is mixed gently to prevent the froth development. The pH of the final sterilised media should be at pH 7.4 ± 0.2. Therefore, sometimes, prior to autoclaving, it may be necessary to adjust the pH of the mixture to approximately 7.6 before autoclaving. This is important because the detection of acid production by coliform bacteria depends on the

pH of the growth media. Therefore the medium needs to be at the recommended pH value. The mixture is then labelled, loosely covered and autoclaved for 15 minutes at 121°C. After autoclaving, the broth is brought out, tightly capped and then allowed to cool down before being stored in the refrigerator pending use.

B.2 Ringer solution, quarter strength ringer solution tablets

Sodium chloride.....2.25g/l

Potassium chloride.....0.105 g/l

Calcium chloride $6H_2O$.....0.12 g/l

Sodium bicarbonate 0.05.....0.05 g/l

pH.....7.0

Two ringer solution tablets are dissolved into reagent grade water to make one litre of quarter strength ringer solution. After dissolving, the solution is autoclaved for 15 minutes at 121°C (pH level should be 7.0 ± 0.2 before autoclaving).

Appendix C

Techniques used for the analysis of physicochemical parameters during the study

C.1 Techniques used for the analysis of physicochemical parameters during the study

C.1.1 Dissolved Oxygen (mg/l) and temperature (℃)

A D.O metre probe (Extech Instruments, Model 407510a) was used to check for D.O. levels within sites. The instrument was first calibrated by first detaching the sensor from the digital meter. The meter was then switched on and then the O_2 mode selected using the O_2 D.O key. To adjust the meter to zero (indicated on the meter by 'OO'), the zero key was pressed and then removing the cap protective the probe, the metre was reconnected to the sensor and allowed to stand until the display of the most stable value (approximately five minutes). On pressing the O_2 Cal button, the digital display window was checked to make sure that the

readings showed approximately 20.9 which is the typical O_2 content in air. To measure D.O within sites, the sensor probe was placed into the water at the site for a short while until the readings stabilised. The D.O and temperature readings were then recorded and the probe rinsed in preparation for measurements at the next site.

C.1.2 Conductivity (μS/cm)

The portable hand meter probe (Hanna Combo HI-98129) was used to measure conductivity within sites during the fieldwork. Firstly, the probe was calibrated by pressing and holding the START/MODE button until the display screen showed CAL. Releasing the key, the probe was placed in the HI7031 (1413 μS/cm) calibration solution. 'OK' appears for a second on the display screen to show that calibration is complete and then the meter returns to the normal mode for measurement. To measure conductivity in sites, the conductivity mode was selected using the SET/HOLD key. The probe was then inserted into the river at the site. On disappearance of the stability symbol, the conductivity reading displayed on the screen was recorded and the probe rinsed.

C.1.3 Flowrate

Flowrates at sample sites were measured using the MJP Geopacks flowmeter (Advanced flow meter model). The measurement was obtained directly without the need for calibration because the required calibration data is already inbuilt in the advanced flow meter version. For measurement, the riser rods of the stream flow impeller were assembled. The jack plug of impeller was then inserted into the socket of the digital meter and the collar tightened. Then using the rotary dial, 'WATER' mode was selected. Inserting the flow impeller into the river, the motion of the sensor was initiated and the ON switch pressed to turn the meter on. After a 10 seconds integration time, the first measurement is displayed. This first value remains on the screen for approximately two seconds and a new average is displayed again.

Before recording flow rate measurements, a couple of averages are obtained and then the last one is selected and recorded.

C.1.4 Nitrates

Nitrate levels were measured using the HACH test kit method 8192. The wavelength of the HACH spectrophotometer was set to 507nm. A cylinder with a 25 ml capacity is filled up to the 15 ml mark with water sample and then the content of one pack of the Nitrate Reagent Powder Pillow was added to the cylinder. The timer on the HACH was set to a 3-minute reaction time. During the reaction period, the sample was shaken vigorously to dissolve the reagent. On the expiration of the 3-minute period, the timer was set again to a 2-minute reaction time at the end of which 10 ml of the sample was poured into a sample cell, taking care to avoid transferring the settled cadmium particles at the bottom of the cylinder. Then the contents of one Nitrite Reagent Powder Pillow is tipped into the sample cell containing the prepared sample and the timer set to a 30-second reaction time. During this 30-second period, the sample is shaken gently (a pink colour indicates the presence of nitrates). The HACH timer is started again and set to a 15-minute reaction time. At the end of the 15-minute period, another sample cell is filled with 10 ml of the original sample. The blank cell is wiped clean and placed into the cell holder and 'Zero' is pressed. This displays a reading of 0.00 mg/l NO_3^-N on the screen. Subsequently, the prepared sample cell is cleaned and placed into the cell holder. Pressing 'Read' displays the results in mg/l NO_3^-N and the displayed result is recorded.

C.1.5 Phosphates (mg/l)

Samples were collected during fieldwork and taken to the laboratory for analysis. Phosphates were also measured using the HACH kit method 8178. The spectrophotometer was set to a wavelength of 530 nm. The water sample and the blank tests were prepared by filling a

measuring cylinder up to the 25ml mark with water sample. Using a pipette, 1ml of the Molybdate Reagent was added to the sample cylinder and 1ml of the Amino acid Reagent solution was also added. Contents of the sample cylinder were thoroughly mixed (the development of a blue colour indicates the presence of phosphates) and the HACH timer set to a 10-minute reaction time. While the timer counts down, the blank is prepared by filling a 25 ml sample cell with deionized water. After the 10-minute reaction time, the blank cell is wiped clean and then placed into the cell holder of the machine. Pushing 'Zero', 0.00 mg/l PO_4^{3-} is displayed on the screen. Another sample cell is filled with 25 ml of the prepared water sample. The cell is then wiped clean and placed into the cell holder of the machine. Pressing 'Read' displays the result on the screen in mg/l PO_4^{3-} and the value is recorded.

C.1.6 Ammonia (mg/l)

Samples were collected in 100 ml plastic sample bottles and taken to the laboratory for analysis. In the laboratory, the Ammonia content of the sample was measured using the HACH kit method 8155. Firstly, the required unit (mg/l) was selected, and then the spectrophotometer was set to a wavelength of 655 nm. Two measuring cylinders (25 ml capacity) were filled up to the 25 ml mark with water sample and deionised water (the blank) respectively. The content of one pack each of the Ammonia Salicyclate Reagent Powder Pillow was tipped into the water sample and deionised water cylinders. Both cylinders were shaken to dissolve the reagent and then the timer on the HACH kit was set to a 3-minute reaction time. After three minutes, the contents of one pack each of Ammonia Cyanurate powder pillow were tipped into each of the cylinders (the blank and the sample cylinders). These were then shaken to dissolve the reagents and the machine timer set to a 15-minutes reaction time (a green colour indicates the presence of Ammonia-N). On expiration of the 15-minute period, the blank was poured into a sample cell, which was wiped clean and then inserted into the sample cell holder in the HACH. Pressing 'Zero' on the machine, the display screen shows 0.00 mg/l

$NH_3 - N$. The sample was then poured into another sample cell, which was also wiped clean and placed into the cell holder in the machine. Pushing 'Read', the result in mg/l $NH_3 - N$ is displayed on the screen and is then recorded.

C.1.7 pH

The pH status of the different sites within the Pymmes Brook study area was also analysed using the Hanna Combo HI-98129 probe. The probe was also calibrated by inserting the probe into the HI 70007P (pH 7.01 buffer) calibration solution. This buffer value is automatically recognised and the meter requests for the second point (pH 4.01) calibration. To perform this calibration, the probe is rinsed in distilled water, dried and placed in the second buffer solution (pH 4.01). On recognition of the buffer value, 'OK' is shown for a second on the display screen and the meter returns to its normal measurement mode. To measure pH, the probe was first rinsed in distilled water at the site after which the probe was inserted into the watercourse for approximately 2-3 minutes. Values for both pH and temperature appear on the screen and are recorded.

Appendix D

Solar radiation data from Kings College

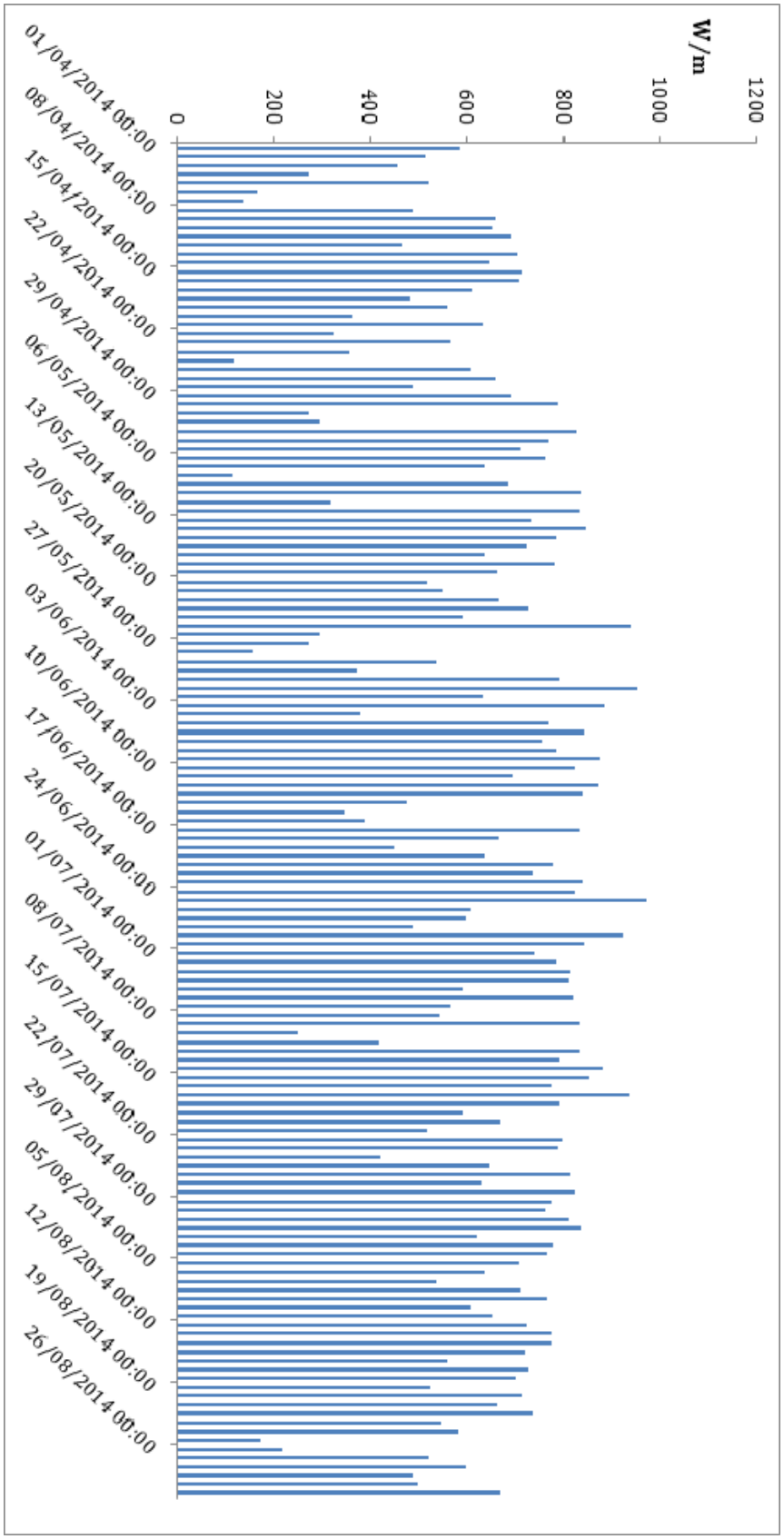


Figure D.1: Solar radiation (W m^2) records (April – August 2014) from London (Data from Kings College, www.londonair.org.uk london

Appendix E

Runoff coefficients for the rational method

Table E.1: Shows runoff coefficients for the rational method

Source of variation	Runoff Coefficient (C)
Lawns	0.05 – 0.35
Forest	0.05 – 0.25
Cultivated land	0.08 – 0.41
Meadow	0.1 – 0.5
Parks, cemeteries	0.1 – 0.25
Unimproved areas	0.1 – 0.3
Pasture	0.12 – 0.62
Residential areas	0.3 - 0.75
Business areas	0.5 – 0.95
Industrial areas	0.5 – 0.9
Asphalt streets	0.75 – 0.95
Brick streets	0.75 – 0.85
Roofs	0.75 – 0.95
Concrete streets	0.75 – 0.95

Appendix F

Analysis of variance (ANOVA) for coliforms at site over the study period

Table F.1: One Way Analysis of Variance (ANOVA) for coliforms at site over the study period

Source of variation	df	SS	MS	F	P-value
Among groups	5	3.66E11	7.32E10	3.054337	0.0028425774
Within groups	24	5.75E11	2.4E10		
Total	29	9.41E11			

Critical value of F (5,24) = 2.63

Appendix G

Leaflet used by the Environment Agency to create awareness on misconnections

How to help

Check your home is connected right.

Are the drains in your home connected to the right sewer? If not fix them.

Only rain down the drain!

Use your local household waste site to dispose of chemicals, paints, cooking and motor oils.

Bin it, don't block it!

Dispose of cooking fats, oils and grease in the bin to avoid pollution from blocked drains and overflowing sewers.

Bag it and bin it!

Dispose of household items such as nappies, cotton buds, wet wipes, razors, tampons and syringes in the bin, as these block drains and cause overflowing sewers.

Report it, don't ignore it!

Report water pollution to the Environment Agency's incident hotline on **0800 80 70 60.**

Connect **with your local environment and do the *right* thing.**
Help keep your local rivers and beaches clean and healthy.

Useful contacts

Water and Sewerage Companies

Anglian Water	08457 919 155
Dŵr Cymru Welsh Water	0800 085 3968
Northumbrian Water	0845 604 7468
Scottish Water	0845 601 8855
Severn Trent Water	08456 016 016
South West Water	0844 346 1010
Southern Water	0845 278 0845
Thames Water	0845 920 0800
United Utilities Water	0845 746 2200
Wessex Water	0845 600 4600
Yorkshire Water	0845 124 2424

Other useful contacts

Environment Agency Enquiry line	03708 506 506
Environment Agency Incident hotline	0800 80 70 60
Natural Resources Wales	0300 065 3000
Local Authority Building Control	020 7091 6860
Chartered Institute of Environmental Health	020 7928 6006
Consumer Council for Water	0121 345 1000
Chartered Institute of Plumbing and Heating Engineering	01708 472791

To find out more visit
www.connectright.org.uk

Environment Agency · WATER UK · ciphe · defra

connect right
Stop pollution

Is your home connected right?



connect right
Stop pollution

Figure G.1: Front Page of Leaflet

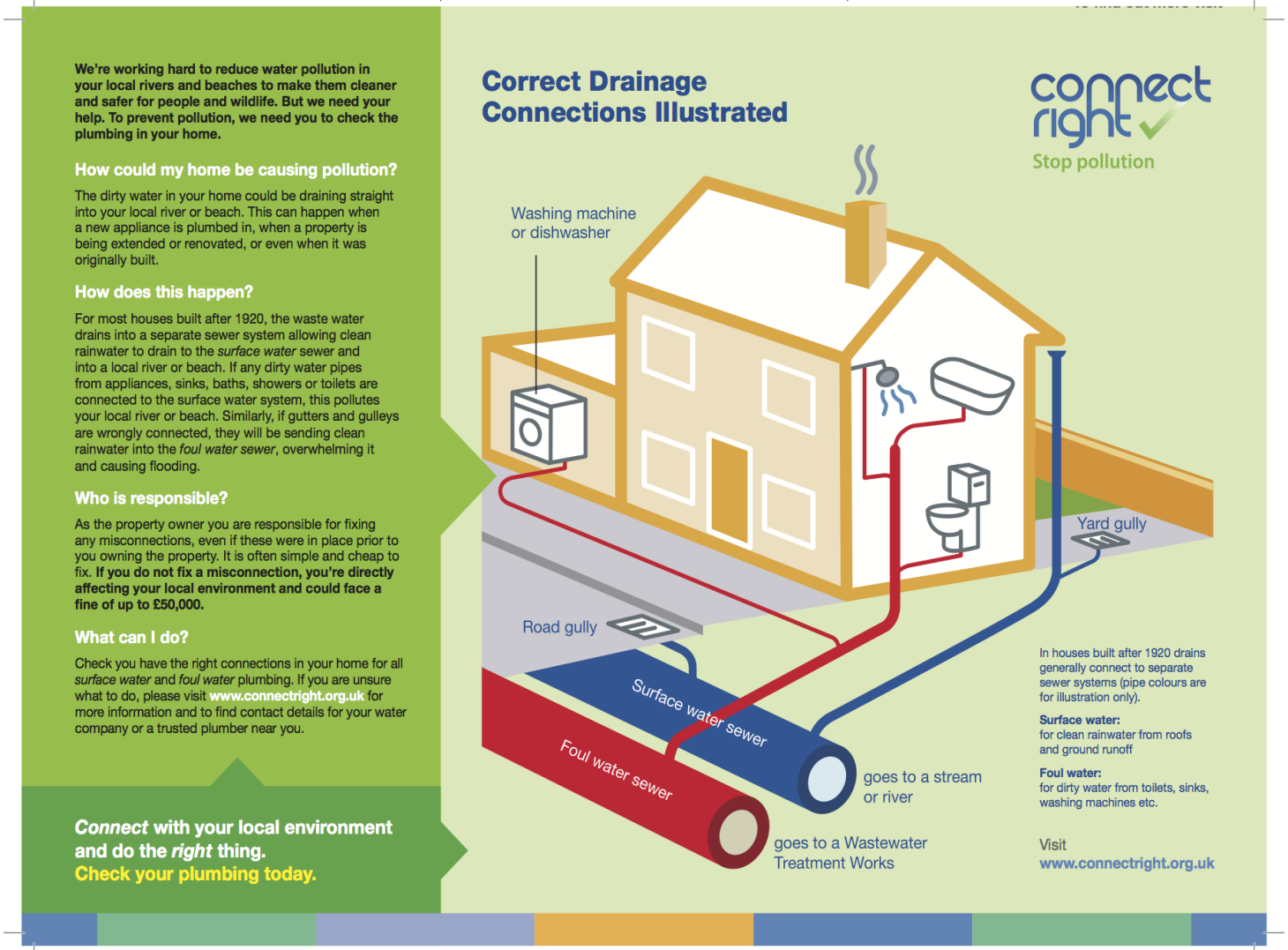


Figure G.2: Back page of leaflet

Appendix H
Risk assessment and COSHH forms

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

Ref No:	
Date:	
Review Date:	

ACTIVITY INFORMATION	
Name of Assessor/ Contact details	Name: Chidinma Chiejina (Nee Nwanekezie) Email address: c.o.chiejina@herts.ac.uk
Title of Activity	River sampling
Location of Activity	River Lee and tributaries, College Lane Laboratories (Biodet and other labs) and Bayfordbury labs
Description of Activity	Water sampling (for coliforms, nitrates, phosphates and ammonia) sampling for biofilms on substrates on the river bed, measurement of flow, D.O. conductivity, temperature and Ph.
Personnel Involved	

TYPES OF HAZARD LIKELY TO BE ENCOUNTERED		
√Animal Allergens ✓Biological Agents (see COSHH) ✓Chemical Compounds (see CoSHH) ☐ Compressed/liquefied gases ☐ Computers ☐ Electricity ☐ Falling Objects ☐ Farm Machinery ☐ Fire ☐ Glassware Handling	☐ Hand Tools ☐ Ionising Radiation ☐ Office Equipment √ Laboratory Equipment ☐ Ladders ☐ Manual Handling ☐ Non-ionising Radiation ☐ Hot or cold extremes ☐ Repetitive Handling ☐ Severe Weather	☐ Sharps √Slips/trips/falls ☐ Stress √Travel ☐ Vacuum systems ☐ Pressure systems ☐ Vehicles ☐ Violence, physical or verbal abuse ☐ Workshop Machinery

HAZARD ASSESSMENT

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

Severity of Consequences	Factor	Risk Classification				
No or minor injury/ health disorder Minor Damage or Loss Insignificant Environmental Impact Group 1 Biological agents	1	Trivial (1)	Trivial (2)	Trivial (3)	Trivial (4)	Tolerable (5)
Injury or Health Disorder – resulting in absence up to 3 days Moderate Damage or Loss Moderate Environmental Impact Group 2 Biological agents	2	Trivial (2)	Trivial (4)	Tolerable (6)	Tolerable (8)	Moderate (10)
Injury or Health Disorder – resulting in absence over 3 days Substantial Damage or Loss Serious Environmental Impact Group 3 Biological agents	3	Trivial (3)	Tolerable (6)	Moderate (9)	Moderate (12)	Substantial (15)
Long Term Injury or Sickness – resulting in permanent incapacity Extensive Damage or Loss Major Long Term Environmental Impact	4	Trivial (4)	Tolerable (8)	Moderate (12)	Substantial (16)	Intolerable (20)
Death Serious Structural Damage Environmental Catastrophe Group 4 Biological agents	5	Tolerable (5)	Moderate (10)	Substantial (15)	Intolerable (20)	Intolerable (25)
Note on Risk Classification: 1-4 Trivial 5-7 Tolerable 8-12 Moderate 13-16 Substantial >20 Intolerable	→ Likelihood	1 Almost Impossible	2 Unlikely – possible exposure every 1-3 years	3 Harm is possible	4 Harm is likely to occur	5 Harm will occur or is very likely to occur.

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

ASSESSMENT OF RISK CLASSIFICATION			
Hazard	Likelihood Score	Severity Score	Risk Classification
Slips trips & falls	2	2	Tolerable
Animal allergy	2	2	Trivial
Drowning	2	5	Tolerable
Weils disease	2	5	Tolerable
Travel	2	2	Trivial

EFFECT OF RISK CLASSIFICATION	
Risk Classification	Action
Trivial	No further action required. Activity can begin.
Tolerable	No additional controls required. Current controls must be maintained and monitored.
Moderate	Reduce risks if cost effective. Implement new controls over an agreed period.
Substantial	Activity cannot begin without major risk reduction.
Intolerable	Activity must not begin.

RISK CONTROL MEASURES
Is the local code of practice or local rules adequate to control the risks identified? Yes If no, list all additional measures required.
Additional Measures:

HEALTH SURVEILLANCE ISSUES	
Persons at Special Risk	n/a
Health Surveillance Measures (including symptoms and signs of exposure)	n/a
Exclusions	n/a

Please think before printing the attached guidance notes – do you really need them?

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

SIGNATURES				
	Staff/PhD student/MSc student/Undergraduate	Name (Print)	Signature	Date
Assessor	PhD student	Chidinma Chiejina		
Supervisor (if Assessor is a student)	Supervisor	Dr Ronni Edmonds-Brown		
Local Health and Safety Advisor	Technical STAFF	Peter Coates	PRE	30/11/15

FACULTY OF HEALTH AND HUMAN SCIENCES
CoSHH ASSESSMENT

Ref No:	
Date:	
Review Date:	

PROJECT INFORMATION	
Name of Assessor/Contact details	Name: Chidinma Chiejina (nee Nwanekezie) Email address: c.o.chiejina@herts.ac.uk
Title of Activity	Phosphate, nitrate, nitrite and ammonium analysis in water samples, Water sample analysis for coliform bacteria
Location of Activity	Bayfordbury, Biodet lab college lane
Description of Activity	Phosphate, nitrate, nitrite and ammonium analysis using powder pillows and cuvettes with a HACH spectrophotometer. Analysis of water samples for coliform bacteria using Oxoid Membrane Lauryl Sulphate Broth (powder).
Personnel Involved	

HAZARD CLASSIFICATION

Hazards involved in project.

☑ Harmful/Irritant 	☑ Corrosive 	☐ Radioactive 	☑ Biological 
☑ Toxic 	☐ Health Hazard 	☐ Explosive 	☐ Oxidising 
☐ Flammable 	☑ Hazardous to the Environment 	☐ Gas under pressure 	

FACULTY OF HEALTH AND HUMAN SCIENCES
CoSHH ASSESSMENT

Severity Classification

Harmful/Irritant 2	Corrosive 3	Radioactive *	Biological **
Toxic 3	Health Hazard 4	Explosive 6	Oxidising 6
Flammable 3	Hazardous to the Environment 4	Gas under pressure 6	

* Radioactive – If any work will involve use of Radioactivity contact the Radiation Protection Supervisor, Janet Evans (ext 4379, j.s.evans@herts.ac.uk) for an accurate severity classification *before* work begins.

** Biological – If any work will involve using biological samples or suspected biohazards contact the Biological Safety Advisor.
For School of Life Sciences contact Di Francis (ext 4527, d.l.francis@herts.ac.uk).
For School of Pharmacy contact James Stanley (ext 4599, j.k.stanley@herts.ac.uk).

Substance Used or Produced.	Concentration or amount used.	Hazard Words and Pictograms	Route of Entry	Severity
NitriVer 3 & PhosVer 3 & LCK 339 A & LCK 349 sample cuvette	<1g in 10ml h_20	Irritant 	Ingestion Inhalation Skin Eyes	2
LCK 339 Nitrate sample cuvette & LCK 348 B & LCK 349 B	< 1 ml	Corrosive 	Ingestion Inhalation Skin Eyes	3
LCK 349 DosiCapZip & LCK 348 DosiCapZip & LCK 305 DosiCapZip	< 1ml	Harmful 	Ingestion Inhalation Skin Eyes	2
LCK 305 DosiCapZip & NitraVer 5	<1g in 10ml h_20 or <1ml	Hazardous to the Environment 		4
NitraVer 5	<1g in 10ml h_20	Toxic 	Ingestion Inhalation Eyes Skin	3
Water sample	10ml	Biological 	Ingestion Open wound Eyes	2
Membrane Lauryl Sulphate Broth (powder)	76.2g in 1 litre	Harmful 	Ingestion Skin Eyes	2
Information sources (eg MSDS)	HACH-Lange website			

CONTROL MEASURES
Additional measures to be used over and above local codes of practice and local rules.
Gloves, goggles, lab coat, well ventilated room Wash hands before breaks and after work
Are alternative less hazardous substances available? If so please list below and state why they cannot be used.
No

EMERGENCY AND DISPOSAL PROCEDURES
Measures to be taken in case of Spillage or Uncontrolled release
Solid spill: Use personal protective equipment. Do not flush into surface water or sewer system. Sweep up and collect into a suitable container for disposal. Liquid Spill: Use personal protective equipment. Do not flush into surface water or sewer system. Soak up with an inert absorbent material (blue towel) and dispose as hazardous waste.
Disposal Measures
Solids: Collect in labelled waste bottle and hold for professional disposal. Liquids: **PhosVer3**, **NitraVer 5** and **NitriVer 3** collect in labelled water bottle and hold for professional disposal. All reagents associated with **LCK 348, LCK 349, LCK 305** and **LCK 339** seal and store in the original packaging and label box as 'used'. These will be collected by HACH-Lange for disposal. Solvents: Biohazard: Pour un-used water samples down the sink and flush with running water.
Measures to be taken in case of Fire
Types of fire extinguisher to be used: Use extinguishing measures that are appropriate to local circumstances and the surrounding environment. Emergency procedure in case of fire: Follow normal emergency procedure. Toxic fumes emitted under fire conditions: **PhosVer 3**: sulphur oxides, carbon monoxide, carbon dioxide, sodium oxide **NitraVer 5**: sulphur oxides and nitrogen oxides **NitriVer 3**: carbon monoxide, carbon dioxide, oxides of phosphorous **LCK 339 sample cuvette**: sulphur oxides **All other reagents** may liberate hazardous vapours
Measures to be taken for First Aid

In contact with eyes: Rinse thoroughly with plenty of water for at least 15 minutes and consult a Doctor. Show the safety data sheet and COSHH to the Doctor.

In contact with skin: Wash off immediately with plenty of water for at least 15 minutes. If irritation persists consult a Doctor. Show the safety data sheet and COSHH to the Doctor.

If inhaled: **NitriVer 3**: Move to fresh air, consult a Doctor.
PhosVer 3: Move to fresh air, consult a doctor after significant exposure
NitraVer 5: Move to fresh air, consult a doctor. Show the SDS to the doctor in attendance.
All other reagents: Move to fresh air.

If ingested:
NitraVer 5: Consult a Doctor, show the SDS. Induce vomiting, only if victim is conscious.
PhosVer 3: Do NOT induce vomiting. Drink 1 or 2 glasses of water if conscious. Call a Doctor immediately and show SDS
NitriVer 3: Clean mouth with water and drink afterwards plenty of water. Consult a Doctor
LCK 348 sample cuvette: Clean mouth with water and drink afterwards plenty of water
LCK 348 DosiCapZip: Clean mouth with water and drink afterwards plenty of water, Consult a Doctor
LCK 348 B: Clean mouth with water and drink afterwards plenty of water. Do NOT induce vomiting, consolt a Doctor show the SDS sheet.
LCK 348 C: Clean mouth with water and drink afterwards plenty of water, Consult a Doctor
LCK 349 sample cuvette: Clean mouth with water and drink afterwards plenty of water
LCK 349 DosiCapZip: Clean mouth with water and drink afterwards plenty of water, Consult a Doctor
LCK 349 B: Clean mouth with water and drink afterwards plenty of water. Do NOT induce vomiting, consolt a Doctor show the SDS sheet.
LCK 349 C: Clean mouth with water and drink afterwards plenty of water, Consult a Doctor
LCK 305 sample cuvette and **LCK 305 DosiCapZip**: Clean mouth with water and drink afterwards plenty of water, Consult a Doctor.
LCK 339 sample cuvette and **LCK 339 A**: Clean mouth with water and drink afterwards plenty of water. Do NOT induce vomiting, consult a Doctor show the SDS sheet.

Signs and symptoms of exposure:

Is hospitalisation required?

Persons at Special Risk:
NitraVer 5: Pregnant women. Possible risk of impaired fertility, possible risk of harm to unborn child.

SIGNATURES				
	Staff/PhD student/MSc student/Undergraduate	Name (Print)	Signature	Date
Assessor	PhD Student	CHIDINMA CHIEJINA		
Supervisor (if Assessor is a student)	Supervisor.	DR. Ronni Edmonds-Brown		
Local health and safety advisor	STAFF	P. GATES	PPE	30/11/15

Appendix I
Coliform and Biofilm primary data for the study pilot and experiment

Table I.1: Coliform data for the River Lee pilot study

Sample Sites	Site number	Easting	Northing	Date	Coliform bacteria levels (cfu/100mls)
Source of the Lee (Five Springs)	1	506100	224800	21st march 2013	200
Bancroft Road (Birdsfoot Lane)	2	507586	224050	21st march 2013	540
Kingsdown Avenue	3	508874	224043	21st march 2013	710
Brooks Street	4	508663	221967	21st march 2013	1,100
Recreation Ground Manor Road	5	509716	220926	21st march 2013	1,800
Cold Harbour Lane Harpenden	6	514101	215585	21st march 2013	2,400
Leaside Court (a)	7	514414	215300	21st march 2013	5,200
Leaside Court (b)	8	514564	215341	21st march 2013	6,100
Leasey Bridge (Cherry Lane)	9	516171	214421	21st march 2013	1,100
Wheathhampstead Carpark, Meadow Lane	10	517889	214071	21st march 2013	2,800
Rye House Gatehouse	11	538397	210009	08th April 2013	200
Fisherman Way (a)	12	538654	209431	08th April 2013	220
Fisherman Way (b)	13	538847	209752	08th April 2013	510
Above Ware lock	14	535891	214008	08th April 2013	110
Mimram Road	15	531807	212326	08th April 2013	300
Bessemer Road	16	526107	214157	08th April 2013	300
Stanborough Park	17	522907	211053	08th April 2013	3,700
Pymmes Park	18	533709	192407	11th April 2013	43,000
Montague Road	19	535159	193329	11th April 2013	32,000
Church Street	20	532898	194629	11th April 2013	22,000
Littlebury	21	532991	194443	11th April 2013	22,000
Opposite Cunard Crescent	22	532604	195100	11th April 2013	610
Park Ridings	23	531998	195437	11th June 2013	150,000
The Chines	24	531877	195302	11th June 2013	44,000
Slades Hill	25	531229	196864	11th June 2013	120,000
Below Jacks Lake	26	527218	197251	15th April 2013	3,200
Baring Road	27	526859	196532	15th April 2013	3,700
Cathill 1	28	527219	195426	15th April 2013	130,000
Cathill 2	29	527283	195041	15th April 2013	310,000
Oakhill Park	30	527751	194832	15th April 2013	46,000
Parkside Gardens	31	528229	194635	15th April 2013	130,000
Eastwalk/ Westwalk	32	528329	194370	15th April 2013	50,000
Waterfall walk	33	529095	192967	15th April 2013	26,000
Vincent	34	538926	191671	11th June 2013	120,000
Winchester	35	538383	191160	11th June 2013	110,000
Studley Road	36	538665	191503	11th June 2013	100,000
Beech Hall Crescent	37	538616	191351	11th June 2013	57,000
Hale End	38	538670	191664	11th June 2013	130,000
Falmouth (The Charles Road)	39	539026	192049	11th June 2013	74,000
Waterhall Avenue	40	539118	192861	11th June 2013	62,000
South Mills	41	549551	220055	29th July 2013	13,000
Piglane (Twyford Lock)	42	549378	219246	29th July 2013	15,000
Tendering Road (Todd Brook)	43	543922	208917	29th July 2013	38,000
Tripton Road (Todd Brook)	44	544500	209045	29th July 2013	28,000
Howard Way (Todd Brook)	45	544886	209431	29th July 2013	97,000

Table I.2: Biofilm data for Site 1 (River Lee pilot study)

List of Individuals	Site 1a	Site 1b	Site 1c	Site 1d	Site 1e	Site 1f	Site 1g	Site 1h	Site 1i	Site 1j
Ulothrix spp.	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	15	21	0	11	21	3	2	13	8	6
Nitzschia sublinearis	15	4	0	0	13	6	0	1	10	5
Stigeoclonium	0	0	0	0	0	0	0	0	0	0
Closterium	0	0	0	0	0	0	0	0	0	0
Chlorella	0	0	0	0	0	0	0	0	0	0
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	3	2	1	0	0
Chlamydomonas	0	0	0	0	0	3	0	0	0	3
Spirogyra	2	0	0	1	0	3	0	0	1	0
Euastrum	1	1	0	1	0	4	0	1	0	2
Scenedesmus	0	0	0	0	0	0	0	1	0	0
Astasia	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0
Melosira	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	30	0	15	6	8	12	27	24	21	0
Navicula gracilis	0	0	19	8	0	0	3	7	8	0
Fragilaria	0	0	0	6	13	3	2	0	2	0
Asterionella	0	0	0	0	3	0	0	0	0	0
Diatoma	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	0	0
Navicula minima	0	0	0	0	0	0	0	0	0	0
Pinnularia	0	0	0	0	0	0	2	0	0	0
Cyclotella	0	0	1	1	0	1	0	1	2	0
Cocconeis	0	0	0	0	0	0	0	4	8	0
Vaucheria	0	4	0	3	3	6	9	0	0	15
Tribonema	0	0	0	0	0	3	3	0	6	5
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0
Mallomonas	2	4	2	3	1	0	0	0	2	2
Dileptus	0	0	0	0	0	0	0	0	2	0
Aspidisca	0	0	0	0	0	0	0	0	0	0
Cinetochilium	0	0	0	0	0	0	0	0	0	0
Epiclintes	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	1	0	0
Vorticella	0	0	0	0	0	0	0	1	0	0
Euplotes	5	0	5	0	4	2	0	1	0	2
Rhynchomonas spp	5	0	0	0	0	0	0	0	0	0
Stentor	0	5	11	0	0	0	0	0	2	0
Ochromonas	0	5	0	0	0	0	0	0	0	0
Lacrymaria	0	0	4	0	3	3	0	2	1	3
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	4	0	0	0	0	0	0	2
Euglena	0	0	4	0	0	0	0	0	0	0
Litonotus	0	0	0	0	11	0	0	0	0	3

Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	0
Rhipidodendron	0	0	0	0	3	3	0	0	0	0
Navicula radiosa	0	0	0	0	0	3	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	3	0	1	0	0
Asellus aquatica	0	0	0	0	0	0	3	0	0	0
Uroglena	0	0	0	0	0	0	0	1	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	2
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0
Mayorella	0	0	0	0	0	0	0	0	0	0
Difflugia	0	0	0	0	0	0	0	0	0	0
Arcella	0	0	4	0	0	0	2	0	0	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	2	0	0	2	0	0	2
Achnanthes granulata	5	13	7	11	0	3	0	3	0	6
Rotifer Bdelloidea	0	8	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	0	0
Rotaria	0	0	0	0	0	0	0	1	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	0
Flatworm	0	0	0	0	0	0	0	0	0	3
Hydroporinae larvae	0	4	4	6	3	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	0	0
Helodidae larva	0	0	0	0	0	3	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	0	0
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	0
Didinium	0	0	0	0	0	0	0	0	0	0
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	0	0
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	2	0	1	3	2	0	0	1	0	0
Gastotrich	0	0	0	0	0	0	0	0	0	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	0
Eunotia	2	2	0	1	3	0	0	2	1	4
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	0
Actinosphaerium	0	0	0	0	0	0	0	0	0	0
Aulacosera	2	0	2	0	4	0	3	0	1	1
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0

Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	0
Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0
Chroococcus	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0
Nitzschia recta	0	0	0	0	0	0	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	0	1	0	3	0	0	2	1	1	0
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	2	2	0	0	3	0	2	2	0	4
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	1	0	1	1	0	0	3	0	2	3
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	0	0	0	0	0	0	0	0	0	0
Gammaridae	2	1	2	4	0	0	0	1	0	0
Rhoicosphenia abrevata	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	2	0	0	4	4	0	0	2	0	0
Actinophrys	3	1	2	1	1	0	5	0	6	0
Nitzschia acicularis	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Suirella ovata	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0
Navicula cuspidata	0	0	0	0	0	0	0	0	0	0
Pelomyxa	0	0	0	0	0	0	0	0	0	0
Rotifer (Philodina)	0	0	0	0	0	0	0	0	0	0
Menoidium	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0
Chamaesiphon	0	0	0	0	0	0	0	0	0	0
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0

Microspora	0	0	0	0	0	0	0	0	0	0
Oscillatoria	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0
Chlorococcum	0	0	0	0	0	0	0	0	0	0
Navicula viridula	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	2	0	4	0	4	0	0	2	2	0
Halteria	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0
Suirella splendida	0	0	0	2	3	3	2	1	0	0
Cymbella ehrenbergii	2	0	4	0	2	0	0	2	1	1
Opercularia	2	2	5	1	4	0	1	1	1	2
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.3: Biofilm data for Site 2 (River Lee pilot study)

List of Individuals	Site 2a	Site 2b	Site 2c	Site 2d	Site 2e	Site 2f	Site 2g	Site 2h	Site 2i	Site 2j
Ulothrix spp.	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	11	21	0	1	23	3	12	13	68	26
Nitzschia sublinearis	13	3	1	0	13	6	0	0	10	5
Stigeoclonium	0	0	0	0	0	0	0	0	0	0
Closterium	0	0	0	0	0	0	0	0	0	0
Chlorella	0	0	0	0	0	0	0	0	0	0
Cladophora	0	0	0	0	0	1	1	0	0	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	7	4	0	4	0
Chlamydomonas	4	0	0	2	0	0	0	0	0	0
Spyrogyra	0	2	3	0	0	1	1	3	0	0
Euastrum	3	3	0	0	1	2	0	3	0	3
Scenedesmus	0	0	0	0	0	0	0	0	0	0
Astasia	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0
Melosira	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	8	0	14	25	11	0	0	15	8	17
Navicula gracilis	4	0	0	16	11	7	0	16	4	8
Fragilaria	0	0	0	0	2	0	0	0	0	0
Asterionella	0	0	0	0	0	0	0	0	0	0
Diatoma	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	0	0
Navicula minima	0	0	0	0	0	0	0	0	0	0
Pinnularia	0	0	0	0	10	0	0	1	0	11
Cyclotella	2	0	2	1	0	3	0	1	0	3
Cocconeis	0	0	0	2	0	0	0	0	0	0
Vaucheria	8	8	0	0	0	1	5	0	2	2
Tribonema	8	1	0	0	0	2	5	1	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0
Mallomonas	0	3	4	4	0	0	3	2	3	0
Dileptus	0	0	4	0	0	0	0	0	0	0
Aspidisca	0	0	0	0	0	0	0	0	0	0
Cinetochilium	0	0	0	0	0	0	0	0	0	0
Epiclintes	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	2	0	0	0	0	0	0
Vorticella	0	0	0	0	0	0	0	0	0	0
Euplotes	2	1	2	0	0	4	0	5	0	0
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	0	0	4	0	0	0	2	0	0	0
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	5	0	4	3	0	5	3	3	0
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	2	2	0	0	0	0	0
Euglena	0	0	0	0	0	0	0	1	0	0
Litonotus	0	3	0	2	2	0	0	0	0	0
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	0

Rhipidodendron	0	0	0	0	0	0	0	1	0	0
Navicula radiosa	0	0	0	0	0	0	4	1	0	3
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0
Mayorella	0	0	0	0	0	0	0	0	0	0
Difflugia	0	0	0	0	0	0	0	0	0	0
Arcella	0	3	7	0	3	0	0	0	4	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	2	0	0	0	1	0	2
Achnanthes granulata	5	13	7	11	0	3	0	3	0	6
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	0	0
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	0
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	0	0
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	0	0
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	0
Didinium	0	0	0	0	0	0	0	0	0	0
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	0	0
Spathidium	0	3	0	0	0	0	0	0	0	0
Chaenia	2	3	0	2	2	0	0	1	0	1
Gastotrich	0	0	0	0	0	0	0	0	0	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	0
Eunotia	0	3	0	1	2	0	0	3	2	2
Busaria	0	0	4	0	0	0	0	0	0	0
Chaetogaster	0	0	4	0	0	0	0	0	0	0
Phialina	0	0	0	2	2	0	0	0	0	0
Anthophysa	0	0	0	2	2	0	0	0	0	0
Cochliopodium	0	0	0	2	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	0	2	4	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	0
Actinosphaerium	0	0	0	0	0	0	0	0	4	0
Aulacosera	0	2	3	0	0	2	0	1	1	8
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	0

Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0
Chroococcus	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0
Nitzschia recta	0	0	0	0	0	0	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	2	0	0	4	0	0	0	3	0	2
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	0	2	0	0	1	1	3	4	0	0
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	1	1	0	0	0	2	0	1	0	0
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	0	0	0	0	0	0	0	0	0	0
Gammaridae	1	0	0	0	1	2	0	0	0	0
Rhoicosphenia abrevata	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	3	0	3	1	0	0	0	4	4
Actinophrys	0	0	1	4	2	0	0	0	1	3
Nitzschia acicularis	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Suirella ovata	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0
Navicula cuspidata	0	0	0	0	0	0	0	0	0	0
Pelomyxa	0	0	0	0	0	0	0	0	0	0
Rotifer (Philodina)	0	0	0	0	0	0	0	0	0	0
Menoidium	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0
Chamaesiphon	0	0	0	0	0	0	0	0	0	0
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0
Microspora	0	0	0	0	0	0	0	0	0	0

Oscillatoria	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0
Chlorococcum	0	0	0	0	0	0	0	0	0	0
Navicula viridula	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	0	0	3	0	0	1	1	7	0	0
Halteria	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0
Suirella splendida	0	0	3	1	1	2	0	0	3	2
Cymbella ehrenbergii	0	3	0	4	0	0	3	3	0	7
Opercularia	6	0	0	3	3	1	1	4	0	2
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.4: Biofilm data for Site 3 (River Lee pilot study)

List of Individuals	Site 3a	Site 3b	Site 3c	Site 3d	Site 3e	Site 3f	Site 3g	Site 3h	Site 3i	Site 3j
Ulothrix spp.	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	1	53	55	0	0	0	72	61	55
Nitzschia sublinearis	11	13	3	6	5	6	26	5	2	16
Stigeoclonium	0	0	0	0	0	0	0	0	0	0
Closterium	0	0	0	0	0	0	0	0	0	0
Chlorella	0	0	0	0	0	0	0	0	0	0
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0	0	0
Chlamydomonas	0	8	1	2	0	3	0	2	0	1
Spyrogyra	4	1	0	0	0	2	0	0	2	4
Euastrum	4	0	0	2	0	1	1	0	2	0
Scenedesmus	4	4	0	3	0	3	0	1	5	1
Astasia	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0
Melosira	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	25	8	0	0	4	0	6	2	2	1
Navicula gracilis	7	13	2	8	0	28	11	0	0	6
Fragilaria	0	0	0	0	0	0	0	0	0	0
Asterionella	0	0	0	4	3	3	0	0	0	1
Diatoma	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	0	0
Navicula minima	0	0	0	0	0	0	0	0	0	0
Pinnularia	7	0	0	0	0	0	0	0	0	1
Cyclotella	4	1	7	5	25	0	0	4	6	1
Cocconeis	7	0	0	0	0	0	0	0	0	1
Vaucheria	0	0	2	0	3	5	0	0	0	0
Tribonema	0	0	0	0	0	0	0	1	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0
Mallomonas	0	1	1	4	0	0	0	2	2	0
Dileptus	0	0	1	0	0	0	1	1	1	0
Aspidisca	0	0	0	0	0	0	0	0	0	0
Cinetochilium	0	0	0	0	0	0	0	0	0	0
Epiclintes	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	1	0	3	0	0	0	0	0
Euplotes	0	0	2	1	2	0	1	1	0	0
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	5	4	1	0	3	0	2	8	0	1
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	2	1	0	0	4	4	0	1	2
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	2	1	1	0	0	0	0	0	0
Euglena	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	0

Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0
Mayorella	0	0	0	0	0	0	0	0	0	0
Difflugia	0	0	0	0	0	0	0	0	0	0
Arcella	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	1	1	3	0	0	0	0	4	0
Achnanthes granulata	2	2	2	0	7	0	2	0	1	1
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	0	0
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	0
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	0	0
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	0	0
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	0
Didinium	0	0	0	0	0	0	0	0	0	0
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	0	0
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	1	0	0	3	0	0	0	0	0	3
Gastotrich	0	0	0	0	0	0	0	0	0	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	0
Eunotia	0	0	0	1	1	0	0	3	3	0
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	0
Actinosphaerium	4	2	0	0	0	0	3	0	0	0
Aulacosera	0	3	4	0	1	1	1	0	0	3
Heterophrys	4	0	0	0	0	0	1	0	0	0
Gomphonema gracili	4	0	0	1	0	0	0	0	1	1
Nuclearia	4	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	0

Pediastrum	0	4	3	1	0	0	0	0	0	0
Volvox	0	8	4	2	6	0	0	1	2	0
Chroococcus	0	0	4	0	0	5	0	0	0	0
Nitzschia palea	0	0	1	0	1	2	0	0	0	0
Nitzschia recta	0	0	0	0	0	0	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0
Prorodon	0	0	0	1	0	0	0	1	0	0
Cosmocladium	0	0	0	0	2	1	1	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	0	1	1	0	0	1	2	2	0	0
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	0	2	0	0	1	0	3	0	0	2
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	0	0	0	0	0	0	0	0	0	0
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	0	0	0	0	0	0	0	0	0	0
Gammaridae	0	3	0	2	1	0	0	0	0	0
Rhoicosphenia abrevata	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	2	2	2	0	0	0	0	0
Actinophrys	0	3	0	0	2	0	2	0	7	0
Nitzschia acicularis	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Suirella ovata	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0
Navicula cuspidata	0	0	0	0	0	0	0	0	0	0
Pelomyxa	0	0	0	0	0	0	0	0	0	0
Rotifer (Philodina)	0	0	0	0	0	0	0	0	0	0
Menoidium	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0
Chamaesiphon	0	0	0	0	0	0	0	0	0	0
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0
Microspora	0	0	0	0	0	0	0	0	0	0

Oscillatoria	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0
Chlorococcum	0	0	0	0	0	0	0	0	0	0
Navicula viridula	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	0	0	0	0	0	0	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0
Suirella splendida	2	1	4	1	0	0	0	2	3	1
Cymbella ehrenbergii	0	2	0	1	1	2	1	0	0	3
Opercularia	1	0	0	1	1	7	0	0	1	2
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.5: Biofilm data for Site 4 (River Lee pilot study)

List of Individuals	Site 4a	Site 4b	Site 4c	Site 4d	Site 4e	Site 4f	Site 4g	Site 4h	Site 4i	Site 4j
Ulothrix spp.	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	9	18	16	22	10	27	0	7	16	0
Nitzschia sublinearis	0	0	8	3	0	8	0	0	0	0
Stigeoclonium	0	0	0	0	0	0	0	0	0	0
Closterium	0	0	0	0	0	0	0	0	0	0
Chlorella	0	0	0	0	0	0	0	0	0	0
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0	0	0
Chlamydomonas	9	6	0	14	0	0	0	0	0	0
Spyrogyra	0	0	0	1	2	0	0	2	1	3
Euastrum	0	14	0	6	6	0	0	2	0	1
Scenedesmus	0	0	0	0	0	0	0	0	0	0
Astasia	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0
Melosira	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	0	0	0	0	0	0	6	0	8	5
Navicula gracilis	0	0	1	4	0	0	0	5	2	0
Fragilaria	3	0	0	0	0	14	6	0	0	0
Asterionella	0	0	0	0	0	0	0	0	0	0
Diatoma	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	0	0
Navicula minima	0	0	0	0	0	0	0	0	0	0
Pinnularia	0	0	0	0	0	0	0	0	0	9
Cyclotella	0	0	6	5	0	0	1	0	12	12
Cocconeis	0	0	0	0	0	0	0	0	0	0
Vaucheria	0	0	0	0	0	0	0	0	0	0
Tribonema	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0
Mallomonas	0	3	4	0	2	2	3	1	0	0
Dileptus	0	0	0	0	0	0	0	0	0	0
Aspidisca	0	0	0	0	0	0	0	0	0	0
Cinetochilium	0	0	0	0	0	0	0	0	0	0
Epiclintes	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	0	0	0	0	0	0	0	0
Euplotes	1	2	0	0	5	2	3	0	0	2
Rhynchomonas spp	0	0	0	0	5	3	2	0	0	0
Stentor	9	18	0	3	13	0	0	2	0	0
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	2	2	0	4	2	0	5	2	5	1
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	0	0	0	0	0	0
Euglena	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0

Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	0
Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	10	3	4	0	0	2
Phacus longicauda	0	0	0	0	0	0	0	0	0	2
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0
Mayorella	0	0	0	0	0	0	6	0	0	0
Difflugia	0	0	0	0	0	0	0	0	0	0
Arcella	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	2	2	0	0	0	0	0	0	0	0
Achnanthes granulata	2	0	0	4	4	2	0	2	0	0
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	0	0
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	0
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	0	0
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	0	0
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	0
Didinium	0	0	0	0	0	0	0	0	0	0
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	0	0
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	1	0	1	0	0	2	2	0	0	1
Gastotrich	0	0	0	0	0	0	0	0	0	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	0
Eunotia	0	1	3	2	0	0	2	2	3	0
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	0
Amoeba	6	3	0	3	0	0	0	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	0
Actinosphaerium	0	0	0	0	0	0	0	0	0	0
Aulacosera	2	0	0	1	0	0	3	0	0	1
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	3	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0

Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	0
Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	5	0	7	6	7
Chroococcus	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0
Nitzschia recta	0	0	0	0	0	0	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	3	0	0	8	8	1	0	0	0	3
Stichoctricha	3	12	0	6	0	0	0	0	0	0
Carteria	9	0	0	0	0	0	0	0	0	0
Chlamydondon	3	0	0	0	8	0	0	0	0	0
Urceolus	3	3	0	0	5	0	0	0	0	0
Acanthamoeba	0	1	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0
Mastigella	9	3	4	0	0	0	0	0	0	7
Cercomonas	0	0	8	0	0	1	0	5	2	0
Chlorogonium	0	0	8	0	0	3	2	0	0	0
Stylonchia	0	0	0	0	0	3	0	0	4	2
Coleps	0	0	3	3	0	0	0	3	2	2
Staurastrum	0	0	0	0	0	0	0	0	10	0
Nematode	1	0	0	2	0	2	0	0	0	0
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	0	0	0	0	0	0	0	0	0	0
Gammaridae	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	1	1
Actinophrys	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Suirella ovata	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0
Navicula cuspidata	0	0	0	0	0	0	0	0	0	0
Pelomyxa	0	0	0	0	0	0	0	0	0	0
Rotifer (Philodina)	0	0	0	0	0	0	0	0	0	0
Menoidium	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0
Chamaesiphon	0	0	0	0	0	0	0	0	0	0
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0

Microspora	0	0	0	0	0	0	0	0	0	0
Oscillatoria	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0
Chlorococcum	0	0	0	0	0	0	0	0	0	0
Navicula viridula	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	0	12	0	0	0	15	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0
Suirella splendida	0	2	0	0	3	5	3	1	0	0
Cymbella ehrenbergii	4	4	0	0	3	2	3	0	1	3
Opercularia	0	0	0	1	3	0	1	1	2	2
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.6: Biofilm data for Site 5 (River Lee pilot study)

List of Individuals	Site 5a	Site 5b	Site 5c	Site 5d	Site 5e	Site 5f	Site 5g	Site 5h	Site 5i	Site 5j
Ulothrix spp.	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	15	0	10	25	22	26	61	53	0	63
Nitzschia sublinearis	0	14	8	17	8	1	11	5	0	9
Stigeoclonium	0	0	0	0	0	0	0	0	0	0
Closterium	0	0	0	0	0	0	0	0	0	0
Chlorella	0	0	0	0	0	0	0	0	0	0
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0	0	0
Chlamydomonas	9	2	0	0	0	2	0	0	0	0
Spyrogyra	5	0	7	1	0	6	1	4	2	2
Euastrum	5	6	0	4	4	3	0	0	2	2
Scenedesmus	0	0	0	0	4	1	0	0	0	2
Astasia	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0
Melosira	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	5	5	16	10	0	0	2	0	18	4
Navicula gracilis	2	6	2	2	7	7	2	3	9	3
Fragilaria	0	0	0	0	0	0	0	0	0	0
Asterionella	0	0	0	0	5	0	0	0	0	1
Diatoma	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	0	0
Navicula minima	0	0	0	0	0	0	0	0	0	0
Pinnularia	0	0	0	0	5	0	0	0	0	1
Cyclotella	0	0	0	0	0	1	0	0	3	0
Cocconeis	4	6	2	5	1	0	2	0	0	5
Vaucheria	7	5	4	0	0	0	0	0	0	0
Tribonema	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0
Mallomonas	4	2	1	0	3	4	1	0	3	0
Dileptus	0	0	0	0	1	1	1	0	0	0
Aspidisca	0	0	0	0	0	0	0	0	0	0
Cinetochilium	0	0	0	0	0	0	0	0	0	0
Epiclintes	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	0	1	0	0	1	0	0	0
Euplotes	0	0	0	0	7	0	0	6	1	1
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	0	0	3	1	4	1	0	1	0	1
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	1	5	4	4	0	2	3	0	2	0
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	4	3	1	0	0	0	3	3	2	0
Euglena	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	0

Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	2	1	0	0	0	3	1	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0
Mayorella	0	0	1	0	0	0	0	1	0	0
Difflugia	0	0	0	0	0	0	0	0	0	0
Arcella	5	0	2	0	0	0	1	1	0	1
Phacus pyrum	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	1	1	0	2	1	0	0	1	1	0
Achnanthes granulata	4	6	2	1	1	0	2	2	2	6
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	0	0
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	0
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	0	0
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	0	0
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	0
Didinium	0	0	0	0	0	0	0	0	0	0
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	o	0	0
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	4	0	2	2	1	3	0	4	4	1
Gastotrich	0	0	0	0	0	0	0	0	0	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	0
Eunotia	0	0	0	0	0	0	0	0	0	0
Busaria	0	0	0	0	0	0	1	1	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	7	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	4
Actinosphaerium	2	0	0	0	0	0	0	0	0	1
Aulacosera	1	1	0	2	2	3	0	2	3	3
Heterophrys	0	0	0	0	0	0	0	0	0	1
Gomphonema gracili	3	4	2	4	0	5	0	1	5	1
Nuclearia	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	0

Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	2	0	1	1	0	0	0	0	0
Chroococcus	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0
Nitzschia recta	0	0	0	0	0	0	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0
Prorodon	0	0	0	0	0	1	0	0	0	0
Cosmocladium	0	0	0	0	8	0	0	0	0	2
Calyptotricha	0	0	0	0	0	0	0	0	0	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	0	1	0	1	0	0	0	1	0	2
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	1	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	2	0	0	0	0	0	0	0	0	2
Mastigamoeba	0	0	0	0	0	0	0	0	0	0
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	5	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	1	0	0	0	0	0	0	1	0
Coleps	6	5	8	3	3	0	1	2	0	2
Staurastrum	0	4	11	8	4	0	0	2	6	1
Nematode	0	1	0	0	2	0	2	2	3	0
Urotricha	3	2	0	0	0	0	0	0	0	0
Erpobdellidae	0	2	2	3	0	1	1	0	2	0
Glossiphoniidae	0	2	2	0	0	1	0	0	1	0
Oligochaete	0	3	3	3	4	1	0	2	4	1
Gammaridae	0	1	1	1	3	1	0	2	0	0
Rhoicosphenia abrevata	0	2	0	2	0	1	0	0	1	0
Baetidae	0	0	1	2	0	0	0	1	0	0
Cymbella naviculiformis	3	8	2	2	1	4	0	3	4	1
Actinophrys	7	0	2	0	4	0	1	1	0	2
Nitzschia acicularis	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Suirella ovata	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0
Navicula cuspidata	0	0	0	0	0	0	0	0	0	0
Pelomyxa	0	0	0	0	0	0	0	0	0	0
Rotifer (Philodina)	0	0	0	0	0	0	0	0	0	0
Menoidium	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0
Chamaesiphon	0	0	0	0	0	0	0	0	0	0
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0
Microspora	0	0	0	0	0	0	0	0	0	0

Oscillatoria	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0
Chlorococcum	0	0	0	0	0	0	0	0	0	0
Navicula viridula	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	5	5	6	9	6	0	2	1	4	0
Halteria	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0
Suirella splendida	2	2	3	2	1	0	0	3	2	4
Cymbella ehrenbergii	2	0	5	1	6	0	7	5	3	0
Opercularia	1	4	1	5	2	1	1	2	0	2
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.7: Biofilm data for Site 6 (River Lee pilot study)

List of Individuals	Site 6a	Site 6b	Site 6c	Site 6d	Site 6e	Site 6f	Site 6g	Site 6h	Site 6i	Site 6j
Ulothrix spp.	2	0	0	0	5	0	2	1	0	9
Amphora ovalis	0	1	0	2	0	0	0	0	1	1
Nitzschia sublinearis	0	0	0	0	0	0	0	0	0	0
Stigeoclonium	5	4	4	5	4	5	2	4	2	10
Closterium	0	17	11	6	0	0	0	0	0	0
Chlorella	1	1	1	1	0	0	2	0	1	0
Cladophora	0	2	0	1	0	1	0	0	0	0
Navicula reinhardtii	0	0	2	1	0	0	1	0	0	2
Oedogonium	0	0	0	3	0	3	2	0	0	5
Chlamydomonas	1	1	1	1	0	0	0	1	1	2
Spyrogyra	0	0	0	0	0	0	0	0	0	0
Euastrum	0	0	0	0	0	0	0	0	0	0
Scenedesmus	0	0	0	0	0	0	0	0	0	0
Astasia	2	0	0	0	4	3	0	7	2	0
Gyrosigma acuminatum	0	0	7	0	0	0	0	0	0	2
Melosira	10	10	4	6	1	17	15	6	6	4
Achnanthes inflata	0	0	0	0	0	0	0	0	0	0
Navicula gracilis	0	0	0	0	0	0	0	0	0	0
Fragilaria	0	0	0	0	0	0	0	0	0	0
Asterionella	0	0	0	0	0	0	0	0	0	0
Diatoma	9	0	1	0	12	0	0	0	2	2
Navicula oblonga	0	0	0	0	0	0	2	7	8	5
Navicula minima	1	13	11	11	13	9	13	12	2	2
Pinnularia	0	0	0	0	0	0	0	0	0	0
Cyclotella	0	0	0	0	1	0	0	0	0	0
Cocconeis	0	0	0	0	0	0	0	8	13	8
Vaucheria	0	0	0	2	1	1	0	0	1	1
Tribonema	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron	0	6	11	6	0	0	0	0	0	0
Mallomonas	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	0	0	0	0	0
Aspidisca	0	0	0	1	2	0	0	2	0	0
Cinetochilium	0	0	6	18	12	2	1	0	2	12
Epiclintes	0	1	2	1	2	0	0	2	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	1	0	1	0	0	0	0	1	0	0
Euplotes	0	0	0	0	0	0	0	0	0	0
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	0	0	0	0	0	0	0	0	0	0
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	4	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	0	2	0	0	0	0
Euglena	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	0	4	0	0	0	0	0	0	2

Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	1	0	1	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	2	0	1	0	3	0	0
Centropyxis	5	0	0	0	0	6	0	0	0	0
Mayorella	5	0	0	0	0	0	2	1	10	0
Difflugia	5	0	0	0	3	2	0	2	0	0
Arcella	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	4	0	2	1	0	0	3	2	2	0
Navicula cryptocephala	0	1	1	1	1	1	0	0	4	2
Achnanthes granulata	0	0	0	0	0	0	0	0	0	0
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	2	0	0	1	1
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	2	4	0	2	0	3	2	3	3	4
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	4	0	2	0	3	2	3	3	4	2
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	2	0	3	4	0	2	0	2
Cymbella lanceolata	4	0	2	0	3	4	0	2	0	4
Dugesia polychroa	2	1	4	0	2	0	3	4	0	2
Gomphonema parvulum	0	2	1	4	0	2	0	3	4	0
Gomphonema angur	1	2	0	2	3	1	1	2	1	2
Didinium	3	3	1	1	4	0	2	1	0	0
Cylindrocapsa	0	0	0	3	3	1	1	4	0	2
Cymbella prostrata	2	3	0	3	0	0	0	0	1	4
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	0	0	0	0	0	0	0	0	0	0
Gastotrich	1	1	1	2	0	0	1	1	1	2
Cymbella cistula	0	0	2	2	5	1	4	0	1	1
Eunotia	0	0	0	0	0	0	0	0	0	0
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	2	2	5	1	4	0	1	1	1	2
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	0	3	4	2	2	2	0	1	1
Actinosphaerium	0	0	0	0	0	0	0	0	0	0
Aulacosera	0	0	0	0	0	0	0	0	0	0
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	4	0	2	0	0	4	3	0	0	6

Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0
Chroococcus	1	2	2	0	8	0	0	0	0	0
Nitzschia palea	21	5	18	4	9	10	10	8	2	16
Nitzschia recta	1	1	1	2	0	0	1	1	1	2
Nitzschia sigmoidea	0	0	2	2	5	1	4	0	1	1
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	2	2	2	7	0	5	5	5	0	0
Nitzschia amphibia	6	8	6	10	6	3	0	13	10	3
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	2	1	4	0	3	8	6	10	6	3
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	0	0	0	0	0	0	0	0	0	0
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	0	1	0	0	0	0	0	0	0	0
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	0	0	0	0	3	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	1	2	0	1	0	3	0	0	0	0
Gammaridae	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	0	0	0	5	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	20	15	18	14	10	10	10	18	22	16
Caloneis silicula	5	0	0	2	3	0	0	1	0	3
Suirella ovata	18	7	5	9	0	10	10	2	0	0
Caloneis bacillum	1	2	0	0	3	5	3	0	0	2
Stephanodiscus	2	0	2	0	1	0	2	0	1	1
Spirostomum	8	7	7	0	0	0	0	0	0	0
Navicula cuspidata	2	1	2	2	5	1	4	0	1	1
Pelomyxa	2	1	1	1	1	1	3	0	0	0
Rotifer (Philodina)	4	2	2	0	0	0	0	0	0	0
Menoidium	1	0	0	0	0	2	0	0	1	0
Hantzchia amphioxys	0	11	0	0	10	0	0	3	10	1
Chamaesiphon	0	8	0	0	3	0	0	3	10	7
Euglypha	0	1	0	0	0	1	0	0	0	0
Phormidium	0	0	0	0	6	1	5	0	0	0
Paramecium caudatum	0	0	0	0	2	0	0	0	0	0
Microspora	1	2	0	0	0	3	0	0	1	0

Oscillatoria	0	0	0	0	0	3	1	1	1	3
Tubifex	0	0	0	0	0	7	5	5	3	3
Anuraeopsis navicula	2	0	0	1	0	0	1	0	0	2
Chlorococcum	0	0	0	0	0	0	0	1	0	0
Navicula viridula	0	0	10	15	6	21	10	6	26	28
Campylodiscus noricus	0	0	0	1	1	0	0	0	1	1
Cocconeis placentula	0	0	0	0	0	0	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0
Aelosoma	2	0	0	2	0	1	1	0	0	0
Suirella splendida	0	0	0	0	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	0	0	0
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.8: Biofilm data for Site 7 (River Lee pilot study)

List of Individuals	Site 7a	Site 7b	Site 7c	Site 7d	Site 7e	Site 7f	Site 7g	Site 7h	Site 7i	Site 7j
Ulothrix spp.	6	0	0	5	3	7	2	9	4	0
Amphora ovalis	1	2	1	1	1	1	1	0	0	3
Nitzschia sublinearis	0	0	0	0	0	0	0	0	0	0
Stigeoclonium	2	4	0	0	0	2	0	0	0	0
Closterium	0	6	0	0	0	2	0	0	0	0
Chlorella	0	0	0	0	0	1	1	0	1	0
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	8	4	0	0	4	0	0	0	6	11
Oedogonium	4	4	0	0	3	3	5	5	5	4
Chlamydomonas	0	0	0	3	3	3	0	0	0	4
Spyrogyra	0	0	0	0	0	0	0	0	0	0
Euastrum	0	0	0	0	0	0	0	0	0	0
Scenedesmus	0	0	0	0	0	0	0	0	0	0
Astasia	0	0	0	0	0	0	3	0	0	1
Gyrosigma acuminatum	0	0	7	0	5	0	0	0	0	0
Melosira	9	10	10	2	0	0	0	14	14	10
Achnanthes inflata	0	0	1	0	0	0	0	0	0	0
Navicula gracilis	0	0	0	0	0	0	0	0	0	0
Fragilaria	0	0	0	0	0	0	0	0	0	0
Asterionella	0	0	0	0	0	0	0	0	0	0
Diatoma	0	0	0	0	0	0	3	2	0	2
Navicula oblonga	6	0	21	2	0	7	0	1	0	3
Navicula minima	12	12	11	18	11	0	0	6	0	14
Pinnularia	0	0	0	0	0	0	0	0	0	0
Cyclotella	0	0	0	0	0	0	0	0	0	0
Cocconeis	1	0	0	0	10	0	5	5	0	9
Vaucheria	0	0	0	0	0	0	0	0	0	0
Tribonema	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron	3	0	3	3	0	2	0	14	14	0
Mallomonas	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	0	0	0	0	0
Aspidisca	2	2	0	0	2	0	0	1	0	1
Cinetochilium	10	9	10	9	8	0	10	13	4	0
Epiclintes	2	2	0	0	1	2	0	0	2	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	0	1	1	1	1	1	1	0	5	0
Euplotes	0	0	0	0	0	0	0	0	0	0
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	0	0	0	0	0	0	0	0	0	0
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	2	0	0	0	0	0	0
Synedra acus	1	0	0	2	0	0	0	0	0	0
Euglena	0	0	0	0	0	0	0	0	3	0
Litonotus	0	0	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	0	0	2	2	0	0	2	0	0

Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	1	0	0	0	0	0	0	0	0	0
Centropyxis	2	0	0	0	2	0	2	0	0	3
Mayorella	0	5	0	0	0	0	0	2	1	1
Difflugia	0	0	0	0	0	2	0	0	0	4
Arcella	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	1	0	0	1	1	0	0	3	4	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0
Achnanthes granulata	0	0	1	1	0	0	0	0	0	0
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	2	0	4	0	3	3	3	4	2	0
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	2	1	0	0	4	0	2	0	3	2
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	1	1	0	1	0	2	0	0	0	3
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	3	4	0	2	0	4	0	0	0
Cymbella lanceolata	0	2	0	3	2	3	0	0	4	0
Dugesia polychroa	0	4	0	2	0	3	4	0	2	0
Gomphonema parvulum	2	0	2	1	4	0	2	0	3	4
Gomphonema angur	0	2	3	0	0	1	2	0	2	3
Didinium	1	0	0	0	3	3	1	1	4	0
Cylindrocapsa	1	0	0	1	1	7	0	0	1	2
Cymbella prostrata	2	3	0	3	0	4	2	0	0	2
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	0	0	0	0	0	0	0	0	0	0
Gastotrich	0	0	2	0	0	1	1	1	2	0
Cymbella cistula	1	2	0	0	2	2	3	1	2	0
Eunotia	0	0	0	0	0	0	0	0	0	0
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	6	0	0	3	3	1	1	4	0	2
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	3	2	0	0	1	1	0	0	3	0
Actinosphaerium	0	0	0	0	0	0	0	0	0	0
Aulacosera	0	0	0	0	0	0	0	0	0	0
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	2	2	0	0	2	0	1	3	0	4

Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0
Chroococcus	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	20	15	18	14	10	10	10	18	22	16
Nitzschia recta	0	0	2	0	0	1	1	1	2	0
Nitzschia sigmoidea	11	12	10	0	2	2	13	11	2	0
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	1	0	1	0	0	0	1	0	0	0
Nitzschia amphibia	12	12	11	0	14	0	20	10	6	0
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	2	6	4	0	3	2	1	4	10	3
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	0	0	0	0	0	0	0	0	0	0
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	0	0	0	0	0	0	0	0	0	0
Urotricha	0	0	0	0	0	0	0	0	2	0
Erpobdellidae	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	1	1	1	0	0	0	0	0	0	0
Gammaridae	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	9	18	34	30	16	18	14	0	22	15
Caloneis silicula	0	0	3	3	0	0	2	1	0	1
Suirella ovata	0	12	0	2	0	1	0	22	10	0
Caloneis bacillum	0	0	0	2	1	0	0	0	2	3
Stephanodiscus	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	3	4	0	0
Navicula cuspidata	11	21	6	0	2	3	3	11	1	4
Pelomyxa	13	29	24	3	7	37	39	30	39	12
Rotifer (Philodina)	0	0	0	0	0	0	0	0	0	0
Menoidium	0	0	0	2	0	0	0	0	0	1
Hantzchia amphioxys	0	0	1	0	2	2	0	0	1	0
Chamaesiphon	8	10	2	5	4	10	9	2	9	5
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	3	0	3	2	1	0	0	0	0	1
Paramecium caudatum	1	0	0	0	0	0	1	4	0	3
Microspora	0	4	4	1	0	2	2	0	2	1

Oscillatoria	2	8	7	0	0	0	7	9	3	3
Tubifex	0	0	1	0	1	1	0	0	0	0
Anuraeopsis navicula	3	0	0	2	2	2	0	0	4	1
Chlorococcum	0	1	0	0	0	0	0	0	5	0
Navicula viridula	20	2	7	3	0	7	0	0	0	3
Campylodiscus noricus	0	0	0	2	3	0	0	0	0	1
Cocconeis placentula	0	0	0	0	0	0	0	0	0	0
Halteria	1	0	0	1	0	0	0	0	0	1
Synedra ulna	0	4	4	0	0	0	0	0	0	2
Aelosoma	1	0	0	1	0	0	0	2	2	1
Suirella splendida	0	0	0	2	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	0	0	0
Pericoma larvae	0	0	0	2	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	4	0	0

Table I.9: Biofilm data for Site 8 (River Lee pilot study)

List of Individuals	Site 8a	Site 8b	Site 8c	Site 8d	Site 8e	Site 8f	Site 8g	Site 8h	Site 8i	Site 8j
Ulothrix spp.	0	0	1	0	2	2	2	1	5	1
Amphora ovalis	0	0	0	0	0	0	0	0	0	0
Nitzschia sublinearis	0	0	0	0	0	0	0	0	0	0
Stigeoclonium	0	1	0	0	0	0	0	5	0	0
Closterium	11	5	0	0	1	0	0	0	2	0
Chlorella	0	6	0	0	0	0	0	5	0	0
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	6	0	0	0	0	0	0	3	0	3
Oedogonium	1	2	2	5	2	3	0	0	0	0
Chlamydomonas	0	0	0	0	5	0	0	0	0	0
Spyrogyra	0	0	0	0	0	0	0	0	0	0
Euastrum	0	0	0	0	0	0	0	0	0	0
Scenedesmus	0	0	0	0	0	0	0	0	0	0
Astasia	1	3	3	0	11	8	0	0	0	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0
Melosira	13	9	17	0	0	7	13	10	11	13
Achnanthes inflata	0	0	0	0	0	0	0	0	0	0
Navicula gracilis	0	0	0	0	0	0	0	0	0	0
Fragilaria	0	0	0	0	0	0	0	0	0	0
Asterionella	0	0	0	0	0	0	0	0	0	0
Diatoma	11	0	2	0	1	0	0	0	0	0
Navicula oblonga	0	11	4	4	2	0	14	3	0	1
Navicula minima	21	0	16	15	6	10	36	0	25	19
Pinnularia	0	0	0	0	0	0	0	0	0	0
Cyclotella	0	0	0	1	1	0	0	0	0	0
Cocconeis	0	3	3	0	0	1	1	0	0	0
Vaucheria	0	0	0	0	0	0	0	0	0	0
Tribonema	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron	2	5	0	0	0	0	0	1	0	2
Mallomonas	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	0	0	0	0	0
Aspidisca	0	0	0	0	1	1	0	0	2	2
Cinetochilium	9	0	9	5	0	8	9	0	13	11
Epiclintes	0	2	2	0	0	2	0	0	1	2
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	0	0	0	0	0	0	0	0
Euplotes	0	0	0	0	0	0	0	0	0	0
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	0	0	0	0	0	0	0	0	0	0
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	2	0	0	0	0	0
Euglena	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	0	0	0	2	0	0	0	1	2

Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	1	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	1	1
Mayorella	0	0	1	0	0	0	0	0	2	1
Difflugia	2	0	5	0	0	0	3	2	0	2
Arcella	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	0	0	2	2	0	3	5	0	0	1
Navicula cryptocephala	3	4	2	0	0	0	3	4	0	2
Achnanthes granulata	0	1	0	0	0	0	0	0	0	0
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	3	4	0	2	0	1	0	1
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	3	3	4	2	1	2	3	3	4	0
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	2	0	7	0	1	7	0	1	2	0
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	2	0	3	4	0	2	0	4	0	0
Cymbella lanceolata	4	0	2	0	3	2	3	4	0	2
Dugesia polychroa	4	0	2	1	1	0	0	0	4	0
Gomphonema parvulum	0	2	1	4	0	2	0	3	4	0
Gomphonema angur	0	0	1	1	2	0	2	1	2	0
Didinium	2	1	0	0	1	0	0	0	2	2
Cylindrocapsa	0	0	0	1	3	0	1	1	2	2
Cymbella prostrata	3	0	3	0	0	0	0	2	1	1
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	0	0	0	0	0	0	0	0	0	0
Gastotrich	1	1	1	2	0	0	2	0	0	1
Cymbella cistula	1	1	1	2	0	0	3	2	1	1
Eunotia	0	0	0	0	0	0	0	0	0	0
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	1	0	0	1	2	2	0	0	1	2
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	2	3	0	0	1	4	0	0	0
Actinosphaerium	0	0	0	0	0	0	0	0	0	0
Aulacosera	0	0	0	0	0	0	0	0	0	0
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	5	5	0	6	8	0	1	0	2	3

Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0
Chroococcus	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	14	30	13	13	0	0	10	21	10	0
Nitzschia recta	1	1	1	2	0	0	2	0	0	1
Nitzschia sigmoidea	4	10	23	3	0	0	0	11	10	0
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	2	5	0	0	0	0	2	4	0	2
Nitzschia amphibia	14	20	2	11	6	3	10	21	10	0
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	4	2	0	0	12	10	2	0	3	2
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	0	0	0	0	0	0	0	0	0	0
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	0	0	0	0	0	0	0	0	0	0
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	2	0	0	3	0	0	0	0	1	2
Gammaridae	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	0	0	19	17	7	0	0	17	16	18
Caloneis silicula	0	2	0	3	0	2	2	0	2	0
Suirella ovata	2	13	9	5	25	20	2	13	2	10
Caloneis bacillum	3	0	0	2	1	0	1	0	0	0
Stephanodiscus	0	0	0	5	2	0	0	0	1	0
Spirostomum	0	0	6	4	1	0	0	0	0	0
Navicula cuspidata	0	2	1	0	0	1	1	7	0	0
Pelomyxa	0	2	3	11	7	4	6	13	2	2
Rotifer (Philodina)	0	0	0	0	0	0	0	0	0	0
Menoidium	0	0	1	0	1	2	0	0	4	1
Hantzchia amphioxys	4	6	2	11	7	4	8	6	2	2
Chamaesiphon	4	6	2	2	1	8	6	0	0	0
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	3	0	0	2	1	0	1	0	0	0
Paramecium caudatum	4	6	2	2	1	8	6	0	0	0
Microspora	0	5	0	2	2	0	0	6	0	0

Oscillatoria	2	3	9	5	5	2	2	3	2	10
Tubifex	0	0	0	0	2	0	0	0	0	0
Anuraeopsis navicula	1	0	0	3	3	0	2	0	2	1
Chlorococcum	0	0	0	0	2	1	4	1	5	2
Navicula viridula	2	13	10	5	15	20	2	13	2	10
Campylodiscus noricus	1	0	0	0	2	2	0	1	0	2
Cocconeis placentula	0	0	0	0	0	0	0	0	0	0
Halteria	1	6	0	0	0	0	0	6	4	4
Synedra ulna	0	2	0	1	0	2	0	1	1	0
Aelosoma	1	0	0	0	0	1	1	0	4	0
Suirella splendida	0	0	0	0	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	0	0	0
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.10: Biofilm data for Site 9 (River Lee pilot study)

List of Individuals	Site 9a	Site 9b	Site 9c	Site 9d	Site 9e	Site 9f	Site 9g	Site 9h	Site 9i	Site 9j
Ulothrix spp.	0	0	4	0	0	0	0	0	0	0
Amphora ovalis	0	0	0	0	0	0	0	0	0	0
Nitzschia sublinearis	0	0	0	0	0	0	0	0	0	0
Stigeoclonium	0	0	0	0	0	0	0	11	2	0
Closterium	0	0	0	0	0	0	0	0	0	0
Chlorella	5	0	0	0	2	1	3	0	6	0
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	12	12	11	0	14	14	0	2	5	0
Oedogonium	6	3	0	0	0	3	1	0	2	3
Chlamydomonas	0	0	6	0	0	3	1	1	3	0
Spyrogyra	0	0	0	0	0	0	0	0	0	0
Euastrum	0	0	0	0	0	0	0	0	0	0
Scenedesmus	0	0	0	0	0	0	0	0	0	0
Astasia	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminatum	0	0	0	0	0	0	4	0	0	0
Melosira	6	6	0	14	15	11	14	14	14	12
Achnanthes inflata	0	0	0	0	0	0	0	0	0	0
Navicula gracilis	0	0	0	0	0	0	0	0	0	0
Fragilaria	0	0	0	0	0	0	0	0	0	0
Asterionella	0	0	0	0	0	0	0	0	0	0
Diatoma	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	10	16	11	0	2	5	2	6	15	0
Navicula minima	15	6	9	0	22	13	13	12	14	17
Pinnularia	0	0	0	0	0	0	0	0	0	0
Cyclotella	0	0	0	0	0	0	0	0	0	0
Cocconeis	0	0	5	0	0	0	10	2	0	0
Vaucheria	0	0	0	0	0	0	0	0	0	0
Tribonema	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron	6	1	0	0	0	6	1	4	0	0
Mallomonas	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	0	0	0	2	2
Aspidisca	3	0	0	4	0	0	0	0	0	0
Cinetochilium	6	3	12	0	0	2	6	5	0	0
Epiclintes	0	0	2	0	0	2	2	1	2	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	5	0	0	0	0	0	0	2	0	0
Euplotes	0	0	0	0	0	0	0	0	0	0
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	0	0	0	0	0	0	0	0	0	0
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	5	0	0	3	0	0	0	0	0
Euglena	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	1	0

Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	2	0	0	0	0	0	1	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0
Mayorella	2	0	0	0	2	0	2	0	0	0
Difflugia	0	0	0	0	0	0	0	2	0	0
Arcella	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	2	0	1	1	0	0	2	2	0	0
Navicula cryptocephala	0	0	3	0	4	2	0	0	0	0
Achnanthes granulata	0	0	0	1	0	0	0	0	0	0
Rotifer Bdelloidea	3	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	3	4	2	2	1	0	0	2
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	2	0	3	2	3	3	4	2	1	0
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	4	3	2	3	0	5	0	1	0	1
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	2	0	3	4	0	2	0	4	0	0
Cymbella lanceolata	0	3	2	4	0	2	0	3	2	3
Dugesia polychroa	2	0	3	1	0	2	0	1	0	2
Gomphonema parvulum	2	0	4	0	0	0	2	1	4	0
Gomphonema angur	2	3	0	0	0	1	2	0	2	3
Didinium	1	1	4	0	2	1	0	0	1	0
Cylindrocapsa	1	4	1	5	2	1	1	2	0	2
Cymbella prostrata	2	3	0	3	0	0	0	0	1	3
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	0	0	0	0	0	0	0	0	0	0
Gastotrich	1	1	2	0	1	1	1	2	0	0
Cymbella cistula	4	0	1	2	1	2	0	0	2	1
Eunotia	0	0	0	0	0	0	0	0	0	0
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	1	3	0	1	1	2	2
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	2	2	5	0	0	2	1	0	0	2
Actinosphaerium	0	0	0	0	0	0	0	0	0	0
Aulacosera	0	0	0	0	0	0	0	0	0	0
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	3	0	0	2	1	0	2	2	0	0

Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0
Chroococcus	0	0	0	7	0	1	0	0	0	0
Nitzschia palea	0	5	0	0	17	0	0	0	7	0
Nitzschia recta	5	11	22	0	1	16	1	2	5	0
Nitzschia sigmoidea	4	0	14	2	1	2	10	0	12	11
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	1	0	4	4	0	5	5	0
Nitzschia amphibia	5	11	12	3	10	16	1	2	5	0
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	1	8	0	2	9	4	0	3	4	1
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	0	0	0	0	0	0	0	0	0	0
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	0	0	0	0	0	0	0	0	0	0
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	1	0	1	1	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0
Oligochaete	0	4	0	0	0	6	0	0	0	0
Gammaridae	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	0	0	0	0	0	0	0	2	4	0
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	16	0	14	23	10	19	19	15	17	18
Caloneis silicula	1	0	2	0	1	1	0	2	0	2
Suirella ovata	2	0	1	0	2	0	1	1	0	2
Caloneis bacillum	1	2	0	0	0	3	3	0	0	4
Stephanodiscus	10	9	0	0	20	27	0	8	16	4
Spirostomum	8	0	0	0	0	2	2	0	0	0
Navicula cuspidata	1	2	0	0	0	1	3	0	1	1
Pelomyxa	3	12	7	4	3	0	8	17	3	0
Rotifer (Philodina)	0	0	0	0	0	2	0	0	0	0
Menoidium	0	0	0	1	0	1	2	0	0	4
Hantzchia amphioxys	1	0	0	6	0	1	0	0	1	3
Chamaesiphon	0	0	3	2	0	0	10	1	0	1
Euglypha	0	1	0	0	0	0	0	0	0	0
Phormidium	0	3	2	0	0	10	0	0	0	13
Paramecium caudatum	0	0	0	3	0	0	0	0	0	2
Microspora	0	4	0	0	0	2	0	0	3	0

Oscillatoria	0	6	5	9	0	0	3	3	0	14
Tubifex	0	5	2	5	4	0	2	2	0	2
Anuraeopsis navicula	1	2	0	0	0	3	2	1	2	0
Chlorococcum	1	1	2	0	2	0	0	0	0	1
Navicula viridula	0	10	28	0	0	11	24	7	8	1
Campylodiscus noricus	0	0	2	1	0	0	2	0	1	2
Cocconeis placentula	0	0	0	0	0	0	0	0	0	0
Halteria	0	2	0	0	0	0	0	0	0	0
Synedra ulna	2	0	2	0	1	0	2	0	0	2
Aelosoma	0	1	2	0	0	0	0	2	2	0
Suirella splendida	0	0	0	0	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	0	0	0
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.11: Biofilm data for Site 10 (River Lee pilot study)

List of Individuals	Site 10a	Site 10b	Site 10c	Site 10d	Site 10e	Site 10f	Site 10g	Site 10h	Site 10i	Site 10j
Ulothrix spp.	3	0	0	0	0	1	0	1	0	0
Amphora ovalis	0	0	0	0	0	0	0	0	0	0
Nitzschia sublinearis	0	0	0	0	0	0	0	0	0	0
Stigeoclonium	0	2	1	1	1	1	0	0	3	0
Closterium	0	0	0	0	0	0	0	0	0	0
Chlorella	8	6	4	0	0	0	0	0	2	8
Cladophora	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	0	0	0	0	1	0	2	6	1	0
Oedogonium	4	0	3	5	0	5	6	5	0	4
Chlamydomonas	1	0	6	0	7	0	7	0	9	0
Spyrogyra	0	0	0	0	0	0	0	0	0	0
Euastrum	0	0	0	0	0	0	0	0	0	0
Scenedesmus	0	0	0	0	0	0	0	0	0	0
Astasia	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminatum	0	1	0	2	3	0	1	0	2	0
Melosira	9	4	9	4	0	0	2	2	0	0
Achnanthes inflata	0	0	0	0	0	0	0	0	0	0
Navicula gracilis	0	0	0	0	0	0	0	0	0	0
Fragilaria	0	0	0	0	0	0	0	0	0	0
Asterionella	0	0	0	0	0	0	0	0	0	0
Diatoma	0	0	0	2	0	0	0	1	1	0
Navicula oblonga	0	21	14	19	3	10	3	1	19	3
Navicula minima	10	21	16	0	0	0	21	19	26	18
Pinnularia	0	0	0	0	0	0	0	0	0	0
Cyclotella	0	0	0	0	0	0	0	0	0	0
Cocconeis	4	0	2	8	8	14	0	2	0	3
Vaucheria	0	0	0	0	0	0	0	0	0	0
Tribonema	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron	2	0	0	0	3	0	3	7	5	0
Mallomonas	0	0	0	0	0	0	0	0	0	0
Dileptus	2	0	0	0	1	0	1	1	0	1
Aspidisca	0	0	0	0	0	0	0	0	0	0
Cinetochilium	15	0	0	6	4	6	0	7	5	0
Epiclintes	0	2	0	0	2	2	0	0	2	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	6	6	5	4	0	5	6	3
Euplotes	0	0	0	0	0	0	0	0	0	0
Rhynchomonas spp	0	0	0	0	0	0	0	0	0	0
Stentor	0	0	0	0	0	0	0	0	0	0
Ochromonas	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	0	5	0	0	0	0
Euglena	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	2	0	4	0	0	2	0	0	2

Rhipidodendron	0	0	0	0	0	0	0	0	0	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0
Uroglena	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	1	0	1	1	0	0	1
Mayorella	2	1	1	2	0	1	0	0	0	2
Difflugia	0	4	2	0	0	0	2	0	1	2
Arcella	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	4	3	3	0	0	0	1	0	0	4
Navicula cryptocephala	1	0	0	2	1	0	0	0	2	2
Achnanthes granulata	0	0	0	0	0	0	0	0	0	0
Rotifer Bdelloidea	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	1	0	0	0	2	2	0	0	4	0
Rotaria	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	1	1	1	4	0	2	0	3	2
Flatworm	0	0	0	0	0	0	0	0	0	0
Hydroporinae larvae	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	2	1	0	0
Helodidae larva	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	2	0	3	4	0	2	0	4	0
Cymbella lanceolata	0	0	4	0	2	0	4	2	3	1
Dugesia polychroa	0	0	1	0	2	0	3	2	0	2
Gomphonema parvulum	2	0	3	4	0	2	0	3	0	0
Gomphonema angur	0	1	2	0	1	3	0	1	2	0
Didinium	3	1	4	0	0	3	3	1	1	1
Cylindrocapsa	0	0	0	4	2	4	0	1	0	1
Cymbella prostrata	2	3	0	3	0	0	0	0	3	2
Spathidium	0	0	0	0	0	0	0	0	0	0
Chaenia	0	0	0	0	0	0	0	0	0	0
Gastotrich	0	0	0	0	1	1	1	1	0	0
Cymbella cistula	2	1	4	0	3	1	1	2	0	0
Eunotia	0	0	0	0	0	0	0	0	0	0
Busaria	0	0	0	0	0	0	0	0	0	0
Chaetogaster	0	0	0	0	0	0	0	0	0	0
Phialina	0	0	0	0	0	0	0	0	0	0
Anthophysa	0	0	0	0	0	0	0	0	0	0
Cochliopodium	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	1	4	1	5	2	1	1	2	0	2
Amoeba	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	3	3	0	4	0	0	0	1	1
Actinosphaerium	0	0	0	0	0	0	0	0	0	0
Aulacosera	0	0	0	0	0	0	0	0	0	0
Heterophrys	0	0	0	0	0	0	0	0	0	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0
Nuclearia	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	2	8	0	0	10	0	2	0	8	8

Pediastrum	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0
Chroococcus	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	5	10	0	10	0	0	12	20	22	0
Nitzschia recta	0	0	0	0	1	1	1	1	0	0
Nitzschia sigmoidea	2	1	4	0	23	1	12	2	7	2
Prorodon	0	0	0	0	0	0	0	0	0	0
Cosmocladium	0	0	0	0	0	0	0	0	0	0
Calyptotricha	5	7	0	0	0	0	4	4	0	6
Nitzschia amphibia	5	5	0	5	7	4	0	4	0	3
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0
Stichoctricha	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	0	0
Chlamydondon	0	0	0	0	0	0	0	0	0	0
Urceolus	0	0	0	0	0	0	0	0	0	0
Acanthamoeba	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	6	5	2	3	2	1	4	0	3	4
Mastigella	0	0	0	0	0	0	0	0	0	0
Cercomonas	0	0	0	0	0	0	0	0	0	0
Chlorogonium	0	0	0	0	0	0	0	0	0	0
Stylonchia	0	0	0	0	0	0	0	0	0	0
Coleps	0	0	0	0	0	0	0	0	0	0
Staurastrum	0	0	0	0	0	0	0	0	0	0
Nematode	0	0	0	0	0	0	0	0	0	0
Urotricha	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	0	0	4	0	4	0	1	5	0	1
Glossiphoniidae	1	4	4	0	4	0	5	0	4	0
Oligochaete	0	0	0	4	0	4	0	0	3	0
Gammaridae	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	3	0	0	0	0	6	0	0	0	5
Baetidae	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	24	12	15	25	18	18	20	0	22	0
Caloneis silicula	0	1	0	2	0	0	2	4	1	0
Suirella ovata	14	24	18	0	10	0	19	0	19	0
Caloneis bacillum	1	0	2	2	0	0	5	1	0	0
Stephanodiscus	0	2	6	2	0	0	1	4	1	0
Spirostomum	0	0	0	0	0	0	0	0	0	0
Navicula cuspidata	2	2	1	4	1	5	2	1	1	2
Pelomyxa	5	1	0	5	3	0	2	2	2	3
Rotifer (Philodina)	0	0	0	0	2	1	0	3	0	1
Menoidium	1	0	1	0	1	2	0	0	4	1
Hantzchia amphioxys	14	14	13	0	10	0	9	8	10	0
Chamaesiphon	0	0	0	1	0	4	0	1	0	4
Euglypha	0	0	0	0	0	0	0	0	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	3	0	0	0	0	5	2
Microspora	2	0	0	6	0	6	0	6	0	6

Oscillatoria	0	0	0	0	11	0	0	4	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	1	1	0	0	3	2	0	0
Chlorococcum	1	2	2	1	4	1	0	0	1	0
Navicula viridula	24	24	10	5	10	0	19	0	19	0
Campylodiscus noricus	3	0	2	0	1	0	3	0	0	1
Cocconeis placentula	0	0	0	0	0	0	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	2	0	2	0	1	0	2
Aelosoma	0	0	1	0	0	0	1	1	0	0
Suirella splendida	0	0	0	0	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	0	0	0
Pericoma larvae	0	0	0	0	0	0	0	0	0	0
Glaucoma	0	0	0	0	0	0	0	0	0	0

Table I.12: Coliform data for the experiment on Pymmes Brook (April – August 2014)

	Apr-14	**May-14**	**Jun-14**	**Jul-14**	**Aug-14**
Site 1a	10	41	60	27	54
Site 1b	38	68	68	13	45
Site 1c	22	62	72	49	41
Site 1d	19	53	64	25	63
Site 1e	27	59	69	36	64
Site 2a	500	800	1,000	2,600	4,200
Site 2b	600	800	800	3,400	3,600
Site 2c	900	1000	900	5,900	3,700
Site 2d	700	800	700	3,400	3,900
Site 2e	700	900	900	3,800	3,900
Site 3a	14,000	15,000	200,000	530,000	700,000
Site 3b	14,000	21,000	130,000	480,000	640,000
Site 3c	17,000	17,000	110,000	170,000	610,000
Site 3d	13,000	20,000	170,000	410,000	710,000
Site 3e	17,000	16,000	120,000	360,000	590,000
Site 4a	150,000	140,000	210,000	490,000	720,000
Site 4b	120,000	130,000	190,000	210,000	690,000
Site 4c	110,000	110,000	130,000	240,000	530,000
Site 4d	120,000	150,000	170,000	320,000	640,000
Site 4e	130,000	170,000	180,000	290,000	620,000
Site 5a	130,000	120,000	160,000	440,000	290,000
Site 5b	120,000	100,000	140,000	360,000	270,000
Site 5c	90,000	85,000	130,000	400,000	310,000
Site 5d	150,000	89,000	180,000	440,000	240,000
Site 5e	100,000	95,000	120,000	410,000	250,000
Site 6a	130,000	110,000	110,000	170,000	400,000
Site 6b	110,000	100,000	110,000	150,000	330,000
Site 6c	90,000	180,000	58,000	85,000	230,000
Site 6d	140,000	130,000	93,000	120,000	290,000
Site 6e	110,000	140,000	91,000	160,000	200,000

Table I.13: Biofilm experiment data for May (M) 2014 (Sites 1 – 6)

List of Individuals	M Site 1a	M Site 1b	M Site 1c	M Site 1d	M Site 2a	M Site 2b	M Site 2c	M Site 2d	M Site 3a	M Site 3b	M Site 3c	M Site 3d	M Site 4a	M Site 4b	M Site 4c	M Site 4d	M Site 5a	M Site 5b	M Site 5c	M Site 5d	M Site 6a	M Site 6b	M Site 6c	M Site 6d
Achnanthes brevipes	2	0	0	10	3	3	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina	1	34	5	23	5	9	9	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthidium minutissimum	6	27	31	16	7	15	13	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Amphora ovalis	2	0	0	13	9	3	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora pediculus	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Arcella	0	0	0	0	0	0	0	0	1	1	0	7	0	0	0	5	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspidisca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Astasia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Aulacosera ambigua	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacosera granulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulbochaete mirabilis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	1	2	3	3
Caloneis silicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Chaemisiphon	0	0	0	0	0	0	0	0	0	0	3	0	0	4	0	5	1	0	5	0	0	0	0	0
Chaenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlamydomonas reinhardtii	0	0	0	0	0	0	0	0	0	0	0	0	4	2	5	0	2	2	4	10	0	3	0	0
Chlamydomonas spp	1	0	0	0	0	0	0	0	0	4	6	3	0	0	0	5	0	0	0	0	0	0	2	0
Chlorella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0
Chlorella	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	1	0	2	0	0	3	0	0

pyrenoidosa																								
Chrysococcus rufescens	1	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	0	1	0	2	0	0	1	2	1	1	1	1	0	0	1	0
Cladophora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorococum	0	0	0	0	0	0	0	0	1	3	11	3	2	0	0	0	1	0	0	6	0	1	1	1
Cinetochilium	0	0	0	0	0	0	0	0	0	0	0	3	4	0	0	0	1	0	3	0	0	2	0	0
Closterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	0	0	0	14	5	0	0	0	2	0	17	13	0	0	4	4	0	0	0	0	0	0	1	0
Coleps	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cyclotella compta	6	0	0	0	13	28	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	4	5	12	0	8	6	14	0	18	3	5	3	1	9	1	1
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	5	0	0	0	3	0	0	0	0	0	2	1	0	0	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	1	0	0	0	0	1	0	0	0
Cymbella naviculiformis	10	9	10	22	18	0	36	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	7	9	9	13	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Diatoma vulgare	0	0	0	0	0	0	0	0	1	0	0	0	3	1	0	0	1	3	0	0	0	4	0	0
Didinium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dinobryon	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epiclintes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erpobdella octoculata	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	1	0	0	0	1	0
Euastrum oblongum	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Euglena viridis	0	0	0	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	2	1	1	2	1
Eunotia bilunaris	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euplotes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria spp	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Gammarus pulex	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastotrich	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossiphonia complanata	0	0	0	0	0	2	1	3	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	3	5	0	0	2	5	4	2	4	3	8	5	1	2	4	7
Gomphonema gracili	23	11	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	26	8	6	13	3	0	0	0	1	4	0	0	1	2	2	2
Gomphonema parvulum	0	0	0	0	0	0	0	0	20	0	14	11	0	5	0	0	1	0	3	0	1	2	0	1
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mallomonas caudata	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mayorella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melosira varians	0	0	0	0	0	0	0	0	0	0	0	0	4	4	8	3	0	4	8	5	2	8	12	3
Menoidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meridion circulare	0	0	0	0	0	6	0	0	0	2	0	7	2	0	0	0	0	3	0	0	1	0	0	0
Microspora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0	0	0	14	16	23	15	17	17	9	8	52	10	8	14
Navicula cuspidata	0	0	0	0	0	0	0	0	0	1	0	0	4	3	0	0	7	4	4	2	1	5	4	7
Navicula gracilis	33	0	0	0	29	26	30	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	0	3	2	0	2	0	0	4	5	7	6	6	0	4	3	10
Navicula radiosa	0	0	0	0	0	0	0	3	0	0	2	0	0	0	0	0	0	0	0	0	1	1	2	4
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	3	0	0
Navicula	0	0	0	0	0	0	0	0	0	0	0	0	13	11	0	8	8	8	13	11	37	10	12	17

viridula																								
Navicula minima	0	0	0	0	0	0	0	0	1	0	0	0	4	5	0	0	4	8	6	3	1	2	1	3
Nematode	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	1	1	1	0	0	1	1
Netrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	1	2	1	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	1	1	2	3	1	0	0	0	1	2	0	0	1	1	5	6
Nitzschia dissipata	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0
Nitzschia frustulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	3	0
Nitzschia hungarica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0	0	0	2	4	5	0	0	3	2	2	0	4	7	3
Nitzschia recta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	9	0	0	2	5	1	1	2	0
Nitzschia sublinearis	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0	0	2	2	3	0	0	0	0	0	2	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Martyana martyii	2	0	0	0	0	3	0	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pandorina morum	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	3	0	0	0	0	0	0	0	1
Paramecium putrinium	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	2	0
Pelomyxa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacotus lenticularis	1	0	0	0	7	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Phagocata vitta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodina	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	2	4	0	0	1	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2	0	0	0	0	0	0	0
Physidae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia viridis	0	0	0	0	0	0	0	5	2	3	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Plagiopyla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prorodon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Raphoneis amphicerous ehr	4	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhipidodendro	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Rhizoclonium hieroglphicum	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	12	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	15	4	6	0	4	9	10	11	4	3	3	8	1	2	0	3
Rotaria rotatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scenedesmus quadricauda	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	2	0	0	0	0
Simulidae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1
Spathidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaerotilus	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0	0	0	0	5	6	0	2	3	0	0	1	1	2	0
Spirogyra spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	4	6	2	1	0	7	5
Stentor	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	3	1	2	0	0	0	1	1	1
Stigeoclonium tenue	0	0	0	0	0	0	0	0	0	0	0	3	2	1	0	4	1	0	0	0	1	0	2	1
Stephanodiscus hantzchii	0	0	0	0	0	0	0	0	2	1	0	5	2	4	9	0	6	6	4	3	1	1	4	3
Stephanodiscus astrae	0	0	0	0	0	0	0	0	0	0	0	0	2	4	0	0	4	0	6	13	0	3	3	0
Stichococcum bacillaris	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella angustata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
Suirella biseriata	0	0	0	0	0	0	0	0	6	2	0	0	0	0	0	0	0	0	0	0	1	2	1	1
Suirella ovata	0	0	0	0	0	0	0	0	4	0	3	0	12	7	2	1	1	3	4	0	1	4	4	7
Suirella robusta	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
Suirella splendida	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	4	3	9	0	5	1	4	5	3
Tabellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	0	1	0
Ulothrix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	4	0	0	2	0	1	0	4	0
Vaucheria	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	3	0	0	0	0	1	2	0	0

Table I.14: Biofilm experiment data for June (J) 2014 (Sites 1 – 6)

List of Individuals	J Site 1a	J Site 1b	J Site 1c	J Site 1d	J Site 2a	J Site 2b	J Site 2c	J Site 2d	J Site 3a	J Site 3b	J Site 3c	J Site 3d	J Site 4a	J Site 4b	J Site 4c	J Site 4d	J Site 5a	J Site 5b	J Site 5c	J Site 5d	J Site 6a	J Site 6b	J Site 6c	J Site 6d
Achnanthes brevipes	15	0	0	0	6	3	16	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina	0	8	12	17	19	8	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthidium minutissimum	19	8	19	17	24	14	16	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinophrys	3	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	6	3	19	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora pediculus	0	0	0	0	0	16	4	0	0	2	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arcella	0	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspidisca	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	4	3	2	0	0	1	0
Astasia	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0
Aulacosera ambigua	0	0	0	0	0	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacosera granulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulbochaete mirabilis	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3	2
Caloneis silicula	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	1	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaemisiphon	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	5	6	4	6	7	0	0	0	0
Chaenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlamydomonas reinhardtii	0	0	0	0	0	0	0	0	0	0	0	0	4	2	4	4	3	3	5	3	0	4	2	8
Chlamydomonas spp	0	0	0	0	0	0	0	0	2	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0
Chlorella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0

Chlorella pyrenoidosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysococcus rufescens	0	0	0	1	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	0
Cladophora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Chlorococum	0	0	0	0	0	0	0	0	2	2	0	0	3	0	10	4	3	0	5	2	0	2	3	4
Cinetochilium	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	2	0	0	3	0	2	0	0
Closterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	0	2	0	0	0	8	0	2	15	3	24	22	0	0	0	0	0	0	0	0	1	0	0	0
Coleps	3	0	0	0	0	0	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	0	0	0	0	0	0	0	0	0	3	2	4	2	0	0	3	0	0	1	1	2
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella compta	4	0	12	2	8	8	10	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	1	2	0	15	7	4	4	6	13	6	4	5	3	9	4	3
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	8	0	0	0	0	3	0	0	0	0	0	0	3	0	0	0
Cymbella cistula	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	2	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	3	4	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Cymbella naviculiformis	3	11	19	5	14	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	13	7	19	10	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Diatoma vulgare	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	3	0	0	0	4	2	0
Didinium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Dinobryon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	0	1	0
Epiclintes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erpobdella octoculata	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	0	1	1	1
Euastrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

oblongum																								
Euglena viridis	0	0	0	0	0	0	0	0	2	0	2	0	0	0	4	0	0	0	1	0	1	0	2	0
Eunotia bilunaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euplotes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria spp	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gammarus pulex	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastotrich	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossiphonia complanata	0	0	0	0	2	1	1	0	1	1	1	0	1	0	0	1	1	0	0	0	0	1	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	4	0	0	3	7	0	4	3	4	8	4	6	3	4	7
Gomphonema gracili	24	19	17	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	12	16	15	9	3	0	0	5	2	3	0	0	3	2	3	2
Gomphonema parvulum	0	0	0	0	0	0	0	0	15	8	8	20	0	4	0	0	2	0	0	2	6	2	0	2
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	2	2	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mallomonas caudata	0	0	0	0	2	3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	3	0	1	0	1	1
Mayorella	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	3	0	0	2	1	0	1	1
Melosira varians	0	0	0	0	0	0	0	0	0	0	0	0	5	5	4	6	0	4	5	7	9	9	11	3
Menoidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meridion circulare	0	0	0	0	6	0	7	3	3	0	11	0	0	0	0	0	0	0	0	0	4	0	0	0
Microspora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0	0	0	12	11	0	13	10	14	11	14	6	9	9	11
Navicula cuspidata	0	0	0	0	0	0	0	0	1	0	0	0	4	6	0	3	6	4	4	3	4	6	4	7
Navicula gracilis	0	0	0	13	13	3	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	4	0	0	0	3	0	0	2	3	6	4	4	7	4	5	3
Navicula radiosa	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	1	2	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	3	0	0

Navicula viridula	0	0	0	0	0	0	0	0	0	0	0	0	12	10	0	13	9	14	11	13	9	9	10	13
Navicula minima	0	0	0	0	0	0	0	0	0	2	0	0	4	4	0	0	6	5	5	8	4	3	2	6
Nematode	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	0	0	0	0	0
Netrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia acicularis	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	3	2	0	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	2	2	0	2	0	5	4	2	2	0	0	1	2	4	4
Nitzschia dissipata	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Nitzschia frustulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0
Nitzschia hungarica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0	0	0	3	3	8	1	0	2	3	3	0	4	4	2
Nitzschia recta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0	0	0	1	0	17	0	0	0	3	1	3	1	1	0
Nitzschia sublinearis	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0	2	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Martyana martyii	0	0	0	1	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pandorina morum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Paramecium putrinium	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	1	0	0	0	0	0	2	0
Pelomyxa	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	3	2	1	3	0	0	0	1	0
Phacotus lenticularis	0	0	0	3	0	6	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Phagocata vitta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodina	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	3	1	3	0	3	0	1	0	0
Phormidium	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Physidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia viridis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiopyla	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	1	0	0	0	3	1	2	1	3
Prorodon	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0
Raphoneis amphicerous ehr	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Rhipidodendron	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizoclonium hieroglphicum	0	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	24	24	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	5	25	0	4	7	8	17	11	9	10	8	8	7	4	0	4
Rotaria rotatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scenedesmus quadricauda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1
Spathidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaerotilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0	0	0	3	4	4	4	3	4	4	0	2	1	2	0
Spirogyra spp	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	1	1	0	0	0	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	2	2	0	1	0	7	5
Stentor	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0	1	1	2	0	0	0	1	0	1
Stigeoclonium tenue	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	1	1
Stephanodiscus hantzchii	0	0	0	0	8	2	0	0	7	11	11	0	5	5	11	6	6	4	4	7	3	2	4	3
Stephanodiscus astrae	0	0	0	0	0	0	0	0	4	0	0	0	3	3	4	0	2	0	0	4	0	3	4	3
Stichococcum bacillaris	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Suirella angustata	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella biseriata	0	0	0	0	0	0	0	0	3	4	0	0	0	0	0	0	0	0	0	0	2	2	2	2
Suirella ovata	0	0	0	0	0	0	0	0	2	3	0	1	5	6	0	4	3	3	0	2	9	6	4	6
Suirella robusta	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	1
Suirella splendida	2	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	2	2	2	0	4	4	1	2
Tabellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0
Ulothrix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	1	0
Vaucheria	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	1	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	0	0	1	1	0

Table I.15: Biofilm experiment data for July (JY) 2014 (Sites 1 – 6)

List of Individuals	Jy Site 1a	Jy Site 1b	Jy Site 1c	Jy Site 1d	Jy Site 2a	Jy Site 2b	Jy Site 2c	Jy Site 2d	Jy Site 3a	Jy Site 3b	Jy Site 3c	Jy Site 3d	Jy Site 4a	Jy Site 4b	Jy Site 4c	Jy Site 4d	Jy Site 5a	Jy Site 5b	Jy Site 5c	Jy Site 5d	Jy Site 6a	Jy Site 6b	Jy Site 6c	Jy Site 6d
Achnanthes brevipes	0	11	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	14	0	6	12	2	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina	0	36	20	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthidium minutissimum	0	11	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	20	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora pediculus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arcella	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspidisca	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	4	0	0	0	1
Astasia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacosera ambigua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacosera granulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulbochaete mirabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	1	0	1
Chaemisiphon	0	0	0	0	0	0	0	0	0	0	0	0	11	3	4	11	1	1	3	2	4	0	0	0
Chaenia	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlamydomonas reinhardtii	0	0	0	0	0	0	0	0	11	2	11	14	2	24	18	9	0	0	0	3	4	0	0	9
Chlamydomonas spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorella spp	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	1	0	1	0	1	7
Chlorella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

pyrenoidosa																								
Chrysococcus rufescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	6	1	1	1	2	0	0	1	1
Cladophora	0	0	0	0	0	0	0	0	0	0	0	0	1	0	6	2	0	0	1	0	0	0	0	0
Chlorococum	0	0	0	0	0	0	0	0	51	43	17	2	0	47	21	6	0	0	0	4	4	0	1	7
Cinetochilium	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	4	1	1	3	0
Closterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Cocconeis placentula	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Coleps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	1	0
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	1	0	0	0	0	0	0	0
Cyclotella compta	0	7	0	14	8	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	0	2	2	7	4	0	0	0	1	1	3	8	0	0	0	1
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	0	1	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Cymbella ehrenbergii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	19	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatoma vulgare	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	6	0	0	2	0	0	0
Didinium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dinobryon	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	2	0	0	0	0	0	0	0	1
Dugesia polychroa	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	0	1
Epiclintes	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Erpobdella octoculata	0	0	0	0	2	0	0	0	1	2	0	1	1	1	2	0	0	1	1	1	0	0	0	0
Euastrum oblongum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Euglena viridis	0	0	0	0	16	0	0	13	13	15	24	9	2	4	9	9	4	7	6	13	2	1	1	7
Eunotia bilunaris	3	0	0	6	16	57	38	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euplotes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria spp	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gammarus pulex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastotrich	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Glossiphonia complanata	0	0	0	0	5	0	0	0	1	0	1	1	0	1	0	2	1	1	1	1	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	3	0	2	3	0
Gomphonema gracili	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	9	0	4	7	5	0
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	9	13	15	2	2	1	6	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	1	0	4	4	0	0	0	0	0	0	0	0	0	1	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	10	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrymaria	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Mallomonas caudata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0	3	2	3	0
Mayorella	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	1	3	0	3	2	1	0
Melosira varians	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	1	9	4	11	5	0	0	0	0
Menoidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Meridion circulare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microspora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	2	4	6	4	8	0	0	0	5	5	3	7	11	20	7	2
Navicula cuspidata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	4	0	0	0	0
Navicula gracilis	26	0	6	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0				
Navicula oblonga	0	0	0	0	0	0	0	0	4	0	4	7	7	0	0	3	2	5	3	2	15	2	12	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	1	0	0	0	0	0
Navicula	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	6	5	3	9	9	7	10	10	5

viridula																								
Navicula minima	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	4	5	8	3	4	9	2	3	4
Nematode	0	0	0	0	4	3	2	4	1	0	1	0	0	0	0	1	1	1	1	0	1	1	1	1
Netrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Nitzschia acicularis	0	0	0	0	0	0	0	0	3	15	9	35	6	0	0	0	2	0	0	2	0	12	3	0
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	0	0	0	3	9	9	7	0	4	0	0	1	0	1	5
Nitzschia dissipata	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia frustulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia hungarica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	0	3	0	10	5	6
Nitzschia recta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0
Nitzschia sublinearis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	8	0	0	0	4	0	0	0	0	0	0	4	0	0	0	0	0	0	0	3
Opercularia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ophephora martyii	0	0	0	0	32	3	38	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pandorina morum	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
Paramecium putrinium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	1	2	0	0	1	0	7	0	1	0	0	3	0	0	0	2
Pelomyxa	0	0	0	0	0	0	0	0	0	0	3	4	2	0	0	0	1	4	3	0	2	1	2	7
Phacotus lenticularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phagocata vitta	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Philodina	0	0	0	0	0	0	0	0	1	2	0	2	2	0	0	4	1	2	0	2	1	1	1	5
Phormidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	5	9	3	2	0	0	0
Physidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia viridis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiopyla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Prorodon	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	0
Raphoneis amphicerous ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhipidodendro	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

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Rhizoclonium hieroglphicum	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	40	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	20	22	12	8	16	13	10	8
Rotaria rotatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scenedesmus quadricauda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Simulidae	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	1	1	0
Spathidium	0	0	0	0	0	0	0	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0	0	0
Sphaerotilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	5	2	0	0	5	5	0	4	0	2	3	0	1	0	1	4
Spirogyra spp	0	0	0	0	0	0	0	0	0	2	0	0	1	2	0	3	1	1	1	0	0	0	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Stentor	0	0	0	0	0	0	4	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Stigeoclonium tenue	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	9	1	0	0	0	0	0	0	0
Stephanodiscus hantzchii	0	0	0	0	0	0	0	0	0	0	0	0	0	4	25	9	0	0	0	0	0	0	2	0
Stephanodiscus astrae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	3	4	1	2	4	0	1	2	0
Stichococcum bacillaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella angustata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella biseriata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella ovata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	4	1	1	1	0
Suirella robusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella splendida	0	0	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	0	0	0	0	0	2	4	0	7	0	0	0	3	0	0	0	2	1	2	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	3	5	5	0	5	15	0
Tabellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	1	1	0	0
Ulothrix	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0	1	1	2	1	1	1	1	0
Vaucheria	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	1
Volvox	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vorticella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	1

Table I.16: Biofilm experiment data for August (A) 2014 (Sites 1 – 6)

List of Individuals	A Site 1a	A Site 1b	A Site 1c	A Site 1d	A Site 2a	A Site 2b	A Site 2c	A Site 2d	A Site 3a	A Site 3b	A Site 3c	A Site 3d	A Site 4a	A Site 4b	A Site 4c	A Site 4d	A Site 5a	A Site 5b	A Site 5c	A Site 5d	A Site 6a	A Site 6b	A Site 6c	A Site 6d
Achnanthes brevipes	0	0	0	18	11	16	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthes inflata	0	0	35	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina	23	0	0	17	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthidium minutissimum	0	0	38	17	11	11	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinophrys	0	4	7	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aelosoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amoeba	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	7	4	0	14	7	6	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora pediculus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anuraeopsis navicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arcella	0	0	0	0	3	3	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asellus aquatica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspidisca	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	4	1	0	0	2
Astasia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacosera ambigua	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacosera granulata	0	0	0	0	0	6	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulbochaete mirabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis bacillum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calyptotricha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Campylodiscus noricus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxis	0	0	0	0	0	0	0	0	0	2	4	3	0	0	0	0	0	0	0	0	0	0	0	0
Chaemisiphon	0	0	0	0	0	0	0	0	0	0	0	0	10	4	12	6	3	3	3	4	0	0	3	0
Chaenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlamydomonas reinhardtii	0	0	0	0	0	0	0	0	19	0	0	0	3	18	0	13	0	0	0	3	0	0	5	5
Chlamydomonas spp	0	0	0	0	0	0	0	0	0	11	27	5	0	0	2	0	0	0	0	0	0	0	0	0
Chlorella spp	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	2	0	4
Chlorella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

pyrenoidosa																								
Chrysococcus rufescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Chironomid larvae	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	1
Cladophora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorococum	0	0	0	0	0	0	0	0	8	16	0	9	1	24	0	4	0	0	0	6	0	0	5	5
Cinetochilium	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	2	0	4	0	3	3	0
Closterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula	0	0	6	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colpidium	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0
Coscinodiscus lacustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptodifflugia	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella compta	0	36	0	0	18	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	0	0	0	0	0	0	0	0	7	2	0	5	6	0	4	0	1	2	3	6	1	0	0	3
Cylindrocapsa	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Cymatopleura elliptica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymatopleura solea	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	2	1	0
Cymbella cistula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella ehrenbergii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	32	16	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella prostrata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella ventricosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatoma vulgare	0	0	0	0	0	0	0	0	0	0	0	0	2	3	3	0	0	4	0	2	0	0	1	2
Didinium	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Difflugia	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dinobryon	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Dileptus	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dugesia polychroa	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	1	0	1	0	1	1
Epiclintes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Erpobdella octoculata	0	0	0	0	2	2	0	0	1	0	2	2	1	1	0	0	0	0	1	1	0	0	1	0
Euastrum oblongum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Euglena viridis	0	0	0	0	7	0	0	5	8	24	18	12	3	4	3	10	7	4	6	6	3	2	1	4
Eunotia bilunaris	0	0	0	0	4	4	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euplotes	0	0	0	0	4	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria spp	0	0	6	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gammarus pulex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastotrich	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossiphonia complanata	0	0	0	0	1	3	5	3	1	1	1	1	0	1	0	1	1	1	1	1	0	0	0	0
Gomphonema angur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	3	3	4	0	0
Gomphonema gracili	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	3	0	8	6	0	0	0	0	0	5	6	1	9	5	3	0
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0	0	0	10	0	9	0	9	12	12	2	1	9	3	0
Gyrosigma acuminatum	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0	0	1	2	0	3	0	0	0
Gyrosigma attenuatum	0	0	0	0	0	0	14	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Halteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hantzchia amphioxys	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	2	0	0
Lacrymaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litonotus	0	0	0	0	3	3	5	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mallomonas caudata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mastigamoeba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	2	0	0
Mayorella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	1	0	4
Melosira varians	0	0	0	0	0	0	0	0	0	0	0	0	6	4	0	3	7	3	9	5	9	6	10	7
Menoidium	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	1	0	0	0	0	0	0	0
Meridion circulare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	3	1
Microspora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	3	11	8	6	2	0	10	0	6	8	7	5	13	7	9	5
Navicula cuspidata	0	0	0	0	0	0	0	0	0	0	11	0	0	0	2	0	4	0	0	4	2	2	3	0
Navicula gracilis	0	0	10	0	0	0	21	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula oblonga	0	0	0	0	0	0	0	0	7	3	6	0	6	0	6	4	8	6	4	4	3	11	15	0
Navicula radiosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula reinhardtii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0
Navicula	0	0	0	0	0	0	0	0	0	0	0	0	6	0	9	7	9	4	7	9	13	10	9	5

viridula																								
Navicula minima	0	0	0	0	0	0	0	0	7	0	6	0	4	0	5	7	7	8	3	4	2	4	7	3
Nematode	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	1
Netrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Nitzschia acicularis	0	0	0	0	0	0	0	0	31	9	0	27	0	0	8	0	3	0	0	2	9	3	0	3
Nitzschia amphibia	0	0	0	0	0	0	0	0	0	0	0	0	5	7	3	7	0	4	0	0	0	2	1.1	4
Nitzschia dissipata	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia frustulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia hungarica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	3	0	0	0	5	1	0	0	0	0	3	2	5	0	4
Nitzschia recta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0	0	0	6	14	0	11	0	4	7	10	7	6	0	10
Nitzschia sublinearis	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	1	0	0	0	0	0	0
Opercularia	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ophephora martyii	0	0	0	0	21	2	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pandorina morum	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Paramecium putrinium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramecium caudatum	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	1	0	0	2	0	0	0	2
Pelomyxa	0	0	0	0	0	0	0	0	5	3	0	3	0	0	2	1	1	4	0	2	1	2	2	4
Phacotus lenticularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus longicauda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus pyrum	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Phagocata vitta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodina	0	0	0	0	0	0	0	0	2	4	0	0	1	0	2	0	1	1	3	0	1	1	2	3
Phormidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Physidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
Pinnularia viridis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiopyla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prorodon	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	4	4	2	1	0	0	0
Raphoneis amphicerous ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhipidodendro	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Rhizoclonium hieroglphicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abrevata	35	30	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	0	0	0	8	0	10	0	26	20	16	11	15	10	17	16
Rotaria rotatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scenedesmus quadricauda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Spathidium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaerotilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirostomum	0	0	0	0	0	0	0	0	0	0	0	0	5	5	6	4	0	3	3	0	1	2	2	4
Spirogyra spp	0	0	0	0	0	0	0	3	0	0	0	5	1	3	0	3	0	1	1	0	0	0	0	0
Stauroneis phoenicenteron	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Stentor	0	0	0	0	0	0	2	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	1
Stigeoclonium tenue	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	1	1	0	0	0	0	0	0	0
Stephanodiscus hantzchii	0	0	0	0	0	0	0	0	0	0	0	0	3	6	0	10	0	0	0	0	0	2	0	0
Stephanodiscus astrae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	5	1	4	2	0	3	0	0
Stichococcum bacillaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella angustata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella biseriata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella ovata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	4	2	2	1	3
Suirella robusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suirella splendida	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synedra acus	0	0	0	0	0	0	0	0	0	4	0	4	6	0	0	0	2	0	0	0	2	0	0	0
Synedra ulna	0	0	0	0	0	0	0	0	0	0	0	0	2	0	8	0	0	3	4	2	5	2	0	0
Tabellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetrahymena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubifex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0
Ulothrix	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0
Vaucheria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Volvox	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vorticella	5	0	0	3	0	3	0	0	0	0	0	1	1	0	1	0	0	0	0	2	0	0	0	1