Investigation of Faecal Indicator Organisms and their controlling factors in the River Wensum Catchment

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Abstract
Research was undertaken to further previous work on Faecal Indicator Organisms (FIOs) and their concentrations in the River Wensum catchment. FIOs are important not only in the Wensum catchment, but also on a national and international scale. They are a good indication of water quality and an important parameter that must be measured and controlled, especially when dealing with bathing and selfishery waters. Research was carried out in conjunction with the Wensum DTC to aid their work in changing agricultural practices to improve water quality.

Samples were collected from the catchment at monthly intervals over a 3 month period, May-July 2013. The samples were processed on the day of sampling in the laboratory, using a MPN method from Quanti-Trays, to obtain concentrations from sites throughout the catchment of total coliforms, faecal coliforms and *E.coli*. This data was then analysed alongside the data from the previous studies to see what current factors control the concentrations of the FIOs and how they have changed over time.

It was established that there are a plethora of factors that are responsible for inputting FIO into the catchment at various concentrations. Landuse, agricultural practices and human activity are all key factors that are dynamic, thus influencing the change of concentrations throughout the year. Natural factors such as precipitation are also important, and control not only natural occurrences such as storm events, but also impact upon STW for example. It was found that STW have a significant effect upon some FIOs, but that it is the presence of a STW, rather than the distance from it that is significant. There have also been changes across the catchment that can be seen from the 3 studies undertaken, again due to several components acting within the catchment.
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Abbreviations

BWD – Bathing Water Directive

DTC – Demonstration Test Catchment

EA – Environment Agency

EC – *E. coli*

EU – European Union

FC – Faecal Coliforms

FIO – Faecal Indicator Organisms

FC – Faecal Coliforms

MPN – Most Probable Number

SAC – Special Area of Conservation

SIMCAT – Simulated Catchments

SSSI – Site of Special Scientific Interest

STW – Sewage Treatment Works

SWAT – Soil and Water Assessment Tool

TMDL – Total Maximum Daily Load

UK – United Kingdom

WFD – Water Framework Directive
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1.1 Background to the project
There is increasing pressure on the UK to improve water quality due to the requirements to comply with the European Union (EU) Directives governing the quality of different water bodies. Many of these Directives are closely linked to agricultural activity in a catchment, so water quality is inherently linked with the type of activity that is undertaken in different catchments. There has been a shift from the traditional point source approach to managing water pollution to an increased understanding that water pollution in a catchment is of more diffuse source in nature. This has led to the shift towards managing water on a catchment scale and examining the activities undertaken within the catchment (Stapleton et al., 2008).

The importance of water quality in a catchment, and managing the catchment as an integrated unit, has been acknowledged by the creation of Demonstration Test Catchments (DTC) in the UK. Three river catchments in the UK are being jointly funded by Defra and the Environment Agency;

- The Eden in Cumbria
- The Wensum in Norfolk
- The Avon in Hampshire

The purpose of these DTC is to determine if adapting agricultural practices, in a cost effective manner, can reduce the impact of agriculture on diffuse water pollution on ecological function whilst still maintaining food security (Wensum Alliance, 2010). The three catchments are acting as pilot areas to help better the understanding of the effects of agricultural activity on diffuse water pollution. This is being achieved through scientific studies examining the physical properties and characteristics of the catchment and rivers, as well as engaging with the local farmers, members of the public and stakeholders. There has been agreement from some farms to adapt the current agricultural practices they use to see if the modifications lead to an improvement in the water quality. If there are positive results at the end of the 5 year trial period, in 2014, then this would be a good basis for encouraging more widespread implementation of these techniques on a national scale.
The Wensum in Norfolk is the chosen area to be examined in this study. As 71km of the river is both a designated Site of Special Scientific Interest (SSSI) and a Special Area of Conservation (SAC), the water quality is very important from an ecological perspective (Wensum Alliance, 2010). Of the 90.6ha of the habitat covered under the SSSI that is in a river or stream, 99.4% is in unfavourable and declining condition. 27% of the water bodies are at risk of failing the phosphorous standards and a source of phosphorous is known to be sewage treatment works (STW) and septic tanks. STW are also a potential source of faecal indicator organisms (FIO) which are an important component of overall water quality. Thus, this study will focus on the FIO concentration in the River Wensum.

The Wensum DTC study area is itself divided into two catchments. There is the larger River Wensum catchment and, within this, the smaller catchment of the River Blackwater which in this study is known as mini-catchment A. Figure 1.1 shows the whole catchment area, as well as the sampling points. Mini catchment A is an area that is predominantly used for intensive arable farming. The Wensum catchment as a whole has a range of different land uses, including the urban areas of Fakenham and Norwich.
Figure 1.1 showing the Wensum catchment. The sampling points are marked with the number/letter. The mini catchment is shaded a darker blue colour. From Wensum Alliance (2010)

1.2 Background to Faecal Indicator Organisms
FIOs are accepted as a good indicator of water quality in a catchment because they indicate the presence of faecal contamination of a water body and there are strict regulations regarding the coliforms and pathogens in water (Britton, 2011). As the pathogens themselves can be difficult to detect, the indicator organisms are used to identify any water bodies where faecal matter is present. The presence of the FIOs indicates there may then be the harmful pathogens present in the water body. There is also an increasing need to monitor microbial pollution, as less attention has been paid to this than other types of pollution such as phosphates and nitrates (Kay et al., 2007). The types of organisms that will be tested in this study are faecal coliforms, total coliforms and *E.coli*.
1.2.1 Total Coliforms (TC)
Total coliforms are a microbial indicator belonging to the family Enterobacteriaceae. They are discharged in high numbers, $2 \times 10^9$ coliforms/day/capita, in human and animal faeces. They are not, however, all faecal in origin. They are a widely used indicator for determining water quality, especially in water treatment plants, to indicate the effectiveness of the treatment that is occurring (Britton, 2011). It is worth noting, however, that their reliability as an indicator of faecal contamination has been questioned due to their ability to grow in both the wider environment and drinking water distribution systems (Tallon et al., 2005).

1.2.2 Faecal Coliforms (FC)
Faecal coliforms include all coliforms that ferment lactose at 44.5°C. The presence of faecal coliforms in water indicates that there is contamination from faecal matter of warm blooded animals. It is not possible to distinguish between human and animal sources however, so this cannot be used to differentiate between agriculture and STW, for example (Britton, 2011). Faecal coliforms are divided into *E. coli* and non *E. coli* species, Klebsiella, Enterobacter and Citrobacter (KEC coliforms). It has since been found that the KEC coliforms can be in existence in the environment and not necessarily of faecal origin, so they cannot be entirely relied on as a true indicator of faecal contamination (Alonso et al., 1999; Tallon et al., 2005).

1.2.3 *E. coli* (EC)
*E. coli* is easily distinguished from other types of faecal coliforms so is useful as an indicator organism in its own right. This is due to its negative impact on human health and also that there has been evidence that not all faecal coliforms are faecal in origin. As *E. coli* is faecal in origin, its presence indicates that there is faecal contamination in the water. *E. coli* also represents 94% of all faecal coliforms so its abundance and ease of detection is also responsible for its increasing importance as an indicator organism (Tallon et al., 2005).

1.3 Aims
The overall aims of the project are to determine the concentration of FIOs in the Wensum catchment, at specific sampling points, and to see how the concentration relates to other features of the catchment, such as distance from STW, land use and precipitation. If possible, the effect of a storm event on the concentrations will also be measured to see if this causes any peaks in the FIO concentrations.
1.4 Specific objectives

1. Establish the concentration of FIO at each sampling point
2. Determine if there is a statistical relationship between distance from STW and FIO concentration
3. Investigate how storm/flushing event affects FIO concentration
4. Establish if there are any additional factors in the catchment that influence FIO concentration
5. Compare the FIO concentrations with the previous studies to see how they vary over time within the catchment
2.1 Overview

It is widely acknowledged that an important indication of the quality of a water body is the concentration of FIOs (Nnane et al., 2011). This importance is increased further when considering water used for drinking and recreational purposes, due to the risk posed to human health. Within lowland catchments, there is little existing knowledge about how the FIOs vary spatially and temporally so, to gain a greater understanding of this and the potential impacts, further work is needed (Crowther et al., 2002). When a water body is used for bathing or drinking water purposes, understanding the fluxes in FIOs is vital to aid with management of the water quality (Kay et al., 2008b). The same can be said for waters that are used for shellfish harvesting, as the ingestion of pathogens by the shellfish and subsequent consumption by humans can have negative health impacts (Wilkinson et al., 2006; Kershaw et al., 2012). There is an increasing need for water companies, under the Water Framework Directive (WFD), to move away from an end of pipe way of remediating pollution, and adopt management practices for reducing the initial pollution. Understanding the sources of FIOs will aid this and allow the implementation of such practices (Council of the European Union, 2000).

In the UK, little attention has been paid to the monitoring of faecal indicators in rivers, limiting the amount of data available. Under the Bathing Waters Directive (BWD), concentrations of FIOs are controlled, which places an increasing pressure on the European competent authorities to implement legally binding plans to manage bacterial faecal inputs, at a catchment scale, in order to protect bathing waters (Kay et al., 2005). An understanding of these inputs into the catchment and how they fluctuate is an important basis for ensuring the standards are met, for as large a time as possible. The standards are 10,000 per 100ml for TC and 2,000 per 100ml for FC (Council for the European Communities, 1976) For drinking water, there can be no FIOs in the water at all (Drinking Water Inspectorate, 2010).

2.2 General inputs

The main sources of FIO inputs from a catchment are human and livestock sources. It is discussed by Tyrrel and Quinton (2003) however, that the dominance of the different sources is dynamic and changes are noted depending on other interplaying factors. During base flow conditions, human sources have a greater impact on FIO concentration than livestock (Wyer et al., 2010). The STW will contribute the majority
of the FIO from treated effluents. At high flow, both human and livestock sources are important but the livestock sources are more dominant (Wyer et al., 2010). Consideration must be given to the land type in the catchment as well as the nature of the livestock farming that is occurring (Chadwick et al., 2008). Dairy farming is seen as the biggest contributor to FIO concentration as the animals are generally kept on high quality grassland and close to farm buildings for milking in the lowlands. The large volumes of slurry produced, as well as animals being housed indoors during the winter, cause a high risk factor for FIO pollution. The suggestion is that FIO pollution measures should be initially targeted to areas of dairy production (Hampson et al., 2010).

Land use was also seen to have an effect on FIO concentration in a study conducted in a large, upland catchment in Wales by Crowther et al. (2003). They found that the proportion of improved pasture in a catchment had an impact on the FIO concentration. Improved pasture was generally used for either intensively grazing livestock or silage production. It was therefore either subject to faecal matter directly from livestock or fertiliser to cultivate the silage crop. This resulted in increased concentrations of FIOs. The relationship between FIO concentration and land use was found to be stronger during high flows. This was thought to be due to the ability of the land use type to generate surface runoff. Improved pasture generated more surface run off so the FIO concentration in sample locations surrounded by pasture were seen to be higher. Because of this, records of the land use around the sample locations will be made so as to take this into account when analysing the results in the Wensum.

When considering the FIO changes on an annual basis, it is important to consider how the climatic factors will affect the hydrological regime and therefore the FIO fluxes. It was advised by Tetzlaff et al. (2012) that five years’ worth of data is prudent for gaining an accurate picture of the fluxes of FIOs over a year. It is also noted that consideration of socio-economic factors may impact on agricultural activity throughout the year, such as stocking densities and crop choice, which may in turn impact on the runoff rates (Davies-Colley et al., 2004). This can affect the pathways for FIOs to enter watercourses which would impact on the fluxes.
2.3 Storm events

The volume of run-off and antecedent weather conditions are important, to understand how the FIOs peaks during wet periods. During prolonged periods of rainfall, diffuse FIO sources (e.g. animal faeces in fields) will become depleted over time. A period of high flow will therefore have higher FIO concentrations if the period preceding it was largely dry as the pollution source will not already have been depleted by wet weather (Crowther et al., 2011). Consideration of weather conditions preceding any sampling will therefore be important to note.

The same acknowledgement, of storm-events being responsible for the largest loads of FIOs, was observed by Sinclair et al. (2009). They studied the Thomas Brook River, the headwaters of a drainage basin in Nova Scotia, Canada. Faecal coliforms and \textit{E.coli} were monitored at five locations over six consecutive growing seasons. It was found that, during baseflow conditions, the site with the highest proportion of \textit{E.coli} was the one where human settlement was located. The contribution from septic tanks was thought to be the cause of this. The results showed that when the preceding rainfall was high or part of a storm event, the FIO concentration was up to 20.1\% of the total seasonal load, again highlighting the significance of storm events for faecal bacteria loading. These results compare to work by Kistemann et al. (2002), who examined three tributaries leading into different drinking water reservoirs in Germany. 99 water samples were taken during differing flow conditions to determine different characteristics, including faecal organism concentrations. It was again found that storm events contributed largely to the annual loading of FIO, at an average 10\% of the total budget.

Wyer et al. (2010) examined the impact of storm events on recreational water quality in Southwest Wales, and the compliance of samples in comparison to EU standards. During the initial hydroFigure phase, lasting about 8 hours, the FIO concentration exceeded the EU levels of 2000 cfu/100ml in six consecutive samples. In some samples, the level exceeded 10,000 cfu/100ml, highlighting the effect that storm events can have on coastal waters. In the remainder of the sampling, the samples were below the legal limits of 10,000 per 100ml for TC and 2,000 per 100ml for FC, (Environment Agency, 2013) but continued to exceed the guideline recommendations.

During the period of the worst water quality where mandatory fails occurred, it was observed there was a rapid movement of increased volumes of freshwater, from the
local streams, which fed into the compliance points. If the inputting water has a high FIO concentration, the rapid transmission of this will cause an exceedence of the standards in coastal waters. It can therefore be seen, that monitoring and understanding the FIO concentration in a catchment is vital to ensure the downstream inputs are limited and appropriate mitigation measures implemented. This is particularly important if the receiving coastal water is a designated bathing or shellfishery water.

Storm events are of particular importance for increasing the FIO concentration; due to the effect they have on overland transport processes, which are seen to contribute high volumes of soil particles to channels. Tyrrel and Quinton (2003) state there are three main ways for microorganisms to enter the channel:

- Attached to a soil particle
- Attached to waste or slurry particles
- As unattached cells or clumps.

Therefore to reduce the number of FIOs entering the channel, either breaking the source-pathway-receptor chain of events or reducing the number of FIOs initially is required. This is why there is increasing pressure on farmers and water companies to ensure they minimise the chance of FIOs entering the channel through such measures as slurry storage and effective waste water treatment processes respectively.

The comparable results from different geographical locations, again highlights that storm events can largely be seen to be of significant importance when considering FIOs in a catchment. Sampling an event of this nature in the Wensum will therefore provide important data to contribute to this field.

2.4 Sewage Treatment Works (STW)

When considering the impact of STW on FIOs it is important to consider the flow conditions. During high flows, FIO concentrations are seen to increase due to increased volumes of sewage flowing through STW which results in a more rapid transmission through treatment plants. This together with the possible increase of FIOs from the increased runoff from agricultural land, can see significant increases in FIOs during high flows. The methods of treatment each STW use can also impact on FIO concentrations. A study by Kay et al. (2008c) has produced indicative results as to which method is most effective. They monitored 162 sewage related discharge
sites in catchments across the UK and Jersey over a 2-3 month period, during a summer bathing season. It highlighted that un-treated sewage and primary treated effluent (physical treatment such as settlement of solids) had little difference in FIO concentration. There are statistically significant reductions in FIO concentration when secondary (biological processes such as an activated sludge plant) and tertiary (final cleaning of effluent, can be specifically designed to remove FIOs e.g. UV disinfection) treatments are used. This is in conjunction with the flow conditions that also have a marked impact on the concentrations. Under high flow conditions, untreated and effluent subject to primary treatment methods have significantly lower FIO concentration than under base flows. The higher levels of treatments are seen to produce an increase in FIO under high flow conditions due to a shorter residence time within the treatment works.

A study by Obiri-Danso and Jones (1999) into the effect of a treatment plant on FIOs in bathing waters around Morecombe, highlighted important considerations when considering the effects of STW on FIOs. A reduction was seen in FIO concentration in 2 of the 3 designated EU bathing waters, with an increase seen in the other. Time of day of the sampling was seen to be important. Samples taken in the morning were found to fail the EU limits where as those taken in the afternoon were found to generally comply. This explains the different between the researchers results compared with the Environment Agency's (EA). The EA conducted their sampling at around midday so their samples passed where as the researchers took their samples at 9am which caused a fail. The detection methods also had an impact, the researches used a most probable number (MPN) method which generally gives higher results then the membrane filtration method used by the EA. Thus both these factors will be considered when collecting samples in the Wensum.

The proximity to the discharge point, of the previously untreated sewage and the new STW, was also thought to be a consideration. The waters that were closest to the old discharge point saw the biggest decrease in FIO concentration where as the waters closest to the new discharge point saw the increase. This is due to the STW discharging treated sewage which may still contain some FIOs.

2.5 Modelling
It has been seen that there is a lack of data regarding FIO concentrations in catchments, which restricts the utilisation of modelling software to predict FIO concentration, at base and high flows. The various modelling tools already in use in
catchments, such as Soil and Water Assessment Tool (SWAT) and Simulated Catchments (SIMCAT), are advanced when considering parameters such as sediments and nutrients. Expanding the data available for both base and high flows would allow this to be adopted in future models which could then help advise future integrated catchment management programmes (Crowther et al., 2011).

A study by Kay et al. (2010) used modelling to identify FIO sources in catchments that were thought to be contributing significant FIO loadings to coastal bathing waters during the summer bathing season, when human use of the coast peaks. This study was conducted in 6 catchments across England, Scotland and Wales. None of the study areas had been subject to any measures designed to reduce FIO input. The study again highlighted the importance of high flow conditions in a catchment and the responsibility for transmitting the FIOs to the receiving waters. It was seen that FIO concentrations increase by an order of magnitude at high-flow, and this together with an order of magnitude increase due to an increase in volume of flows, led to a two order of magnitude increase in FIO delivery. Improved grasslands used for grazing were seen to be a key contributor of FIO at high-flow. Identifying areas surrounding the sample points in the catchment that may be of this type will therefore be important. Further work was found to be needed on the model in this study before it could be used widely to help catchment management programmes. The importance of increasing data on high-flow conditions was stressed however in order to help shape future policies. Routine monitoring of rivers in the UK do not target high-flow events so the catchments that have the highest FIO pollutant loadings are unknown. This further highlights the importance for continuing this nature of work and any data able to be gathered during high-flow conditions in the Wensum catchment will be vitally useful to the wider understanding of FIOs.

Tetzlaff et al. (2012) undertook a study in two Scottish highland catchments, the Dee and Esk, and took weekly samples over a year to develop a model determine the FIO concentrations from landuse. Hydrochemical and tracer studies were also being completed which allowed the base flows and storm flows to be characterised. Their findings supported the results from other studies that grazing densities and human activity are important factors in FIO concentrations. The results were high, in comparison to other catchments, considering the low levels of urbanisation and improved pastures in the study area. They found that the spatial patterns of FIO concentrations using the model were relatively straightforward but that the temporal dynamics were more complex. This was in part due to the weekly sampling strategy
that was adopted, as there was a rather extended period of time in-between samples being taken. The highest FIO concentrations were observed in higher discharge periods, but a statistically significant link between flow and concentration was not possible at the seasonal level, again due to the weekly sampling. The importance of the antecedent conditions was highlighted and that for the results to be put into context, the sampling periods should where possible be taken into account with the antecedent conditions and where applicable specific storm event characteristics such as rising/falling limb. This will be important to consider when sampling in the Wensum and if possible the antecedent conditions will be established when interpreting the data collected.

2.6 Total maximum daily load (TMDL)
Total maximum daily load is the total pollutant a water body can receive and still remain compliant with relevant water quality standards. Understanding the FIO loading of a water body is important to see how it changes under different flow regimes, to ensure that it remains compliant with relevant water quality regulations for as large a time as possible. It is difficult to measure accurately from sampling, the loading of a river, as FIO changes with climatic conditions, flow volumes and land use for example. This is why modelling is an increasingly important tool when considering this aspect of FIO in river catchments. A study was undertaken by Schulz and Childers (2011) on the Tangipahoa river in Louisiana which is located in a rural catchment, with agriculture as the dominant activity, along with small settlements using the river for recreational purposes. They used non-parametric multiple regression to model the possible future faecal loading of the river as well as using it retrospectively. They concluded modelling was only accurate if faecal loading was from a predominantly runoff/diffuse source and not from specific point sources. The modelling method adopted used minimal variables that were widely available, and allowed creation of a model that was deemed reasonably representative through examining past data. Considering catchment characteristics before creating such models was found to be important to understand the inter-linkages between all the factors controlling FIO concentration. A feature of this type of model is the reliance of the inputs of FIOs being diffuse source. Any deviation from the predicted values could be used to detect possible point source inputs and speed up the remediation of these before further exceedences are caused.

Identifying the main sources of FIO in the Wensum will be important for if future modelling work is conducted that focuses on FIOs. Having the background data for
given periods of time could also help aid the validation of any models and understanding their accuracy of predicting changes in FIO loads under different conditions.
Chapter 3: Methodology

3.1 Overview
The data required to answer the aims and objectives are the quantity of the different FIO types at each sample location. In order to obtain these data, samples were taken in the field and then processed in the laboratory in order to obtain the quantity of FIOs. Statistical analysis was then undertaken to determine if the results were significant.

3.2 Field Method
Initial observations were made regarding the site, land use around the site was recorded, including the presence of any livestock or evidence there had been livestock in the surrounding area. Vegetation type was also noted along with any human activity or settlement in the vicinity. Photographs were then taken at each site to refer back to later if required.

Before the samples were taken, the river channel was examined to determine the safest way to enter and optimum location to collect the water sample from. The river was entered downstream to avoid sediment disturbance affecting the sample taken. The 2 sample bags were labelled with permanent marker to identify the site from which they related to. The first was opened and immediately immersed into the water, taking a sample from the middle of the river channel, below the surface of the water in an upstream direction. The sample was taken so it just over the 100ml volume, and taken in one scoop. The whirl-pak sample bag was sealed by whirling it round 3 times to ensure a secure seal. The process was then repeated for both sample bags. Once the samples were obtained, they were placed into a cool box, containing ice packs, to maintain the sample’s quality until it was processed back in the lab.

3.3 Laboratory Method
To ensure the results obtained from the samples were as accurate as possible, they were processed within 6 hours of collection. In the lab, the worktop was initially wiped with 70% ethanol solution to minimise risk of cross-contamination. Gloves were also worn both for health and safety reasons and to minimise contamination. The Quanti Tray sealer was switched on and left to warm up for 10 minutes. The incubators were checked to ensure they were at the correct temperatures of 35°C and 44.5°C. The first sample was removed from the cool box; an ampoule of Colilert substrate was
opened and poured into the sample. This was thoroughly mixed until all the Colilert substrate was dissolved, before being poured into a Quanti Tray. The Quanti Tray was then sealed in the sealer and labelled as to which site it originated, and temperature of incubator it was to be put into. This process was repeated until all sites had a tray corresponding to each incubation temperature. The Quanti Trays were then placed into the appropriate incubator and left for 24 hours.

After the incubation period, the Quanti Trays were removed. For the trays in the 35°C incubator, a record was made of the number of large and small wells in the tray that were yellow in colour. The numbers of large and small wells that fluoresced under UV light were also recorded. For the 44.5°C temperature, just the number of the large and small wells that were coloured yellow were recorded. The numbers of wells were checked against the MPN table to determine the concentration of the associated FIOs in each sample.

The use of the Colilert and Quanti Tray method is recognised by the United States Environmental Protection Agency as an alternative to membrane filtration and multi-tube fermentation methods (Sercu et al., 2011). It was chosen in this instance as the previous studies on the Wensum had been undertaken using it, so the results would be comparable. It is also a relatively quick and simple method to use, so ideal when needing to process high numbers of samples within the 6 hour time window.
Chapter 4: Results

4.1 Examination of 2013 data

As can be seen from the results, there are differences across the catchment in the concentrations of FIOs. **TC** were found to be consistently high across the catchment in the majority of the sampling sites. 18 of the sampling sites for May had concentrations of over 800 MPN/100ml and in June the number of sites over 800 MPN/100ml, were 17. In July this was considerably higher, with 27 of the sites having concentrations over 800 NPN/100ml. The 27 sites did in fact have the highest number achievable using the MPN lab method of 1011.2 MPN/100ml. **FC** and **EC** were seen to have more variation in concentrations across the catchment and this was observed in all three months where samples were taken. All the data collected, as well as that from previous studies, can be found in Appendix A.

Table I shows a breakdown of the means, minimums and maximums for the different indicators in May, June and July. It can be seen that for all three indicators, there was an increase in mean concentration for each successive month.

With regards to sites with high concentrations of **FC** & **EC**, sites 9, 14 and 21 are immediately obvious when examining figures 4.1 - 4.9. This observation relates to May and June. July’s results displayed an increased number of sites with high concentrations of **FC** & **EC**. Site 14 for example had the highest concentration that was possible from the MPN Quanti-tray method, 1011.2 MPN/100ml, for both **FC** and **EC** in May. In June, sites 9 and 14 remained high but site 21 had a decrease in concentration of **FC** and **EC**. In July, for **EC**, site 10 also demonstrated a concentration of 1011.2 MPN/100ml. It is not possible to compare the value for site 21 as this sample was contaminated so the results were invalid. When examining **FC** for July, sites 9, 14, 16 and E demonstrated the highest value of 1011.2 MPN/100ml.

When examining the results for sites that displayed low values in comparison to the other sites, the field drains were immediately obvious and having either very low or zero concentration of FIOs in all three months. With regards to other sites exhibiting low concentrations, the sites do not remain the same when making a comparison across months. In May, sites 2, 4 and 20 had low concentrations for **FC** & **EC**, whereas in June the concentrations had increased at all these sites. The sites with the lowest concentrations in June were 8, 15 and 18. In July the sites that exhibited the
lowest concentrations were 4, 18 and M. In all indicator categories, it can be seen from the maximums that at least one site achieved the maximum possible concentration that is detectable by the Quanti-Tray method. The large range for each indicator again highlights the extremes in differences in concentrations across the catchment at the various sampling points.

Table I comparing the min, max, mean and std. deviation across the monthly samples.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC_May</td>
<td>33</td>
<td>4.10</td>
<td>1011.20</td>
<td>659.8121</td>
<td>386.26493</td>
</tr>
<tr>
<td>FC_May</td>
<td>32</td>
<td>1.00</td>
<td>1011.20</td>
<td>306.0125</td>
<td>284.12907</td>
</tr>
<tr>
<td>EC_May</td>
<td>26</td>
<td>18.90</td>
<td>1011.20</td>
<td>351.1308</td>
<td>311.78738</td>
</tr>
<tr>
<td>TC_June</td>
<td>31</td>
<td>18.90</td>
<td>1011.20</td>
<td>397.6862</td>
<td>294.57980</td>
</tr>
<tr>
<td>FC_June</td>
<td>29</td>
<td>2.00</td>
<td>1011.20</td>
<td>397.6862</td>
<td>301.89316</td>
</tr>
<tr>
<td>EC_June</td>
<td>27</td>
<td>27.50</td>
<td>1011.20</td>
<td>390.9926</td>
<td>308.14646</td>
</tr>
<tr>
<td>TC_July</td>
<td>30</td>
<td>31.80</td>
<td>1011.20</td>
<td>869.9300</td>
<td>325.14354</td>
</tr>
<tr>
<td>FC_July</td>
<td>27</td>
<td>11.50</td>
<td>1011.20</td>
<td>593.9815</td>
<td>302.21239</td>
</tr>
<tr>
<td>EC_July</td>
<td>26</td>
<td>101.70</td>
<td>1011.20</td>
<td>552.5038</td>
<td>315.66526</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.1 showing the Total Coliform concentration in MPN/100ml for each of the sample sites in May.
Figure 4.2 showing the Faecal Coliform concentration in MPN/100ml for each of the sample sites in May.

Figure 4.3 showing the *E.coli* concentration in MPN/100ml for each of the sample sites in May.
Figure 4.4 showing the Total Coliform concentration in MPN/100ml for each of the sample sites in June.

Figure 4.5 showing the Faecal Coliform concentration in MPN/100ml for each of the sample sites in June.
Figure 4.6 showing the *E.coli* concentration in MPN/100ml for each of the sample sites in June.

![Graph showing E.coli concentration]

Figure 4.7 showing the Total Coliform concentration in MPN/100ml for each of the sample sites in July.

![Graph showing Total Coliform concentration]
Figure 4.8 showing the Faecal Coliform concentration in MPN/100ml for each of the sample sites in July.

Figure 4.9 showing the *E.coli* concentration in MPN/100ml for each of the sample sites in July.
4.2 Antecedent weather conditions
Data was obtained from the Met Office to determine the average monthly precipitation for Marham. Marham was chosen as it was the closest location to the Wensum catchment. Calculating the monthly average allowed a comparison to be made between the precipitation during the sampling period, and the average conditions. This then allowed a conclusion to be drawn as to if the results obtained were usual for the month or if they may have been different due to a change in the climatic regime. The data is the average for the period 1981-2010.

4.2.1 Previous studies sampling – 15th June & 11th July 2011
The average precipitation for June is 59.2mm and July is 52.1mm (Met Office, 2013). As can be seen from Table II, the precipitation preceding June’s sampling was low and would not have been classed as a storm event. July saw slightly more rainfall before sampling occurred. The total precipitation for June and July in 2011 was 82.8mm and 63.9mm respectively, both above the average for the months. When examining May 2011, the total rainfall was 16.4mm, well below the average of 53.3mm for the month. It can therefore be seen that June’s sampling was preceded by a dry month of May with minimal precipitation in June. July’s sampling was preceded by a wet second half of June with some further precipitation in July.

Table II showing the precipitation that occurred prior to the sampling taking place. Only days where precipitation was recorded are shown. Data from Met Office (2013).

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Date</th>
<th>Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/06/2011</td>
<td>06/06/2011</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>07/06/2011</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>10/06/2011</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>11/06/2011</td>
<td>4.6</td>
</tr>
<tr>
<td>11/07/2011</td>
<td>01/07/2011</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>06/07/2011</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>07/07/2011</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>08/07/2011</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>09/07/2011</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>10/07/2011</td>
<td>9.1</td>
</tr>
</tbody>
</table>
4.2.2 Previous studies sampling – 19th December 2011, 24th January & 21st February 2012

From the Met Office (2013) data, the average precipitation for December, January and February is 52.7mm, 56.1mm and 39.3mm respectively. Prior to sampling taking place in December, there had already been 48.7mm of precipitation during the month which is a large proportion of the average monthly total. 70.8mm fell in total during December 2011 which is considerably higher than the average highlighting that December was a wet month. This is in contrast to the preceding November, which had precipitation of 22.5mm fall, compared with the average of 62.2mm, so November was drier than usual. January in was in total slightly drier than average, with total precipitation of 42.5mm. 28.7mm had fallen prior to sampling on 24th January. The February was a lot drier than average, 17.1mm of precipitation was the monthly total, with 15.4mm falling prior to the sampling occurring. A summary of the precipitation falling on each day prior to sampling is show in Table III.
Table III showing the precipitation that occurred prior to the sampling taking place.

Data from Met Office (2013)

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Date</th>
<th>Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19/12/2011</td>
<td>01/12/2011</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>03/12/2011</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>05/12/2011</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>07/12/2011</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>09/12/2011</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>10/12/2011</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>12/12/2011</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>13/12/2011</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>15/12/2011</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>16/12/2011</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>17/12/2011</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>18/12/2011</td>
<td>8.1</td>
</tr>
<tr>
<td>24/01/2012</td>
<td>01/01/2012</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>02/01/2012</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>03/01/2012</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>04/01/2012</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>05/01/2012</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>06/01/2012</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>13/01/2012</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>18/01/2012</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>19/01/2012</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>20/01/2012</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>21/01/2012</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>22/01/2012</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>23/01/2012</td>
<td>0.1</td>
</tr>
<tr>
<td>21/02/2012</td>
<td>02/02/2012</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>05/02/2012</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>07/02/2012</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>12/02/2012</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>13/02/2012</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>14/02/2012</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>15/02/2012</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>19/02/2012</td>
<td>1.8</td>
</tr>
</tbody>
</table>

4.2.3 Sampling 13th May 2013

From data obtained from the Met Office for the settlement of Marham, the antecedent weather conditions can be gauged for the general Wensum area. Up until 9th May, there was no recorded precipitation in May and then small amounts were measured
in the following days. The amounts are shown in Table IV. As seen, the rainfall before and during the sampling period was low, so the results obtained do not reflect those of a storm event. The average precipitation for May is 53.3mm. In May 2013, 41.4mm of precipitation fell in total, slightly less than the average. In the days prior to sampling, 23.4mm had fallen, just over half of the monthly total.

Table IV showing the amount of precipitation for May, June and July recorded in Marham. Data from Met Office (2013).

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Date</th>
<th>Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13/05/2013</td>
<td>09/05/2013</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>10/05/2013</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>11/05/2013</td>
<td>3.8</td>
</tr>
<tr>
<td>17/06/2013</td>
<td>12/05/2013</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>13/06/2013</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>14/06/2013</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>15/06/2013</td>
<td>0.6</td>
</tr>
<tr>
<td>02/07/2013</td>
<td>27/06/2013</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>28/06/2013</td>
<td>4.2</td>
</tr>
</tbody>
</table>

4.2.4 Sampling 17th June 2013
In June there was no recorded precipitation until the 12th, when 4.4mm fell. Further precipitation then fell in the days preceding sampling on the 17th. As stated before, the average precipitation for June is 59.2mm. In June 2013, 14.4mm fell in total so considerably lower than the average. This is also much drier than June 2011 when sampling occurred previously enabling a comparison between a wet and dry summer. In the days prior to sampling, 5.2mm of rain had fallen, approximately a third of the monthly total.

4.2.5 Sampling 2nd July 2013
As sampling in July was undertaken at the beginning of the month, it is not possible to examine the weather conditions for the days preceding. As the data for July’s total precipitation is not yet available also, a comparison cannot be made between July 2011 and 2013. It can be said though, that due to the higher temperatures experienced at the beginning of July, the precipitation for the month is likely to be below the average of 52.1mm and almost certainly below that of July 2011 when 63.9mm fell. At the end of June, there was 4.8mm of precipitation that fell.
As it was not possible to sample during a storm event, the data was analysed to see if the amount of rainfall in the preceding 30, 14 and 7 days before sampling had a significant effect on the concentration of FIOs that were collected. This helps indicate when higher volumes of precipitation would have an impact on the FIO concentration. It may indicate if they would cause an increase in the few days after the precipitation event, or if there would be a delay on the effects being seen. From a preliminary visual inspection of the data in Table V, there seemed to be no consistent pattern as to when the higher concentrations were recorded. From June 2011 to July 2011 there was increase in precipitation which led to a decrease in some concentrations of FIOs and an increase in others. The majority of the precipitation occurred more than 14 days before the sampling occurred. In January 2012, when approximately a third of the precipitation fell in the 14 days prior to sampling, this led to an increase in concentrations when compared to the previous month.

Table V showing the average concentrations across the catchment for each indicator and the average precipitation for 30, 14 and 7 days prior to sampling.

<table>
<thead>
<tr>
<th></th>
<th>Average FIO</th>
<th>Ppt 30 days</th>
<th>Ppt 14 days</th>
<th>Ppt 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jun-11</td>
<td>TC</td>
<td>982.77</td>
<td>28</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>120.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>141.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul-11</td>
<td>TC</td>
<td>150.73</td>
<td>103.2</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>327.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>401.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec-11</td>
<td>TC</td>
<td>614.40</td>
<td>56</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>28.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-12</td>
<td>TC</td>
<td>790.75</td>
<td>38</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>373.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>331.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb-12</td>
<td>TC</td>
<td>634.50</td>
<td>30</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>159.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>173.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May-13</td>
<td>TC</td>
<td>835.08</td>
<td>31.6</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>378.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>359.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun-13</td>
<td>TC</td>
<td>881.52</td>
<td>21.4</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>437.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>403.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul-13</td>
<td>TC</td>
<td>1011.20</td>
<td>14.4</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>633.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>566.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A Pearson’s correlation was run, to determine how the average for each indicator in all sampling months was affected by when the precipitation occurred. As can be seen
from Table VI, the only indicator that was significantly affected by when the precipitation occurred was TC. It was seen that it was affected by the total precipitation that fell in the 30 days prior to the sampling, rather than if it fell just prior to sampling. This evaluation is based on the monthly average for all sites compared with the precipitation recorded in the days preceding sampling. The negative relationship indicates that as precipitation increases, the concentration of the indicator decreases. As all indicators have a negative value, this relationship is apparent in all cases but it is only significant when considering TC and the precipitation in the 30 days preceding sampling.

Table VI showing the Pearson’s correlation results. Significant results are highlighted in yellow. Significant ad 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>30 days</th>
<th>14 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>-0.942</td>
<td>-0.632</td>
<td>-0.543</td>
</tr>
<tr>
<td>FC</td>
<td>-0.194</td>
<td>-0.134</td>
<td>-0.222</td>
</tr>
<tr>
<td>EC</td>
<td>-0.118</td>
<td>-0.468</td>
<td>-0.533</td>
</tr>
</tbody>
</table>

4.3 Sewage Treatment Works

The data regarding the distance of each sampling site was obtained from a map with both the sampling locations and the STW location marked on. The scale was then used to calculate the distance and is what this analysis is based upon.

Upon initial analysis of the FIO concentration data, it was confirmed that they were not normally distributed. A visual inspection of the histogram showed no bell shaped curve and this was confirmed by the Sharipo-Wilk's test (p > 0.5) which had a value of 0.001, showing the data were not normally distributed.

The initial test to be conducted on the data was a Mann-Whitney test as the data were not normally distributed, to determine if there was a significant difference between a STW being present upstream of a sample point compared where there was no STW upstream on the concentration of FIOs. The null hypothesis is that there is no difference between upstream presence of a STW on FIO concentration.

From examining the summary table it is seen that the presence of STW have an effect on the concentration of some indicators, but not all. It can be seen that the null hypothesis is accepted
The null hypothesis is rejected for TC for May and June and FC for May as they had p scores of 0.021 (TC May), 0.038 (FC May) and 0.028 (TC June) with a significance level of 0.05, as seen in Table VII.

Table VII showing the results from the Mann-Whitney test to see if upstream presence of a STW significantly affects the concentration of FIO. Confidence level is 0.05.

<table>
<thead>
<tr>
<th>Null Hypothesis</th>
<th>Sig</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 TC concentration in May is not related to presence of an STW upstream</td>
<td>0.021</td>
<td>Reject null hypothesis</td>
</tr>
<tr>
<td>2 FC concentration in May is not related to presence of an STW upstream</td>
<td>0.038</td>
<td>Reject null hypothesis</td>
</tr>
<tr>
<td>3 EC concentration in May is not related to presence of an STW upstream</td>
<td>0.203</td>
<td>Retain null hypothesis</td>
</tr>
<tr>
<td>4 FC concentration in June is not related to presence of an STW upstream</td>
<td>0.879</td>
<td>Retain null hypothesis</td>
</tr>
<tr>
<td>5 TC concentration in June is not related to presence of an STW upstream</td>
<td>0.028</td>
<td>Reject null hypothesis</td>
</tr>
<tr>
<td>6 EC concentration in June is not related to presence of an STW upstream</td>
<td>0.981</td>
<td>Retain null hypothesis</td>
</tr>
<tr>
<td>7 FC concentration in July is not related to presence of an STW upstream</td>
<td>0.017</td>
<td>Retain null hypothesis</td>
</tr>
<tr>
<td>8 TC concentration in July is not related to presence of an STW upstream</td>
<td>0.261</td>
<td>Retain null hypothesis</td>
</tr>
<tr>
<td>9 EC concentration in July is not related to presence of an STW upstream</td>
<td>0.959</td>
<td>Retain null hypothesis</td>
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</tbody>
</table>

The data where it was shown that presence of a STW was significant was then further tested, to determine if the distance from the STW had an affect on those sites that had an STW located upstream. Sites that did not have an STW were excluded from this test. A Spearmans rank test was chosen for this as it is a measure of strength of the relationship between two continuous variables. This would then indicate if there was a relationship between STW distance and FIO concentration and how strong this relationship was.

It was seen in the case of TC for May and STW distance that the results were not significant. A weak negative relationship was found, $r_s = -0.146$. $P=0.603$ which at the 0.05 confidence level is not statistically significant showing that STW distance did not affect the TC concentration in May.
For FC in May, there was a weak positive relationship found, \( rs = 0.038 \). Again, the relationship between STW and FC concentration was not statistically significant, \( P = 0.893 \) at the 0.05 confidence level.

For FC in June, there was a weak negative relationship between STW distance and FC concentration, \( rs = -0.185 \). This was again not statistically significant at the 0.05 confidence level with \( P = 0.509 \).

The results therefore indicate that it is the upstream presence of a STW that has the affect on the concentration of FIOs, rather than the distance from the STW itself.

4.4 Change from previous data

To see how the FIO concentration has varied over time, the data was compared with that collected in previous studies by Zheng (2011) and Walkerley (2012).

Each of the indicators, TC, FC and EC were compared against the other samples of the same type over the whole time range of which there is data. This period is June and July 2011, December 2011, January and February 2012, May, June and July 2013.

A repeated measures ANOVA test was deemed most suitable for comparing the changes over time of the FIOs. It is to be noted that whilst the data is not normally distributed, the ANOVA test is deemed robust enough to deal with this violation of normality. Outliers in the data were also included as they were not thought to be due to either an input error or equipment malfunction for example. This is to be taken into consideration when considering the results of the test. The ANOVA test will identify which months have seen a change in the population means of the FIOs and will indicate which months require closer examination to try and identify the possible reasons for the increase/decrease.

The null hypothesis is that the group means are equal so there is no difference between the sampling sites.

The alternative hypothesis is that the group means are not equal so there is a statistically significant difference between sampling sites.

Each of the FIO types were examined separately from the others. Each months results were compared against every other month in order to identify if there were
any significant differences between the months and in which months they occurred. The results are presented below:

4.4.1 Total coliforms
When the ANOVA test was conducted, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(27) = 143.799, p = <0.0005$. As this was violated, the Greenhouse-Geisser correction was used to determine if the results of the ANOVA test were still reliable. As $p = <0.0005$, the results were significant indicating there were differences between the means in some groups so the null hypothesis was rejected.

Post-hoc analysis with a Bonferroni adjustment revealed which sites had significant differences in concentrations are shown as the highlighted values in Table VIII. The table also shows if the change was an increase or decrease by examining the values in the mean difference column.
Table VIII showing the results for the repeated ANOVA for TC. The months where a significant change occurred is highlighted. P <0.05

<table>
<thead>
<tr>
<th>Month</th>
<th>Month</th>
<th>Mean Difference (I-J)</th>
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<th>Sig.a</th>
<th>Increase/decrease</th>
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4.4.2 Faecal Coliforms
Again, When the ANOVA test was conducted, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 82.51$, $p = <0.0005$. The Greenhouse-Geisser correction was used to determine if the results of the ANOVA test were still reliable as sphericity had again been violated. As $p = 0.009$, the results were significant indicating there were differences between the means in some groups.
and lead to the null hypothesis being rejected. Post-hoc analysis with a Bonferroni adjustment revealed which sites had significant differences in concentrations are shown as the highlighted values in Table IX.

Table IX showing the results for the repeated ANOVA for FC. The months where a significant change occurred is highlighted. P <0.05

<table>
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4.4.3 *E. coli*

Again, when the ANOVA test was conducted, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(27) = 112.589, p = <0.0005$. The Greenhouse-Geisser correction was used to determine if the results of the ANOVA test were still reliable as sphericity had again been violated. As p = 0.007, the results were significant indicating there were differences between the means in some groups so the null hypothesis was again rejected. Post-hoc analysis with a Bonferroni adjustment revealed which sites had significant differences in concentrations are shown as the highlighted values in Table X.
Table X showing the results for the repeated ANOVA for EC. The months where a significant change occurred is highlighted. P <0.05

<table>
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<th>Month</th>
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<th>Increase/decrease</th>
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Chapter 5: Discussion

5.1 Findings from previous studies
It can be seen from the previous studies of FIOs in the Wensum catchment, that possible inputs have already been identified, and reasons for the changes in concentrations across the catchment postulated. Zheng (2011), undertook the initial study in June and July 2011 and concluded that the main contributors to FC and EC were STW. STW were less important contributors to the TC. During a storm event, the STW were the main inputs into the catchments and livestock provided the background FIO contribution. In addition to this, pig farms and use of fertilisers on the land and were shown to be important contributors. Pig farms were found to be greater contributors than other livestock farms to FIO levels, perhaps due to there being large areas in Norfolk devoted to pig farming rather than other types of pastoral agriculture. With regard to the contribution of fertilisers, both the application of them without allowing exposure to UV light and the length of time they were on the field for before precipitation occurred affected the FIO concentration. This is because UV light breaks down FIOs, and the strength of the UV light together with temperature affects the survival period (Oliver et al., 2007; Hodgson et al., Unknown). If precipitation occurs during the organism’s survival period, then they will be transported into the river.

Walkerley (2012) undertook their study during December, January and February 2011/12. It was again identified that STW have an important influence over the FIO concentrations, and that sites affected by an STW upstream had little variation across the months sampled, due to the constant inputs. It was also seen that although the Wensum is a largely arable catchment, the presence of Bernard Matthews turkey farm led to a high proportion of fields being fertilised with turkey manure. This led to higher FIO concentrations generally than would have been seen in an arable catchment. The influence of the weather was explored further. June and July 2011 were wetter than average where as the winter 2011/12 experience drought conditions. This led to a lower concentration of FIOs during the winter due to decreased flows which increased the die off rate of the organisms.

5.2 Results compared to legislated maximums
It is necessary to compare the results obtained to the limits and guidelines for FIOs that are set by the EU. As there are no specific legislations regarding river waters,
the most appropriate limits to compare to are those of the BWD (Council of the European Union, 2006). As already stated, the Wensum eventually discharges into the North Sea at Great Yarmouth, a popular tourist destination, so the waters there must conform to the BWD. The limits are 10,000 per 100ml for TC and 2,000 per 100ml for FC. This must be equal to or less than the limit for 95% of the year. The guidelines for what waters should aim to achieve are 100 per 100ml for FC and 500 per 100ml for TC (Council of the European Union, 2006; Environment Agency, 2013).

As can be seen from the results collected in this study in May, June and July 2013, none of the sampling sites exceeded the maximum values permitted for TC and FC. This could in part be attributed to the maximum detection limit of the Quanti Tray used to ascertain the MPN of FIO in the samples collected. As this was 1102.2 MPN/100ml and on numerous occasions sites reached this maximum, it is not possible to know if the detection limit was higher, if the concentration would have exceeded these limits.

The guideline limits however, were included in the measurable range of the Quanti Trays and they were breached on a number of occasions, in all of the months sampled. In all months, between 24-27 sites exceeded the guidelines which could have a significant effect on the receiving waters at the coast.

This data on the concentrations of FIOs and how they vary over a given time is important when considering implementing a ‘programme of measures’ under the WFD. For this to be successful, at risk water bodies need to be identified and then using the data, a TMDL can be calculated. Incorporating this in future policy and working with landowners and users will allow catchments such as the Wensum to manage the pollutants that are input into the river so that water quality targets are not affected, both in the receiving water body and downstream of it; a key consideration of the Wensum catchment (Kay et al., 2008a).

5.3 Examination of 2013 data
A notable observation of the data collected was the values of the FIOs recorded in the field drains. The values were largely negligible but with higher concentrations of TC than FC or EC. The field drains demonstrated the lowest concentrations across the catchment. This is likely to be due to the restricted area that the water from the drains is originated from. Unlike the main river channel which has multiple inputs from a wide area, the water from the drains had a catchment in the scale of an
individual field. As the drains were draining arable land on the Salle estate, there was no livestock present in the fields. It is said that livestock in fields increases the concentration of FIOs in water flowing from field drains and that field drains by-pass any mitigation put in place to reduce the entry of pollutants/contamination into the channel (Edwards et al., 2012). If animal manure is also used as a fertiliser, this would also cause an increase in the concentration (Vinten et al., 2004).

There is a further reason for the low concentrations found in the field drains. Catchments are thought to have two main stores: the channel itself (e.g. within sediments) and those on or near the land’s surface (Adrian McDonald et al., 1982). If there are limited FIOs stored below the land surface and in the soil, then the transport through the soil and to the drains would be minimal, thus reflecting the results observed.

As a precaution, the fields were checked for the presence of animal fertiliser. Observations showed that there was no evidence of animal fertilisers having been applied recently during the sampling period. It was also noted that the drains were not flowing at a high rate so the transmission time for the FIOs to reach the drain output were increased. As FIOs have a die off period of 20 days, organisms in the soil would have been more likely to have died before they were transmitted to the drain exit.

Whilst sampling at site 15, it was noted that there were horses present in the field adjoining the river. Previously, they had been able to access the river via a shallow bank but, during the sampling in May, the stream was fenced off do direct access to the river was no longer possible. During the sampling in June, the fence was no longer across the entire river bank, so the horses were able to access the river once again. This remained the case for the sampling conducted in July. When examining the data, this appears to have had little effect on the FIO levels. TC remained the same across the sampling period at 1011.2 MPN/100ml. FC and EC decreased from May to June and then increased again in July to similar concentrations see in May when there was no direct river access.

It was expected that allowing direct river access to livestock would have caused an increase in FIO concentrations due to the faecal material being excreted directly into the river channel. As this was not reflected in the results, it is likely there are other factors affecting the FIO concentrations. When considering further the likely impact of
the livestock, it is apparent that the horses would not have had a significant impact on the FIOs concentration. The stocking level was low, only 3-4 horses in a field that was approximately 2-3 acres in size. As has been seen in other studies, large numbers of stock are required to have a significant impact on FIO levels (Vinten et al., 2008). It is thought by Dampney et al. (2002), however, that access to the river is of greater importance than stocking density. It is still unlikely that a significant increase in FIO concentration would be seen with so few livestock, even with direct river access. As the sampling was conducted at the top of the field where the river enters and flows downstream through the field, any impact of the livestock would have been seen at a point further downstream in the field. Sampling would have needed to be repeated at this location to understand further what impact the stock was having if any.

This stocking density relationship is reflected at site 20 where there were cows in the field adjoining the river and upstream of the sampling location. At this site, there was an increase in EC and FC over the sampling months whilst TC decreased from May-June then increased from June-July. There were other sites where livestock were absent, that exhibited higher concentrations than this site. This indicates that if the livestock were a significant factor, the concentrations would have been amongst the highest at sites where they were present. At site 20, the livestock were again not densely stocked in the field and the flat topography, together with the lesser rainfall over the sampling period, would have resulted in less transport into the river. Land used for grazing is also less likely to have fertiliser applied to it, so sites with arable land surrounding the river may exhibit higher concentrations than those fields used for livestock but at a low density.

Another observation made from the results is the overall high concentrations for TC in July. All of the sites apart from site M and the field drains, had concentrations that reached the maximum detectable of 1011.2 mpn/100ml. Initially, it was thought that a sampling error had occurred and the samples had become contaminated. However, if this were the case then all the samples would have had the same concentrations. As all samples were transported in the same cool box and processed in the same lab area, a contamination was not thought to be responsible. Upon examining the past TC data for 2013, the TC concentrations in the river were generally the highest of the indicators tested for.
Site 14 was of particular interest. For summer 2013 sampling, the maximum levels of 1011.2 MPN/100ml were recorded for all three indicators for all samples taken. This demonstrates a marked increase since summer 2011, when all concentrations were under 200 MPN/100ml. By the Winter 2011/12 sampling period, the concentrations had risen and were higher than seen in the previous summer. Site 14, Foulsham Drain, Twyford runs under a road and there are several properties both upstream and downstream. As can be seen from figure 5.1, it is also downstream of 3 STW. The high concentrations at this site are likely to be due to the cumulative effect of these 3 STW discharging into the river. Also it could also be possible that the properties are not connected to the mains sewerage system, as they are not within close proximity to an urban area. If this is the case, then it is likely the properties would have septic tanks, which if not properly lined can increase concentrations of FIOs through leakage into the surrounding soil. Ultimately, this will then be transported into the river (Kay et al., 2008c). Additionally, the increase from previous studies could be due to a change in treatment method or an increase in the volumes of waste that the STW now deals with. Further investigation into this would be necessary to understand if there has been a change in activity of the STW.

Figure 5.1 showing the location of STW in relation to sampling points in the catchment. From Wensum Alliance (2010).
5.4 Effects of Sewage Treatment Works
As has been found in the previous studies concerning the Wensum catchment and FIOs, STW were seen to be an important factor when examining the concentration of the indicators at the various sampling points along the river. As seen in the results section, the presence of a STW upstream of a sampling point was seen to have a significant effect on the concentrations of some indicators found downstream. When this relationship was investigated further by comparing the distance from the STW and the FIO concentrations, the results proved to be insignificant. A possible reason for this could be the nature of the STW upstream and the level and type of treatment that is undertaken at these works. As already discussed by Kay et al. (2008c), STW that have a higher level of treatment for FIOs will discharge fewer organisms into the river than a STW with a lower treatment level. If the STW on the Wensum have differing methods, then a site closer to a STW with higher levels of treatment (e.g. activated sludge plant or disinfection) may in fact have lower levels of FIOs than a site further away from a STW that only uses settlement, for example. As it is unknown which types of treatment each STW use, further studies would be required to investigate this relationship.

Another factor assessed, whilst examining the data, was the effect of increased flow due to high volume rainfall events on STW. Transmission of the water through the STW is increased during high flows in response to storm events. This increased transmission and connected reduction in treatment time for the water can lead to increases in FIO concentrations. This may then impact upon the significance of the distance of the STW from the sampling location. Sites located closer to the STW at this time may see a higher concentration than sites further downstream. This is because the downstream sites will be subject to a dilution due to the continuing rising volumes of water, as seen on the rising limb of the storm hydroFigure. As during sampling there were no storm events and precipitation was low, this could explain why distance was not a significant factor when considering the relationships between distance from STW and FIO concentration.

5.5 Antecedent weather conditions
The results indicate that there is a negative relationship between the amount of precipitation and how long prior to sampling it fell, on the concentration of FIOs. Generally, when the amount of precipitation that fell in the different time periods prior to sampling was higher, the concentration of the indicators was lower. This could be attributed to an initial dilution effect of the levels of precipitation on the organisms. As
none of the samples were collected either during or just after a storm event, then the probable flushing effects, and therefore increases in concentrations of FIOs, were not seen. This pattern appears consistent with elements, such as phosphates and nitrates, that have an initial dilution when precipitation falls and then, if the precipitation is prolonged, the concentration is seen to increase due to the increased transmission from the land.

The volumes of precipitation that occurred during the sampling period indicate that low volumes of rainfall in the 7 and 14 days prior to sampling do not significantly impact on the concentrations of FIOs. Only the volume of rainfall that fell in the 30 days prior to sampling in May significantly affected the concentration of the TC.

The data was varied and this, in part, could be attributed to the variety of weather conditions experienced during the sampling. None of the samples have been collected during a ‘typical’ year. Summer 2011 was unusually wet followed by a dry winter in 2011/2012. The sampling in summer 2013 has been conducted during dryer conditions than average, when comparing rainfall to the averages. The lack of long term record for FIO concentrations in the Wensum catchment makes it difficult to understand how the concentrations vary over time, and what levels are considered ‘normal’. As has been seen, the land use surrounding the sampling site has an impact upon the concentration. The land use, particularly in the sites within agricultural land, is strongly related and reliant upon the weather conditions.

What has been noted from the weather data is that when 9.1mm of precipitation fell the day before sampling in July 2011, this led to a decrease in the average TC concentration and an increase in the EC and FC concentrations from the previous month’s average. The day before sampling in December 2011, there was 8.1mm of rain which produced different concentrations from those seen in July, TC was higher than EC which was only 28.154 MPN/100ml compared to 401.949 MPN/100ml in July. As no data is available for November 2011, it cannot be seen how this has changed. From this basic analysis it is clear that simply having a higher volume of rainfall in the days before sampling will not produce the same concentrations as expected. Further factors are involved which would link back to land use at that time as well as longer term weather conditions.

From 2013 data, when the precipitation was low, concentrations of FIOs remained high. This again highlights that high concentrations of FIOs are not simply due to high
volumes of precipitation having a flushing effect and increasing transport to the river channel. The background inputs in the Wensum appear to be dominant, particularly during the low flows which were seen during sampling. This is consistent with Oliver et al. (2009a) where during low flows, washing of dairy machinery twice daily still provided an influx of the FIO even when there was an absence of rainfall to transport the organism to the river. Low flows also reduce the dilution effect that higher flows would produce. The results indicate that during times lower precipitation and therefore lower flows, the concentrations of the FIOs remain high, which in part could be attributed to the lack of dilution occurring.

5.6 Change from previous studies
From an initial examination of the data, from all sampling periods in this and previous studies, it was apparent that there had been changes in the concentrations recorded across the catchment. The ANOVA test only examined the averages for each indicator in each sampling month to identify where the significant changes had occurred. This was used then to focus down and undertake a visual inspection of the data to see which sampling sites had seen the largest differences in concentrations between the months identified.

5.6.1 Total coliforms
When looking at a comparison between the summer sampling periods, July 2011 and July 2013 had a mean difference of 866.243 MPN/100ml. The site with the greatest difference was site 7, Langor Drain, with an increase from 9.87 MPN/100ml in 2011 to 1011.2 MPN/100ml in 2013. This site was located adjacent to a motor garage and there was evidence of people having dumped rubbish and waste on the river banks. As the site is situated next to a main road and motor garage, the land use is unlikely to have vastly changed since 2011, it is possible the nature or volume of the rubbish being dumped may have changed but this is unlikely to account for the significant increase in TC concentration. As FIOs originate from warm blooded mammals, if the rubbish was accountable for the marked increase in concentration, it would have to either be contaminated with or be of faecal matter origin. From visual observations of the waste, it was seen to be mainly household waste e.g. old carpets. It is therefore extremely unlikely that there would have been a large enough amount of faecal contamination, if any at all, to be responsible for the increase in concentration at this site.
A significant difference was also noted between December 2011 and June 2013, winter and summer. The mean difference was 207.195 MPN/100ml. The largest increase was at site 4, the River Wensum at Whissonsett, where it increased from 178.5 MPN/100ml to 960.6 MPN/100ml. This stream is groundwater fed so it is likely that in December, when the water table would have been higher after the wet summer period, the contribution from the groundwater would make up a larger proportion of the total volume in the channel, so the TC would be diluted. During the summer when the stream flows are lower, the TC would be more concentrated in the smaller flows and when the groundwater contribution is less so there is less dilution from water which would have fewer FIOs.

5.6.2 Faecal coliforms
There were fewer statistically different months when examining FC compared with TC, but the results still demonstrate some fluctuations. When comparing the summer months of June 2011 and June 2013, the two means had a difference of 305.248 MPN/100ml. The site with the largest difference in FC was 16, Blackwater, Alderford. The FC increased from 6.32 MPN/100ml in June 2011 to 328.32 MPN/100ml in June 2013. From the field observations, it was seen that the site was located next to agricultural land which was used for grassland in June 2013. There was no evidence of livestock in the field during sampling but, as it was grassland, it is possible it was used for livestock previously. This could explain the increase in FC from 2011 to 2013. If the land use had changed from arable to pastoral, then the increase in animal faeces in the field adjoining the river would explain the higher concentration of FC found during sampling in 2013.

When comparing the winter and summer months of February 2012 and June 2013, the difference in the means was 246.922 MPN/100ml. The site with the greatest difference was site 9, Penny Spot Beck, Elsing, which saw a change from 47.9 MPN/100ml in Feb 2012 to 1011.2 MPN/100ml in June 2013. When comparing the rainfall in the previous 30 days, both sites received similar of between 20-30mm so it is unlikely that the precipitation was a contributing factor. The observations conducted during the sampling process showed that the land adjacent to the sampling point was pastoral grassland. It is again possible that livestock used the field prior to sampling in June, and this is why there was such a large increase from February, when livestock may have been housed indoors for the winter period. By February time, any faecal organisms that had built up in the soil would already have died or been transported into the river, thus depleting the store. Then, after the winter
when the livestock would have returned to the fields for grazing, the stores would have built up and increased the number of organisms transported into the river.

5.6.3 *E. coli*

EC had the fewest statistical differences between sampling months, indicating that it is a less variable organism in the Wensum. There was a significant difference identified between the mean concentration of EC in June 2011 and July 2013. The difference between the means was 414.451 MPN/100ml, with the means of the sites generally increasing from June 2011 to July 2013. The site that exhibited the largest difference actually had a decrease in the EC concentration from 1299.65 MPN/100ml in June 2011 to 298.7 MPN/100ml in July 2013. This was at site 13, the Wensum in Fakenham. The site is located in an urban area so it is possible that a change in land use in the area could be responsible for the decrease in EC concentration. For example a change in a discharge permit may mean less effluent is being discharged into the river which would cause the decrease. As the site is also downstream of three STW, their activity may have also changed which would contribute to the change in EC concentration.

When comparing summer and winter concentrations, there was a significant change between January 2012 and July 2013. There was generally an increase in concentrations from January to July with the mean difference being 236.632 MPN/100ml. The site with the greatest difference was site 10, Blackwater at Whitwell, where there was an increase from 68.9 MPN/100ml to 1011.2 MPN/100ml. It was noted that there were horses in a field upstream from the site so this could partly explain the increase in concentration. If the horses were stabled more during the winter months, there would be less faecal matter in the field, so a lower concentration seen than in the summer when it is likely they were spending increased amounts of time in the fields, grazing.

It can be seen from this overview analysis of how the concentrations in the catchment has changed over time, that there is not one seasonal pattern for the organisms. Largely, the significant changes show that the concentrations of the organisms have increased from the first sampling period in 2011 to 2013. There have been some months where decreases in concentrations have occurred but these were not statistically significant. A range of factors are therefore thought to be important in contributing to the overall concentration of FIOs in the Wensum, such as climatic conditions, land use and human activity within the catchment. This is consistent with
work done by Oliver et al. (2009b) and Dorner et al. (2007) who acknowledge that there are a large number of sources of FIOs in catchments and these sources vary both in space and time, as has again been seen in this and previous studies.
Chapter 6: Conclusion

6.1 Conclusion

It can be seen from this study that FIOs are of great importance in a catchment and understanding the concentrations and how they vary can aid with determining the overall ‘health’ of the catchment.

With regards to the specific objectives, they can be summarised as follows:

- Objective 1 was met as the concentration at each point was measured 3 times at monthly intervals. This was then the basis for the further analysis.

- Objective 2 was to establish if there was a statistical relationship between distance from an STW and concentration. The results indicated and were shown statistically to only be significant if there was a STW present upstream, the distance was found to not have an effect on the concentration.

- Objective 3, to determine the effect of a storm event upon concentrations was not possible to achieve. This was due to there being no storm events over the sampling period, examination of the rainfall data prior to sampling indicated that the timing during the month when low volumes of precipitation fall, does not usually have a significant effect on the concentration of the FIO.

- Objective 4, to determine if there were any other factors in the catchment affecting the concentration of the FIOs was a more difficult one to complete. It was seen that the factors that affect the concentrations are wide ranging including land use, weather conditions and STW presence. These vary seasonally so one factor may be more dominant at one time of the year than another. It was seen that an amalgamation of factors are at work in the dynamic environment that is the Wensum catchment.

- Objective 5, the comparison of results with other studies has shown that there have been some significant differences in concentrations. This was attributed to a range of factors including land use changes, changes in livestock grazing and seasonal variations.
This study has therefore highlighted that FIO concentrations are complex and cannot easily be predicted. Whilst it is positive that none of the sites exceeded the legal limits for the BWD, a large number did exceed the recommended guidelines of what is ideal for health and ecological standards. This indicates that to reduce the potential impact the catchment is having on the coastal waters, further work is needed in the catchment to reduce the faecal inputs. As parts of the Wensum are also used as a source of drinking water, keeping the concentrations of the FIOs low will aid with the treatment of the water by lessening how much the water is needed to be treated.

Continuing the monitoring and study of the FIOs in the Wensum catchment will ensure there is a greater understanding of how the FIOs respond to changes in the catchment. This will not only help aid the Wensum DTC’s work but will also aid catchments elsewhere as FIOs are present in all of them.
Chapter 7: Limitations & future study

7.1 Limitations & future study

A limitation to this study, and the reason one of the aims was not able to be completed, was that sampling was not able to be undertaken during a storm event. Originally, the fieldwork was to be started March-April time, which would have given an increased window of time for sampling, and therefore more chance of catching a storm event. This was not possible due to the risk assessment approval taking longer than initially anticipated. The sampling could therefore not begin until the go ahead was received and approval was given in May. During the sampling period, there were no significant storm events so the catchments response with regard to FIO concentration during one of these events was not able to be recorded. Completing the study over the winter months would, in theory lead to increased opportunity for sampling during a storm event. This would then provide important information for the catchment response and also how this might impact on the receiving waters further downstream and at the coast, in particular waters covered by the Bathing Waters Directive and Great Yarmouth.

Another limitation with regards to the actual data output element of the study is the maximum MPN concentration the Quanti Trays are able to detect. This is particularly prevalent when considering the results for Total Coliforms collected in July, as the majority of the sites sampled were recorded as having the highest measurable concentration. This therefore cannot be used as a true reflection as to the concentration of the FIOs at those sites, only an indication that the levels are high and used as a comparison with the results from the other sites. For future investigation, in order to gain an accurate figure of the concentration of FIOs at the sites that exhibited that maximum concentration, a different method could be employed that did not and an upper limit on what detection is possible. This would allow a greater understanding as to the actual concentrations at these sites and if and how far they deviated from the permissible concentrations written into legislation.

The use of a grab sample in one point in the river, is also restrictive as to if the sample is representative of the whole water column at that point. However, due to the number of sampling points across the catchment, taking multiple samples at each point was not practical from a time perspective when the samples had a short optimum time period in which they had to be processed when back in the lab. The
results should therefore only be used as an indication as to the FIOs at each sampling point and aid in comparison across the catchment to see where the peaks and troughs are in the concentrations. Further work could be undertaken to see how the FIOs vary in the cross sectional profile of the channel, perhaps at fewer sampling points to get a deeper understanding.

Another area for further study would be to continue sampling down the River Wensum, to see how the concentrations of the FIOs vary downstream. Then continuing this to coastal waters and comparing the results with those at the coast would allow a greater understanding how activity upstream in the Wensum catchment is impacting upon the bathing waters in the Great Yarmouth area. Considering that the guideline maximums for FIOs are exceeded at the majority of the sampling sites in the Wensum, this could have a considerable affect downstream not only for compliance with the Bathing Waters Directive, but also when awards such as Blue Flag beaches are considered which may have a knock on effect with the tourism industry and local economy.
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### Appendix A

Results from all sampling sites & days.

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