Cranfield University

Natalie Sarah Teresa Lamb

Controlling *Cryptosporidium* in Vulnerable Catchments used for Drinking Water Supply

Cranfield Water Science Institute

MSc Thesis

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Supervisor: Dr. Peter Jarvis

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List of Nomenclature

AISE- The International Association for Soaps, Detergents and Maintenance Products

BOD- Biochemical Oxygen Demand

COD- Chemical Oxygen Demand

Cumecs- Cubic Metres per Second (cumecs)

DAF- Dissolved Air Flotation

DAPI- 4’6-diamidino-2-phenylindole

EPA- Environment Protection Agency

GAC- Granular Activated Carbon

GACF- Granular Activated Carbon Filter

HPA- Health Protection Agency

NHS- National Health Service

PHE- Public Health England

STW- Sewage Treatment Works

WTW- Water Treatment Works

Abstract

The overall aim of this project was to assess the potential to control *Cryptosporidium* in vulnerable catchments, using the Louth area in the Anglian Water region as a case study. This was completed through a literature review, a critical analysis of existing data and a four month sampling programme.

It was found that *Cryptosporidium* oocysts which could potentially contaminate humans who get their drinking water from the downstream Covenham Water Treatment Works (WTW), were originating from Louth Sewage Treatment Works (STW). The oocysts from human or sheep hosts were entering Louth STW in the crude sewage. The STW collects and builds up the oocysts in the sludge holding tanks. When dewatering occurs, the sludge holding tanks release a large amount of *Cryptosporidium* into the STW, which in turn passes through the rest of the works, relatively untreated, into Louth Canal. The oocysts in Louth Canal were abstracted from, and added to, Covenham Reservoir. The oocysts from the reservoir occasionally passed through to Covenham WTW, where there is potential for human contamination.

The literature review identified that treatment processes at Louth STW were less effective at oocyst removal than other research has indicated. Trickling filters and humus tanks removed a lower percentage of *Cryptosporidium* oocysts (17% and 44%) than literature suggested (91%). Overall, it appeared that during the sampling period, the works added 18 oocysts/l, when the influent and the final effluent of the works were compared. This is because of the episodic nature of oocysts and the way that they were being recycled in the works. Oocysts entering Louth STW seemed to be being concentrated in the sludge holding tanks and then recirculated in the sludge supernatant from the dewatering process, back to the primary settlement tanks. This meant that primary settlement at Louth STW was not as effective (-1299%) as literature suggests (54% removal) because of the additional input of oocysts to this treatment process. The concentration increase of *Cryptosporidium* oocysts within primary settlement tanks has not been observed previously. Not only did this appear at Louth STW, but also at Stamford STW, which was sampled as an additional STW.

Because of this research, the operation and monitoring of the sludge at Louth STW is to be further investigated. Additional treatment options are to be considered at the STW and WTW, such as sand filtration at Louth STW and the installation of a permanent UV system or ultrafiltration at Covenham WTW.

Further work would be to complete a more in depth analysis of more STWs to determine whether other sites have the same potential to accumulate and release *Cryptosporidium*.

Another area for further study would be to look at the different combinations of treatments that STW use. This would help us understand why there are discrepancies in *Cryptosporidium* removal rates between sites. This would help to determine the most effective combination of treatment methods for the removal of *Cryptosporidium* during wastewater treatment.

Acknowledgements

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Executive Summary

Cryptosporidium is a widespread protozoan of humans. When it is ingested in oocyst form through contaminated food or water, the host can become infected by *Cryptosporidium* which leads to Cryptosporidiosis, an intestinal condition. In particular, it is a challenge to the water industry because the protozoa are resilient and difficult to treat, with chlorine being ineffective. This means that the potential is there for *Cryptosporidium* oocysts to be able to survive through the water treatment process and pass through into peoples' homes. An example of this was that of a *Cryptosporidium* outbreak in 2008 in Anglian Water, which infected 22 people with cryptosporidiosis through their drinking water (DWI, 2009).

On 03/02/10 *Cryptosporidium* was detected in the effluent of Covenham Water Treatment Works (WTW). Even though oocysts were only detected once, this one instance meant that the 120,000 people who had their drinking water supplied from Covenham WTW were at risk of being infected with *Cryptosporidium*, resulting in Cryptosporidiosis. After this event, *Cryptosporidium* was monitored more closely and it was found that the source of abstraction, Louth Canal, had a high prevalence of *Cryptosporidium* and that Covenham Reservoir, the reservoir that holds the abstracted water before treatment, also had heightened levels of *Cryptosporidium* at times.

The host of the oocysts is most likely to be human (from domestic sources) or sheep in origin, identified from typing results. The Louth area does not have an unusually high number of human cases of cryptosporidiosis or high number of anti-diarrhoeal sales for a population of its size or location. Trade effluent is unlikely to play a part. Sheep are common in the catchment but diagnosis is difficult and rarely completed.

It was found that the oocysts which could potentially contaminate humans who get their drinking water from Covenham WTW were originating from Louth Sewage Treatment Works (STW). The oocysts from human or sheep hosts are entering Louth STW in the crude sewage. The STW collects and builds up the oocysts in the sludge holding tanks. When dewatering happens, the sludge holding tanks release a large amount of *Cryptosporidium* into the works, which passes through it relatively untreated into Louth Canal. The oocysts in Louth Canal are abstracted from and added

to Covenham Reservoir. The oocysts from the reservoir are sometimes passed through to Covenham WTW, where there is potential for human contamination.

The literature review found the following to be the most effective treatment types for *Cryptosporidium*: Ultrafiltration, Microfiltration, UV, Dissolved Air Flotation + Filtration, Slow Sand Filtration, Chlorine Dioxide + Free Chlorine, DAF, Ozone, Tertiary Treatment, Coagulation and Flocculation, Chlorine Dioxide, Final Settlement and Sedimentation.

The literature review also shows that treatment processes at Louth STW are less effective than other research has suggested, at oocyst removal. Trickling filters and humus tanks removed a lesser percentage of *Cryptosporidium* oocysts (17% and 44%) than literature suggested (91%). Overall, it appeared that during the sampling period, the works added 18 oocysts/l, when the influent and the final effluent of the works were compared. This is because of the episodic nature of oocysts and the way that they were being recycled in the works. Oocysts entering Louth STW seemed to be being concentrated in the sludge holding tanks and then recirculated in the sludge supernatant from the dewatering process, back to the primary settlement tanks. This meant that primary settlement at Louth STW was not as effective (-1299%) as literature suggested (54% removal) because of the additional input of oocysts to this treatment method. Therefore no accurate value on this processes' removal rate was found. The increase of *Cryptosporidium* oocysts within primary settlement tanks has not been observed previously. Not only did this appear at Louth STW, but also at Stamford STW, which was sampled as an additional STW.

Because of the research, an alteration in the methodology and monitoring of the sludge holding process is to be completed. Further treatment options are to be considered, such as sand filtration at Louth STW, the installation of a permanent UV system or ultrafiltration at Covenham WTW.

Interesting further work would be to complete a more in depth analysis of the chosen control works, with an additional two sampling programmes. This would potentially explain why Stamford and Louth STW had similar *Cryptosporidium* prevalences but Stowmarket was able to remove the *Cryptosporidium* it encountered more effectively.

1 Introduction

1. 1 An Overview of *Cryptosporidium*

Cryptosporidium is a protozoan that is found in the intestines of many mammals, reptiles and birds. It has a complex life cycle, which can consist of both sexual and asexual stages but to reproduce, the protozoan must be within a host organism.

Cryptosporidium enters hosts by being ingested in oocyst form through contaminated food or water. The host can then become infected by *Cryptosporidium* which leads to cryptosporidiosis, an intestinal condition. Diarrhoea is the main symptom of cryptosporidiosis and other symptoms include abdominal discomfort, dehydration, nausea and vomiting (DuPont, et al., 1995).

Certain strains of *Cryptosporidium* are a public health concern due to the infectivity of the parasite. Ingesting only five oocysts can cause the clinical infection to present itself in humans. Once infected, the host can shed 10 million oocysts per gram of faeces. This oocyst excretion can occur for more than 10 days and the symptoms will occur longer in individuals with compromised immune systems. This data demonstrates the extent to which humans are susceptible to *Cryptosporidium*.

Cryptosporidium poses a challenge for the water industry. The discharges of sewage treatment works (STW), as well as the run off from farmland and the environment, results in *Cryptosporidium* entering bodies of water, such as reservoirs and rivers, that are abstracted from for clean water treatment works (WTW). Rose et al. (1991) completed a survey of 257 surface water samples from 17 states in America. They found that *Cryptosporidium* was detected more frequently in waters that received sewage and agricultural discharges, rather than pristine waters.

Cryptosporidium is resilient and difficult to treat using conventional water and wastewater treatment processes because the oocyst phase of the organism is highly resistant to chlorine. Oocysts are small cylindrical cells housed within a protective multi-layered cell wall. Disinfection is the main way by which micro-organisms are killed and controlled in drinking water. This means there is potential for

Cryptosporidium oocysts to be able to survive through the water treatment process and pass through into peoples' homes. An example of this was that of a *Cryptosporidium* outbreak in 2008 in Anglian Water, which infected 22 people with cryptosporidiosis through their drinking water (DWI, 2009).

1. 2 Importance of the Research

The research of Rose et al. (1991) stated that *Cryptosporidium* was detected more frequently in waters that received sewage discharges, such as the final effluent discharges from STW. This is an issue when WTW abstract downstream of these flows. This research will explore this in more detail using a case study site in the Anglian Water region.

Anglian Water is a water company that operates in the East of England. The area of particular interest for this study on *Cryptosporidium* is that of Louth, a small market town in Lincolnshire. Louth has an interesting arrangement of water and wastewater treatment systems consisting of Louth STW, Louth Canal, Covenham Reservoir and Covenham WTW. Louth STW discharges into Louth Canal. Approximately 12km downstream is the intake to Covenham Reservoir, which feeds into Covenham WTW [Figure 1].

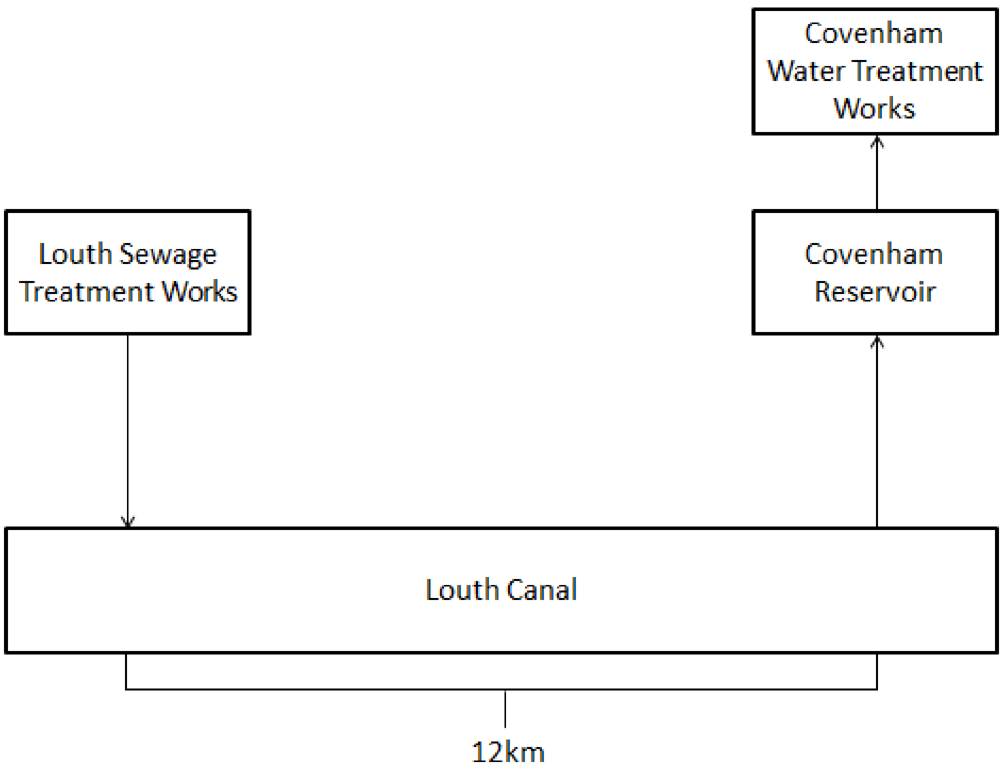

Figure 1 A schematic of the water and wastewater treatment assets in Louth and the relationship between them

In 2009, heightened levels of *Cryptosporidium* were found in Covenham Reservoir [Figure 2]. This was of concern because *Cryptosporidium* can pass through water treatment processes. An example of this has been seen at Covenham WTW. The WTW consists of in-line strainers, primary ozone, secondary ozone, microstrainers, rapid gravity filters and UV. On one occasion, *Cryptosporidium* was detected in drinking water on 03/02/10. Even though oocysts were detected only once, this instance meant that the 120,000 people who had their drinking water supplied from Covenham WTW were at risk of being infected with *Cryptosporidium*, which would lead to cryptosporidiosis. After this event, *Cryptosporidium* was monitored more closely and it was found that Louth Canal had a high prevalence of *Cryptosporidium*.

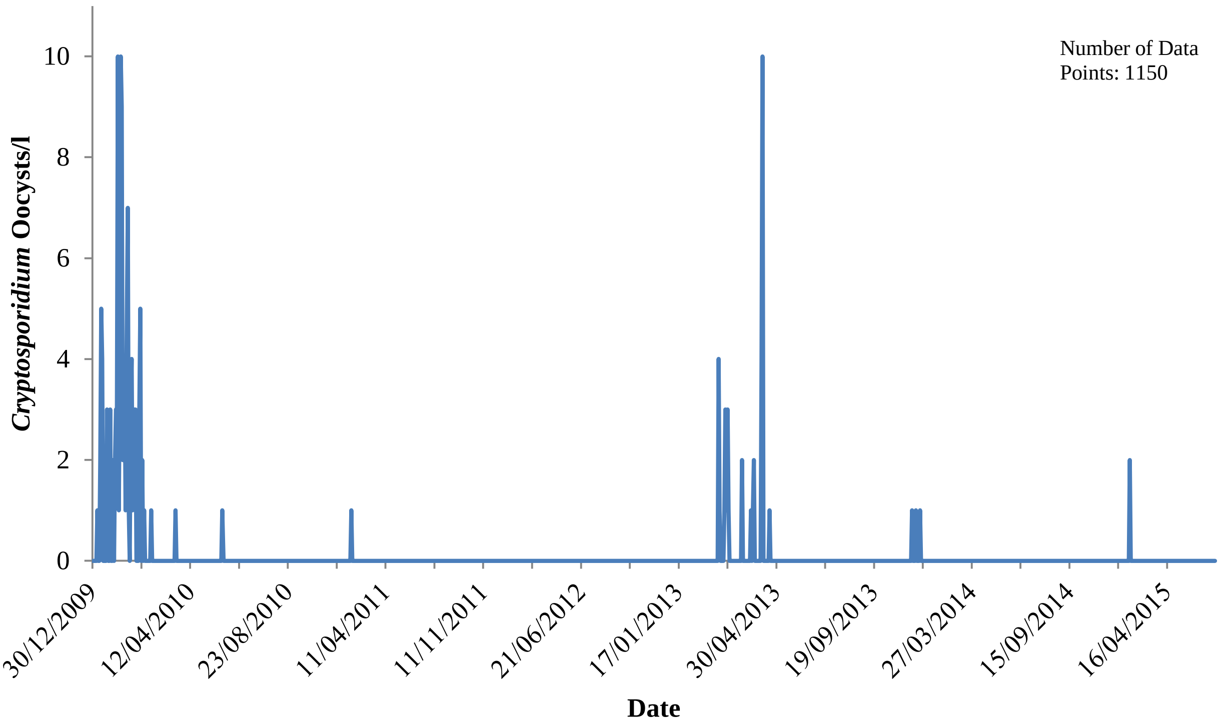

Figure 2* Cryptosporidium *oocysts/l found in the Covenham Reservoir2009-2014

Data for this figure extracted from: Anglian Water (2015)

The *Cryptosporidium* issue at Covenham caused additional problems because its presence resulted in the abstraction of water from Louth Canal ceasing, in an attempt to prevent the organism entering Covenham Reservoir. This lowers the reservoir levels, meaning that unless there is a large amount of precipitation, the future water supply to Covenham is at risk. An example of this was during the summer of 2014, when there was no abstraction for five weeks and reservoir water levels became very low. Furthermore *Cryptosporidium* causes other water industry problems to be amplified. Not only is abstraction at Louth Canal cut off when 3 oocysts/l are detected, but also when metaldehyde is present in the water. The result of this is that abstraction is frequently stopped, resulting in lowered water reserves in the reservoir.

The *Cryptosporidium* at Covenham Treatment Works has currently been even more an area of concern because it will now also be supplying the area of Boston, increasing the amount of water used by 15.6Ml/day. Therefore not only are the 120,000 people who currently get their water from Covenham at risk, but also the 64,000 extra people which the Covenham-Boston Network will now be supplying.

Consequently, the Louth area in which Anglian Water operates has a recurring *Cryptosporidium* prevalence which was in need of investigation, hence why this project was prioritised.

1. 3 Research Questions, Aims and Objectives

The overall aim of this project was to assess the potential to control *Cryptosporidium* in vulnerable catchments, using the Louth area as a case study. If the source of the *Cryptosporidium* could be found at that specific site, the most effective and feasible solution to prevent outbreaks could be decided on.

This aim was split up into the following objectives:

- To determine the possible sources of *Cryptosporidium* and research its progress through the water treatment and wastewater treatment processes
- Analyse data from *Cryptosporidium* and other water quality samples which have been collected by Anglian Water since 2009
- Compare data on *Cryptosporidium* from similar sites to determine whether the same issues are observed elsewhere
- Propose solutions to control *Cryptosporidium* from entering the sewage water supply

2 Literature Review

The aim of this literature review was to collate research which has tested the *Cryptosporidium* removal efficiencies of different treatment processes in both STW and WTW. The reason for doing this was to identify the most and least useful treatment methods for oocyst inactivation and removal.

2. 1 The Removal and Progress of *Cryptosporidium* through Sewage Treatment Works

There appears to be high variability in the removal efficiency of *Cryptosporidium* at different STW, with 10-90% removal recorded by Lim et al. (2004). This large variation is not only due to the differences between different sites in sampling and processing and the properties of the cysts themselves but also because it is less of a concern, resulting in a lack of urgency surrounding STW and *Cryptosporidium.* People do not directly consume effluent from STW and so *Cryptosporidium* is less of a treatment priority at STW, since infection is less probable.

Cryptosporidium in clean water is a major concern for the water industry. Cryptosporidiosis is an unpleasant and virulent infection, meaning that mention of it in the media can cause apprehension in the public. Furthermore *Cryptosporidium* events at WTW are highly publicised. One example of this was the *Cryptosporidium* found in Anglian Water customers' drinking water in 2008 (BBC News, 2008). A further example is the United Utilities *Cryptosporidium* outbreak in 2015 (United Utilities, 2015). Similar focus is not seen in STW because there is not a direct connection between contamination and customers. This means that *Cryptosporidium* levels are not regulated at STW, staff are not educated about the organism and the technology available is either not present or is not as developed (for example WTW have *Cryptosporidium* cartridges to continuously monitor the *Cryptosporidium* present but STW have no equivalent equipment). There is therefore a paucity of information about the fate of *Cryptosporidium* through a STW. The limited information available has been reviewed here by analysing research carried out at the different stages of the sewage treatment process (including preliminary treatment, primary treatment, secondary treatment, final settlement and tertiary treatment), illustrated by Figure 3.

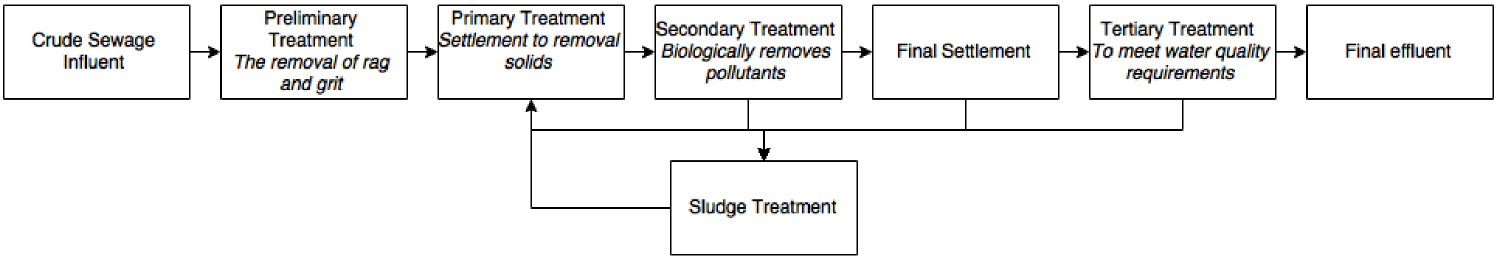

Figure 3 A schematic diagram of general sewage treatment works processes

2. 1. 1 Preliminary Treatment

Preliminary treatment is the process where rag (such as plastic, toilet paper and wood) and grit are removed from the crude sewage, [Figure 4]. Rag must be removed so it does not cause downstream blockage or damage. It is usually achieved using a 6mm screen. Grit is usually removed by settling in a grit chamber with a flow rate of ~0.3m/s. If *Cryptosporidium* was removed during this process, it would be through physical separation of particles, especially when the oocysts are attached to larger particles in the water.

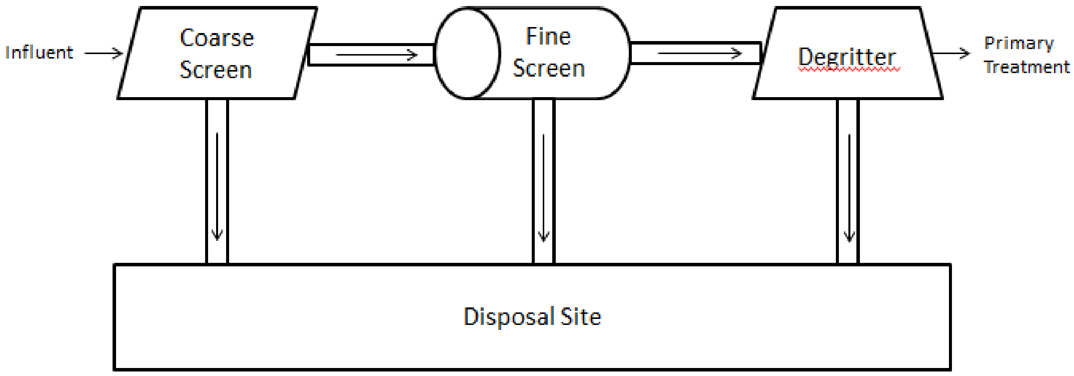

Figure 4 A schematic diagram of the preliminary treatment process

Zhang et al. (2008) investigated *Cryptosporidium* removal across a STW in Beijing. They found that the preliminary treatment process removed few protozoans. The study stated that the removal efficiencies for *Cryptosporidium* were 26% in this part of the process, when other processes had higher removal rates (for example secondary sedimentation removed 97% of oocysts). It was clear that oocyst viability did decrease a little, however this could have been due to causes not linked to the treatment.

It is unsurprising that preliminary treatment does not significantly reduce the viability of *Cryptosporidium* oocysts. This process generally only involves physical removal apparatus, with coarse screens designed to remove materials of 6mm and fine screens for 1.5-6mm. *Cryptosporidium* oocysts, however, are only 4-6µm in diameter, so they easily enter the works through the screens, unless they are attached to larger solids.

2. 1. 2 Primary Settlement

Primary settlement is the process which removes the settleable organic solids from wastewater [Figure 5]. If this process removed *Cryptosporidium* oocysts, it would be due to the oocysts settling to the bottom of the tank, a physical separation method, especially when the oocysts are attached to larger more heavy particles in the water.

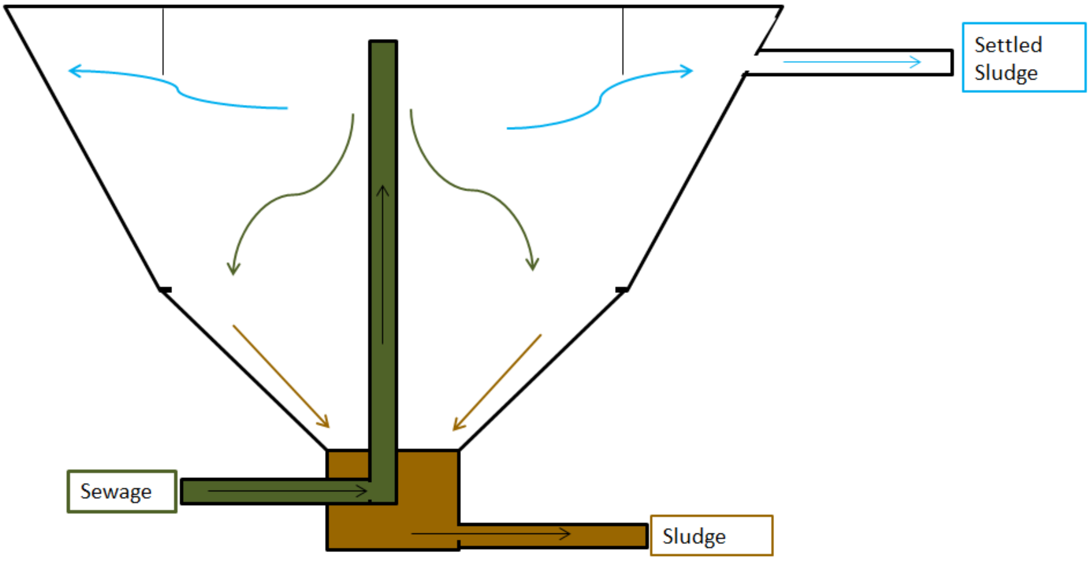

Figure 5 A diagram of the primary settlement process

The ability of *Cryptosporidium* to settle can vary depending on environmental conditions. *Cryptosporidium* normally has a low hydrophobicity, but this is increased in suspensions with high conductivity (Bonatti & Franco, 2014). High conductivity occurs with the presence of inorganic dissolved solids, such as chloride, nitrate, sulfate, and phosphate anions, sodium, magnesium, calcium, iron, and aluminum cations. Low conductivity occurs when there is a low ion content and in the presence of uncharged compounds like organics such as oils, phenol, alcohol, and sugar (United States Environmental Protection Agency, 2015). This means that depending on what is dissolved in the water, *Cryptosporidium* oocysts can behave differently.

There is some evidence to suggest that primary settlement has little effect on *Cryptosporidium.* The research of Bonatti and Franco (2014) suggests that *Cryptosporidium* is less likely to be removed by sedimentation, since the adhesion of oocysts to sediment is prevented. This reduced settlement is due to the oocysts being negatively charged, causing the repulsive electrostatic forces that increase the load of inorganic particles to become more negative also.

Other research disagrees, saying that *Cryptosporidium* is effectively settled during the primary settlement process. Medema et al. (1998) showed how *Cryptosporidium* efficiently attaches to biological particles in effluent. After a few minutes of mixing, 30% of cysts had attached to suspended particles and after 24 hours this figure had increased to 75%. Searcy et al. (2005), too, stated how *Cryptosporidium* is likely to attach to particles and settle out. The research states that oocysts were not likely to remain suspended in natural aquatic systems with low-turbulence, they are likely to be removed from the water column because of attachment to the suspended matter. However, although the oocysts settle out faster when attached to particles than alone, it still takes longer than the settlement process required by the other matter in the water.

This difference in the ability of *Cryptosporidium* to attach to suspended particles means that there is also discrepancy about whether primary settlement is an effective treatment type for *Cryptosporidium*. Robertson et al. (2000) found that primary settlement alone was relatively inefficient at removing *Cryptosporidium* cysts. When two different works were compared, the works which had primary settlement had no difference in numbers of *Cryptosporidium* oocyst removal as the works that did not have it. Whitmore and Robertson (1995) found similar low rates of *Cryptosporidium* removal in a laboratory simulation of primary sedimentation at a sedimentation velocity of 2.2-2.8cm/h. Their work, therefore, suggests that *Cryptosporidium* is unlikely to be removed at this stage in the sewage treatment process, since primary sedimentation tanks are designed to have velocities of 0.5-1.5m/h. Flow rates do, however, depend on the time of day and the weather.

Even if settlement is not the most effective treatment method, it may make other processes more effective. Stadterman et al. (1995) found that trickling filters removed 37% of encountered oocysts but when the process was combined with sedimentation, a 50% removal rate was achieved.

Other studies found that *Cryptosporidium* is removed successfully at the Primary Settlement stage. One example is the study of a STW in Beijing completed by Zhang et al. (2008), who found that the primary settlement process resulted in a 24.7%

removal of oocysts (from 238 oocysts/l in untreated wastewater to 179 oocysts/l in primary sedimentation), although secondary treatment was more successful (97% removal from 179 to 6 oocysts/l). Stadterman et al. (1995) found similar successful results in a continuous flow model. There was an oocyst removal rate of 83% during primary settlement at a flow rate of 24.5 l/day. This figure is greater than many secondary treatment methods (for example 55% with settled activated sludge, 62% with raw settled sludge, 37% with trickling filters and 40% with biodiscs) but secondary settlement caused a 90.7% removal. The fact that this research was completed in a model, rather than a works, may be why such high removals were seen.

A further point to consider is that since primary settlement is one of the first methods of treatment at a STW, so they encounter the greatest *Cryptosporidium* load. Consequently the percentage removal of oocysts at different stages in a works may are not directly comparable with one another and the effectivity of primary settlement may be underestimated.

The existing literature therefore appears to provide conflicting evidence as to whether primary settlement is or is not an effective treatment type. This could be because of what compounds are dissolved in the water at each different test site or it could be because of how the works is set up and operated and where the samples have been taken. As can be seen by Figure 3, STW generally feed their returned liquors back to the head of the works, at the primary settlement tanks. If *Cryptosporidium* oocysts are accumulated in the sludge holding tanks this could mean oocysts are being pumped back into this process, making it appear less effective. The addition of this sludge may also affect the rate of settling and the composition of the water, which in turn affects settlement.

2. 1. 3 Secondary Treatment

This stage in the process removes unwanted pollutants (such as ammonia and organisms that cause biochemical oxygen demand) biologically, usually in the presence of oxygen and a food source. Secondary treatment includes biological (trickling) filters, rotating and submerged biological contractors, activated sludge and biological aerated filters.

Stott (2003) described that the combination of primary and secondary treatment significantly increases protozoa removal efficiencies, when primary sedimentation alone is not effective. There have been numerous other studies that detail the effectiveness of the secondary treatment in *Cryptosporidium* cyst removal.

Zhang et al. (2008) reported that the secondary process removed 97% of oocysts (from the following oocyst concentration in cysts/l at 6 STW: untreated wastewater 238, primary sedimentation 179, secondary sedimentation 6, flocculation-sedimentation 1, sand filtration effluent 0.3). As can be seen, secondary treatment was the most successful at reducing *Cryptosporidium.*

Similar results were also found by Montemayor et al. (2005). This study found that secondary treatment resulted in a 96% removal (from 124 oocysts/l to 3), as an average from three different STW. It is important to note, however, that tertiary treatment was the most successful, with a 99.8% *Cryptosporidium* removal rate. Furthermore those cysts that survived the process were still viable. The mean viability of cysts only reduced from 37% to 30%. Viable oocysts were additionally detected by Robertson et al. (2000), even though they found that more parasites were removed by secondary treatment (60%) than by primary settlement (40%).

Thus it seems that secondary treatment is one of the most effective methods of *Cryptosporidium* cyst removal, although cysts are able to survive the process. There are many variations in the technology that can be used at a STW for secondary treatment (such as trickling figures, biological contractors, activated sludge treatment and biological aerated filters). These different methods can have different levels of *Cryptosporidium* removal rates, as the work of Stadterman et al. (1995) shows, since activated sludge removed 55% of oocysts, trickling filters removed 37% and biodiscs removed 40%. Therefore, each individual technology needs to be assessed on its efficiency of *Cryptosporidium* oocyst removal, separately.

Trickling Filters

Trickling filters are one method of secondary treatment in STW that reduce the amount of residual organic pollution in the partially treated wastewater. They are a biological filter method which is commonly used, for example Louth STW has seven trickling filter systems. They consist of a fixed bed over which the wastewater flows, forming a biofilm on the bed, which removes organic matter from the passing water [Figure 6]. In this process, *Cryptosporidium* would be removed biologically by the microorganisms present in the media and through attachment onto the media/biofilm.

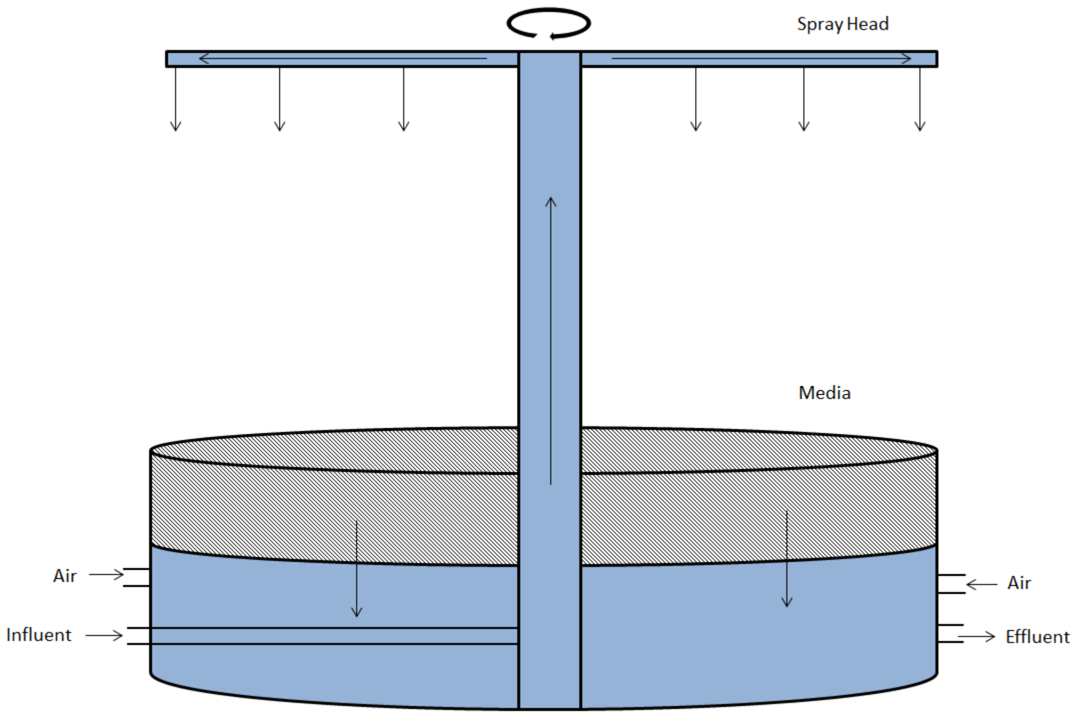

Figure 6 A diagram of the trickling filter process

There are discrepancies between different sets of results about the effectivity of this method, compared with others. One study, completed by Stadterman et al. (1995), found that, over 3.5 hours, trickling filters had an oocyst removal efficiency of 37%, or 50% following sedimentation. Activated sludge treatment, however, had a considerably higher removal rate of 55% or 92% following sedimentation. These results make it appear that trickling filters are relatively effective at reducing *Cryptosporidium* oocyst viability, but more successful secondary treatment types exist, such as activated sludge plants. This, however, is not shown by other results.

Some studies suggest that trickling filters are as equally effective as other methods of secondary treatment for *Cryptosporidium* removal. Kitajima et al. (2014) found that there were no statistically significant different reductions between two STW, where one used trickling filters and the other activated sludge. Robertson et al. (2000) reported similar findings, stating that there was no significant parasite removal difference between activated sludge treatment and trickling filters.

Results for the efficiency of *Cryptosporidium* removal in trickling filters may vary because the filter performance can be affected in different ways. The performance of trickling filters can be diminished by the following: feed sewage organic strength, distribution mechanism, ventilation system, the frequency/rate of dosing and weather/season. Even when they are functioning at their optimum, cysts detected after being treated by trickling filters have been shown to have sufficient viability to infect the mice they were inoculated into (Villacorta-Martinez de Maturana, et al., 1992). This means that such filters alone are not sufficient enough to remove *Cryptosporidium* from wastewater.

Although it is unclear whether or not trickling filters are the most effective method of cyst removal, as part of the secondary treatment, it is clear that they are an efficient method for *Cryptosporidium* removal, especially when used with sedimentation.

Activated Sludge Plants

Activated sludge plants are moving biofilm systems. The microorganisms flocculate to form a microbial mass known as activated sludge. It is a biological process which treats wastewater using air and a biological floc. It can achieve the following:

- Oxidisation of carbonaceous and nitrogenous biological matter
- Removal of: phosphate, entrained gas, dissolved and suspended material
- Production of a biological floc that can settle easily

The primary treated water is combined with organisms, creating the biological floc. The organisms found in activated sludge plants can vary between STW but includes saprophytic bacteria (such as micrococcus) and protozoans (such as rotifers and cladocera). It is these organisms that remove *Cryptosporidium* from the waters by consuming them. When the mixed liquor is formed, the surplus activated sludge is removed for further digestion and to increase the efficiency of the process. Returned activated sludge is removed from the process for a short time to be aerated and to continue back to the activated sludge process.

Neto et al. (2006) found high levels of *Cryptosporidium* removal from the activated sludge process, with a 99.7% oocyst reduction when the influent and effluent samples were compared. Villacorta-Martinez deMaturana et al. (1992), too, found the process had an 80-84% removal efficiency after 2.8 hours. Stadterman et al. (1995) also found that activated sludge treatment removed 55% or 92% *Cryptosporidium* oocysts with and without sedimentation, which was greater than the rates found in trickling filters (37-50%) or biodiscs (40-44%).

However other studies have found evidence of cysts in sludge samples. Regardless of Stadterman et al.'s (1995) findings, Kitajima et al. (2014) found that there was no statistically significant difference in *Cryptosporidium* oocyst numbers in the effluents of trickling filters and activated sludge. Furthermore, the Foundation for Water Research (1996) found cysts in activated sludge samples from five different STW on at least one occasion each, meaning that full removal was not achieved by any of the works.

Consequently there is much evidence that supports the effectiveness of activated sludge for *Cryptosporidium* removal, however there is some debate about whether other methods may be equally as successful. A further area of interest is that of combined treatment methods, which can have a synergistic effect. One such example was identified by Madore et al. (1987), who found that two plants that had sand filters after having activated sludge had the lowest number of oocysts. This would be an interesting area for further study and may explain, looking at the different combinations of treatments that STW use, why there are such discrepancies in *Cryptosporidium* removal rates between plants. This research would help to determine the most effective combination of treatment methods for *Cryptosporidium* eradication.

Biological Aerated Filters

Aeration is required to promote the oxidation of wastewaters for the organisms that complete biological processes within the STW. Aeration is provided in this treatment process by using a blower at the bed, where there is a relatively small filter media for a biomass to grow [Figure 7]. This process could remove *Cryptosporidium* oocysts by allowing them to settle out but also they can be removed biologically. Aeration provides microorganisms the oxygen they require to survive and potentially consume oocysts.

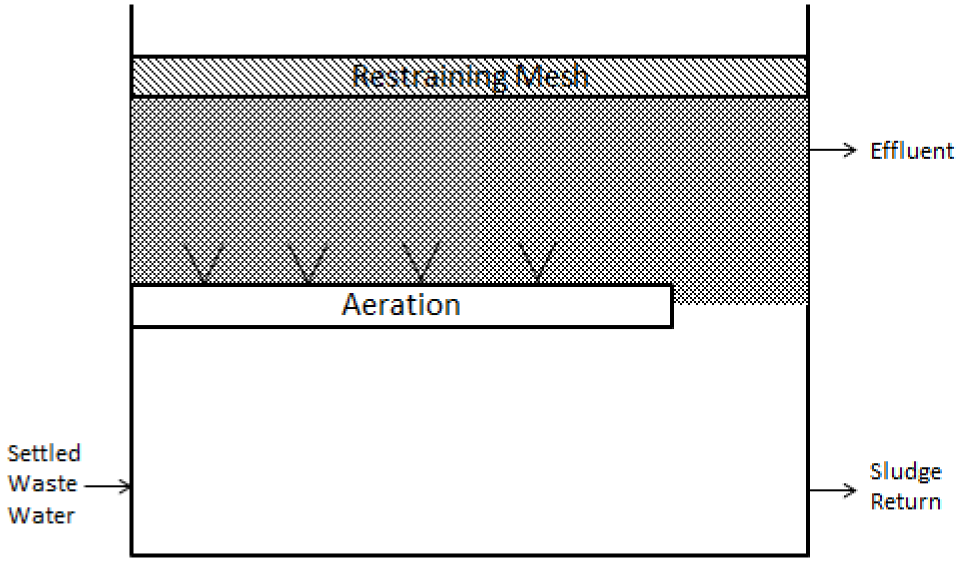

Figure 7 A diagram of the biological aerated filter process

For this process there seems to be limited specific research on *Cryptosporidium* removal. One study, completed by Hill et al. (2002), found that biological aerated filters are an effective treatment of flushed swine waste. The process was found to significantly remove enteric microbes (including: *Escherichia coli*, enterococci, somatic coliphages, and male-specific coliphages), but did not include the study of *Cryptosporidium.*

There is, however, research surrounding *Cryptosporidium,* aeration systems and the aeration technique deployed in biological aerated filters. Castro-Hermida (2008) described *Cryptosporidium* issues associated with aeration systems. They stated that aeration (BAFs in general) re-suspends the oocysts, favouring their exit in the effluent.

Further research by Lim et al. (2007) investigated the aeration methods at two different STW to see how effective they were at removing *Cryptosporidium.* They found that an extended aeration system was much more effective (73% removal rate) than the STW that employed an aerated lagoon system, at 33%. This was probably due to the way the former used fine bubbles through submerged diffusers, which has greater efficiency of oxygen transfer. The lagoon used surface aerators which do not have as high efficiency rates. This would not be a suitable method to use for average-sized STW, such as Louth STW, however, because extended aeration is less efficient and requires more energy per unit of oxidised waste. The relatively poor operating efficiency of this system is due to the way that the combined sludge used starts with a higher concentration of inert solids. This causes digestion of these solids to take more time, meaning that more energy is required to mix this waste for a longer period of time. Thus the transfer from a lagoon system to an extended system is not a practical method of aiding *Cryptosporidium* removal at larger works; it is only suitable for smaller waste loads.

To conclude, there is little specific research into *Cryptosporidium* and biological aerated filters and it would be a useful area for further work. It does seem that different aeration techniques will have an effect on the *Cryptosporidium* removal at a works, even though it may be slight.

2. 1. 4 Final Settlement

The purpose of this treatment process is to settle out the biological floc in a humus tank and produce effluent that has low organic material and suspended matter, using a methodology highly similar to primary settlement. *Cryptosporidium* oocysts can also be settled out during this process.

The effectivity of settlement has previously been discussed in this thesis, however Robertson et al. (2000) found further results. The research found that a secondary sedimentation for 3.6 hours resulted in a 91% oocysts removal, whereas primary settlement for 2 hours removed only 83%. Since the process is the same, though, this percentage difference may be due to the length of time *Cryptosporidium* oocysts are settled for.

2. 1. 5 Tertiary Treatment

This is the final step in the treatment of wastewater. It is used to ensure the final effluent is of the optimum condition for discharge into the environment. It includes: filtration, lagooning, nutrient removal, disinfection and odour control.

Montemayor et al. (2005) found that three plants which had tertiary treatment methods had removals of 99.9, 99.8 and 99.8%, when secondary treatment had removed 98, 99 and 96%. Furthermore all of the secondary effluent samples contained cysts but only three quarters of the samples had cysts after tertiary treatment. It does however seem that different treatment types have different levels of effectivity, for example Lim et al. (2007) found removal rates of 73% in extended aeration systems but 33% in aerated lagoons.

Filtration

Tufenkki et al. (2004) studied different physical straining types. They found that physical straining was an important capture mechanism for *Cryptosporidium.* Straining and physicochemical filtration were found to control the removal of *C. parvum* oocysts.

Sand filtration is used to remove residual suspended material. Filtration can also be carried out using activated carbon for adsorption to remove toxins and micropollutants. The carbon is processed to have small pores that increase its surface area for adsorption.

Enriquez et al. (1995) investigated the efficiency of tertiary sand and coal filtration for the removal of *Cryptosporidium.* They found that the *Cryptosporidium* was not affected by the process. Fu et al. (2010) also describes how sand filtration was not as successful as membrane ultrafiltration at *Cryptosporidium* removal, although there is potential that this method could get blinded too quickly for use at a STW. The work of Stott (2003) said that although oocyst concentrations were significantly reduced after sand filtration, oocysts were still detected in the final effluent and thus are not completely eradicated.

The efficiency of sand filtration was supported by Madore et al. (1987). This research found that *Cryptosporidium* cysts were more effectively removed when sand filtration was used in combination with activated sludge treatment, rather than activated sludge alone. More recent research, completed by Stott (2003), found the same results. This work showed that the combination of secondary treatment and sand filtration can reduce *Cryptosporidium* concentration by 99-99.9%. The success of sand filtration treatment was shown by Timms et al. (1995) too. They found that slow sand filtration was highly efficient and resulted in a more than 99.997% reduction in oocyst levels. This success may be due to *Cryptosporidium* attaching to debris, which then gets entrapped in the filter matrix.

Therefore sand filtration seems to be a successful method which helps *Cryptosporidium* removal, even if it does not completely eradicate it. There are, however, controlling variables such as the media used because sand grain size will affect the success of the sand filter at removing *Cryptosporidium*. Similarly the speed of filtration alters the effectiveness, for example, slow sand filtration is more successful than rapid gravity filtration at removing oocysts. Ultrafiltration is a more successful process for removal of *Cryptosporidium* because it offers a complete barrier to the passage of the protozoan oocyst, although wastewater requires significant pre-treatment before it is passed through a membrane.

Lagooning

The lagooning process provides further settlement and biological improvement of wastewater from passage through large man-made lagoons. These bodies of water usually contain aerobic macrophytes (such as reeds) and filter feeding invertebrates (such as Daphnia and Rotifers) to remove fine particles. *Cryptosporidium* could be removed in this process biologically, from the microorganisms within the water, or by attaching to larger particles and settling out.

Previous research has shown how the lagooning process is effective at removing microorganisms. Godfree (2013) found that this method was able to remove 99.999% of encountered *E. coli*. With *Cryptosporidium* there have been poorer results. Lim et al. (2007) remarked how, although aerated lagoons could reduce the concentration of *Giardia* cysts, they could not significantly reduce *Cryptosporidium* oocysts. Bowman et al. (2008) found that *Cryptosporidium* oocysts were more resistant to inactivation by lagoons than *Salmonella*, enteric bacteria and viruses.

Research from Bowman et al. (2008) found that 15 of the 18 lagoons contained oocysts at quantities from 34-431 oocysts/ml, when the effluent of only 6 lagoons contained viable oocysts, suggesting that it was effective. Jenkins et al. (2013) also found that after 13.1 and 20.1 weeks in two different lagoons, there was a 99% *Cryptosporidium* oocyst reduction. These successes, however, were not based at tertiary treatment lagoons but at agricultural lagoons, which may mean the water treated and the processes used are different.

Other work has shown the analysis of *Cryptosporidium* removal at this stage is difficult because the *Cryptosporidium* may already have been removed at this point in the sewage treatment process. Salter et al. (1999) focused their research on two tertiary lagoons at Holmwood STW but were unable to conclude much on *Cryptosporidium* removal, since only one oocyst was found over the whole sampling period (including both the influent and effluent).

Stott et al. (2003) investigated whether filter feeders are effective at reducing *Cryptosporidium* oocyst numbers. It was found that rotifers, which are present in lagoons, consumed 1.6 oocysts per individual. Although this was not the highest removal rate observed by microorganisms (for example after one hour, *Paramecium caudatum* consumed 1.9 oocysts per cell), it could be a significant way by which *Cryptosporidium* could be removed from lagoons.

Therefore research suggests that the success of lagooning at removing *Cryptosporidium* is difficult to observe, even though agricultural lagoons are successful and the microorganisms present are able to consume oocysts.

<u>Disinfection</u>

Disinfection is used to remove or reduce the viability of microorganisms in the water. The effectiveness of this process can be diminished by the type and dosage of the disinfection used, environmental variables and the water's quality, for example pH and turbidity. Disinfection can be achieved using: ozone, UV and chlorine. *Cryptosporidium* is extremely tolerant to halogens such as chlorine or iodine due to the protozoan being housed within a sturdy multi-layered wall. The wall protects the protozoan from the harsh external environments it encounters to enable it to survive.

This includes protection against natural breakdown, as well as protection against many disinfection methods, such as chlorine.

Ozone

Ozone is the reactive form of oxygen which can be very toxic to many pathogens. It is produced by passing oxygen through a high voltage potential. It is a highly efficient disinfection process, produces few by-products, can be produced on site when needed, has no long-lasting residual effects and can be used to treat large volumes of water (Bowman, 2007). An example of its effectiveness was shown by Corona-Vasquez et al. (2002), who found that ozonisation of tap water at 1°C caused a 99% cyst reduction in 10 minutes, with a concentration of 4mg/l. Ozone inactivates *Cryptosporidium* in two ways, through direct contact with ozone gas bubbles and through association with free radicals.

The effectivity of ozone has, however, been questioned in other studies. Blackburn et al. (2006) described the case of a *Cryptosporidium* outbreak from apple cider that had been ozonated. The study suggested that ozone was not effective when water has a high turbidity or high sugar content. It must be noted, however that this study was completed on drinking water, rather than sewage water.

Consequently it seems that ozone is a relatively effective method of *Cryptosporidium* removal, however it is only efficient under certain environmental conditions.

UV Light

UV disinfection is a physical process which inactivates microorganisms, including *Cryptosporidium*, by denaturing or damaging the organism's DNA, preventing the organisms from replicating. This is usually achieved by submerging an ultraviolet lamp in the water to be treated [Figure 8].

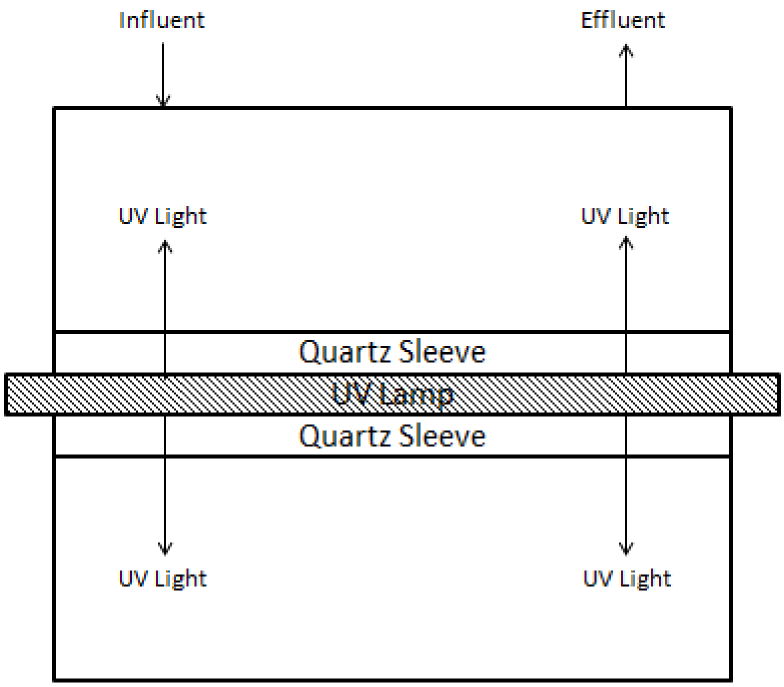

Figure 8 A diagram of the UV process

Shin et al. (2001) found that *Cryptosporidium* does not regain its infectivity after UV light has deactivated it. Oguma et al. (2001) found that *Cryptosporidium* can begin to photo-repair itself, however its infectivity was not recovered. Thus it seems that UV light is one of the most effective disinfection methods of eliminating *Cryptosporidium*, but its susceptibility to turbidity may decrease its ability to work optimally at STW.

Like ozone, UV is susceptible to interference by the presence of turbidity and other materials that reduce the UV transmissivity through the water. Wolyniak DiCesare et al. (2012) investigated this further, saying that biofilms reduce the solar disinfection of *Cryptosporidium* oocysts, which may be a problem in STW, which have a greater quantity of suspended solids than WTW. It is important that the final effluent has been treated to a high quality, since solids in the water may shield microorganisms from the UV radiation. This may be why Neto et al. (2006) detected *Cryptosporidium* oocysts in 1/38 samples of UV-treated effluent.

Chlorine and Chlorine Dioxide

Chlorination is a frequently used method for disinfection but it is not effective at eliminating *Cryptosporidium*, for example Finch (1997) found a removal rate of 0%. Chlorine dioxide, therefore, can be an adjustment to this method that does produce reduction in oocyst numbers. This method consists of the addition of a highly reactive water-soluble gas with high oxidative potential that does not have a chlorinating action.

Li et al. (2001) suggested that a disinfection basin with a retention time of 150 minutes could achieve 99% inactivation at 22°C with 1.1mg/l of chlorine. Korich et al. (1990) also found it an effective method. This research found +90% infectivity inactivation using the following methods: 1 mg/l of ozone (5 minutes), 1.3 mg/l of chlorine dioxide (1 hour) and with both 80 mg/l of chlorine and 80 mg/l of monochloramine (90 minutes).

Although there are issues with this method in terms of water containing high particulates or BOD, it seems that chlorine dioxide is equally as effective as ozone. It is not as effective as UV, although it could be used in combination with it (Bowman, 2007).

2. 1. 6 Other Treatment Methods

Reed Beds

Reed beds are used to remove nitrogen through biological oxidation using Nitrosomonas species, producing ammonia and nitrate. The nitrate is then oxidised, facilitated by *Nitrospira* and *Nitrobacter* species, producing nitrogen gas that is released from the water into the atmosphere.

Sidhu et al. (2010) found positive results when they found that planted reeds reduced *Cryptosporidium* oocysts by 90% after 33 days. They did say however, that constructed reedbed usually have a mean residence time of 10 days so that it would not be an effective method in practise. Redder et al. (2010) also studied the use of planted reeds (*phragmites spp.)* and willows (*salix spp*.) in the wastewater treatment process. It was found that these methods were of minor importance in the removal of *Cryptosporidium.* Thus, it seems this planted reeds are not an effective method of *Cryptosporidium* removal.

Other Processes with no Specific Data

No specific data was found for a range of other processes including: phosphorus removal, biological contractors and odour control.

The removal of phosphorus can be achieved through biological (enhanced biological phosphorus removal using polyphosphate-accumulating organisms) or chemical (chemical precipitation using iron salts, aluminium or lime) means. There is probably little research into the area of the effectiveness of phosphate removal techniques in the removal of *Cryptosporidium* because it does not usually occur as a separate practice, but is usually combined with the other stages of the treatment process. Consequently it is difficult to determine the effectiveness of this process.

Biological contractors consist of a rotating disk which builds up a biofilm. It would be interesting to see the survival rates through this process if *Cryptosporidium* cysts were tested for before and after a biological contractor.

Odour control is required because of the anaerobic procedures used by the sewage treatment process, resulting in odours such as those produced by hydrogen sulfide. If plants are located in an area where people may find complaint with these smells, they will be treated. Methods include: the use of certain pipes, carbon reactors, bio-slime media, small doses of chlorine, use of iron salts or hydrogen peroxide or calcium nitrate and fluid circulation. There is little research into this area, potentially because this is not a vital step in the STW process so it is not present at all sites. Also when it is present there are so many different methods that it must be difficult to compare them accurately.

2. 1. 7 Sewage Treatment Works Cryptosporidium Removal Conclusion

To conclude, the STW processes and their effectiveness at removing *Cryptosporidium* oocysts can be summarised.

Preliminary treatment was found to not be an effective method of removing oocysts, with only 26% removal (Zhang, et al., 2008). Primary settlement had better removal rates, but research found that this rate varied considerably from between 25 and -97% (Stadterman, et al., 1995; Zhang, et al., 2008)

The effectiveness of secondary treatment varied from 40 to 97%, depending on the treatment methods used (Robertson, et al., 2000; Montemayor, et al., 2005; Zhang, et al., 2008). Trickling filters removed 37-50% of encountered oocysts (Stadterman, et al., 1995). Activated sludge treatment removed 55-99.7% of oocysts (Villacorta-Martinez de Maturana, et al., 1992; Stadterman, et al., 1995; Neto, et al., 2006). A combination of activated sludge and biological aerated filters was found to remove 33-73% (Lim, et al., 2007). But no research was found for the effectiveness of biological contractors.

Final settlement was found to be, on average, more effective than primary settlement, with a rate of 91% (Robertson, et al., 2000).

Finally, tertiary treatment was relatively successful at inactivating *Cryptosporidium* oocysts, with a removal rate of 99.8% (Montemayor, et al., 2005), however that does depend on the treatment method used. Filtration removed 99-99.997% of oocysts (Timms, et al., 1995; Stott, 2003). Lagooning, however, was found to be an ineffective tertiary treatment method, with only 33% removals (Lim, et al., 2007). The effectiveness of processes which are designed to remove nutrients such as nitrogen and phosphorus, is unknown due to a lack of research.

One method of disinfection, ozone, was found to be effective, with 92.25-99% removal rates (Corona-Vasquez, et al., 2002). Another method, chlorine, is not effective, with 0% removal rates (Finch, 1997). Chlorine dioxide, was more successful, with rates of 90-99% (Korich, et al., 1990; Li, et al., 2001).

2. 2 The Removal and Progress of *Cryptosporidium* through Water Treatment Works

As previously noted, *Cryptosporidium* in drinking water is a major concern for the water industry. Thus this has been an intensely studied area. An overview of the processes used in drinking water production at a surface water/reservoir source WTW can be found in Figure 9.

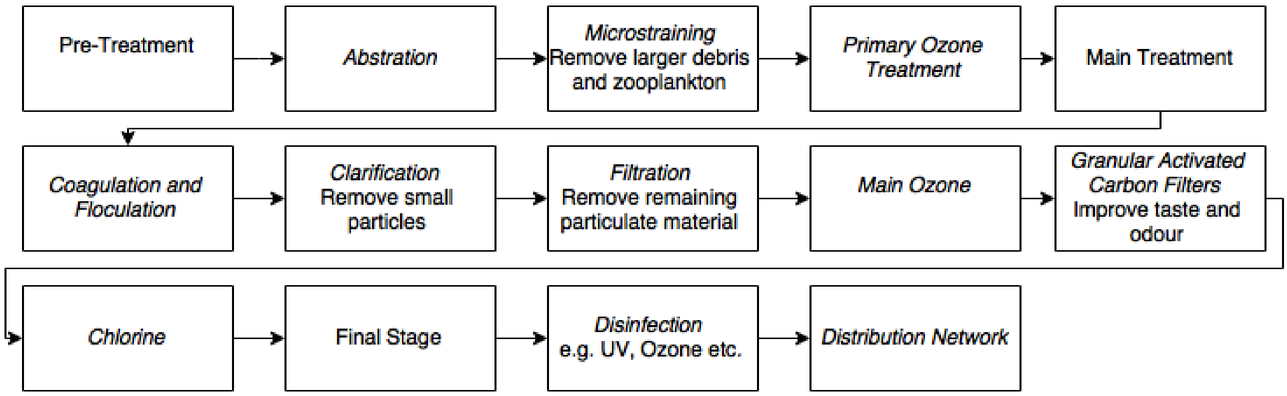

Figure 9 A schematic diagram of a surface water/reservoir source water treatment works

2. 2. 1 Pre-Treatment

Water Quality and Abstraction

There are numerous sources from where water can be abstracted. This includes aquifers which are underground stores of fresh water trapped in porous rock (for example chalk, limestone and sandstone). Boreholes are dug into these groundwater aquifers to abstract freshwater for drinking. Surface water sources of drinking water include reservoirs and water bodies, such as rivers. River water can be pumped directly to WTW for purification (which happens for 5% of Anglian Water WTWs) or into storage reservoirs (50%).

It is imperative that the water from these sources have had the necessary treatment to prevent the spread of *Cryptosporidium.* One demonstration of the importance of this was researched by Willocks et al. (1998), who tracked a *Cryptosporidium* outbreak to a borehole that only used rudimentary filtering techniques.

One effective way to minimise the risk of *Cryptosporidium* is to protect the raw water sources from contamination by catchment management (United Utilities, 2014). This was also shown by Bryan et al. (2009), who used an adaptive management framework, using knowledge from past management schemes, to reduce *Cryptosporidium* outbreaks in South Australia. This can be difficult, however, since water companies do not usually own all of the land surrounding the water source. For this reason, we usually have to rely on treatment processes have to be relied upon for the removal of *Cryptosporidium* from drinking water sources.

Microstraining

This stage removes the larger debris and zooplankton from the water. A microstrainer is used, which is a rotating drum with a fine mesh. However the mesh of microstrainers is not fine enough to remove all algae and does not remove bacteria, viruses, *Cryptosporidium* or *Giardia* (Pizzi, 2010).

Primary Ozone Treatment

Primary ozone is used to breakdown compounds of the water that require oxidation, such as pesticides and micropollutants. It is also often used for other applications including the removal of particles, algae and bacteria.

This review has already shown the effectiveness of ozone in the removal of *Cryptosporidium* from wastewater, but it is important to review this information again for WTW and, specifically, for primary ozone treatment. Li et al. (2001) tested the efficiency of primary ozone treatment, in terms of *Cryptosporidium* removal. It was found that ozone pre-treatment increased the efficacy of free chlorine by 4-6 times. The ozone would primarily kill 97% of oocysts and the free chlorine would then have a 99.9% removal rate of *Cryptosporidium*. Similar results were found by Corona-Vasquez et al. (2002), who established that ozonisation of tap water at 1°C caused a 99% cyst reduction in 10 minutes with a concentration of 4mg/l.

Temperature was one factor which altered the effectivity of this treatment type. Li et al. (2001) found that the efficiency of the combination of primary ozone and free chlorine treatment decreased by a factor of 1.8 for every 10°C temperature decrease.

Therefore although this treatment method is susceptible to environmental conditions, it is a useful method of removing *Cryptosporidium* from the drinking water.

2. 2. 2 Main Treatment

<u>*Coagulation and Flocculation*</u>

Coagulation is required for flocculation to occur. It occurs when a chemical is added to the water which causes impurities to destabilise and stick together, forming larger clumps which can be removed through methods such as sedimentation, flotation and sand filtration [Figure 10]. It is likely this process may help oocysts stick to particles, making them be more likely to settle out or be removed physically through filtration.

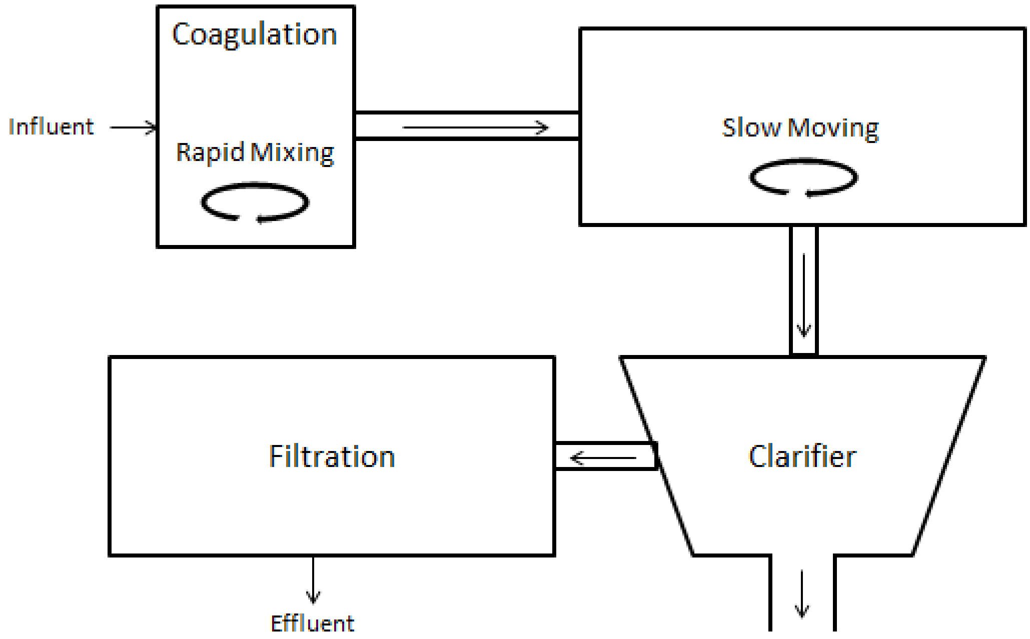

Figure 10 A schematic diagram of the coagulation, flocculation and clarification processes

Coagulation and filtration is a useful method of oocyst removal (United Utilities, 2014). Hall et al. (2000) found that well operated chemical coagulation-based treatment, using either dissolved air flotation or floc blanket clarification, were capable of achieving removal of *Cryptosporidium* oocysts of over 99%. Edzwald and Kelley (1998) agreed when they stated that coagulation provides effective control of *Cryptosporidium* by clarification and filtration. Thus it seems that coagulation is required, along with other processes, for the removal of *Cryptosporidium.*

Coagulation is also an important process for downstream possesses. Edzwald et al. (2001) identified that coagulation creates conditions which aid the downstream removal of *Cryptosporidium* through the reduction of: turbidity, particle count, and natural organic matter. Croll (2000) said that even with normal drinking water filtration rates (5-10m/hr), without a coagulant, *Cryptosporidium* oocyst removal was 90% and with sub-optimal coagulation and flocculation, there was 95% removal. When a coagulant (for example aluminium sulphate) was used, *Cryptosporidium* removal increased to 99.9-99.99%.

To conclude, coagulation and flocculation is highly important for the removal of *Cryptosporidium* from drinking water, since it improves the efficiency of clarification and filtration downstream.

Clarification

This part of the process is for the removal of particulates by physical separation of the floc from the water. It is a process which can either be completed using sedimentation or dissolved air flotation. Sedimentation is where water flows through the clarifier and the floc are allowed to settle by gravity. The accumulated solids are removed from the water. Dissolved air flotation (DAF) is where microscopic air bubbles attach to the floc, making it buoyant so it floats to the surface [Figure 10]. *Cryptosporidium* would be removed in this process by floating to the surface, especially when associated with other particles.

Edzwald and Kelley (1998) researched the success of different clarification methods. They found that DAF achieved *Cryptosporidium* removals of 99.9%, while sedimentation achieved only 90%. In a later study by Edzwald et al. (2001), they found similar results. This research found *Cryptosporidium* removals using DAF to be 99% ± 50% and by settling 88-92% ± 50%.

DAF and filtration combined are a highly effective barrier to *Cryptosporidium* when combined. Cumulative removals of 99.99-99.999% compared to removals of 99.9-99.99% by sedimentation and filtration were found (Edzwald & Kelley, 1998).

Therefore clarification seems to be an important and highly successful part of the removal of *Cryptosporidium* for drinking water, although DAF is the most effective method, rather than sedimentation and is even more effective when combined with filtration.

Filtration

Filtration, in the form of sand filtration, has already been researched as part of the sewage water treatment process and it was found that it is a useful method which helps (even if it does not completely eradicate) *Cryptosporidium* removal, but it should be used in combination with other methods. It is the case that the same is true for WTW treatment, for example the way that a combination of DAF and filtration can result in removals of 99.99-99.999%. There are additional filtration methods available for WTW as well as sand filters.

Microfiltration and ultrafiltration are pressure-dependent membrane filtration processes which cause very small solids and colloids to be removed from water. Microfiltration uses pores with a size of 0.1-10µm and ultrafiltration membranes have a pore size of 0.001-0.1µm. Since *Cryptosporidium* oocysts are 4-6µm, it means they are able to pass through some microfiltration pores but not ultrafiltration pores, so ultrafiltration is one of the most effective controls for *Cryptosporidium*.

Microfiltration and ultrafiltration resulted in a *Cryptosporidium* removal efficiency of > 99.99999% in a study done by Hirata and Hashimoto (1999). The research regarded both methods as being suitable processes for producing safe drinking water. Fu et al. (2010) also described how membrane ultrafiltration was successful at *Cryptosporidium* removal, more so than conventional flocculation sedimentation and sand filtration. Regardless, the Hirata and Hashimoto (1999) study also noted that some oocysts appeared in the filtrate in both methods, which does contradict these results. This could have potentially occurred using ultrafiltration because of contamination or a break in the membrane fibres.

Consequently it seems that microfiltration and sand filtration are useful methods which help *Cryptosporidium* removal, but they should be used in combination with other methods of disinfection to ensure any surviving oocysts do not progress to other sections of the WTW. Also ultrafiltration is highly effective at removing oocysts, but the membrane must be monitored for potential contamination and damages.

Main Ozone

The application of ozone has already been studied as part of this review. It has been found that it is a relatively effective method of *Cryptosporidium* removal, with removals of 97-99% (Li, et al., 2001; Corona-Vasquez, et al., 2002).

Bukhari et al. (2000) found less than a 90% reduction in three out of four *Cryptosporidium* tests with 0.4mg/L of ozone for 2 minutes at 22°C, although one of the tests found a 99% reduction in viability.

Granular Activated Carbon Filters

Granular activated carbon filters (GACF) improve the taste and odour of the drinking water by removing dissolved components, micropollutants and taste and odour-causing compounds. It is carbon which has been 'activated' by heat and steam treatment. This activation produces a highly porous and high surface area media onto which pollutants can adsorb. GAC is usually added into a filter bed and water is allowed to percolate through the media [Figure 11]. Because of this, GAC filters do also remove some remaining solids from the water, including the potential removal of *Cryptosporidium*.

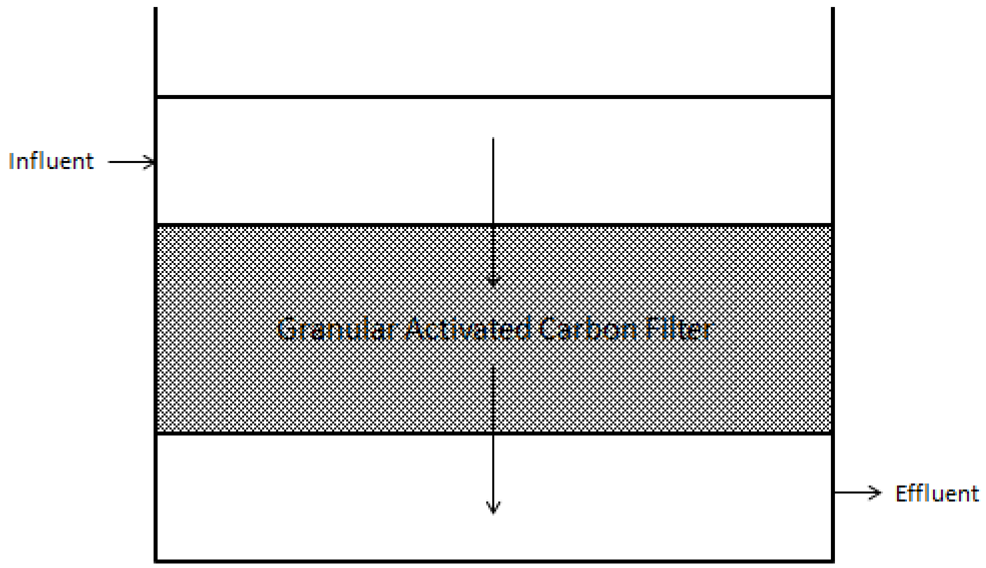

Figure 11 A diagram of the granular activated carbon process

GACF is a good technique to use as part of the water treatment process because it is cost effective and is the most effective method of removing organic compounds (such as volatile organic compounds and radon). It also seems to have some positive contributions towards the removal of *Cryptosporidium.* Bichai et al. (2010) found that this method had some successes in the removal of *Cryptosporidium.* They found an average mass reduction of *Cryptosporidium* oocysts of 66.2% and 32.1% in the upper and lower parts of the GAC filter beds. Hijnen et al. (2010) found this method had some success in *Cryptosporidium* removal too. They found GACF resulted in *C. parvum* removal rates of 92-99.8%. This is in agreement with some older research by Patania et al. (1995), who found *Cryptosporidium* removal rates of 60–95% at filtration rates of 12-18m/h.

Other research has comes to different conclusions. Bichai et al. (2010) found that predation by zooplankton during GACF can cause *Cryptosporidium* oocysts to be retained in the filter beds. This means that the *Cryptosporidium* is more likely to be transmitted into drinking water. There are other disadvantages to this process, including the requirement for frequent filter changes and bacteria can accumulate in the filter, reducing the contaminant/carbon contact and so the efficiency decreases.

Therefore it seems that this technique does aid in the removal of *Cryptosporidium.* However when compared to other processes used in drinking water treatment, it is not as effective.

2. 2. 3 Final Stage

<u>Disinfection</u>

The different methods of disinfection were reviewed in other parts of this document for treatment of wastewater. However, this process is most routinely used for treatment of water used for drinking.

Chlorination is used for disinfection against bacteria and viruses, control algal growth and to prevent biological growth. It is not, however, as discussed, an effective method of *Cryptosporidium* removal. For example, there are some examples of no *Cryptosporidium* removal being observed at all (Finch, 1997).

UV

UV light is a useful disinfectant because it does not require the addition of extra chemicals to the water and produces fewer known by-products. However, it has no lasting residual disinfection capacity.

As can be seen from Table 1, UV light is an effective method of decreasing the viability of pathogens such as *Cryptosporidium*, achieving 68-99.99% deactivation, depending on the UV dose used (Environment Protection Agency, 2006). This high level of removal was also seen by Betancourt and Rose (2004), who found a disinfection rate of 97-99.9% removal at 1-3 mJ/cm^2 of UV light. Furthermore, Shin et al. (2001) found that *Cryptosporidium* does not regain its infectivity after UV light has deactivated it. Oguma et al. (2001) found that *Cryptosporidium* can begin to photo-repair itself, however its infectivity was not recovered.

Table 1 UV dose requirements (mJ/cm2)

Target Pathogen	**Log and % Inactivation**							
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
	68%	90%	97%	99%	99.7%	99.9%	99.97%	99.99%
Cryptosporidium	1.6	2.5	3.9	5.8	8.5	12	15	22
Giardia	1.5	2.1	3.0	5.2	7.7	11	15	22
Virus	39	58	79	100	121	143	163	186

Data extracted from: Environment Protection Agency (2006), 'Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule', Report Number EPA 815-R-06-007, Available at http://www.epa.gov/ogwdw/disinfection/lt2/pdfs/guide_lt2_uvguidance.pdf (Accessed: 17/12/14).

Consequently it was found that UV was the most effective method for *Cryptosporidium* removal with 68-99.99% (Betancourt & Rose, 2004; Environment Protection Agency, 2006), followed by ozone with 92.25-99% (Bhukari et al., 2000; Corona Vasquez et al., 2002), and chlorine dioxide with 90-99% (Korich, et al., 1990; Li, et al., 2001).

Distribution Network

This is the process by which water from the treatment works and storage points enter the public water supply zones.

This part of the process has no mechanisms in place for the removal of *Cryptosporidium,* however the integrity of the pipes prevent *Cryptosporidium* entering the water from external sources. Current technology allows pipes to be much longer, reducing the number of breaks and so reducing the areas at risk for contamination.

The pipes themselves are made out of materials which are subjected to temperatures of 200-220°C, decontaminating them. They are also encased in an aluminium foil barrier which would prevent the movement of microorganisms. The integrity of the barrier is checked every 1h30 on the production line.

Contamination is possible, however, where there are distribution network failures. Examples of this include: pressure-drops, repair requirements and the effects of peak events (such as heavy rainfall, snow melt and oocyst-contaminated discharges). These events can enhance the risk of *Cryptosporidium* being in drinking water (Savioli, et al., 2006).

2. 2. 4 Water Treatment Works Cryptosporidium Removal Conclusion

To conclude, the WTW treatments and their effectiveness at removing *Cryptosporidium* oocysts were found to be:

The most effective method of inactivating oocysts during the pre treatment stage was the use of a combination of chlorine and ozone, with 99.9% (Li, et al., 2001). This was closely followed by primary ozone, which had removals varying from 97-99% (Li, et al., 2001; Corona-Vasquez, et al., 2002. Abstraction and microstraining, however, were not effective.

During the main treatment stage, literature found that many water treatment methods successfully inactivated oocysts in the water. Coagulation and flocculation were found to have inactivation rates of 90-99.99% (Croll, 2000; Hall, et al., 2000). Similar rates were found during the clarification process, depending on what treatment method was used. DAF alone was found to remove 99.9% (Edzwald & Kelley, 1998) but when combined with filtration, this increased to 99.9-99.999% % (Edzwald & Kelley, 1998). Sedimentation, too, had a good percentage removal, of 88-90% (Edzwald & Kelley, 1998) (Edzwald, et al., 2001). Other methods of filtration were the most successful though. Microfiltration and ultrafiltration had removal rates of 99.99999% (Hirata & Hashimoto, 1999), while GACF had 32.1-99.8% (Patania, et al., 1995; Bichai, et al., 2010; Hijnen, et al., 2010).

The final stage of water treatment was relatively effective at removing oocysts. UV is one effective method of disinfection and it has a removal rate of 68-99.99% (Betancourt & Rose, 2004; Environment Protection Agency, 2006). Another method of disinfection, ozone, has an inactivation rate of 97-99% (Li, et al., 2001; Corona-Vasquez, et al., 2002). Although chlorine dioxide had a high rate of 90-99% (Korich, et al., 1990; Li, et al., 2001), chlorine alone is not effective, with some evidence of 0% removal being seen (Finch, 1997). No information was found for the effectiveness of the distribution network.

2. 3 Literature Review Conclusion

Cryptosporidium is removed more efficiently through the WTW process than through STW, because it has more processes that disinfect the water.

Numerous treatment methods were found to have high levels of *Cryptosporidium* removal. Ultrafiltration and microfiltration had removals of 99.99999% (Hirata & Hashimoto, 1999). Dissolved air flotation combined with filtration achieved removal rates of 99.9-99.999% (Edzwald & Kelley, 1998). Slow sand filtration removed 99-99.997% of encountered oocysts (Timms, et al., 1995; Stott, 2003). A combination of chlorine dioxide and free chlorine removed 99.9% (Li, et al., 2001). DAF achieved the same rate, 99.9% (Edzwald & Kelley, 1998). UV disinfection removed 68-99.99% (Betancourt & Rose, 2004; Environment Protection Agency, 2006). Disinfection using ozone was also found to be successful, with 92.25-99% (Bukhari, et al., 2000; Li, et al., 2001; Corona-Vasquez, et al., 2002). Coagulation and flocculation removed 90-99.99% (Croll, 2000; Hall, et al., 2000). Chlorine dioxide removed 90-99% (Korich, et al., 1990; Li, et al., 2001). Final settlement removed 91% (Robertson, et al., 2000). And, finally, sedimentation removed 88-90% (Edzwald & Kelley, 1998; Edzwald, et al., 2001).

Some treatment methods had medium levels of *Cryptosporidium* removal. Activated sludge removed 55-99.7% of encountered oocysts (Villacorta-Martinez de Maturana, et al., 1992; Stadterman, et al., 1995; Neto, et al., 2006). GACF removed 32.1-99.8% (Patania, et al., 1995; Bichai, et al., 2010; Hijnen, et al., 2010). Secondary Treatment also had highly varying results, of 40-97% (Robertson, et al., 2000; Montemayor, et al., 2005; Zhang, et al., 2008). Primary settlement removed from 24.7 to 97% of oocysts (Stadterman, et al., 1995; Zhang, et al., 2008). Biological aerated filters removed 73% (Lim, et al., 2007). And finally, trickling filters had removals of 37-50% (Stadterman, et al., 1995).

The least effective methods of *Cryptosporidium* removal were found to be lagooning, preliminary treatment and chlorine, with removal rates of 33, 26 and 0% (Finch, 1997; Lim, et al., 2007; Zhang, et al., 2008).

3 Materials and Methods

The project was split into two stages of work, an analysis of existing data and a *Cryptosporidium* sampling programme through Louth STW.

3. 1 Data Analysis of Existing Data

Historic *Cryptosporidium* data from Anglian Water was analysed to determine the source of the oocysts to better focus the sampling programme. *Cryptosporidium* sampling results from different sections of Louth Canal were analysed in an attempt to find the source of the oocysts, where they were entering the canal [Figure 12]. This data was collected over the period from 2009-2013. Then an analysis of *Cryptosporidium* typing results were used to narrow the potential hosts which were spreading the infection. The typing analysis was carried out by Swansea *Cryptosporidium* Referencing Unit.

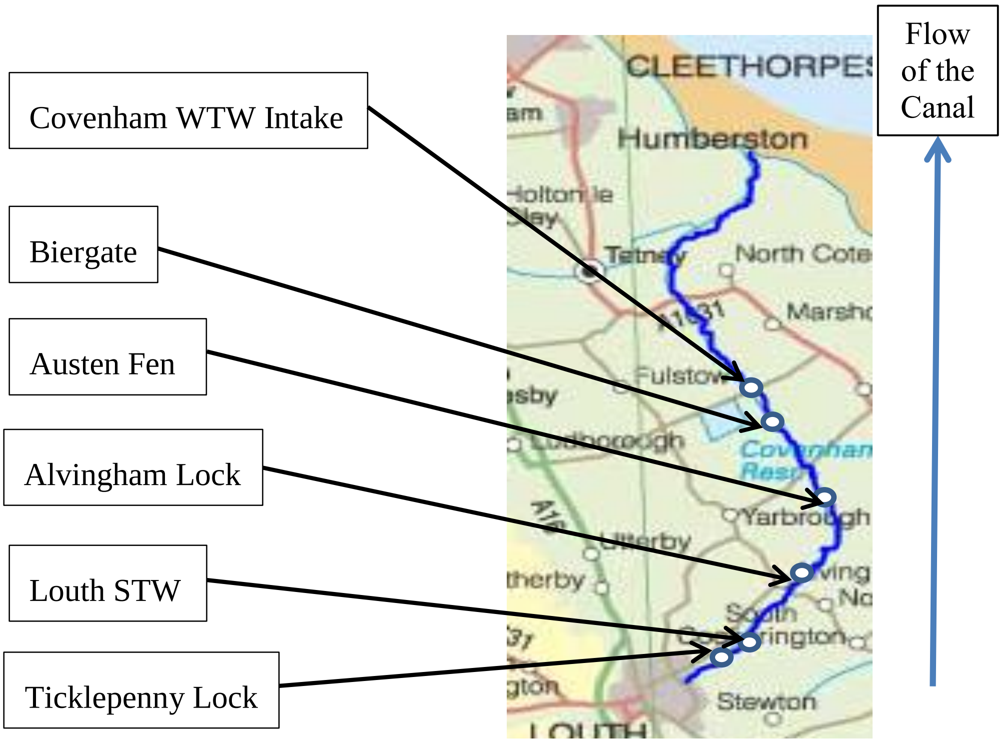

Figure 12 Louth Canal segmented sample locations

Potential human sources of *Cryptosporidium* were then investigated. This involved comparing epidemiological data of the number of cryptosporidiosis cases identified by Public Health England in the Louth area (postcode LN11) with two other locations with a similar population size to Louth and in a similar rural environment. The LN11 cryptosporidiosis data was also contrasted with Lincolnshire as a whole (the county to which Louth belongs). Then data from Lincolnshire and the epidemiological data from other nearby counties were also compared. This analysis was done in an attempt to identify if Louth had a normal number of cryptosporidiosis cases for a place with a population of its size and its environment. The LN11 cases were then contrasted with the oocysts identified in Louth Canal, to see if there was a relationship.

To investigate the potential animal sources of *Cryptosporidium*, the results of a walkover study of Louth Canal were scrutinised to see if sheep were present in the Louth area (Hewson-Fisher, 2014). Local veterinary practises were contacted to determine the ease of cryptosporidiosis detection in the area, the frequency that this happens in Louth and to see if they had any record from past outbreaks. A review of literature was completed to see the accuracy of cryptosporidiosis detection methods and to support the feedback from the vets.

Trade effluent sources of *Cryptosporidium*, from both humans and animals, were also considered. The licenses that companies have to discharge into Louth STW were obtained. Using this, the different companies were researched to determine how likely it was that oocysts could be entering the STW from these sources.

It is a challenge to have confirmation of all human cases of *Cryptosporidium* because in many cases people do not go to the doctor when they are sick. Instead, during their illness people may take over the counter anti-diarrhoeal medication. For this reason, other sources of information were collated and correlated with the *Cryptosporidium* results in an attempt to find a link between the two sets of information. Local pharmacies were contacted to see if there was an increase in anti-diarrheal medications, such as Imodium, at certain times of the year. Patterns of absences at certain times of year may also have shown when people had had cryptosporidiosis but may not have visited a doctor. Thus, nursing homes, day care centers, schools and large employers in the area were contacted for a list of residence illness or staff or pupil absentees.

A further important part of the data analysis phase was to compare precipitation and climate information at the time of data collection, since this can help identify patterns in the prevalence of oocysts in the environment. This data was confirmed with a sample taken from the storm holding tanks in Louth STW.

3. 1. 1 Laboratory Methodology

Water samples were sent to the Anglian Water labs for oocyst detection, where the Environment Agency procedures for *Cryptosporidium* monitoring were followed (Environment Agency, 2005).

The first stage of counting the number of oocysts is that of separation of useful and waste materials and liquid. The water sample was passed through a filter to remove the supernatant water and collect the materials. This material was then centrifuged to form a pellet, removing further supernatant fluid. Magnetic beads, which are connected with anti-*Cryptosporidium* antibodies, are added to the pellet. A magnet is used to separate the material which associates with the magnetic beads and the material was discarded, as well as the magnetic bead complex. The remaining liquid is then retained for oocyst enumeration.

Enumeration is completed by staining the oocysts with fluorescently labelled monoclonal antibodies and DAPI (4’6-diamidino-2-phenylindole). Fluorescence and differential interface contrast (DIC) microscopy determines the numbers of oocysts per slide.

4 Results and Discussion

4. 1 Data Analysis of Existing Data

4. 1. 1 Louth Canal Segmented Samples

Through analysis of historical data from Anglian Water for Louth Canal between 2009 and 2013, the evidence strongly indicated that the source of the *Cryptosporidium* was Louth STW. This was because 0.2 oocysts/l were detected before the STW, at Ticklepenny Lock, and 43.18 oocysts/l were found after the STW's final effluent was discharged into the canal [Figure 13].

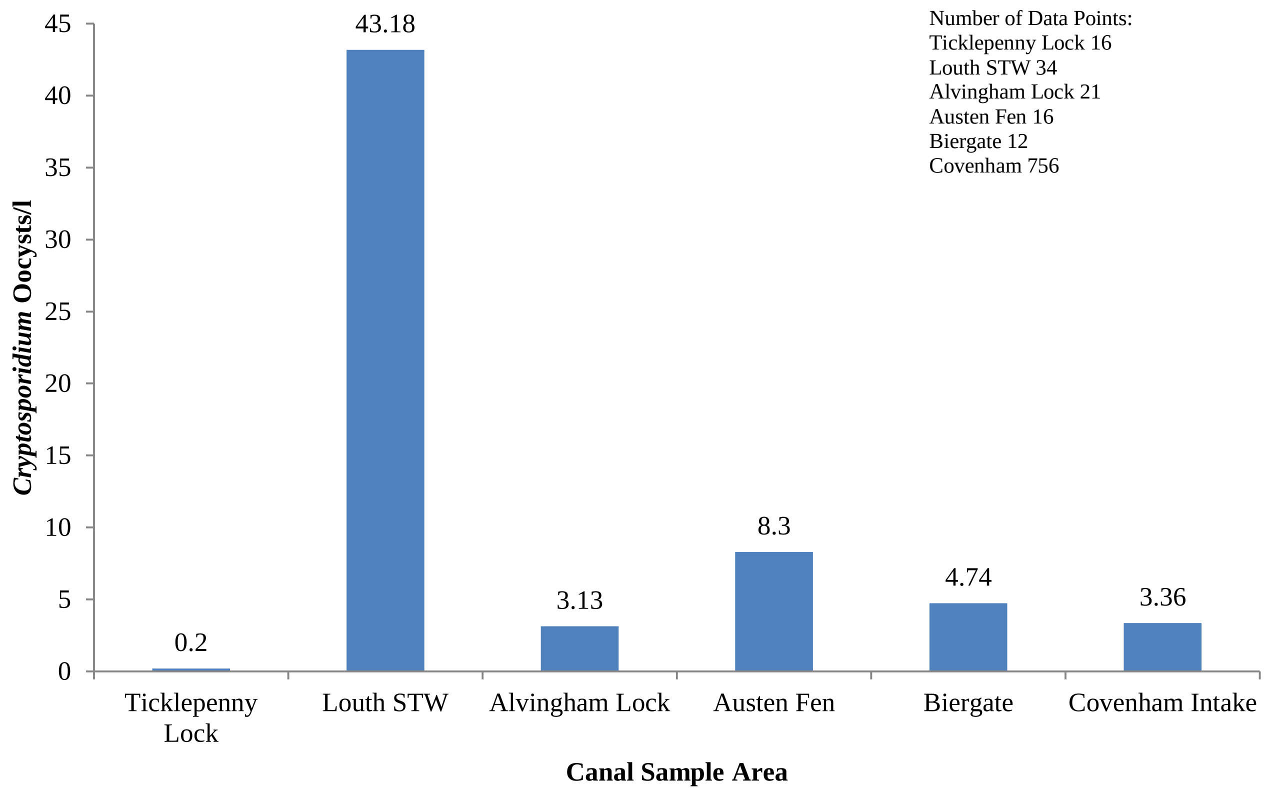

Figure 13 Mean* Cryptosporidium *oocysts/l found at different Louth Canal sample areas 2009-2013

Data extracted from: Anglian Water (2015)

The contribution of STW effluent to Louth Canal was further explored through a comparison of the oocysts found entering Louth Canal from the effluent of Louth

STW and the oocysts found in Louth Canal at the point of abstraction for Covenham [Figure 15]. These results further support the view that the STW effluent was the most probable source of *Cryptosporidium*.

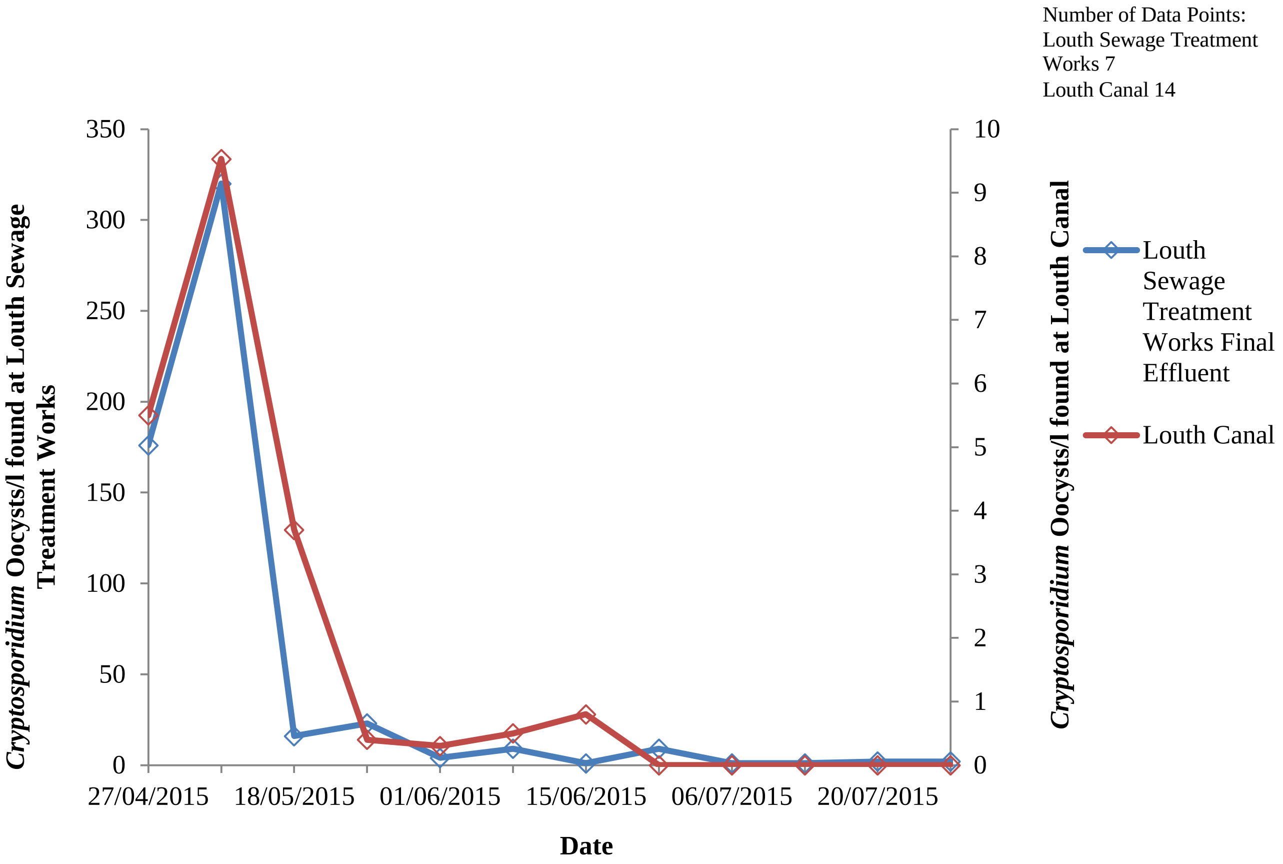

Figure 14 A comparison of* Cryptosporidium *oocysts in Louth Sewage Works effluent and Covenham Water Treatment Works abstraction

4. 1. 2 Cryptosporidium Taxonomy and Typing

Typing of the *Cryptosporidium* found in the samples from the Louth system identified that *C. hominis*, *C. andersoni* and *C. parvum* were detected in Louth Canal [Figure 15]. The major hosts of the strains found are: sheep, humans and cattle [Table 2].

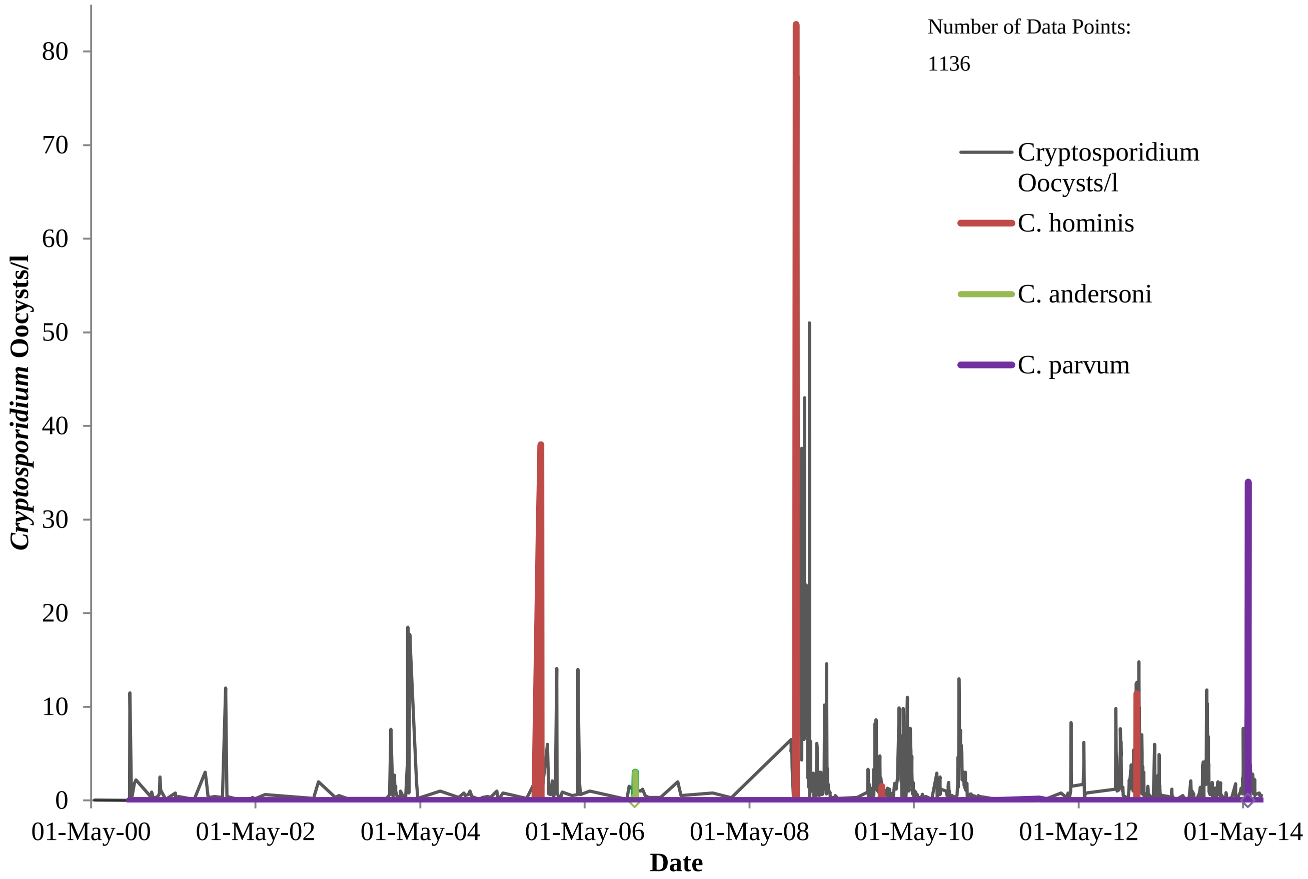

Figure 15* Cryptosporidium *oocysts/l found at Covenham Reservoir intake from Louth Canal 2000-2014

Data extracted from: Anglian Water (2015)

Table 2 The* Cryptosporidium *Species found in Louth Canal and the likely host based on literature

Species	**Major Host**	**Minor Host**
C. andersoni	Cattle	Deer, Mice, Pigs, Humans, Sheep, Goats, Bactrian Camels
C. hominis	Humans, Monkeys	Dugongs, Sheep, Cattle, Goats, Marsupials
C. parvum	Cattle, Sheep, Goats, Humans	Deer, Mice, Pigs, Other Mammals

Data extracted from: Šlapeta, J. (2013); Xiao et al. (2004) and Fayer (2010).

4. 1. 3 Human Sources of Cryptosporidium

The two largest peaks that were found when *Cryptosporidium* samples from Louth Canal were typed were *C. hominis* [Figure 16]. Humans are a major host of *C. hominis* and *C. parvum*, with the latter posing a major public health concern (Rochelle, et al., 2005). If humans were the source of the *Cryptosporidium,* it could be from numerous different places, including domestic waste and trade effluent. In an attempt for the source to be identified, both domestic and trade effluents in the area were studied to see if the oocysts were originating from a specific location.

Domestic Wastes

If the *Cryptosporidium* was human in origin and from the residents of Louth, it would enter the STW from the sewage collected from the Louth area. There was an approximate 24 hour travel time from residential areas to the canal through the STWs.

It seems that the people who live within Louth, or more particularly the LN11-postcoded area, do not have an unusually high level of oocyst infection. This was established by comparing the cases of *Cryptosporidium* in the Louth postcode area (LN11) with areas of similar populations [Table 3]. Further comparison was made between the LN11 area and the county of Lincolnshire [Table 4] and the Lincolnshire county with other counties in the East Midlands [Figure 16].

Within the local area there had been very few people reporting to doctors with illnesses that have the same symptoms as *Cryptosporidium.* Public Health England (PHE) monitor surveillance data closely to identify outbreaks or infectious clusters which require public health actions. Table 3 shows the *Cryptosporidium* cases in the LN11 area compared with two randomly selected areas within Lincolnshire (PE10, SK13) which have similar population sizes. It was clearly seen that the LN11 area did not have unusually high levels of *Cryptosporidium,* and that the results were relatively 'normal' for a population of this size.

Table 3 The regional analysis of the average number of **Cryptosporidium** *oocyts per year*

Place	Population	Males	Females	% Males	% Females	Area Hectares	Density	Average *Cryptosporidium* Oocysts/year 2010-2014
LN11	2350	1128	1222	48.00%	52.00%	62	37.9	3.6
SK13	2531	1191	1340	47.06%	52.94%	84.87	29.9	2.2
PE10	2224	1085	1139	48.79%	51.21%	8811	0.3	4.2

When the cases of *Cryptosporidium* in the LN11 area and Lincolnshire as a county were compared, the LN11 PHE statistics were not significantly high. The area only accounted for 7.8% of the *Cryptosporidium* cases within the Lincolnshire area from 2010-2014 [Table 4] (Gilijohann, 2014). Furthermore, PHE have not seen an increase in the number of notifications of *Cryptosporidium* for Louth area over the last few years, when the *Cryptosporidium* peaks in Louth Canal were identified.

Table 4 The total number of PHE **Cryptosporidium** *notifications in the LN11 area and Lincolnshire area*

Cryptosporidium Notifications	2010	2011	2012	2013	2014	Grand Total
LN11 Cases	<5	0	9	<5	<5	15
Lincolnshire Grand Total (Inc. Louth)	48	28	73	35	28	212

*Data extracted from: Gilijohann, C. (2014), '*Cryptosporidium *in the LN11 Area and the Lincolnshire Area', Public Health England.*

Lincolnshire had some of the lowest rates of *Cryptosporidium* cases per 1000 people for 2009-2012 when compared with other nearby counties (Derbyshire, Nottinghamshire, Leicestershire, Northamptonshire and the East Midlands) [Figure 16].

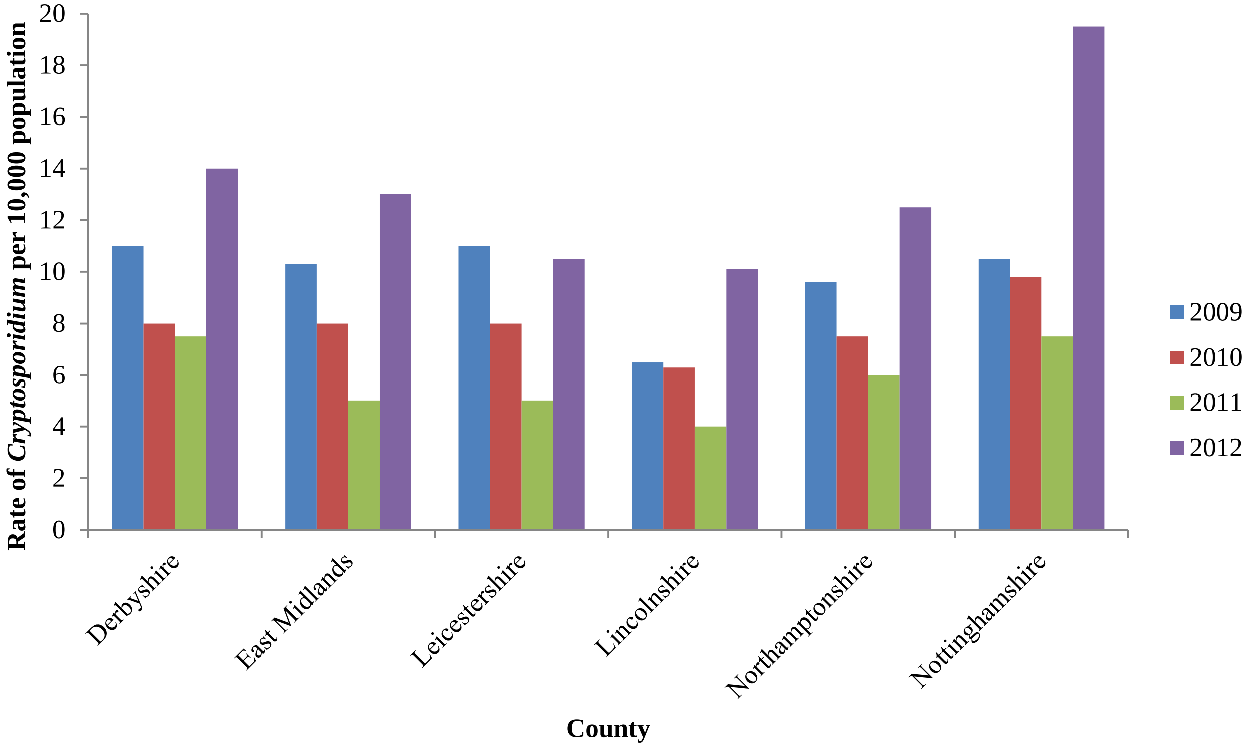

Figure 16 Rates of cases of* Cryptosporidium *per 100,000 people 2009-2012 in East Midlands counties

Data extracted from: PHE (2014), East Midlands Cryptosporidium Surveillance, 2012.

The *Cryptosporidium* cases detected in the population of Louth in 2012 were compared with the oocysts detected at the Covenham intake [Figure 17]. It was found that some outbreaks in Louth Canal did coincide with cases of cryptosporidiosis in the population (such as in April 2014), however most cases did not. This may be because not all cases of *Cryptosporidium* are reported to their doctors, but it could equally be likely that the *Cryptosporidium* was coming from another location or source.

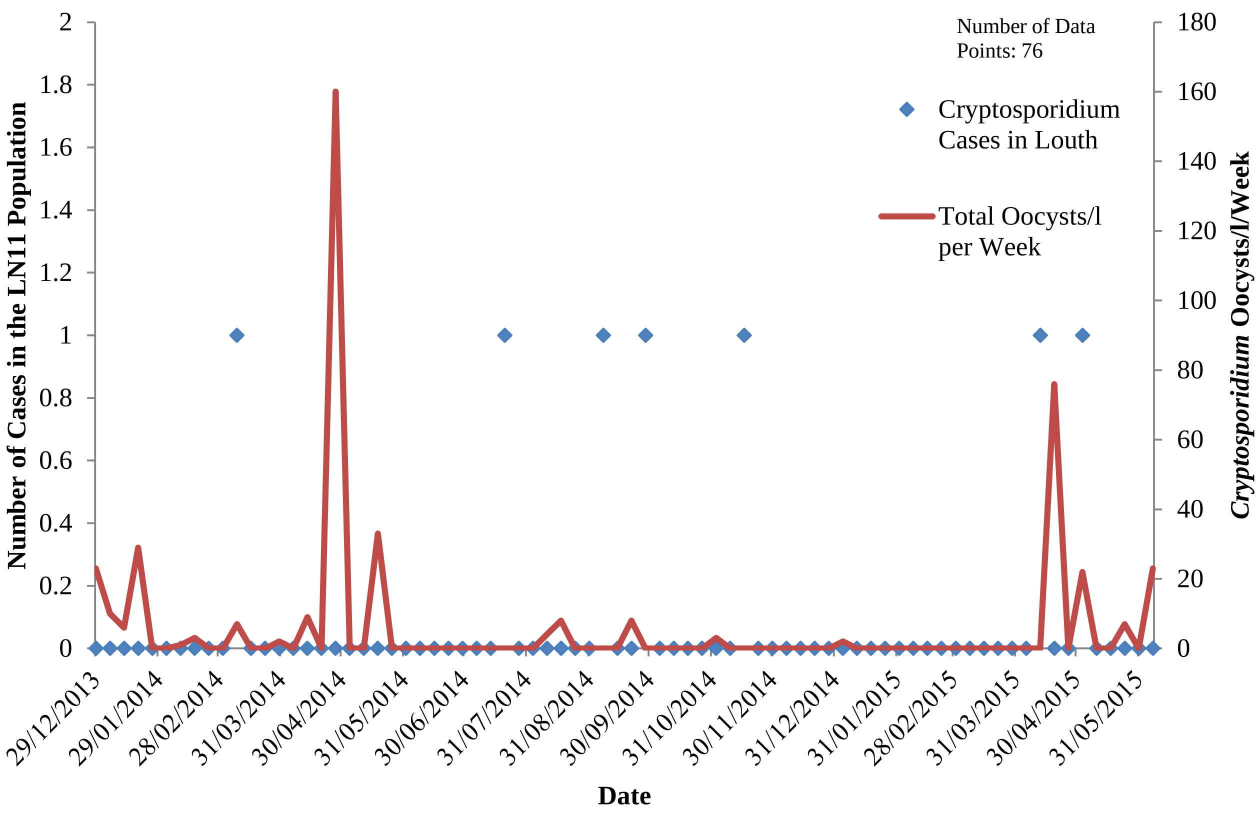

Figure 17 **Cryptosporidium** ***oocysts/l found at the Covenham Reservoir intake and the number of detected cases of cryptosporidiosis in the LN11 population 2014-2015***

Data extracted from: Anglian Water (2015) *and Public Health England* (2015)

Alternative Sources of Information

PHE data cannot be taken as absolute proof that there was not a local outbreak of cryptosporidiosis because this type of *Cryptosporidium* surveillance is usually underestimated. Not all patients seek medical attention when ill and may instead take over the counter medication, such as Imodium, since the main symptom is diarrhoea. If people do visit their doctor, the doctor still may not submit samples for analysis and *Cryptosporidium* detection. Tam et al. (2012) estimated that, in infectious intestinal diseases in the UK, for every case reported to surveillance, there are 147 unreported cases in the community.

Correspondence between Cryptosporidium *found in Louth Canal and Anti-Diarrheal Sales in the LN11 Area*

No relationship was found between anti-diarrhoeal medications purchased and *Cryptosporidium* cases in the LN11 area from 2012-2014 [Figure 18].

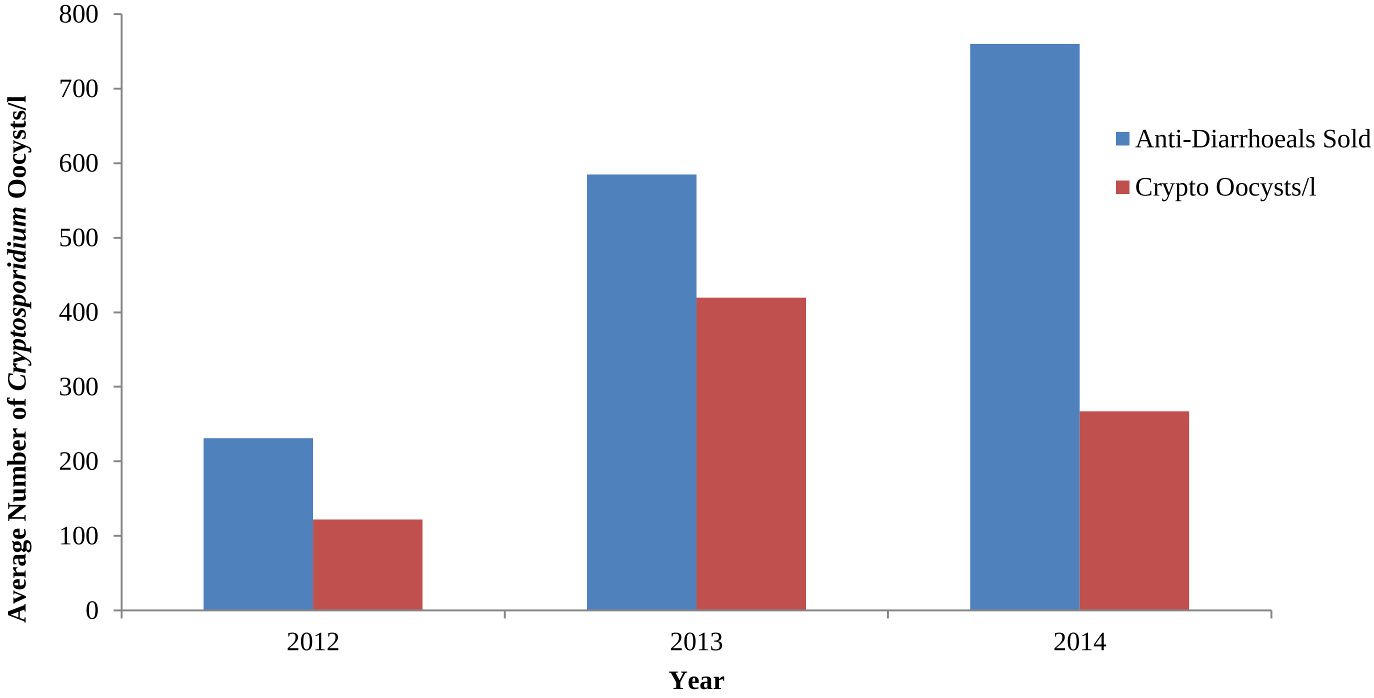

Figure 18 Anti-diarrhoeal sales in the LN11 area and* Cryptosporidium *oocysts/l found at Covenham Reservoir intake from Louth Canal 2012-2014

Data extracted from: Co-operative Pharmacies (2015)

The data used for this analysis, however, was only for one group of pharmacies in the local area (Co-operative pharmacies) and was provided on a per year basis, rather than per month. As such, it cannot be considered conclusive.

It could be that the lack of illness in Louth may be due to asymptomatic individuals. Körkoca et al. (2013) found that 5 out of 393 (1.27%) people who tested positive for *Cryptosporidium,* were asymptomatic. This rate is too low to suggest that the people of Louth are causing the STW issues without showing symptoms.

Correspondence between Cryptosporidium *found in Louth Canal and Absence Rates*

Since people do not always visit the doctor when infected with *Cryptosporidium*, staff and pupil absence rates were investigated to try and find peaks in illness seasonality. Seasonal outbreaks of *Cryptosporidium* are common in the UK (Public Health Wales, 2012). *C. parvum* is usually the most common strain in the spring peak. This is associated with animal contact on farms, followed by human-human contact. The autumn peak is normally *C. hominis* and is contracted through overseas travel and recreational water usage (Public Health Wales, 2012). If there had been a seasonal pattern of illness which links with seasonal *Cryptosporidium* outbreaks, there would have been a greater number of sources of data to determine the source.

East Lindsey District Council, as one of the major employers in the LN11, investigated their staff sickness absence rates and found no particular peaks or troughs according to specific times of the year (Waddell, 2015).

Pupil absences were also researched. When *Cryptosporidium* outbreaks occur in an area, it affects nurseries the most. Children who go to nurseries are young and so are less likely to follow hygienic procedures, meaning that infections can spread more easily. In particular, children aged one to five are more susceptible to being infected by *Cryptosporidium,* (Public Health Wales, 2012). Outbreaks in childcare settings are most common in August-September. Unfortunately, however, nurseries within the LN11 area would not disclose information of this type.

Illegal Dumping of Septic Tanks

A further point to consider is that the *Cryptosporidium* could be due to an unknown sewerage output, such as tanker drivers illegally disposing of septic tank contents into the STW. An example of where septic tank dumping has occurred, causing numerous problems, was in 2014 at the Northern Ireland Water sewage network in Lisnarick, where the extra waste caused a blockage and resulted in a pollution incident at Lisnarick STW (Northern Ireland Water, 2014). This would explain how *Cryptosporidium* is in Louth STW but the people of Louth do not seem to be ill.

Since Louth and the surrounding areas are in a relatively rural environment, it is more likely that people would have septic tanks at their homes. The septic tank companies cover a large rural area, with villages between Lincoln, Grimsby and Skegness (Brown, 2015).

There have been past issues with illegal dumping in the area. Louth STW used to be a reception site for septic tank waste and would receive large numbers of vehicles every day. This was stopped when the *Cryptosporidium* issue first became apparent. This means that the only sites which can officially accept septic tank waste now are in Grimsby and Ingoldmells. Consequently tank drivers now have to travel greater distances to discharge their septic tank waste, costing them more money. One particular contractor complained that the change had ruined his business (Brown, 2015). This demonstrates the disapproval that tanker drivers have for the new regulations, potentially making illegal dumping a more attractive option.

Anglian Water have taken a crude sample from a tanker and it contained a very large number of oocysts. However it was only a single sample and the number of *Cryptosporidium* oocysts/l can often be inaccurate in crude sewage. It was useful data, however, because it confirmed that oocysts are able to survive in crude sewage in tankers and that they are present in these sorts of environments (Brown, 2015). This confirms the results of other studies which have stated that *Cryptosporidium* is present in septic tanks (Gamba, et al., 2000).

If illegal dumping has a part to play, its reduction is difficult because of the numerous manholes (and so points of entry) in the LN11 area. The employees at Anglian Water are very vigilant about illegal dumping and are encouraged to report anything that looks like an illegal discharge into the sewers. It is difficult, however, since it would be most likely to occur somewhere remote and at night, when fewer people are nearby. It could potentially be monitored through the use of CCTV but it is too large an area for thorough coverage.

Survival of Cryptosporidium *in Septic Tanks*

Factors which affect the likelihood of oocysts surviving in septic tanks include: the nature of the soil, pathogen cell size and shape, the degree of water saturation and clogging. When macropores are present, preferential flow is increased, decreasing the filtering of microorganisms. It is also more difficult to filter out smaller microorganisms, like *Cryptosporidium,* although long rod-shaped cells are also filtered less effectively (Weiss, et al., 1995). Higher flow rates and clogging result in a reduction in filtering efficiency (Stevik, et al., 2004).

Oocysts can accumulate in septic tanks for the duration of an individual's illness. This would explain how oocyst numbers were so high periodically but not present at other times. It does not, however, explain the continual issues with *Cryptosporidium,* over many months.

A further question is 'Is it normal for septic tanks to contain *Cryptosporidium*?'. Frey et al. (1998) studied 14 cases of *Cryptosporidium* outbreaks from 1984-1996. Two of the cases (14%) were potentially due to septic tank contamination and leakage. These results make it seem as though it is possible for septic tanks to cause outbreaks. It is unlikely, however, for *Cryptosporidium* to be a 'normal' finding in a tank because active oocysts should not normally be present in the home. There are only a few ways by which active *Cryptosporidium* could enter the septic tank, since hot water from showering, washing machines, washing hands, washing dishes etc. should be of a high enough temperature to kill oocysts. The most likely way this would occur is if individuals within the property were infected and so their faecal matter would cause the septic tank to be contaminated. As identified, rates of infection within the general population is relatively low. Blanshard et al. (1992) tested HIV-positive patients in London. Although these people are highly sensitive to such an infection, only 5% of the 128 patients had *Cryptosporidium.*

Therefore, although literature suggests that it is not normal for septic tanks to contain *Cryptosporidium*, Anglian Water samples have found that they do.

Trade Effluent Sources of Cryptosporidium

If the *Cryptosporidium* is human in origin, it may have entered the STW through means other than domestic waste, namely trade effluent. The companies who are permitted to discharge their waste into the STW were identified.

Industrial Launderettes

The company with the permit for the largest effluent allowance was a commercial laundering company. It has a maximum daily volume of 480m^3 and a maximum flow rate of 20m^3 per hour. The company is not likely to be a source of the *Cryptosporidium* because the laundry cleaned is fully decontaminated to Class C of the ASTM F51/00 standard for garment particulate control (Microclean, 2014). This standard is achieved through the use of barrier washers, which have separated clean and soiled laundry inlets and outlets, reducing the risk of contamination.

That specific laundering company building is classified as IOS6 under ISO 14644-1 cleanroom standards (Microclean, 2014). This means that very low levels of particles are present at the site (for example 1.0×106 at ≥0.1 µm, 237,000 at ≥0.2 µm, 102,000 at ≥0.3 µm, 35,200 at ≥0.5 µm, 8,320 at ≥1 µm and 293 at ≥5 µm) (Pharma Journal, 2014). The laundering company is additionally regularly audited by Pharmaceutical and NHS customers to ensure that it meets these standards (Microclean, 2014).

Other Industrial Laundrettes had wastewater permits to Louth STW too. One such company washes the laundry of companies such as nursing homes, hotels and other industrial businesses (The Laundry Room Ltd., 2014). Even without the high regulations that other companies are under, it was unlikely that *Cryptosporidium* would survive the high temperatures used by automatic machines such as those used by these companies. Fayer (1994) found *C. parvum* infectivity was removed after warming oocysts to 45°C for five to twenty minutes. This makes it seem incredibly unlikely that *Cryptosporidium* would survive the process. Regardless, as McCulloch (2000) said, cross-infection risks can occur at all stages of the laundry process: handling; packaging; transporting; laundering and delivery of clean supplies.

To conclude, the trade effluent from industrial laundrettes is unlikely to contain *Cryptosporidium* oocysts.

Commercial Launderettes

There is some debate as to whether or not *Cryptosporidium* can survive in a domestic washing machine environment.

Fayer (1994) found *C. parvum* infectivity was removed after warming oocysts to 45°C for five to twenty minutes. This makes it seems unlikely that *Cryptosporidium* would survive in a washing machine environment since most washing cycles are at or around this temperature. This research agrees with work done by Casanova et al. (2001). Casanova's study on greywater found no *Cryptosporidium* in any laundry effluent tested. However this study only focused on 20 households so it is likely no-one was infected at the time of the study.

Washing of clothes is not always done at high temperatures, especially with the current environmental 'I Prefer 30' campaign for people to wash their clothes at 30°C to save energy. AISE (the International Association for Soaps, Detergents and Maintenance Products) commissioned a study to determine the appropriate guidelines for low temperature washing. An example of this is that guidelines suggest that the following should be washed at 60°C: the clothing of the ill or vulnerable people and their carers, clothing contaminated with faeces, vomit, blood etc. (The International Association for Soaps, Detergents and Maintenance Products, 2013). Although the 30°C campaign suggests that people should separate higher risk items from normal daily laundry and wash them separately in a 60°C wash, people often do not read the additional clauses and just acknowledge the main message of the advertising campaign- to wash on a lower temperature. This is an issue because, as Surl et al. (2011) identified, *Cryptosporidium* can survive being submerged in laundry detergent.

It could be said that in normal domestic households, the soiling of clothing and thus the spreading of *Cryptosporidium* through the washing of this clothing, is unlikely. Nevertheless, households that contain young children, incontinent people, older people and people who work with faeces (such as farmers, septic tank cleaners, care workers etc.) could still potentially have washing that contains *Cryptosporidium* oocysts. If a regular 60°C wash is not done, a biofilm could build up, allowing the passage of infection.

To conclude, the trade effluent from commercial laundrettes is fairly unlikely to contain oocysts but it could be possible if washing machines can be altered for lowered temperatures. Regardless, this is unlikely to have caused all of the *Cryptosporidium* issues found within the area over the time period being studied.

Swimming Pool

The second largest maximum flow rate per day effluent allowance for a trade mostly likely to produce *Cryptosporidium* oocysts was that of an East Lindsey District Council swimming pool. It has a maximum daily volume of $14m^3$ and a maximum flow rate of 46l per second permitted but this can be increased to allow drain down of the pool (after the formal approval from Anglian Water Services has been received). The wastewaters arise from swimming pool filter backwash operations.

Cryptosporidium contamination is unlikely to go undetected in swimming pools, since the source of the infection is easily traced back to the source. This is because the people who get infected are of similar demographics, are usually based in a similar area and may know each other, so may report the issue after more than one household becomes ill. Since PHE has not recorded any pool closures due to *Cryptosporidium* contamination in this area, it is unlikely to be the cause.

4. 1. 4 Animal Sources of **Cryptosporidium**

The results from the typing found that most animals would not be a major host for the *Cryptosporidium* strains found in Louth STW. Sheep could be the cause because, as Table 2 shows, they are a minor host of *C. hominis* and a major host of *C. parvum* and *C. andersoni*. This was demonstrated by Connelly et al.'s study (2013), which typed *Cryptosporidium* from a remote population of Soay sheep in Scotland. The study found that 11.4% of the samples tested contained *C. hominis*, 9% contained *C. parvum* and 2.7% contained *C. andersoni*.

Are Sheep Common in the Catchment?

The presence of sheep along the canal is likely. Hewson-Fisher (2014) found, on a Louth Canal walk-over study, that approximately 80% of the canal banks were grazed. Some of the grazing animals included sheep, with there being a definite sighting of 25-30 sheep grazing along 0.6 miles of the canal bank at one side. Sheep defecating near the canal is not the cause of the *Cryptosporidium,* since it is being found in the STW effluent. It could be that sheep waste is entering the STW (for example from an abattoir) which could be the source, especially if sheep along the canal are drinking from the waters to get the infection.

Cryptosporidium *Diagnosis in Sheep*

A local vet in the LN11 area was contacted to gather further information about the likelihood of *Cryptosporidium* being present in animals. The veterinary scientists who work at the clinic said they could not recall any recent outbreaks. However, the veterinary scientists also said that it was difficult for them to check their past records because their farm files are paper based. Another issue was that *Cryptosporidium* is not a very common diagnosis in pets. Scouring animals, therefore, are generally treated the same and the zoonotic aspect is rarely recorded in the notes. Laboratory testing, as in human sources of *Cryptosporidium*, would be needed, either in house with SureCheck (a Nimrod product) or externally (Moore, 2014). This extra work which the vet would have to do makes it unlikely that *Cryptosporidium* would be diagnosed. Furthermore Davies et al. (2003) found that *Cryptosporidium* oocyst recovery rates in sheep faeces samples were only 4-62%.

Furthermore, sheep can be carriers of *Cryptosporidium* but present no symptoms. Uluats and Voyvoda (2004) investigated a sheep farm in Turkey. The study found that, out of the lambs infected with *Cryptosporidium*, 79% had diarrhoea and 21% were asymptomatic. It was noticed, however, that 21% of the animals which had diarrhoea tested negative for *Cryptosporidium.* This infers that not only does the absence of diarrhoea in sheep not necessarily mean there is no *Cryptosporidium* infection but also that the presence of diarrhoea in sheep does not necessarily mean there is a *Cryptosporidium* infection.

Animal Trade Effluent Sources of Cryptosporidium

There was an investigation into the trades which currently have consent to discharge their effluents into Louth STW. It was found that there were no agricultural-type businesses that are licensed to discharge their waste into the STW that continuously have sheep present on site, such as farms, abattoirs etc. This greatly reduces the likelihood of *Cryptosporidium* being from animals. Regardless, illegal dumping could still be occurring.

There was one company with a permit to discharge into the STW, a livestock auctioneers, which operates roughly every week. The data for the sheep present and sold on each date was collated and compared with the *Cryptosporidium* oocyts/l discovered at that time. As Figure 20 shows, there was no clear relationship between the two. If this company had been the source of the *Cryptosporidium*, it would be expected that after a peak in animals sold, roughly 24 hours later, a peak in *Cryptosporidium* oocysts would have presented themselves. This source of data was, however, unlikely to produce successful results since there may have been animals present on the day that were not sold. Also, it is possible that only a few infected animals are present within a herd, and that the others are healthy individuals. Another consideration is that waste does not flow in a predictable manner. The faeces could stay within the drainage system until a precipitation event, accounting for the irregular *Cryptosporidium* results found. Thus number of animals sold is not commensurate with the number of animals infected.

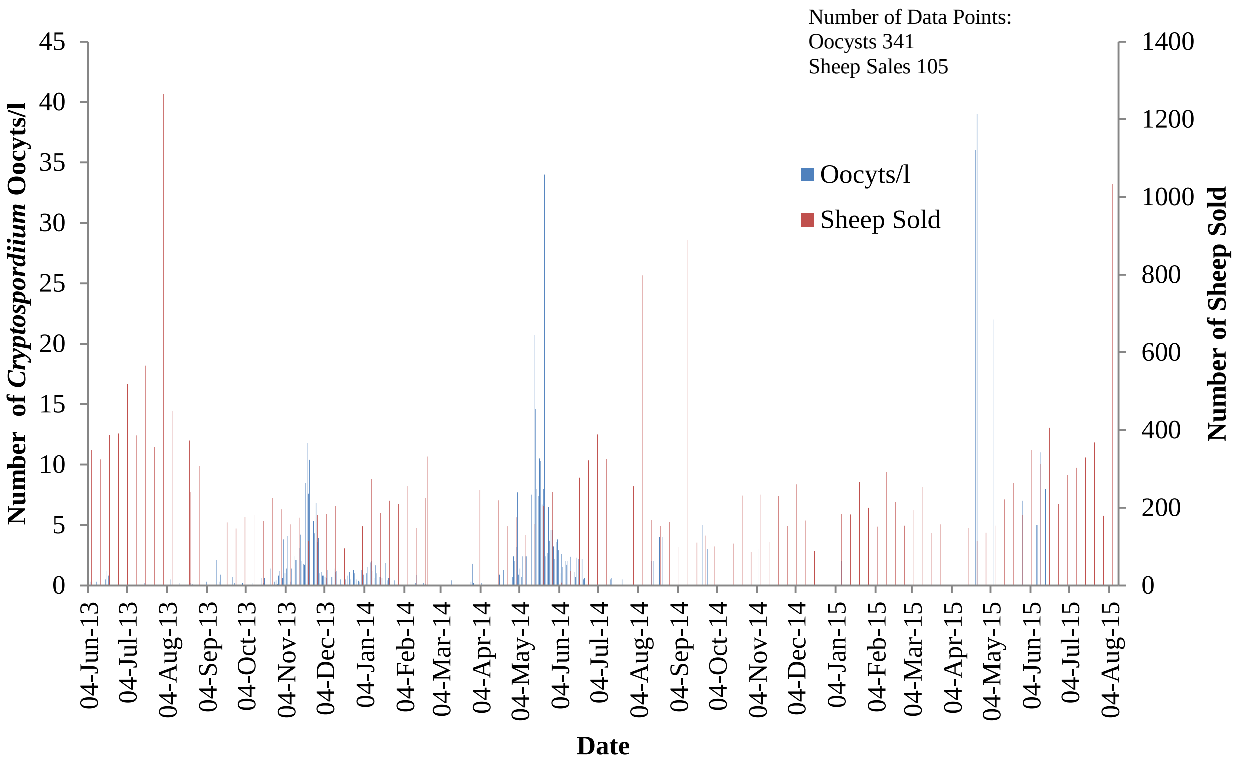

Figure 19 Total number of sheep sold and number of **Cryptosporidium** ***oocysts/l per month***

Data extracted from: Anglian Water (2015) *and* (2015)

4. 1. 5 Climate and Precipitation

Regardless of whether the *Cryptosporidium* found in the Louth area was due to a sheep or a human host, it is likely that climate and precipitation may play a part. Changes in the climate and precipitation can have many different effects which influence the treatment of water, both through environmental alterations and by causing changes in the works themselves. One such example is that after large temperature changes (for example between seasons), mass shedding of the bio filter biofilm occurs. Another example is that seasonal variation in water levels can become apparent, such as the case at Tetney Lock.

Drought and High Temperatures

Drought can lower groundwater water tables, which causes changes in underground water flows. It also means that surfacewater and groundwater can become mixed when it next rains again, as in the case of *Cryptosporidium* outbreak studied by Bridgman et al. (1995). In *Cryptosporidium* incidences where groundwater plays a part, several factors contribute to groundwater exposure (Balthazard-Accou, et al., 2014). It is reliant upon variations in: geological structures, geomorphology, rock types, and precipitation (Knowles, et al., 1999). However, *Cryptosporidium* is more common in surface water than groundwater because these sources are less vulnerable to direct contamination from sewage discharges and runoff (United States Environmental Protection Agency, 2001). Furthermore, this is unlikely to be the issue at Louth because the *Cryptosporidium* has been found in the STW effluent. Besides, there is little chance of groundwater contamination causing further infection because drinking water is abstracted from Louth Canal (and rainwater) and not the groundwater.

A lack of rain water can also cause less diluted sewage effluent and animal wastes in surface waters. Therefore if *Cryptosporidium* is present, it is more likely to be spread in the water and is more likely to be identified by sampling. Although this has potential to alter the amount of *Cryptosporidium* found at warmer times of the year, such results have not been found in the Louth case study. Figure 20 shows how average temperatures and seasons appear to not affect the occurrence of *Cryptosporidium*.

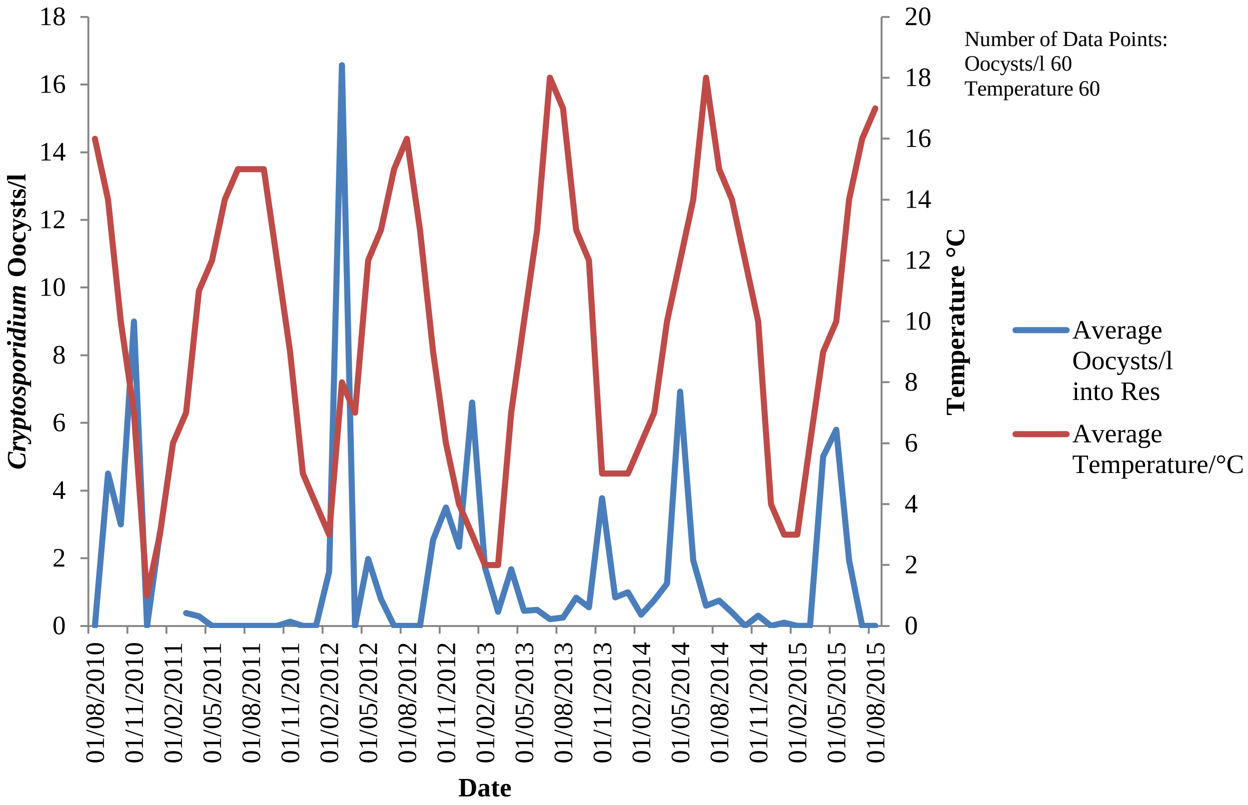

Figure 20 Average temperature and average oocysts/l in the reservoir intake in Louth 2010-2015

Data extracted from: Anglian Water (2015) *and Weather Underground* (2015)

Consequently, drought is unlikely to play a part in the *Cryptosporidium* issue. When droughts do occur, additional water is pumped into Louth Canal, along a pipeline from the Great Eau, meaning that the canal is not exposed to these conditions for long. Furthermore, as Figure 20 shows, there is no clear relationship between periods of high temperature and *Cryptosporidium* oocysts.

Precipitation

An investigation into the relationship between precipitation and the number of oocysts found at Covenham Reservoir inlet showed that the two variables do seem to have a relationship [Figure 21]. There were three peaks in oocysts and rainfall that coincided with one another but two peaks of increased precipitation which seemingly had no effect on the *Cryptosporidium* levels.

Number of Data Points: 27

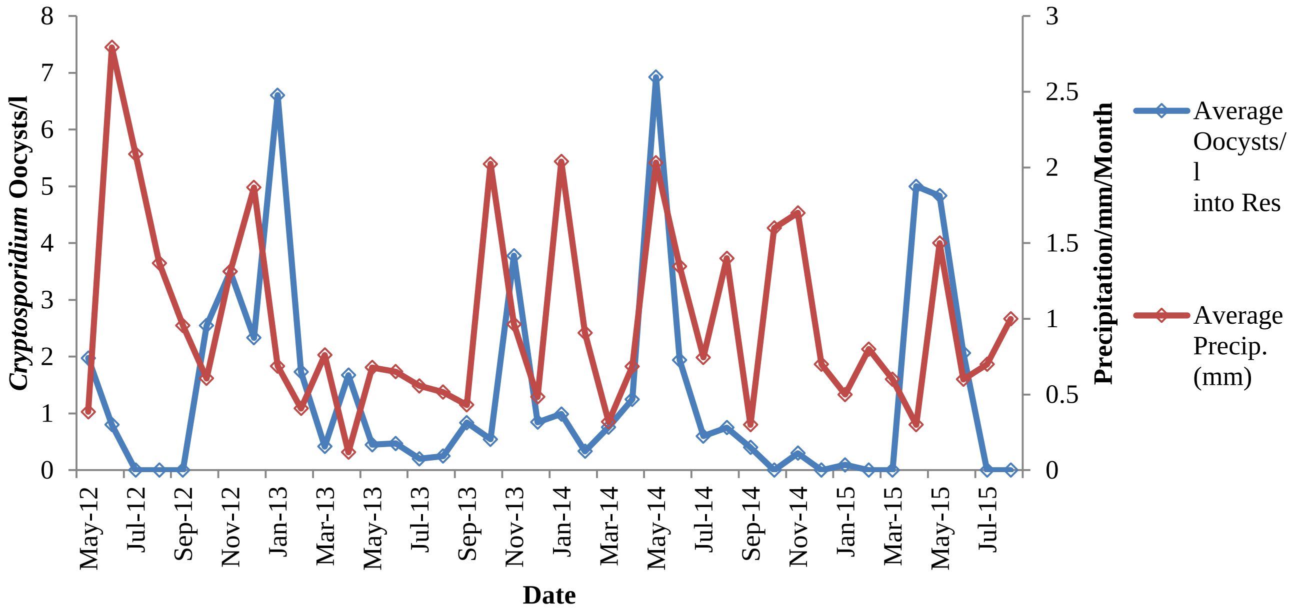

Figure 21 Average precipitation/mm and average oocysts/l in the reservoir intake in Louth 2012-14

Data extracted from: Anglian Water (2015) *and Weather Underground* (2015)

This was investigated further through analysis of rainfall intensity and *Cryptosporidium* occurrence [Figure 22]. However rain intensity had no clear relationship with *Cryptosporidium* found between 2012 and 2014.

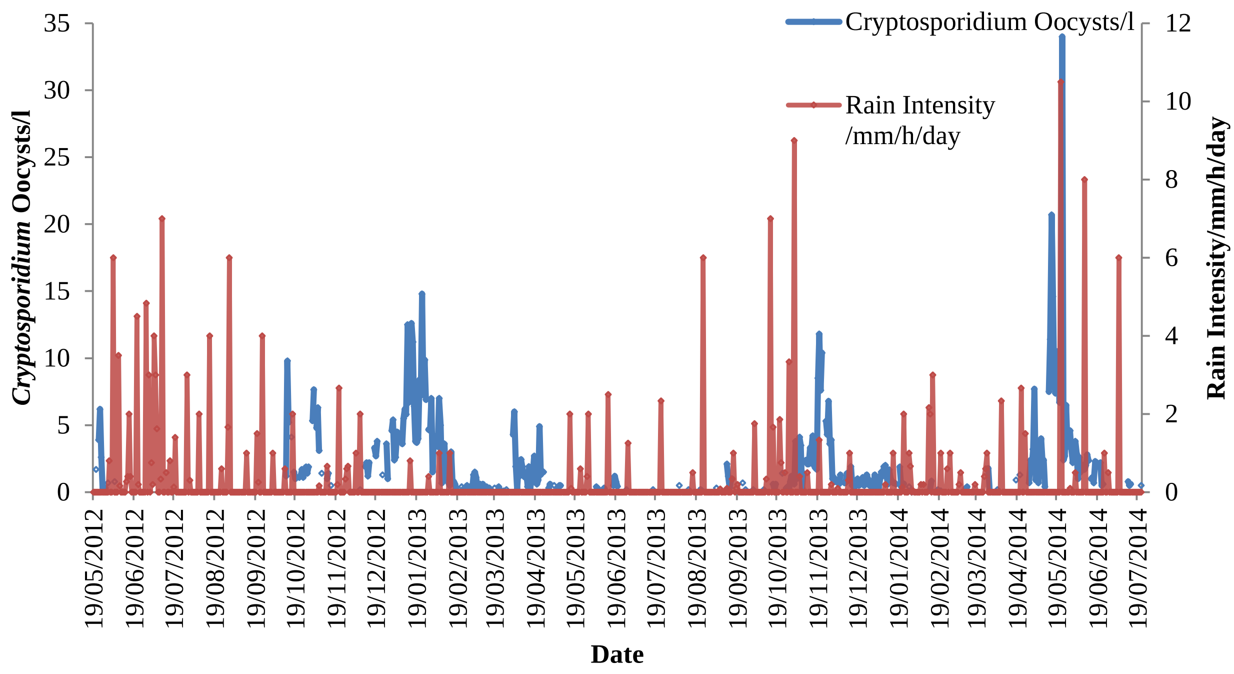

Figure 22 **Cryptosporidium** ***Oocysts/l and rain intensity/mm/h/day***

Data extracted from: Anglian Water (2015) *and Weather Underground* (2015)

To further investigate precipitation, average rainfall either side of *Cryptosporidium* spikes were investigated. In 8/13 *Cryptosporidium* peaks of >20oocysts/l there was higher than average total rainfall 5 days before the oocyst event.

The average rainfall when low levels of *Cryptosporidium* were detected was also investigated. On 9/9 occasions when lower than average *Cryptosporidium* was detected (less than 2.4 oocysts/l) for a period of 7 days or greater, there was lower than average total rainfall during this period.

Seasonal rainfall can also cause *Cryptosporidium* issues or make current problems worse. In the UK seasonal outbreaks may also be caused between spring run-off events, as in the case of Lake et al. (2005). If the *Cryptosporidium* had been found mostly in Louth Canal, it may have been due to run-off from the Lincolnshire Wolds because the area surrounding the canal is at the bottom of a slight slope. This is not, however, the case. Because the *Cryptosporidium* is concentrated at the STW it is unlikely to be due to run off. However it is still possible because gravity-fed as well as pumped inlets exist as Louth STW.

The fact that *Cryptosporidium* is concentrated at Louth STW also means that flooding is unlikely to play a part. Flooding can be a major reason why *Cryptosporidium* outbreaks occur. An example of this is from research in the USA that found 51% of 548 outbreaks of *Cryptosporidium* were due to flooding (Curriero, et al., 2001). This happens because high-risk flood areas often have latrines and septic tanks equipped with infiltration wells. These wells can then overflow and the sludge can be discharged to alluvial formations. The area surrounding LN11 is at risk of flooding, however measures are in place to stop this.

Consequently it appears that *Cryptosporidium* at Louth STW is exacerbated by high levels of precipitation and can be absent when precipitation is low but it does not appear to be specifically affected by rain intensity/type (i.e. mm/hour). Seasonal rainfall and flooding are unlikely to have caused the oocysts detected in Louth Canal, although they do affect the works because storm flow can be produced.

Storm Flow through the STW

After prolonged rainfall, Louth STW overflows due to storm-water inflow, resulting in the works going into storm flow. This means that the STW discharges untreated effluent into Louth Canal under these conditions. When this happens, the abstraction from the canal for Covenham Reservoir is shut down for four days. Regardless, there may be a delay between the heavy rain and the shutdown.

To explore this as a potential contributor, storm flow from Louth STW from 2012-2015 was correlated with *Cryptosporidium* oocyts found at the [Figure 23]. There did not seem to be a relationship.

Number of Data Points:
Oocysts 484
Flow 1191

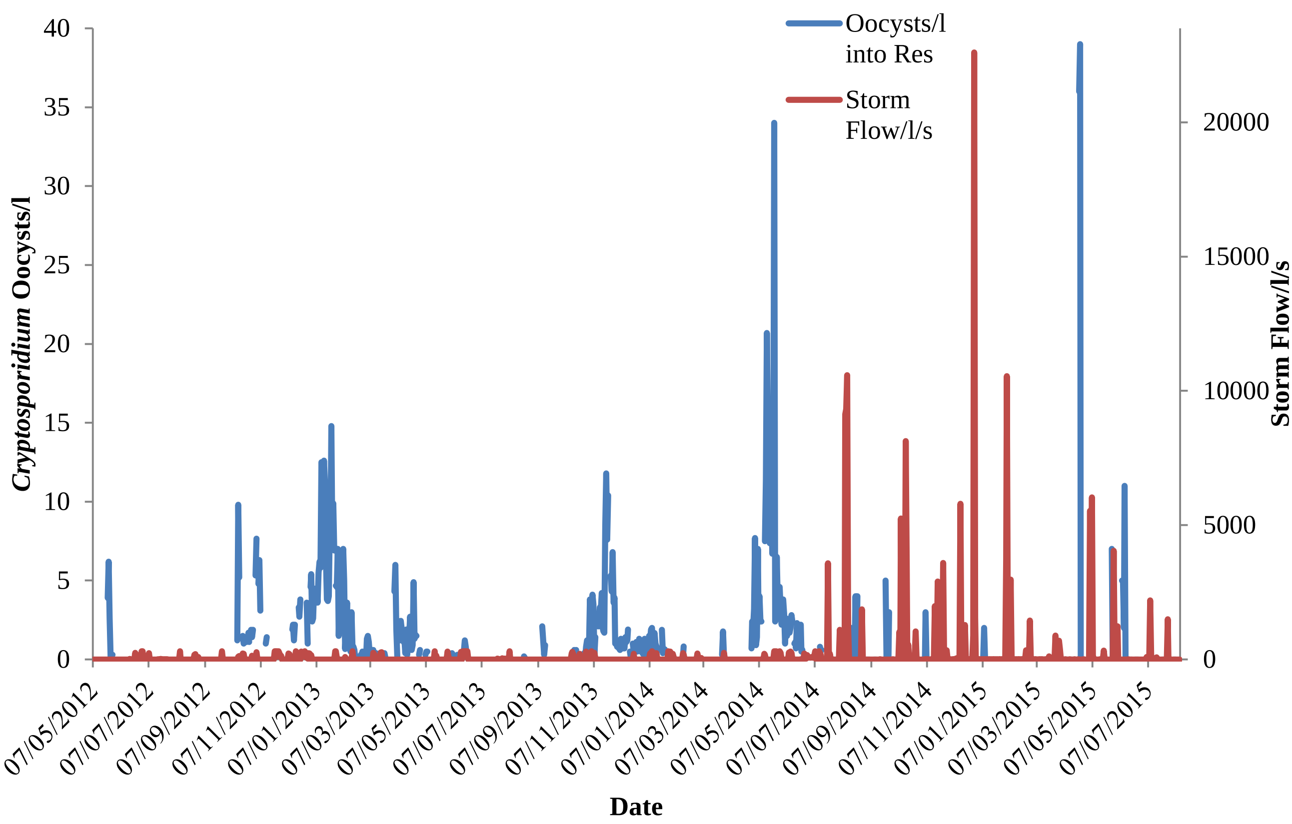

Figure 23 **Cryptosporidium** ***oocysts/l at Covenham Reservoir intake and Louth storm flow/l/s 2012-2015***

Data extracted from: Anglian Water (2015)

A sample from the storm flow tank was taken to see if *Cryptosporidium* oocysts were present in greater than expected numbers. In this sample 10 oocysts/l were detected on 27/07/15, which is perfectly normal for water of this quality, since it contains great amounts of agricultural run off. This data, combined with Figure 23, makes it seem that storm flow is not particularly influencing the *Cryptosporidium* in Louth Canal.

4. 1. 6 Other Causes and Issues

Comparisons of Sewage Works Discharge on Abstraction

Louth WTW's abstraction point is influenced by the STW discharge. Therefore, work using GIS was completed by Anglian Water to compare Louth with two other abstraction points which are influenced by STW (Evangelidou & Berry, 2014).

It was found that the discharge into Louth Canal was more diluted than at the other sites (The River Great Ouse with the Offord intake at St Neots and the River Gipping with the Sproughton intake at Needham Market and Stowmarket STW). The sewage effluent entering Louth Canal is diluted by 11.8%, whereas the River Great Ouse and the River Gipping had figures of 0.7 and 4.4%, due to the flow of the canal and the size of the catchment area draining to it, as well as the sewage effluent (Evangelidou & Berry, 2014). This may occur because the other abstraction points have STW discharges closer to them and one of the STW is larger than Louth.

Another difference between the sites was that the other abstraction points have a relatively larger catchment area draining to the rivers (256km^2 and 2570km^2), compared with Louth Canal (55km^2). This means that Louth Canal has higher flows (potentially due to smaller pipe sizes and so higher flow rates), which causes higher mixing, resulting in greater dilution. This means that Louth Canal is especially likely to be highly diluted during storm events, which is when the worst quality water discharges from the STW.

Cryptosporidium *in Canals*

The *Cryptosporidium* in the canal may behave differently than *Cryptosporidium* in a river because it is an artificial environment. Thus, it could be that when canals are used for disposing of STW effluents, *Cryptosporidium* spikes are a normal occurrence.

One such difference is that of the slower flow rate found in the canal. Flow in the canal is likely to be plug flow rather than mixed flow. Mixed flow is where the fluid mixes in a continuous and uniform way and so contamination would be found throughout the volume of the water. Plug flow describes the movement of water and contamination when it is in a state of continuous flow, without backwash or mixing (although in natural environments, such as rivers, this is less common) (Levenspiel, 2011). These types of systems usually produce a biofilm, where the water has been passing over a surface consistently. Louth Canal is a more plug flow system, although some mixing could occur.

Other research on *Cryptosporidium* occurrence in canals indicates that its presence in canals is not normal. For example two sampling sites in the Grand Union Canal (Duston Bridge and Flore Bridge) had no *Cryptosporidium* found between May and July 2012. This data was based on a total of 22 samples over the sampling period.

Cryptosporidium oocysts are able to move laterally through a river either freely or when attached to particles within the water. Dai and Boll (2006) suggested that although oocysts travel freely in water, it is more difficult for them to do so when not attached to sediments. Bonatti and Franco (2014) said that *Cryptosporidium* oocysts have a low attachment rate to suspended particles in water bodies because of their low hydrophobicity, as well as their slight negative charge (when soil particles, too, generally have a negative charge). This makes it seem as though *Cryptosporidium* oocysts are more likely to be transported laterally than vertically in the water column.

Oocysts settle out very slowly because they are so small and light, at a rate of approximately $0.67\ \mu ms^{-1}$ (Dai & Boll, 2006). This is altered, however, when the oocysts attach to particles, since the rate of sedimentation is increased as the particle size increases. Regardless it must be remembered that there are many other contributing factors, such as the degree of mixing of the water body or its flow rate.

The average daily flow of Louth Canal (1968-2004) is 0.12 m^3/s (Maunsell, 2004). It can be seen how small a flow this is when compared with the mean flow of the River Great Ouse which has a flow rate of 15.7 $^3/s$. Generally, the faster a body of water is, the more likely it is that oocysts will be transported laterally and that a greater distance will be travelled.

Final settlement experimentation completed by Medema et al. (1998) showed how 30% of *Cryptosporidium* oocysts became attached after minutes of mixing. This then increased to 75% after 24 hours.

Zhang et al. (2001) found that oocysts that get adsorbed to sediment may get detached and re-suspended in the flow because of the flow's increased shear stress. This means that when flow is greater (for example due to storm flow or just rainfall events themselves), the *Cryptosporidium* oocysts previously settled at the base of the canal are re-suspended and able to move once more through the water column. This is a potential explanation for how *Cryptosporidium* oocysts found were more numerous when precipitation was greater.

Dredging describes the process where the canal bed is cleared of accumulated materials. The Environment Agency periodically dredges Louth Canal using draglines (which are positioned on top of the channel banks) approximately every 10 years. It is completed when there is increased deposition which is likely to affect the level of flood risk (Maunsell, 2004). The procedure could effect how *Cryptosporidium* survives and interacts with its environment, since it is disturbed, making it not settled in the water. Regardless, since this process happens every 10 years, it is unlikely to have had a major impact on *Cryptosporidium* levels in this particular case study.

4. 2 *Cryptosporidium* sampling across Louth STW

In order to determine that Louth STW was effective at *Cryptosporidium* removal, a sampling campaign was carried out across the STW between April and September 2015. Planned sampling occurred every two weeks for five months. During a spike in *Cryptosporidium*, sampling intensified to once per week.

The sampling consisted of grab samples using sampling cups on telescopic poles and 1l or 10l bottles and autosamplers. The grab samples were useful when the *Cryptosporidium* outbreak in the population just began, meaning that the samples could be taken immediately, without the need to ask other departments for equipment. 1l of sample was taken from the influent, after primary sedimentation and after the trickling filters and 10l from the effluent.

On each visit, samples were taken from the influent to the STW, the effluent from the STW into Louth Canal and the effluent of the primary settlement tanks, trickling filters and the humus tanks. Further samples were taken when unexpectedly high primary settling tank oocysts were being detected. These samples were taken from the sludge holding tanks, the sludge supernatant, the humus return and the storm tank [Figure 13].

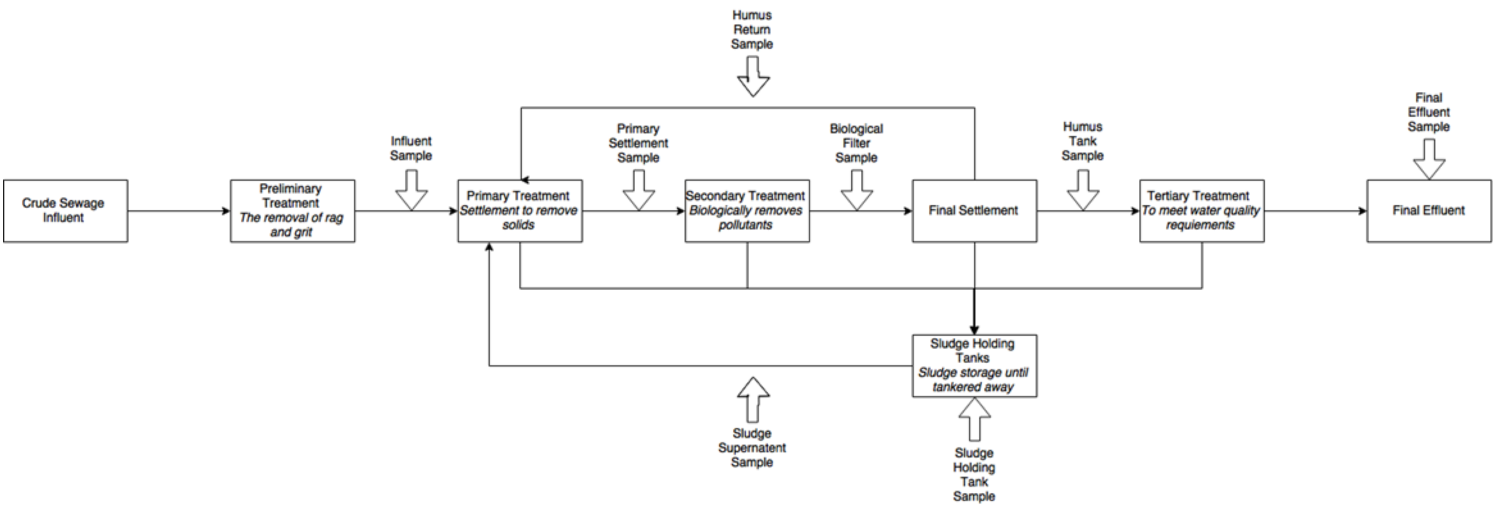

Figure 24 Louth sewage treatment works sampling map

A further important part of the sampling phase was to compare similar STW to see if this issue has been seen or is common in other STW which share similar characteristics. These characteristics include the similarity of the treatment processes used at Louth [Figure 13], the numbers of oocysts encountered, the rural environment of the STW, the water quality and the size of the works, the population capacity it was built for and the current population number served. This was completed by sampling Stamford and Stowmarket STW.

4. 2. 1 The Effectiveness of Louth Sewage Treatment Works' Current Treatment Methods

The sampling of Louth STW found that the number of oocysts entering the works varied weekly, with a range from 5 oocysts/l to 235 oocysts/l over the sampling period [Figure 25]. It was also noted that there were no samples which had 0 oocysts/l in the influent of Louth STW, meaning that oocysts were found to be continuously entering the works, although the numbers found did vary significantly.

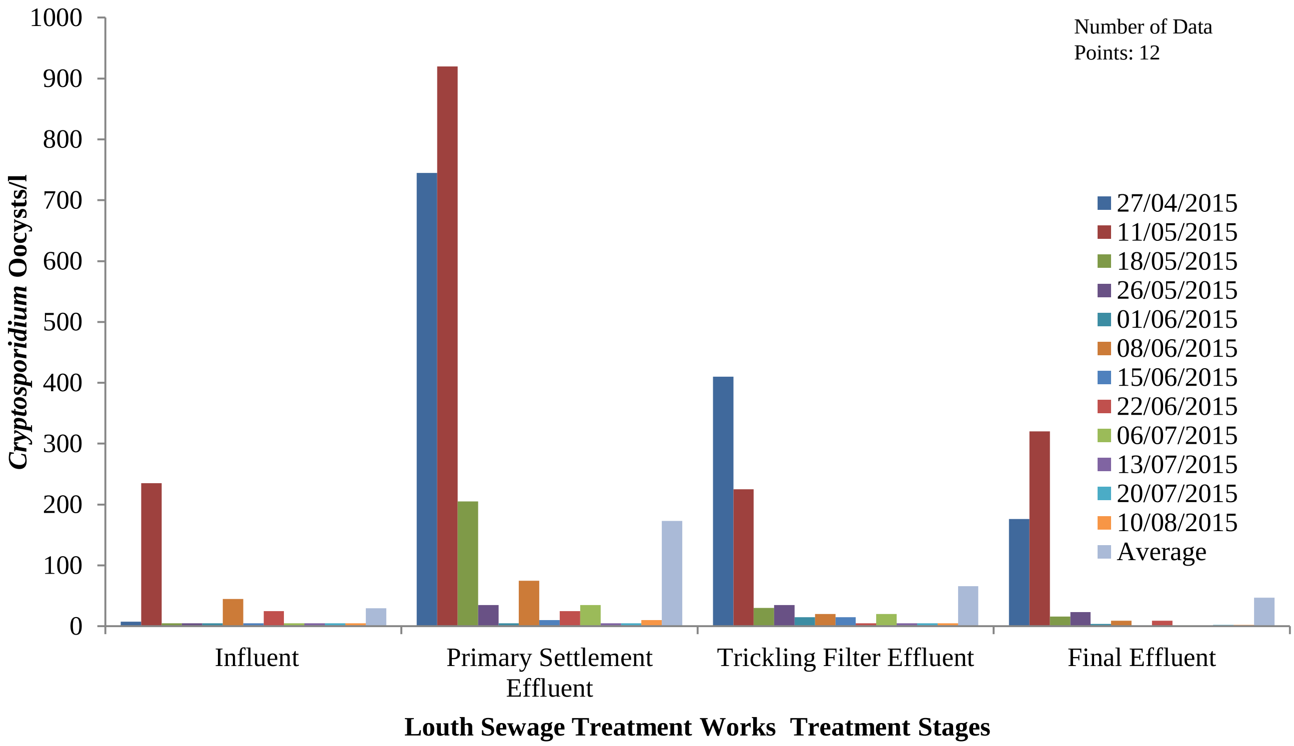

Figure 25 The* Cryptosporidium *oocysts/l at different treatment processes in Louth Sewage Treatment Works

The effectiveness of the trickling filters and the humus tanks for *Cryptosporidium* removal were also investigated and it was found that they were a relatively good treatment process for removing *Cryptosporidium* [Figure 25; Figure 27]. Over the sampling period, trickling filters removed 17% of the oocysts encountered and humus tanks removed 44%. One point to note is that as the oocysts progress through the STW, and as they are removed, fewer oocysts were encountered by later treatment processes. A more appropriate means of comparison therefore may not be between different treatment processes but for specific processes and past studies on them. Thus Figure 26 shows that the trickling filters and humus tanks did remove oocysts from Louth STW but they did not do so as effectively as literature suggests is possible. One example is a 17% removal by trickling filters at Louth STW but a literature average of 53% removal rates was found.

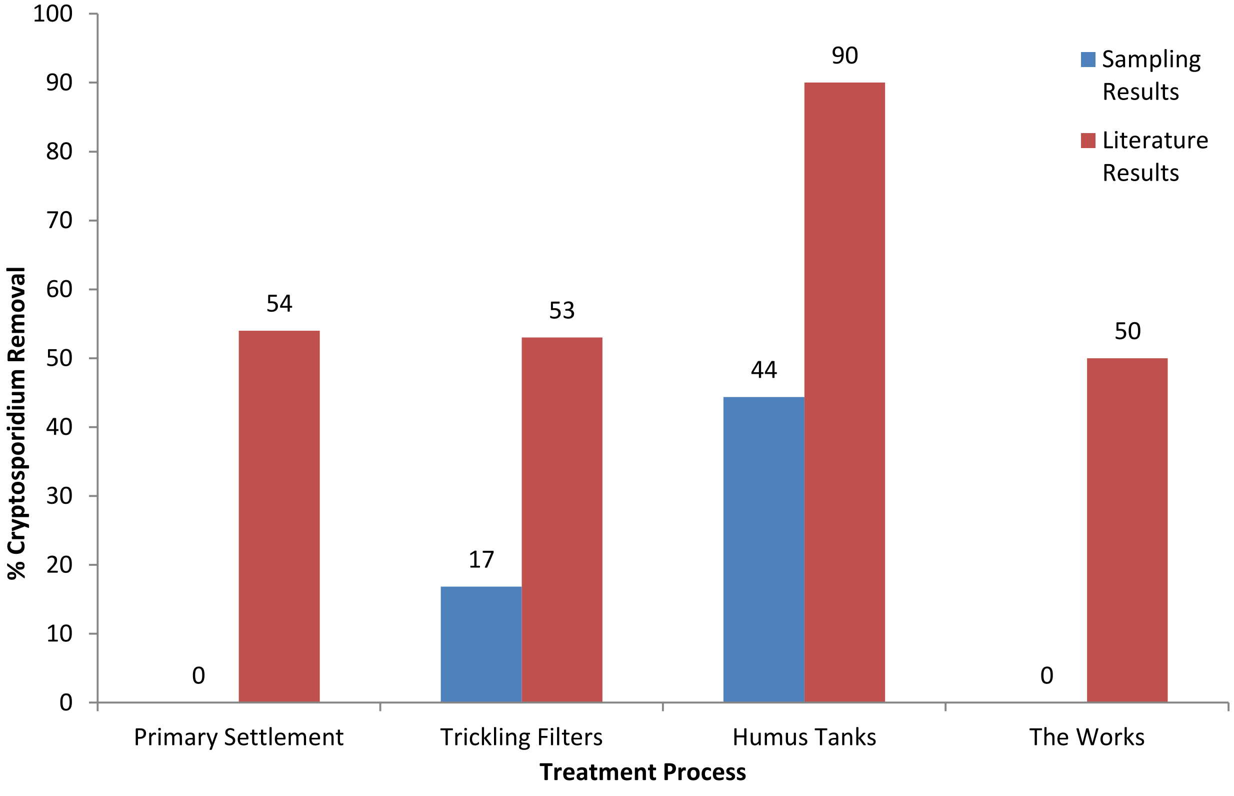

Figure 26 A comparison between the sampling results and the literature review results

Over the sampling period, it was generally found that more oocysts were found in the final effluent than in the influent to Louth STW. This is why Figure 26 shows that the works had a 0% oocyst removal rate. One example of this is that on 11/05/15, 235 oocysts/l were detected in the influent and 320 oocysts/l were in the final effluent

[Figure 25]. Similar results were also found during the primary settlement process, for example on 18/05/15, 5 oocysts were detected before the primary settlement tanks and 205 oocysts/l were in the effluent of this process. Since *Cryptosporidium* can only reproduce within a host, these results indicated either that there was contamination in Louth STW or that the oocysts were being concentrated somewhere in the process. Therefore a more in-depth analysis of Louth STW needed to be completed, resulting in an investigation of the sludge holding tanks, sludge supernatant and humus return well [Figure 27].

It was found that large numbers of oocysts were present in the sludge holding tank, for example the sample from 11/05/15 had 3333 oocysts/l [Figure 27]. It seemed that the oocysts were concentrating in the sludge holding tank, accounting for the low works influent numbers but high works effluent numbers. The sludge supernatant and humus return well samples found that *Cryptosporidium* was surviving in these conditions and being passed through to the beginning of the works at the primary settlement tanks. It is this recycling of oocysts which account for the increase in oocysts at the primary settlement tanks which was detected in Figure 25 and Figure 27.

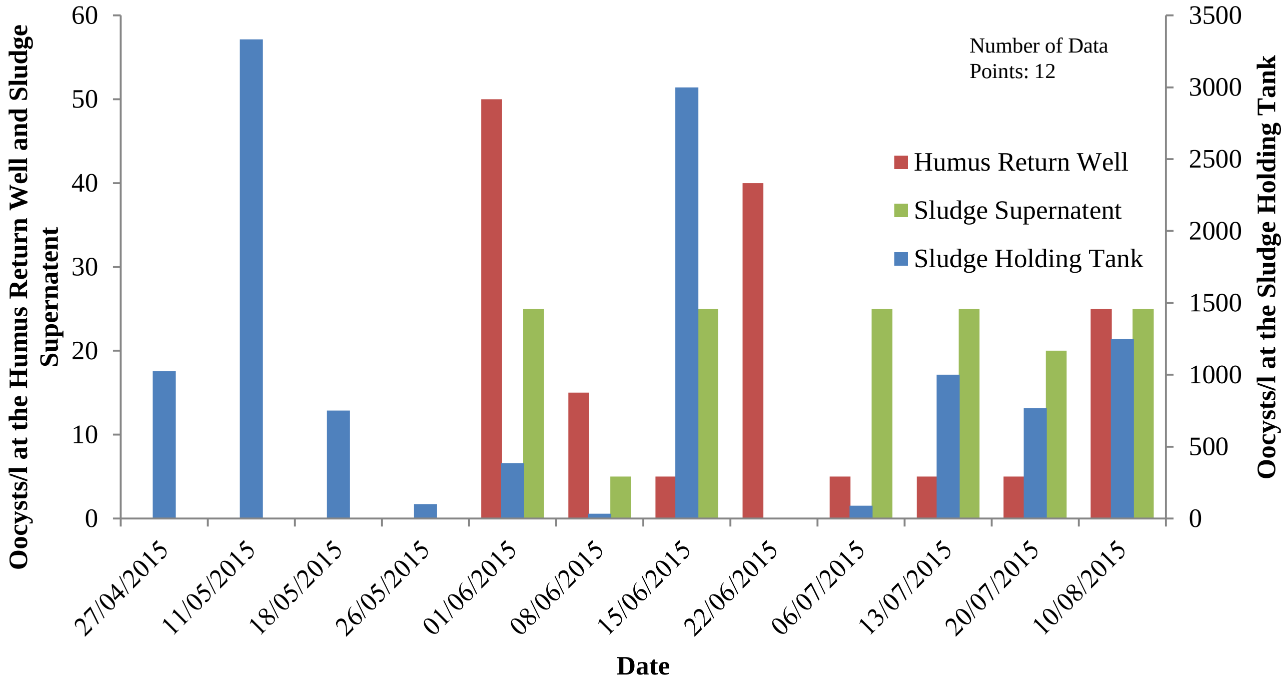

Figure 27 The* Cryptosporidium *oocysts/l at the sludge holding tank, humus return well and Sludge supernatant in Louth Sewage Treatment Works

Primary Settlement

The effectiveness of primary settlement in terms of *Cryptosporidium* deactivation was poor, with a 1299% oocysts increase at Louth STW. This is because many more oocysts were detected after the process (an average of 29 oocysts/l in the influent) than before it (an average of 173 oocysts/l after primary settlement), resulting in an increase of 1299%. This high figure is likely to be the result of the sludge holding tank supernatant being recirculated at this point in the works. It should be noted that this increase is not likely to be due to *Cryptosporidium* replicating within the works because it cannot reproduce outside of a host. It was most likely due to the episodic accumulation of oocysts in the sludge holding tank being recycled back into the STWs.

The studies of Robertson et al. (2000) and Whitmore and Robertson (1995) both found that primary settlement alone was relatively inefficient at removing *Cryptosporidium* cysts. Zhang et al. (2008) also found a relatively low removal rate of 24.7% oocysts. But these results are still much higher than the sampling results from Louth STW.

A model completed by Stadterman et al. (1995) detected an oocyst removal rate of 83%, far greater than that seen at Louth STWs. This great variation may be because the work of Stadterman et al. (1995) was completed in a model rather than a works. Regardless, there are discrepancies between this data and other research that has been done about the effectiveness of primary settlement.

As shown in Figure 3, STW generally feed their returned liquors back to the head of the works, at the primary settlement tanks. If *Cryptosporidium* oocysts are accumulated in the sludge holding tanks this could mean oocysts are being pumped back into this process, making it appear less effective. The addition of this sludge may also affect the rate of settling and the composition of the water which affects settlement. Thus the sludge holding tanks, the humus return and the sludge supernatant were investigated to find the numbers of oocysts in each of these stages and see how they effect primary settlement.

Secondary Treatment

At Louth STW secondary treatment did not have a very high percentage removal of *Cryptosporidium* oocysts (17% removal, rather than 44% removal after final settlement), but it did remove the greatest number of oocysts/l (108 oocysts/l with a range of 695-0 oocysts/l removed). Similar results were found by Zhang et al. (2008), Montemayor et al. (2005) and Robertson et al. (2000), who stated that secondary treatment was the most successful at reducing the viability of *Cryptosporidium.*

Trickling Filters

At Louth STW the method of secondary treatment used is that of trickling filters. The trickling filters at Louth STW had a removal rate of 17%. This removal rate was lower than the results from other studies. One study, by Villacorta-Martinez de Maturana et al. (1992), found that when waste water had a residence time of over 3.5 hours in trickling filters, there was an oocyst removal efficiency of 56%. A model completed by Stadterman et al. (1995) found removal rates of 50% following sedimentation.

There is currently an issue with increased phosphorus at Louth STW, resulting in increased plant growth on the filters. The performance of trickling filters can be diminished by the following: feed sewage organic strength, distribution mechanism, ventilation system, the frequency/rate of dosing and weather/season. This increased plant growth and the lack of regular weeding may be reducing how effective the trickling filters are at removing *Cryptosporidium* oocysts.

Therefore the trickling filters at Louth STW successfully removed the most oocysts than any other treatment process Figure 26]. But in terms of percentage removal they are not as effective as other studies suggest, nor as effective as the humus tanks.

Humus Tanks

At Louth STW a 44% removal rate was observed across the final settlement tanks (although this did vary between -80-95% during the study). Robertson et al. (2000) found that a secondary settlement for 3.6 hours resulted in a 91% oocysts removal.

The effluent from the final settlement process is sent back to the head of the works at the primary settlement tanks. Since final settlement occurs in the humus tanks, this process is known as humus return. The humus return was also sampled for *Cryptosporidium* and it was found that it contained an average of 19 oocysts/l (with a range of 5-50 oocysts/l). This means, therefore, that *Cryptosporidium* oocysts are able to survive through the final settlement process and so are able to pass back to the beginning of the STW.

Sludge Holding Tank

The solids at Louth STW are collected in the sludge holding tanks until they can be transported by road to Pyewipe Sludge Treatment Centre. When in the holding tanks, excess water in the sludge is removed and sent back to the primary treatment process. This dewatering procedure is done to minimise transportation costs.

In an attempt to explain the *Cryptosporidium* peak at the primary sedimentation tanks, the oocysts/l in the sludge holding tanks began to be sampled at Louth STW too. An average of 1067 oocysts/l were found during this stage of the process (with a range of 33-3333 oocysts/l). This part of the process had the largest number of oocysts, suggesting that they were being contained and concentrated here.

Research completed by Robertson et al. (2000) found that sludge stored in holding tanks had reduced viable *Cryptosporidium* oocysts over time. After 0 hours there was 72% viability, after 66 hours it was 47% and after 162 hours it was 36%. This was a much greater reduction when sludge was in different environments to the holding tanks, for example raw sewage's *Cryptosporidium* oocyst viability only decreased from 72-68%. However the research at Louth does not necessarily present the same findings, since so many oocysts were found on each of the sampling times over the 4 months. This may be because in the study there was continuous flow in the sludge holding tanks. Since more sludge was added continually to the top while sludge was continually removed from the base, the sludge itself in Robertson et al.’s (2000) experiment was in a semi-solid state. This meant that the oocysts could potentially have been reducing in viability through desiccation. However this is not likely at Louth STW, where this continuous flow system is not in place. At Louth the sludge in the tanks builds up until tankers remove it. This means that the sludge in the tanks has varying levels of solids concentration, since the amount of sludge and the frequency of sludge removal varies. Therefore *Cryptosporidium* oocysts may not act in the same way as was seen in the Robertson et al. (2000) study.

If the oocysts are able to survive in the sludge holding tanks, as it seems from the number of cysts found in this work, they will then be transferred to a sludge treatment centre (Pyewipe). Any *Cryptosporidium* oocysts entering Pyewipe Sludge Treatment Centre would be inactivated by the processes used. The centre pasteurises the sludge at a temperature of 57°C for 5 hours. Since *C. parvum* infectivity is removed after warming oocysts to 45°C for 5-20 minutes, the oocysts would no longer be viable after this process (Fayer, 1994).

Regardless of the way *Cryptosporidium* oocysts are neutralised at Pyewipe Sludge Treatment Centre, it is clear that they accumulate in the Louth STW’s Sludge Holding Tanks. To investigate how or if this impacted the works, sampling of the sludge supernatant was carried out.

Sludge Supernatant

When the sludge gets dewatered in the sludge holding tanks, the excess water is passed back to the head of the works at primary settlement to allow it to be re-treated. Since many oocysts were being found in the sludge holding tanks, further sampling was carried to see if the oocysts were being settled out into the sludge for neutralisation or if they were being removed from the tank in the supernatant and re-contaminating Louth STW.

The results of sampling the sludge supernatant at Louth STW found 21 oocysts/l (with a range of 5-25 oocysts/l). This means that *Cryptosporidium* oocysts were able to survive in the sludge holding tank and were, along with the humus return's oocysts, re-contaminating the works at the primary settlement tanks.

4. 2. 2 Sewage Treatment Works Comparisons

Anglian Water currently carry out approximately 150 Louth Canal *Cryptosporidium* samples per year. There was the suggestion, therefore, that *Cryptosporidium* was only being found in the area, more so than in other areas, because it was being looked for. Consequently samples of other STW have been taken to act as a control. The controls were selected because they had the same treatment processes and approximate population size as Louth, as well as all of them being in relatively rural environments.

Even though a sample could only be taken once at each control site, as can be seen by Figure 28, all three of the STW sampled had *Cryptosporidium* through the process.

It was found that Louth STW had similar results as Stamford STW [Figure 28; Figure 30]. Stamford STW had a higher number of oocysts in the primary settlement effluent than in the influent to the works. It also had oocysts present in the sludge supernatent and the humus return, suggesting that *Cryptospoiridum* was recirculating through the works like at Louth STW. This process is not of concern at Stamford STW because a WTW does not use this discharged water.

Stowmarket STW showed different results to Louth STW [Figure 28; Figure 29]. Although this works had oocysts present in the humus return and the sludge supernatent, it seemed that they were being settled out and removed by the works because few oocysts were in the final effluent.

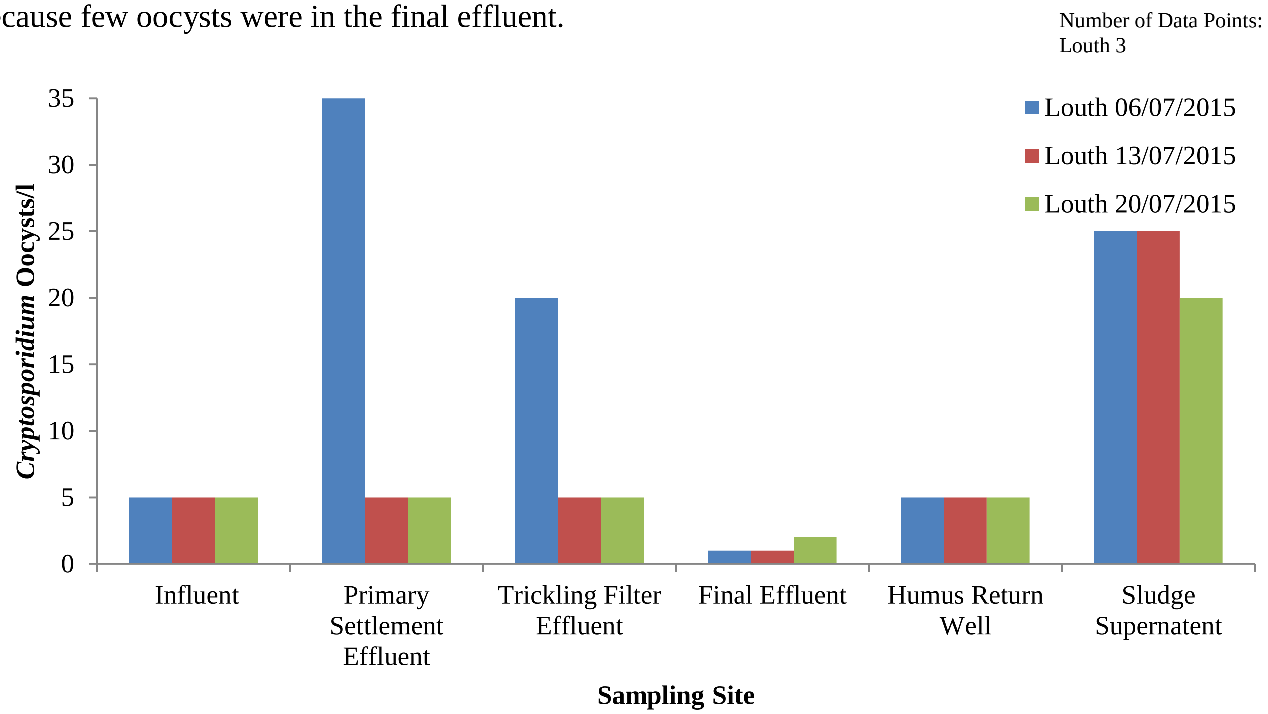

Figure 28* Cryptosporidium *oocysts/l at Louth Sewage Treatment Works

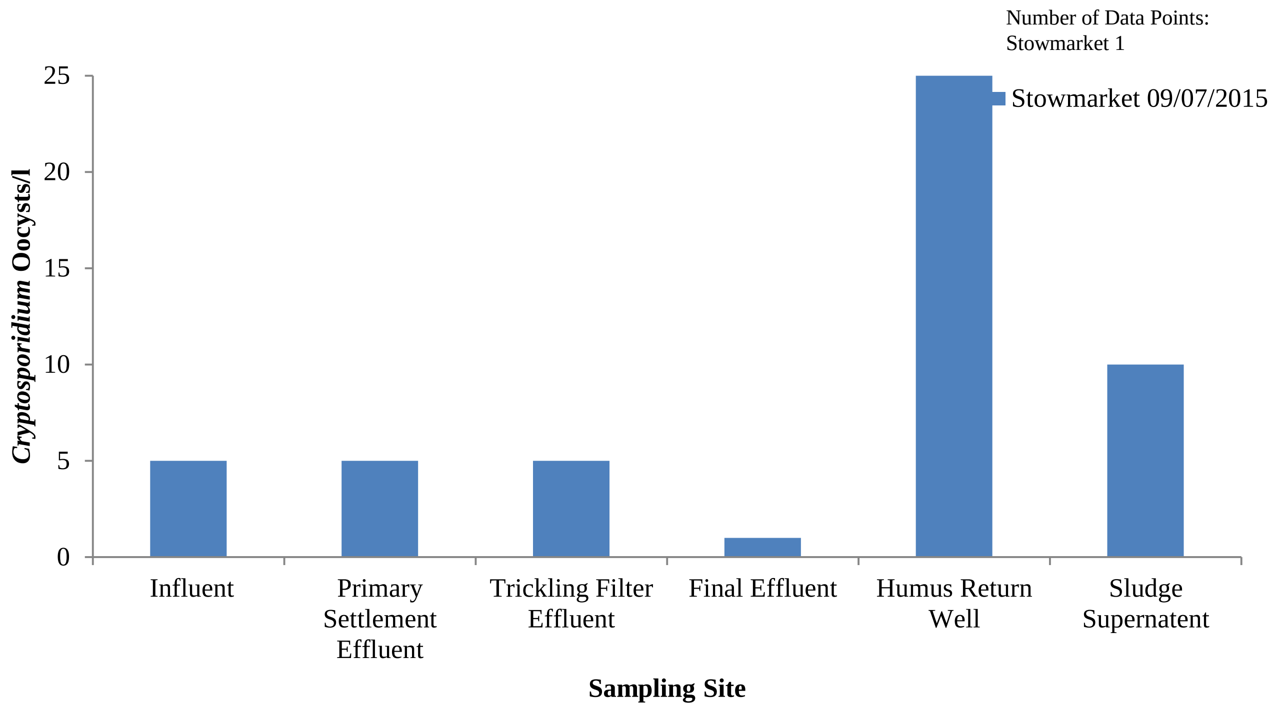

Figure 29 Cryptosporidium *oocysts/l at Stowmarket Sewage Treatment Works*

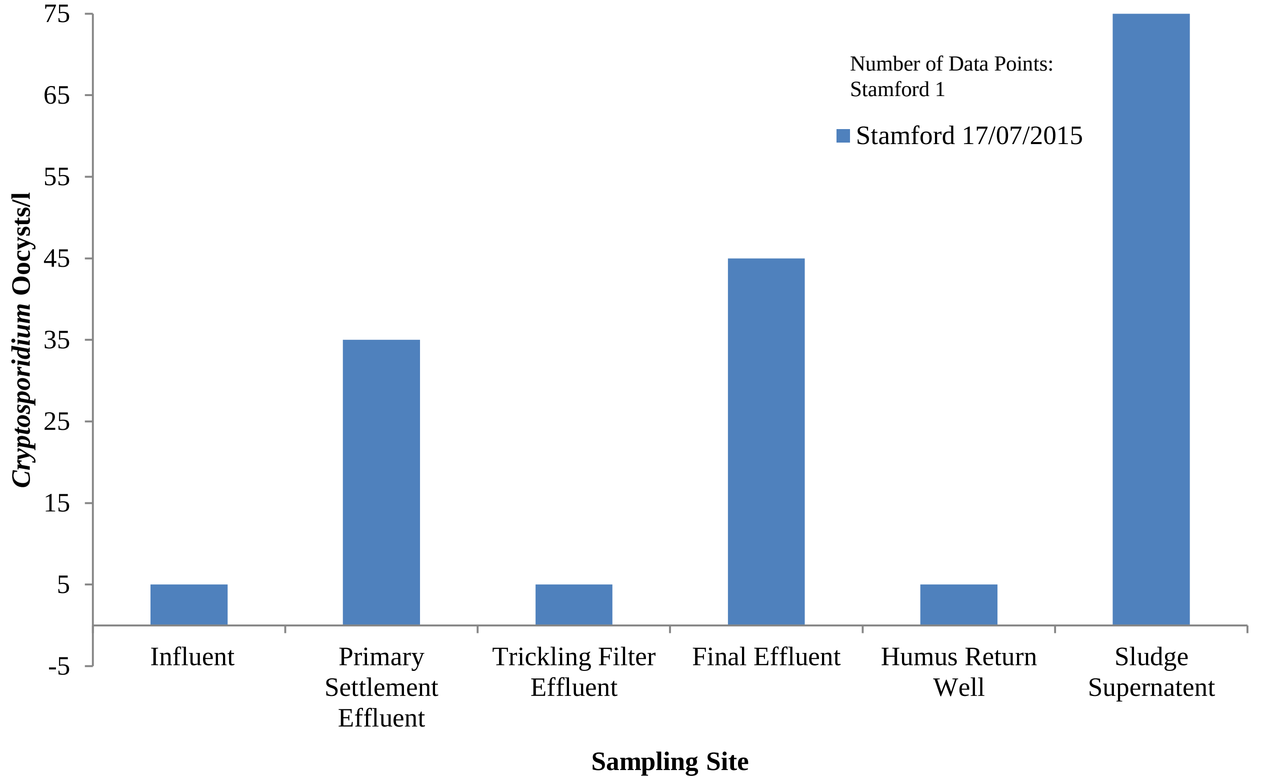

Figure 30 Cryptosporidium *oocysts/l at Stamford Sewage Treatment Works*

The past performance of each works was also researched. It was found that the ammonia, turbidity, phosphorus, BOD, COD and suspended solid levels were below consent for each STW (Appendix 1).

The population equivalent design for the STW was investigated to see if any works are currently operating at a rate greater than what they were commissioned to do. It was found that Stamford STW was working 4% over what it should be and Louth still had 30% capacity remaining for its size [Table 5].

Table 5 The intended and current population sizes of Louth, Stowmarket and Stamford Sewage Treatment Works

	Sewage Treatment Works		
	Louth	*Stowmarket*	*Stamford*
Population Size STWs Built for	30528	22428	21000
Current Population Size	19846	21515	21908
Available Capacity	35%	4%	-4%

Data extracted from: Anglian Water (2015)

5 Overall Discussion and Implications of the Findings

Cryptosporidium is a problem in the Louth area because in 2010 it was shown able to survive through the WTW process, resulting in the potential for the public to be infected with *Cryptosporidium* and so contract cryptosporidiosis from their drinking water. The oocysts seem to be entering Covenham WTW from Louth STW, which is discharging the organism into Louth Canal. When *Cryptosporidium* oocysts have been entering Louth STW continuously but in low numbers, they seem to become concentrated in the sludge holding tanks, and are then released back into the STWs via the return water from the sludge dewatering stage. These oocysts then pass through the STWs and into the canal. The solution, therefore, seems to be either reducing the *Cryptosporidium* leaving Louth STW or/and by fully disinfecting the clean water at Covenham WTW.

There might not be one particular solution for the *Cryptosporidium* issue and perhaps a combination of methods would be the best approach. There is not just the issue of what the new treatment process should be, but also there is consideration required as to where the system should be placed. One option is that focus should be placed at Louth STW, since this is the source of the problems. However, it may make more sense to put the treatment as one of the final treatment stages at Covenham WTW, since this is where the presence of *Cryptosporidium* is the greatest issue. Even if treatment at Louth STW stops the *Cryptosporidium* issue there, it will not prevent more of the organism from entering the water as it passes through the rural environment where Louth Canal is situated. However, this risk is likely to be small. Analysis of a similar system, the river Great Eau, which flows through agricultural land in the same area as Louth Canal, shows that it has far less *Cryptosporidium*. There were 10 samples taken from 05/01/11 to 19/05/14 and they show an oocyst/l range of 0.1-0.2, with an average of 0.13 oocysts/l detected. During the same time frame Louth Canal had an average of 3.5 oocysts/l, with a range of 0-83. This therefore suggests that the surrounding agricultural environment has very little impact on the *Cryptosporidium* within Louth Canal. Consequently, STW treatment is able to be used without WTW treatment in addition to it.

A further point to consider is that of costs of treatment for *Cryptosporidium* removal both at the STW and the WTW [

Table ***6***]. A costing exercise was undertaken using the Asset + software. Data had to be inputted (for example, the required capacity of that specific treatment) and an approximation of the cost that would be required for each new treatment type was generated.

Table 6 The capital and operational costs of different potential treatment options

Treatment	Capex	Opex	Totex
Dilution of Louth Canal with Great Eau Water	£7000	£0	£7000
Digestion at Louth Sewage Treatment Works Supernatant	£200,000	£30,000	£230,000
Sludge Pump at Louth Sewage Treatment Works	£300,000	£10,000	£310,000
UV at Louth Sewage Treatment Works	£400,000	£60,000	£460,000
Sand Filtration at Louth Sewage Treatment Works Supernatant	£900,000	£20,000	£920,000
Extra Sludge Tank at Louth Sewage Treatment Works	£1,000,000	£40,000	£1,40,000
Permanent UV at Covenham Water Treatment Works	£1,000,000	£200,000	£1,200,000
Ozone at Louth Sewage Treatment Works	£2,000,000	£60,000	£2,060,000
Ultrafiltration at Covenham Water Treatment Works	£7,000,000	£10,000	£7,010,000
Replacement of the Louth Canal with River Eau Water	£0	£12,000,000	£12,000,000

Treatment may be needed at both works, in an attempt to both stop the *Cryptosporidium* at the source and as an extra safety barrier between the environment and the public at Covenham WTW. A discussion about the best treatment options to use is below.

5. 1 Review of Processes

5. 1. 1 Filtration

Filtration has been found to be one of the most effective methods of *Cryptosporidium* removal. It is a safe treatment process which requires no further chemical treatment.

Microfiltration and ultrafiltration resulted in a removal efficiency of >99.99999% in a study completed by Hirata and Hashimoto (1999). Fu et al. (2010) described how membrane ultrafiltration was particularly successful at *Cryptosporidium* removal, more so than conventional flocculation sedimentation and sand filtration. It is also capable of removing viruses but it is more expensive than microfiltration. Micro and ultrafiltration have the combined effect of near complete removal of suspended solids from the water. However one issue with all membranes is that of membrane fouling. Buteyn (2010) said how fouling can arise from: surface materials (such as partially charged macromolecules), biofilm growth due to the microbiological activity of accumulated pathogens, suspended solids and precipitation of insoluble salts. Because of the small pore sizes of microfiltration (0.1-10 microns) and ultrafiltration (0.001 to 1.0 microns), they would not be suitable methods for use at Louth STW due to solids loading, although they would be suitable for Covenham WTW.

Ultrafiltration could be potentially be used at Covenham WTW if clarification was replaced by it, but still some upfront treatment would be needed to prevent the membrane being overloaded with particles. The clarification design at Covenham is currently not as efficient as it could be because of the age of the equipment. This is, though, a big business expense and so would need full justification, when other treatment options are available and less expensive.

The success of sand filtration treatment was shown by Timms et al. (1995). The study found that slow sand filtration was highly efficient and resulted in a more than 99.997% reduction in oocyst levels. Stott (2003) found that the combination of secondary treatment and sand filtration can reduce *Cryptosporidium* concentration by 99-99.9%. Unfortunately, slow sand filtration requires large amounts of land, meaning that it is better where less water needs to be treated, such as the sludge supernatant, rather than the effluent of Louth STW or Covenham WTW. However, the quality of the supernatant water is less than that of the final effluent, meaning that it may get blinded easily and so be ineffective.

Consequently, slow sand filtration would be a suitable option for implementation at the end of Louth STW. Microfiltration would be more suitable to use at Covenham WTW effluent rather than ultrafiltration because of its high cost (for example ultrafiltration would cost £6,722,971.92, whereas sand filtration would cost £895,183.42). It is not only an expensive treatment process but it is also costly because of the size of the membrane that would be required to reach the amount of water which needs to be treated per day. It may be feasible, however, if other processes, such as clarification, could be removed to compensate for this cost.

5. 1. 2 UV

The effectiveness of *Cryptosporidium* removal by UV has already been explored in this research, with Betancourt and Rose (2004) finding a 68-99.99% (Betancourt & Rose, 2004) (Environment Protection Agency, 2006) oocyst reduction when this method was used. At Covenham WTW there is currently a UV process. This system is designed to deliver a target dose of 40mj/cm, enough for a removal rate of over 99.99% for *Giardia* and *Cryptosporidium* and a 68% removal rate for viruses. The current dose of the temporary UV is 60mj/cm, meaning it removes over 99.99% of *Giardia* and *Cryptosporidium* and 90% of viruses. The UV, however, was only supposed to be a temporary means and only covers the current flow. The system would therefore need to be fully upgraded in order to meet the required increase in flow of 15.6Ml/day.

When heightened levels of *Cryptosporidium* were first identified at Covenham Reservoir on 06/02/10 - 07/02/10, a permanent UV treatment stage was due to be implemented. However, after the initial concern and interest in *Cryptosporidium* had passed, UV was not installed due to the expense of its implementation. As can be seen by

Table 6, UV at the site would cost Anglian Water £1388477.38 to implement, as well as £180,801.00 in operational costs. The capital cost, however may be overestimated because some of the construction has already been completed. At Louth STW it would cost less, at a total of £502,996.07, because fewer lamps would be required due to lower flows that would need to be treated.

UV light is also a useful disinfectant since it does not require the addition of extra chemicals to the water, has no lasting residual effects and produces no by-products. It is susceptible to interference by reduction of transmittance, typically caused by turbidity or residual organic matter present in the water. This means that UV is likely to be more effective at Covenham WTW than Louth STW due to the improved UV transmittance of treated drinking water compared to wastewater. However, UV may be less effective at Covenham WTWs than it should be. The clarifiers at Covenham are precipitators of poor design, reducing the water quality and so reducing the effectiveness of UV.

Thus it seems that UV light would be an effective, although costly, method to use at the end of the Covenham WTW process flow sheet. It may not be the best option to use at Louth STW because if the oocysts were deactivated by the UV, they would still be seen in the *Cryptosporidium* samples in the laboratory. Therefore Anglian Water staff would be unable to distinguish between deactivated oocysts from Louth STW and live oocysts from other sources and Louth Canal abstraction would be unable to be stopped in an accurate way.

5. 1. 3 Ozone

Corona-Vasquez et al. (2002) found that ozonisation of tap water at 1°C caused a 99% cyst reduction in 10 minutes with a concentration of 4mg/l. However Blackburn et al. (2006) described the case of a *Cryptosporidium* outbreak due to ozonised apple cider, suggesting that ozone has reduced effectively when water has a high turbidity, like in sewage waters.

It is a highly efficient disinfection process, produces few by-products, can be produced on site when needed, has no long-lasting residual effects and can be used to treat large volumes of water (Bowman, 2007). Regardless, the equipment and the requirements are expensive at WTWs (£2,533,978.54), even more so than UV (£502,996.07) [

Table ***6***]. Also, ozone is currently used at Covenham WTW but *Cryptosporidium* is still able to survive. This is because high amounts of ozone are required to deactivate the oocysts, especially at STW. Furthermore this treatment increases bromate, which is part of the discharge license of STW, when *Cryptosporidium* is not.

Consequently it seems that ozone is a relatively effective method of *Cryptosporidium* removal, however it is only efficient under certain environmental conditions. This, combined with the expensive and the requirements needed, make it an unsuitable method for controlling the *Cryptosporidium* in Louth.

5. 1. 4 Chlorine Dioxide

Chlorine dioxide is a relatively effective method of *Cryptosporidium* removal. Li et al. (2001) suggested the effectiveness could be 99% inactivation under favourable conditions.

The use of chlorine dioxide does not produce by-products such as ammonium compounds and chlorinophenols, although chlorite, chlorate, and organic DBPs are produced. A further negative aspect of this disinfection type is that it is explosive under pressure so it must be generated on site.

To conclude, this method would be a relatively effective method, as effective as ozone (but not as much so as for UV), for both the end of Louth STW and Covenham WTW, as well as the Louth STW supernatant from the sludge dewatering.

5. 1. 5 Canal Dilution

One alternative method of helping reduce the *Cryptosporidium* entering Covenham WTW is through canal dilution. Louth Canal could be diluted using flows from the Great Eau at a greater rate than the current amount. Structural re-routing of the intake would need to be completed to ensure the water was added directly to the point of abstraction, rather than downstream of it.

This would be a useful method of reducing the *Cryptosporidium* because dilution is one of the issues which makes the canal an exceptional case. The sewage effluent entering Louth Canal is diluted by 11.8%, whereas the River Great Ouse and the River Gipping had figures of 0.7 and 4.4%, due to the flow of the canal and the size of the catchment area draining to it, as well as the sewage effluent (Evangelidou & Berry, 2014).

It is a potentially achievable option because the abstraction license for this grid reference is already in place. For water companies to be able to abstract water from the environment, they must apply for an abstraction licence from the Environment Agency. When the application has been applied for, potential rejections can happen. However, an abstraction license is already present in the River Great Ouse, so there is no possibility of rejection. Furthermore the license is high, to a maximum of 97.7Ml/d and 9000Ml/y, when the current abstraction license for Louth Canal is 159.Ml/d and 23,230Ml/y. Regardless, these flows are not always achievable for example in the summer the River Great Eau has a much reduced amount of water in it. Louth Canal would display the same effects if it did not have the effluent from Louth STW discharging into it. As shown by Figure 31, approximately 20% of Louth Canal’s flow is due to Louth STW effluent being discharged into it.

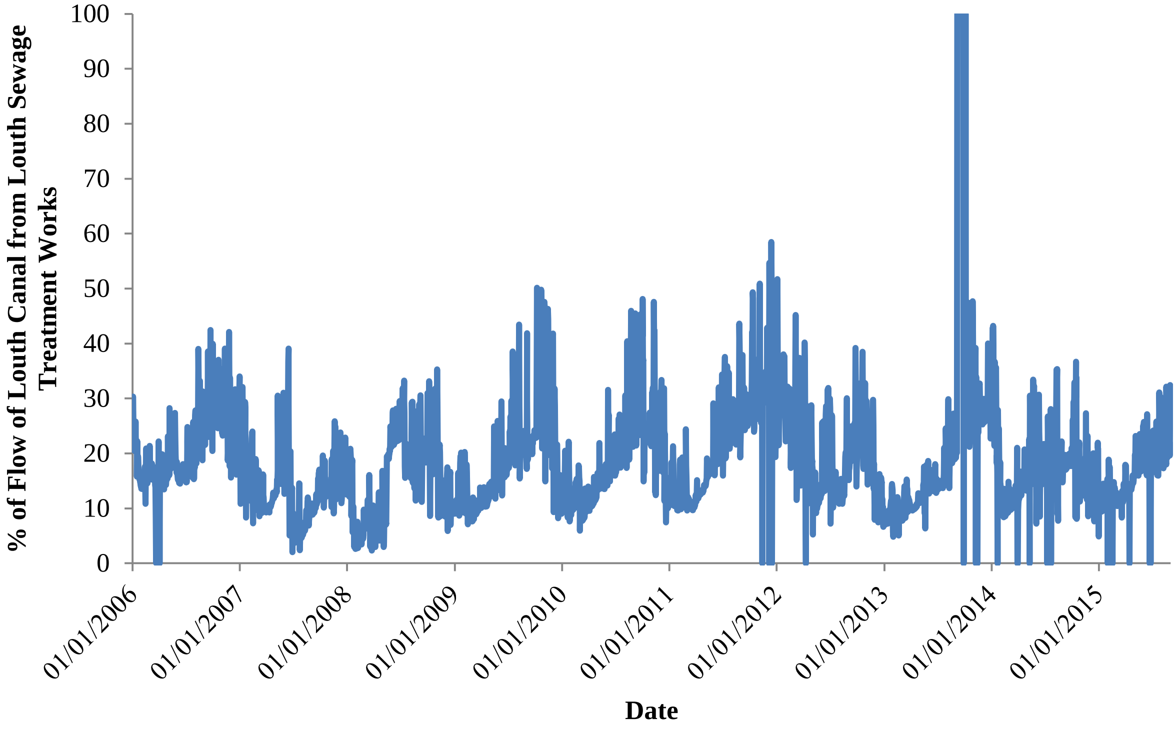

Figure 31 The percentage of flow of Louth Canal which is from Louth Sewage Treatment Works

Data extracted from: Anglian Water (2015)

It is unknown how successful this method would be, since there is a lack of research on the success of dilution in solving or aiding the *Cryptosporidium* issue. However, although it may help reduce the intensity of oocysts, it does not decrease the number of oocysts present. Regardless, the River Great Eau has relatively low levels of *Cryptosporidium* oocysts. However, irrespective of the low oocyst numbers in the River Great Eau, it has high levels of the pesticide metaldehyde present. This means that other problems would arise if it was implemented.

Therefore, this method may seem like an attractive one because the license is already in place and abstraction currently occurs on a smaller scale. However, it would be very expensive (£12,000,000.00) to make this a permanent occurrence and even when continuous dilution occurs it is still costly (£7,093.00), because a new infrastructure would be needed.

5. 1. 6 Catchment Surveillance

Catchment surveillance is a scheme which Anglian Water is currently participating in. As part of this, employees report environmentally-related issues concerning abstraction routes. An example of this is that supply teams need to routinely inspect land which is adjacent to abstraction points and report back to the water resources team for investigation. This process of reporting during surveillance helps to prevent incidents from occurring, effectively avoiding events.

Catchment surveillance would therefore be a useful tool to use for controlling *Cryptosporidium* because if land use changes were noticed, they could be reported for future investigation. If these incidences coincided with *Cryptosporidium* outbreaks, a pattern could be found and a potential source investigated further. Furthermore, regular notifications of *Cryptosporidium* cases from Lincolnshire could be reported to Anglian Water from the Health Protection Agency. This would allow the company to monitor Louth and respond to reported cases by altering the way the STW operates or ceasing canal abstraction earlier. This catchment management would reduce the risk of Covenham WTW being contaminated, although there would be a time delay between confirmed cases and Anglian Water reports.

A positive impact the research into *Cryptosporidium* at Covenham has caused is that Anglian Water and Public Health England now work in collaboration with one another more than they had in the past. Public Health England have said that they will continue to monitor *Cryptosporidium* notifications as part of their routine surveillance for all our counties in the East Midlands. They have also been encouraged to send off positive *Cryptosporidium* results for species typing.

Although this method helps reduce the consequences of *Cryptosporidium* outbreaks, it does not help prevent it. Therefore, catchment surveillance should be an on-going occurrence but is not a viable ‘treatment’ method.

5. 1. 7 Emptied Sludge Holding Tanks

If the *Cryptosporidium* is getting recirculated by the supernatant from the sludge holding tanks, either stopping the supernatant being recirculated or making recirculation happen on a more frequent basis would be a potential solution. This is because sludge builds up in the tanks and new wastewater entering the tanks has nowhere to go. Therefore the solids do not settle before the effluent leaves the tank.

Sludge holding tanks could be kept as empty as possible to stop supernatant sludge being recirculated. This could happen with a pumping system being added to the existing sludge tanks or by increasing the number of tanks present. This would mean that the oocysts would not be building up and entering the works in one plug flow but instead, in manageable amounts. Another potential way is too have another sludge holding tank, yet this is expensive (£1,40,000).

Another way of reducing *Cryptosporidium* in being recirculated is by not dewatering at all. All of the sludge could instead be collected and sent to a sludge treatment centre. This could potentially be carried out during an outbreak in the environment (i.e. when 3 oocysts/l or more have been detected in Louth Canal), if it is not cost efficient to be done at all times, since it would increase tankering costs by at least three fold. A potential issue with this solution is that it relies on staff to continue with it. If staff change and knowledge is lost, the cost of tankering may encourage them to reduce the frequency, which may result in the *Cryptosporidium* findings which are currently being experienced.

Therefore stopping the desludging process or making it more efficient would be a viable treatment option. However if the *Cryptosporidium* is coming in from somewhere else, such as the humus return tanks too, the same problems would still be seen. Furthermore stopping this process would result in high expense, increasing tankering costs by three fold. Further sampling would be required in this area, to see how the *Cryptosporidium* levels throughout the works are affected by each of these alterations.

5. 2 Recommendations and Progress

A new pump system is going to be installed at Louth STW. This will pump sludge from the current holding tanks into a larger circular concrete holding tank which is currently at the works but is out of operation. This will give the sludge a greater residence time, meaning oocysts will have more time to allow for settlement, as well as increasing the regulation of the dewatering process. This process will be carried out and the *Cryptosporidium* will be monitored to see how this alteration affects its concentration. If an improvement is not found, other treatment options will be considered.

Further potential treatment options include the use of sand filtration at the end of Louth STW, the installation of a permanent UV system at Covenham WTW or the use of ultrafiltration, instead of the current clarification system that is in place at Covenham Treatment Works. This is as well as the on-going monitoring that will be occurring. *Cryptosporidium* samples will be taken more regularly at Louth STW to provide Covenham WTW more time to detect a potential outbreak and react quicker to it. Tankering more sludge during times when there are outbreaks will also be considered as a continuing measure to protect Covenham against the *Cryptosporidium*.

5. 3 Impacts of the Research

This research project has contributed to current research, since an increase of *Cryptosporidium* oocysts within primary settlement tanks has not been detected in the past, as far as I know. It is something which is likely to be occurring at other STW because there were similar findings at one of the STW that was used as a control. This finding may be impacting other STW that discharge into a slow moving body of water or those which discharge into a river (for example) where the effluent makes up a great proportion of the water in the river. Areas where this happens may require further risk management downstream.

The research has also been of use to Anglian Water, since it has highlighted the source of the *Cryptosporidium* problem, meaning that it can be treated at source, rather than at the end of Covenham WTW.

A positive impact of this research into *Cryptosporidium* at Covenham is that it has caused greater collaboration. Anglian Water and Public Health England now work in partnership with each other more. Public Health England have said that they will continue to monitor *Cryptosporidium* notifications as part of their routine surveillance for all our counties in the East Midlands. They have also been encouraged to send off *Cryptosporidium*-positive results for species typing. The Environment Agency and Anglian Water will also be working together, potentially sharing data samples to help stop environmental sources of contamination.

6 Conclusions

The aim of this project was to assess the potential to control *Cryptosporidium* in the catchment of Louth. This involved determining potential sources of *Cryptosporidium*, researching its progress through treatment works, collecting and analysing data and proposing solutions for the problem.

The following conclusions have been made from the study. The host of the *Cryptosporidium* is either humans or sheep. The oocysts from the hosts are entering Louth STW in the crude sewage and being concentrated in the sludge holding tanks. When dewatering happens, the sludge holding tanks release a large amount of *Cryptosporidium* into the works, which passes through it relatively untreated into Louth Canal. The treatment processes at Louth STW are less effective than other research has suggested at oocyst removal, for example trickling filters and humus tanks removed 17% and 44%, compared with the literature results of 91%. Overall, the STW added 18 oocysts/l, when the influent and the final effluent of the works were compared over the period studied. The oocysts in Louth Canal are abstracted from and added to Covenham Reservoir. The oocysts from the reservoir are sometimes passed through to Covenham WTW, where there is potential for human contamination.

Currently, a new pump system is going to be installed at Louth STW to give the sludge a greater residence time. This will provide oocysts with more time for settlement and will make the regulation of the dewatering process easier. Tankering more sludge during times when there are outbreaks will also be considered as a continuing measure to protect Covenham against the *Cryptosporidium*.

Anglian Water are also deciding which additional treatment methods will reduce the risk of drinking water contamination. The following treatments have been recommended to them: sand filtration at the end of Louth STW, a permanent UV system at Covenham WTW and the use of ultrafiltration, instead of the current clarification system that is in place at Covenham Treatment Works. Preferably, sand filtration and ultrafiltration will be used, however if the business decides this option is too expensive, full UV will be chosen instead.

On-going monitoring of *Cryptosporidium* in Louth Canal will continue. *Cryptosporidium* samples will be taken more regularly at Louth STW to provide Covenham WTW more time to detect a potential outbreak and react quicker to it.

7 References

Advantage Environment, 2012. *Energy Efficient Water Treatment with Ozone.* [Online]
Available at: http://advantage-environment.com/livsmedel/energy-efficient-water-treatment-with-ozone/
[Accessed 29 December 2014].

Anglian Water, 2015. *Our Company.* [Online]
Available at: http://www.anglianwater.co.uk/about-us/our-company.aspx
[Accessed 07 July 2015].

Anglian Water, 2015. *Regular Monitoring Water Sample Data.* s.l.:s.n.

Balthazard-Accou, K., Bras, A. & Clervil, E., 2014. Urban Groundwater Contamination by Cryptosporidium Oocysts in Haiti: Analysis of Risk Factors for Human Health. *Haïti Perspectives,* 3(1).

BBC News, 2008. *Sickness Bug found in Tap Water.* [Online]
Available at: http://news.bbc.co.uk/1/hi/england/northamptonshire/7472619.stm
[Accessed 27 May 2015].

Berry, M., 2015. *Personal Communications.* s.l.:s.n.

Betancourt, W. Q. & Rose, J. B., 2004. Drinking Water Treatment Processes for Removal of Cryptosporidium and Giardia. *Veterinary Parasitology,* 126(1-2), pp. 219-234.

Bichai, F., Barbeau, B., Dullermont, Y. & Hijnen, W., 2010. Role of Predation by Zooplankton in Transport and Fate of Protozoan Oocysts in Granular Activated Carbon Filtration. *Water Research,* 44(4), pp. 1072-1081.

Blackburn, B. G. et al., 2006. Cryptosporidiosis Associated with Ozonated Apple Cider. *Emerging Infectious Diseases,* 12(4), pp. 684-686.

Blackwelder , R. E., 1940. Some Aspects of Modern Taxonomy. *Journal of the New York Entomological Society,* 48(2), p. 470.

Blanshard, C. et al., 1992. Cryptosporidiosis in HIV-Seropositive Patients. *An International Journal of Medicine,* Volume 85, pp. 813-823.

Bonatti, T. R. & Franco, R. M. B., 2014. Real Scale Environmental Monitoring of Zoonotic Protozoa and Helminth Eggs in Biosolid Samples in Brazil. *Journal of Parasitic Diseases,* pp. 1-10.

Bowman, D. D., 2007. Waste Management. In: R. Fayer & L. Xiao, eds. *Cryptosporidium and Cryptosporidiosis.* Boca Raton: CRC Press, pp. 371-385.

Bowman, D. D., Liotta, J. L. & Jenkins, M., 2008. *Survival Kinetics of Cryptosporidium in Swine Facility Wastes in Southern Piedmont and Coastal Plain Watersheds.* s.l., s.n.

Bridgman, S. A. et al., 1995. Outbreak of Cryptosporidiosis Associated with a Disinfected Groundwater Supply. *Epidemiology and Infection,* 115(3), pp. 555-566.

British Waterways, 2004. *The Economic Impact of Restoring the Huddersfield Narrow and Rochdale Canals,* s.l.: s.n.

Brown, A., 2015. *Personal Communication.* s.l.:s.n.

Bryan, B. A. et al., 2009. Adaptive Management for Mitigating Cryptosporidium Risk in Source Water: A Case Study in an Agricultural Catchment in South Australia. *Journal of Environmental Management,* 90(10), pp. 3122-3134.

Bukhari, Z. et al., 2000. Comparison of Cryptosporidium parvum Viability and Infectivity Assays following Ozone Treatment of Oocysts. *Applied and Environmental Microbiology,* 81(2), pp. 2972-2980.

Buteyn, J., 2010. *Microfiltration with A Macro Future.* [Online]
Available at: http://www.watertechonline.com/articles/microfiltration-with-a-macro-future
[Accessed 05 June 2015].

Casanova, L. M., Little, V., Frye, R. J. & Gerba, C. P., 2001. A Survey of the Microbial Quality of Recycled Household Graywater. *Journal of the American Water Resources Association,* 37(5).

Castro-Hermida, J. A. et al., 2008. Contribution of Treated Wastewater to the Contamination of Recreational River Areas with Cryptosporidium Spp. and Giardia duodenalis. *Water Research,* 42(13), pp. 3528-3538.

Chalmers, R. M. et al., 2011. Comparison of Diagnostic Sensitivity and Specificity of Seven Cryptosporidium Assays used in the UK. *Journal of Medical Microbiology,* Volume 60, pp. 1598-604.

Connelly, L., Craig, B. H., Jones, B. & Alexander, C. L., 2013. Characterising The Genetic Diversity of Cryptosporidium species Within a Remote Population of Soay Sheep on St. Kilda Islands, Scotland. *Applied and Environmental Microbiology,* 81(11).

Co-operative Pharmacies, 2015. *Anti-Diarrhoeal Medication Sales Data.* s.l.:s.n.

Corona-Vasquez, B., Samuelson, A., Rennecker, J. L. & Mariñas, B. J., 2002. Inactivation of Cryptosporidium parvum Ocysts with Ozone and Free Chlorine. *Water Research,* 36(16), pp. 4053-4063.

Croll, B., 2000. *Coping with Cryptosporidia.* Nottingham, Safeguarding Safety in Swimming Pools.

Curriero, F. C., Patz, J. A., Rose, J. B. & Lele, S., 2001. The Association Between Extreme Precipitation and Waterborne Disease Outbreaks in the United States, 1948–1994. *American Journal of Public Health,* 91(8), pp. 1194-1199.

Dai, X. & Boll, J., 2006. Evaluation of Attachment of Cryptosporidium parvum and Giardia lamblia to Soil Particles. *Journal of Environmental Quality,* Volume 32, pp. 296-304.

Dai, X. & Boll, J., 2006. Settling Velocity of Cryptosporidium parvum and Giardia lamblia. *Water Research,* 40(6).

Davies, C. M., Kaucner, C., Deere, D. & Ashbolt, N. J., 2003. Recovery and Enumeration of Cryptosporidium parvum from Animal Fecal Matrices. *Applied Environmental Microbiology,* Volume 69, pp. 2842-2847.

DuPont, H. L. et al., 1995. The Infectivity of Cryptosporidium parvum in Healthy Volunteers. *The New England Journal of Medicine,* Volume 332, pp. 855-859.

DWI, 2009. *Drinking water 2008: Eastern Region of England,* London: DWI.

Edzwald, J. K. & Kelley, M. B., 1998. Control of Cryptosporidium : From Reservoirs to Clarifiers to Filters. *Water Science and Technology,* 37(2), pp. 1-8.

Edzwald, J. K. et al., 2001. *Impacts of Filter Backwash Recycle on Clarification and Filtration.* s.l.:American Water Works Association.

Enriquez, V., Rose, J. B., Enriquez, C. E. & Gerba, C., 1995. Occurrence of Cryptosporidium and Giardia in Secondary and Tertiary Wastewater Effluents. In: *Protozoan Parasites and Water.* Cambridge: The Royal Society of Chemistry, pp. 84-86.

Environment Agency, 2004. *Louth Canal Cross Section Survey Tetney Lock to Austen Fen,* s.l.: s.n.

Environment Agency, 2004. *Summary Report Fisheries Survey ,* s.l.: s.n.

Environment Agency, 2005. *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA.* [Online]
Available at: http://www.epa.gov/microbes/documents/1622de05.pdf
[Accessed 21 July 2015].

Environment Agency, 2010. The Microbiology of Drinking Water: Part 14- Methods for the Isolation. In: *Identification and Enumeration of Cryptosporidium Oocysts and Giardia Cysts.* s.l.:s.n.

Environment Protection Agency, 2006. *Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule,* s.l.: s.n.

Evangelidou, S. & Berry, M., 2014. *STW in the Proximity of SW Intakes- 3 Examples.* s.l.:s.n.

Fayer, R., 1994. Effect of High Temperature on Infectivity of Cryptosporidium parvum Oocysts in Water. *Applied and Environmental Microbiology*, 60(8), pp. 2732-2735.

Fayer, R., 2010. Taxonomy and Species Delimitation in Cryptosporidium. *Experimental Parasitology,* Volume 124, pp. 90-97.

Fayer, R., Trout, J. M. & Jenkins, M. C., 1998. Infectivity of Cryptosporidium parvum Oocysts Stored in Water at Environmental Temperatures. *Journal of Parasitology,* 84(6), pp. 1165-1169.

Finch, G. R., 1997. *Control of Cryptosporidium Through Chemical Disinfection: Current State of the Art.* Portland, Oregon, s.n.

Foundation for Water Research, 1996. *Occurrence of Cryptosporidium sp. Oocysts and Giardia sp. Cysts in Sewage Effluents and Sludges from Sewage Treatment Plants in England,* s.l.: s.n.

Foundation for Water Research, 2011. *A Review of Current Knowledge: Cryptosporidium in Water Supplies,* s.l.: s.n.

Fournet, N. et al., 2013. Simultaneous Increase of Cryptosporidium Infections in the Netherlands, the United Kingdom and Germany in Late Summer Season. *Eurosurveillance,* 18(2).

Fox, L. M. & Saravolatz, L. D., 2005. Nitazoxanide: A New Thiazolide Antiparasitic Agent. *Clinical Infectious Diseases,* 40(8), pp. 1173-1190.

Frey, M., Hancock, C. & Logsdon, G. S., 1998. *Cryptosporidium : Answers to Questions Commonly Asked by Drinking Water Professionals.* s.l.:American Water Works Association.

Fu, C. Y. et al., 2010. Monitoring and Evaluation of Removal of Pathogens at Municipal Wastewater Treatment Plants. *Water Science and Technology,* 61(6), pp. 1589-1599.

Gamba, R. D. et al., 2000. Detection of Cryptosporidium Sp. Oocysts in Groundwater for Human Consumption in Itaquaquecetuba City, S. Paulo-Brazi. *Brazilian Journal of Microbiology,* Volume 31, pp. 151-153.

Gilijohann, C., 2014. *Cryptosporidium in LN11 Area and Lincolnshire Area,* s.l.: Public Health England.

Godfree, A., 2013. Health Constraints on the Agricultural Recycling of Wastewater Sludges. In: *Handbook of Water and Wastewater Microbiology.* s.l.:Academia Press.

Hall, T., Pressdee, J., Gregory, R. & Murray, K., 2000. Cryptosporidium Removal during Water Treatment using Dissolved Air Flotation. *Water Science and Technology,* 31(3-4), pp. 125-135.

Harp, J. A., Fayer, R., Pesch, B. A. & Jackson, G. J., 1996. Effect of Pasteuraization on Infectivity of Cryptosporidium parvum Oocysts in Water and Milk. *Applied Environmental Microbiology,* 62(8), pp. 2666-2867.

Harrison, M., 2014. *Personal Communication.* s.l.:s.n.

Hewson-Fisher, K., 2014. *Louth Canal Walk Over Survey,* s.l.: s.n.

Hijnen, W. A. M. et al., 2010. GAC Adsorption Filters as Barriers for Viruses, Bacteria and Protozoan Oocysts in Water Treatment. *Water Research,* 44(4), pp. 1224-1234.

Hill, V. R., Kantardjieff, A., Sobsey, M. D. & Westerman, P. W., 2002. Reduction of Enteric Microbes in Flushed Swine Wastewater Treated by a Biological Aerated Filter and UV Irradiation. *Water Environment Research,* 74(1), pp. 91-99.

Hirata, T. & Hashimoto, A., 1999. Experimental Assessment of the Efficacy of Microfiltration and Ultrafiltration for Cryptosporidium Removal. *Water Science and Technology,* 38(12), pp. 103-107.

Hlavsa, M. C., 2014. Infectious Diseases Related to Travel. In: *CDC Health Information for International Travel 2014.* Oxford: Oxford Press, p. 162.

Jenkins, M. B., Liotta, J. L. & Bowman, D. D., 2013. Inactivation Kinetics of Cryptosporidium parvum Oocysts in a Swine Waste Lagoon and Spray Field. *Journal of Parasitology,* 99(2), pp. 337-342.

Kitajima, M., Haramoto, E., Iker, B. C. & Gerba, C. P., 2014. Occurrence of Cryptosporidium , Giardia, and Cyclospora in Influent and Effluent Water at Wastewater Treatment Plants in Arizona. *Science of The Total Environment,* Volume 484, pp. 129-136.

Knowles, R. B., Markley, B., Buckalew, J. O. & Roebuck, L. W., 1999. Water Resources Assessment of Haiti. *Army Corps of Engineers.*

Korich, D. G. et al., 1990. Effects of Ozone, Chlorine Dioxide, Chlorine, and Monochloramine on Cryptosporidium parvum Oocyst Viability. *Applied and Environmental Microbiology,* 56(5), pp. 1423-1428.

Körkoca, H. et al., 2013. Prevalence of Cryptosporidium spp. in Asymptomatic Food Workers. *Turkish Journal of Parasitology,* Volume 37, pp. 241-244.

Lake, I. R., Bentham, G., Kovats, R. S. & Nichols, G. L., 2005. Effects of weather and river flow on cryptosporidiosis. *Journal of Water and Health,* Volume 3, pp. 469-474.

Levenspiel, O., 2011. Two Ideal Flow Models: Plug Flow and Mixed Flow. *Tracer Technology,* Volume 96, pp. 27-34.

Li, H., Finch, G. R., Smith, D. W. & Belosevic, M., 2001. Sequential Inactivation of Cryptosporidium parvum using Ozone and Chlorine. *Water Research,* 35(18), pp. 4339-4348.

Lim, Y. A. L., Wan Hafiz, W. I. & Nissapatom, V., 2007. Reduction of Cryptosporidium and Giardia by sewage treatment processes. *Tropical Biomedicine,* 24(1), pp. 95-104.

Lim, Y. A. L., Wan Hazif, W. I. & Nissapatorn, V., 2004. Reduction of Cryptosporidium and Giardia by Sewage Treatment Processes. *Tropical Biomedicine,* 24(1).

Louth Market Auctioneers, 2015. *Sale Reports,* s.l.: s.n.

Madore, M. S. et al., 1987. Occurrence of Cryptosporidium Oocysts in Sewage Effluents and Selected Surface Waters. *The Journal of Parasitology,* 73(4), pp. 702-705.

Maunsell, F., 2004. *Restoration of the Louth Canal,* s.l.: s.n.

McCulloch, J., 2000. Laundry Issues. In: *Infection Control: Science, Management and Practise.* London: Whurr Publishers.

Medema, G. J., Schets, F. M., Teunis, P. F. M. & Havelaar, A. H., 1998. Sedimentation of Free and Attached Cryptosporidium Oocysts and Giardia Cysts in Water. *Applied and Environmental Microbiology,* 64(11), pp. 4460-4466.

Medscape, 2014. *Cryptosporidiosis Medication.* [Online]
Available at: http://emedicine.medscape.com/article/215490-medication#showall
[Accessed 05 December 2014].

Medscape, 2014. *Cryptosporidiosis Work Up: Approach Considerations.* [Online]
Available at: http://emedicine.medscape.com/article/215490-workup#showall
[Accessed 04 December 2014].

Microclean, 2014. *Louth Site.* [Online]
Available at:
http://www.micronclean.co.uk/MobileDetails/CleanroomLocationInfo.aspx?Local=Louth
[Accessed 21 January 2015].

Montemayor, M., Valero, F., Jofre, J. & Lucena, F., 2005. Occurrence of Cryptosporidium spp. Oocysts in Raw and Treated Sewage and River Water in North-Eastern Spain. *Journal of Applied Microbiology,* 99(6), pp. 1455-1462.

Moore, P., 2014. *Personal Communication.* s.l.:s.n.

Neto, R. C., Santos, J. U. & Franco, R. M. B., 2006. Evaluation of Activated Sludge Treatment and the Efficiency of the Disinfection of Giardia Species Cysts and

Cryptosporidium Oocysts by UV at a Sludge Treatment Plant in Campinas, South-East Brazil. 54(3), pp. 89-94.

Northern Ireland Water, 2014. *Illegal Dumping at NI Water Site in Lisnarick Causes Concern.* [Online]
Available at: http://www.niwater.com/news-detail/?News_ID=10828&FriendlyID=
[Accessed 08 July 2015].

Oguma, K. et al., 2001. Determination of Pyrimidine Dimers in Escherichia coli and Cryptosporidium parvum During UV Light Inactivation, Photoreactivation, and Dark Repair. *Applied and Environmental Microbiology,* 67(10), pp. 4630-4637.

Okhuysen, P. C. et al., 1998. Susceptibility and Serologic Response of Healthy Adults to Reinfection with Cryptosporidium parvum. *Infection and Immunology,* 66(2), pp. 441-443.

Parker, J. F. W., Smith, H. V. & Girdwood, R. W. A., 1993. *Survey of Loch Lomond to Assess the Occurrence and Prevalence of Cryptosporidium sp. oocysts and Their Likely Impact on Human Health,* s.l.: Foundation for Water Research.

Patania, N. L. et al., 1995. Optimization of Filtration for Cyst Remova. *American Water Works Association.*

Pavlasek, I. & Ryan, U., 2008. Cryptosporidium varanii Takes Precedence over C. saurophilum. *Experimental Parasitology,* 118(3), pp. 434-437.

Pharma Journal, 2014. *Clean Room Classifications.* [Online]
Available at: http://www.pharmacistspharmajournal.org/2010/02/clean-room-classification-aspects-of.html#.VL-SKfnA_4Y
[Accessed 21 January 2015].

Pizzi, N. G., 2010. Water Treatment: Principles and Practices of Water Supply Operations.

Public Health England, 2014. *East Midlands Cryptosporidium Surveillance.* [Online].

Public Health England, 2015. *Cryptosporidium Cases in the Louth Population.* s.l.:s.n.

Public Health Wales, 2012. *Cryptosporidium : Epidemiology.* [Online] Available at: http://www.wales.nhs.uk/sites3/page.cfm?orgid=457&pid=48350 [Accessed 05 December 2014].

Redder, A. et al., 2010. Constructed Wetlands- Are They Safe in Reducing Protozoan Parasites?. *International Journal of Hygiene and Environmental Health,* 213(1), pp. 72-77.

Robertson, L. J., Campbell, A. T. & Smith, H. V., 1992. Survival of Cryptosporidium parvum Oocysts under Various Environmental Pressures', Applied and Environmental Microbiology. *Applied and Environmental Microbiology,* pp. 3494-3500.

Robertson, L. J. et al., 2000. Giardia Cysts and Cryptosporidium Oocysts at Sewage Treatment Works in Scotland, UK. *Water Research,* pp. 2310-2322.

Rochelle, P. A., Marshall, M. M. & Mead, J., 2005. *Comparing Cell Culture and Mouse Assays for Measuring Infectivity of Cryptosporidium,* s.l.: Awwa Research Foundation Reports.

Rose, J. B., Gerbs, C. P. & Jakubowski, W., 1991. Survey of potable water supplies for Cryptosporidium and Giardia. *Environmental Science Technology,* 28(5), pp. 1393-1400.

Salter, H. E. et al., 1999. The Performance of Tertiary Lagoons in the United Kingdom. *Water Research,* , 33(18), pp. 3775-3781.

Savioli, L., Smith, H. & Thompson, A., 2006. Giardia and Cryptosporidium join the 'Neglected Diseases Initiative'. *Trends in Parasitology,* 22(5).

Searcy, K. E., Packman, A. I., Atwill, E. R. & Harter, T., 2005. Association of Cryptosporidium parvum with Suspended Particles: Impact on Oocyst Sedimentation. *Applied and Environmental Microbiology,* Volume 71, pp. 1072-1078.

Shin, G. A. et al., 2001. *DNA Repair of UV- Irradiated Cryptosporidium parvum Oocysts and Giardia lamblia Cysts.* Washington DC, s.n.

Sidhu, J. P. et al., 2010. Pathogen Inactivation during Passage of Stormwater through a Constructed Reedbed and Aquifer Transfer, Storage and Recovery. *Water Science Technology,* 62(5), pp. 1190-1197.

Šlapeta, J., 2013. Cryptosporidiosis and Cryptosporidium Species in Animals and Humans: A Thirty Colour Rainbow?. *International Journal for Parasitology,* Volume 43, pp. 957-970.

Stadterman, K. L., Sninsky, A. M., Sykora, J. L. & Jakubowski, W., 1995. 'Removal and Inactivation of Cryptosporidium Oocyst by Activated Sludge Treatment and Anaerobic Digestion. *Water, Science and Technology,* 31(5-6).

Stevik, T. K., Ausland, K. A. G. & Hanssen, J. F., 2004. Retention and Removal of Pathogenic Bacteria in Wastewater Percolating through Porous Media: A Review. *Water Research,* Volume 38, pp. 1355-1367.

Stott, R., 2003. Fate and Behaviour of Parasites in Wastewater Treatment Systems. *Handbook of Water and Wastewater Microbiology.*

Surl, C., Jung, B. D., Park, B. P. & Kim, H., 2011. Resistance of Cryptosporidium parvum Oocysts Following Commercial Bleach Treatment. *Korean Journal of Vetinary Research,* Volume 51, pp. 101-105.

Tam, C. C. et al., 2012. Longitudinal Study of Infectious Intestinal Disease in the UK (IID2 Study): Incidence in the Community and Presenting to General Practice. *Gut,* 61(1), pp. 69-77.

The International Association for Soaps, Detergents and Maintenance Products, 2013. *The Case for the AISE Low Temperature Washing Initiative: Substantiation Dossier,* s.l.: s.n.

The Laundry Room Ltd., 2014. [Online]
Available at: http://www.laundryroomukltd.co.uk/shopcontent.asp?type=Aboutus
[Accessed 21 January 2015].

Timms, S., Slade, J. S. & Fricker, C. R., 1995. Removal of Cryptosporidium by Slow Sand Filtration. *Water Science and Technology,* 31(5-6), pp. 81-84.

Tufenkki, N. et al., 2004. Transport of Cryptosporidium Oocysts in Porous Media: Role of Straining and Physicochemical Filtration. *Environmental Science and Technology,* 38(22), pp. 5932-5938.

Uluats, B. & Voyvoda, H., 2004. Cryptosporidiosis in Diarrhoeic Lambs on a Sheep Farm. *Turkish Journal of Parasitology,* 28(1), pp. 15-17.

United States Environmental Protection Agency, 2001. *Cryptosporidium : Drinking Water Health Advisory,* s.l.: s.n.

United States Environmental Protection Agency, 2015. *5.9 Conductivity.* [Online]
Available at: http://water.epa.gov/type/rsl/monitoring/vms59.cfm
[Accessed 20 August 2015].

United Utilities, 2014. *Cryptosporidium.* [Online]
Available at: http://www.unitedutilities.com/documents/Cryptosporidium .pdf
[Accessed 06 January 2015].

United Utilities, 2015. *Boiled water notice for customers in parts of Lancashire.* [Online]
Available at: http://www.unitedutilities.com/lancashire-boiled-water.aspx
[Accessed 20 August 2015].

Villacorta-Martinez de Maturana, I., Ares-Mazás, M. E., Duran-Oreiro, D. & Lorenzo-Lorenzo, M. J., 1992. Efficacy of Activated Sludge in Removing Cryptosporidium parvum Oocysts from Sewage. *Applied and Environmental Microbiology,* 58(28), pp. 3514-3516.

Waddell, A., 2015. *Personal Communication.* s.l.:s.n.

Weather Underground, 2015. *Historic Weather.* [Online]
Available at: http://www.wunderground.com/history/
[Accessed 03 November 2015].

Weiss, T. H., Mills, A. L., Hornberger, G. M. & Herman, J. S., 1995. Effect of Bacterial Cell Shape on Transport of Bacteria in Porous Media. *Environmental Science and Technology,* 29(7), pp. 1737-1740.

Whitmore, T. N. & Robertson, L. J., 1995. The Effect of Sewage Sludge Treatment Processes on Oocysts of Cryptosporidium parvum. *Journal of Applied Microbiology,* 78(1), pp. 34-38.

Willocks, L. et al., 1998. A Large Outbreak of Cryptosporidiosis Associated with a Public Water Supply from a Deep Chalk Borehole. *Communicable Disease and Public Health,* 1(4).

Wolyniak DiCesare, E. A., Hargreaves, B. R. & Jellisonb, K. L., 2012. Biofilms Reduce Solar Disinfection of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology,* 78(12), pp. 4522-4525.

Xiao, L., Fayer, R., Ryan, U. & Upton, S. J., 2004. Cryptosporidium Taxonomy: Recent Advances and Implications for Public Health. *Clinical Microbiology Reviews,* 17(1), pp. 72-97.

Zerpa, R. & Huicho, L., 1994. Childhood Cryptosporidial Diarrhea Associated with Identification of Cryptosporidium sp. In the Cockroach Periplaneta americana. *Pediatric Infectious Disease Journal,* 13(6), pp. 546-548.

Zhang, Q., Mankin, K. R. & Erickson, L. E., 2001. *Modelling Transport of Cryptosporidium parvum Oocysts in Overland Flow.* s.l., s.n.

Zhang, T., Hu, H. Y., Zong, S. Y. & Xie, X., 2008. 'Removal Characteristic of Pathogenic Protozoan in Wastewater Treatment and Reclamation Process. 29(7).

8 Bibliography

Drinking Water Directorate (2006), '*Cryptosporidium* Epidemiology', Available at http://dwi.defra.gov.uk/research/completed-research/reports/DWI70_2_201.pdf (Accessed: 15/12/14).

Lamb, N. (2014), 'The Prevalence of Giardia in the River Taff', *University of South Wales*, Available at https://www.researchgate.net/publication/269573382_The_Prevalence_of_Giardia_in_the_River_Taff (Accessed: 07/01/15).

Hunter, P. R. and Nichols, G. (2002), 'Epidemiology and Clinical Features of *Cryptosporidium* Infection in Immunocompromised Patients', *Clinical Microbiology Reviews*, Vol. 15 (1) (pp. 145-154), Available at http://cmr.asm.org/content/15/1/145.full (Accessed: 11/02/15).

Anglian Water (2014), *Our Plan 2015-20*, Available at http://www.anglianwater.co.uk/_assets/media/ICT_Final.pdf (Accessed: 06/01/15).

Centre for Disease Control and Protection (2012), *Cryptosporidium : Child Care Facilities*, Available at http://www.cdc.gov/parasites/crypto/daycare/index.html (Accessed: 04/12/14).

9 Appendix

9. 1 Ammonia

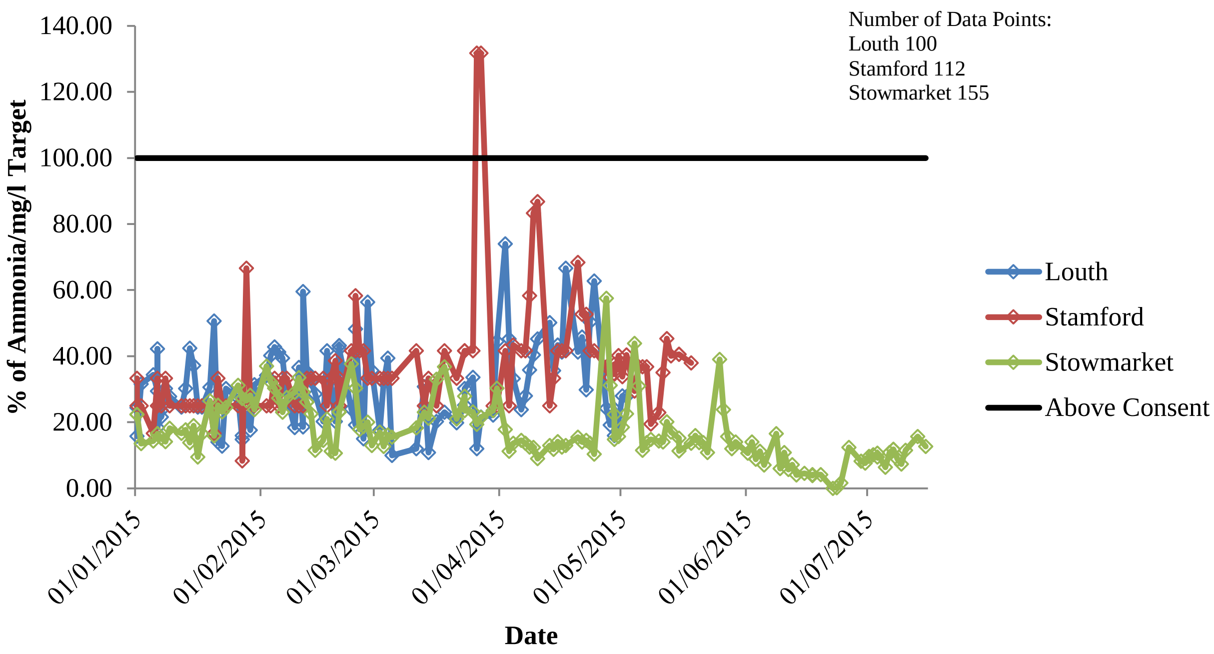

Figure 32 The percentage of Ammonia/mg/l target for Louth, Stamford and Stowmarket Sewage Treatment Works

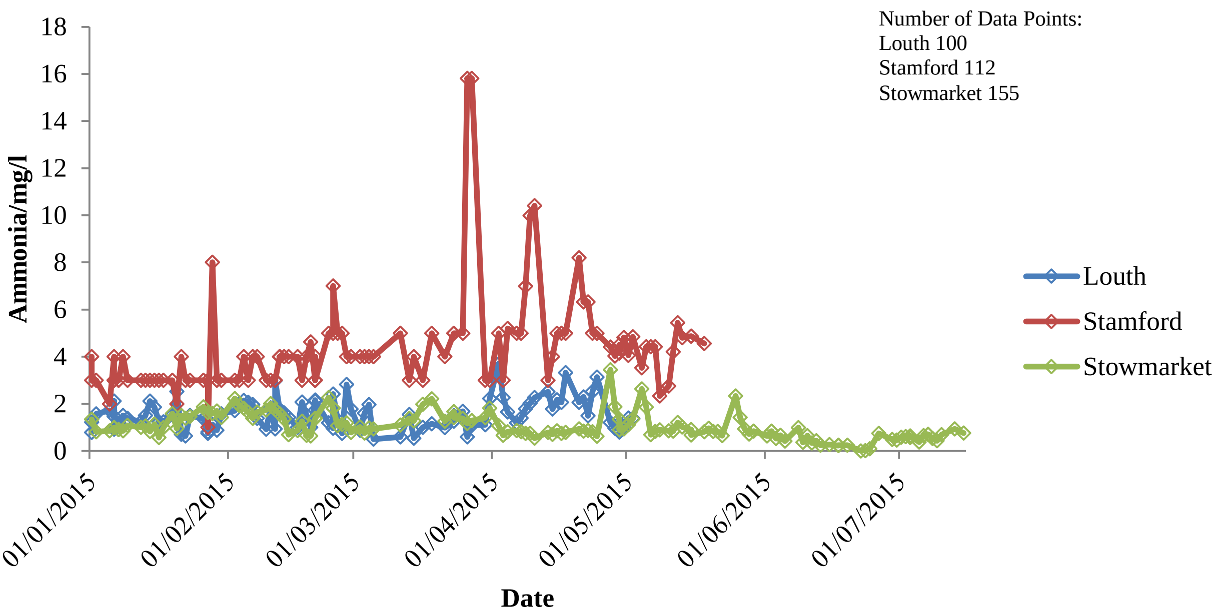

Figure 33 The Ammonia/mg/l produced at Louth, Stamford and Stowmarket Sewage Treatment Works

9. 2 Turbidity

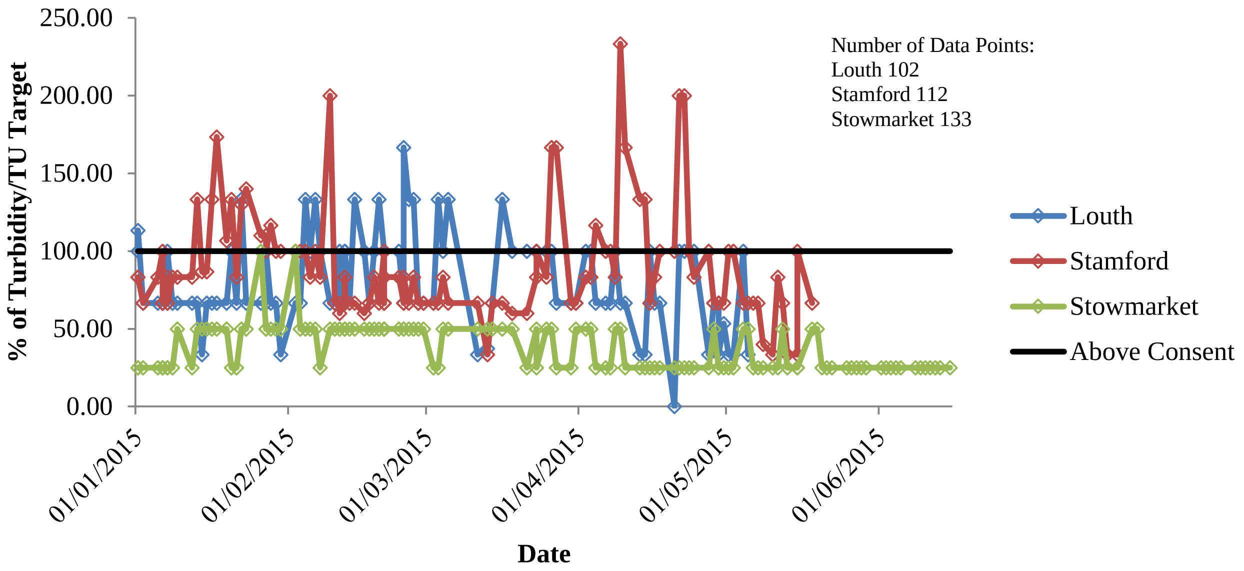

Figure 34 The percentage of Turbidity/TU Target for Louth, Stamford and Stowmarket Sewage Treatment Works

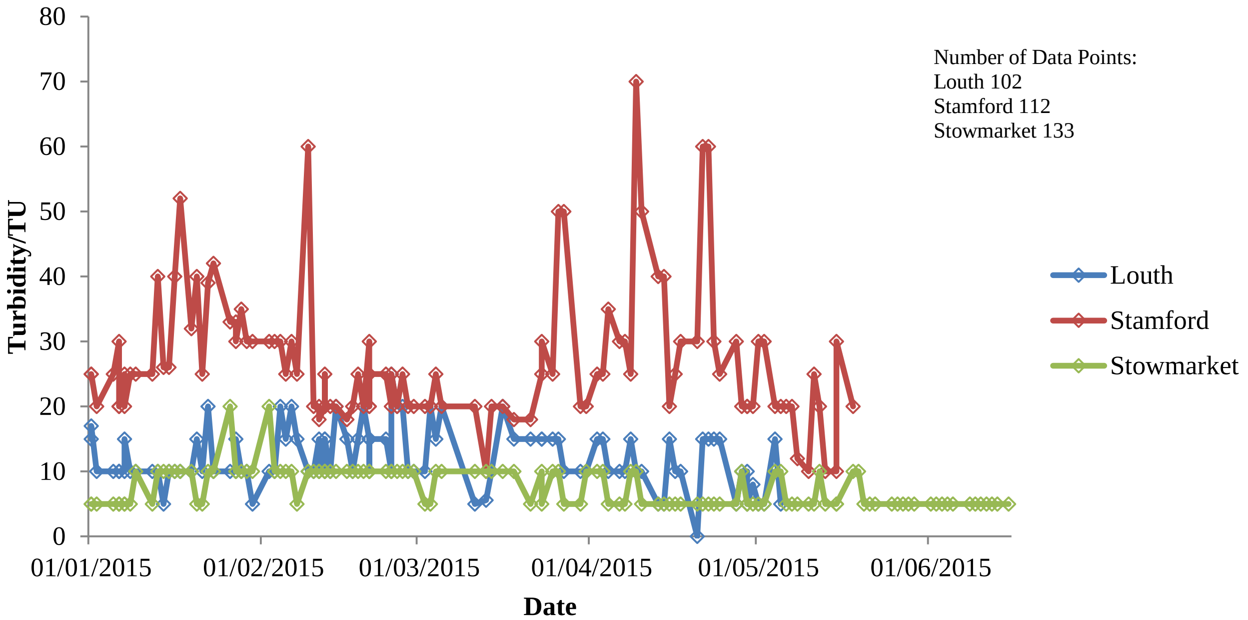

Figure 35 The Turbidity/TU produced at Louth, Stamford and Stowmarket Sewage Treatment Works

9. 3 Phosphorus

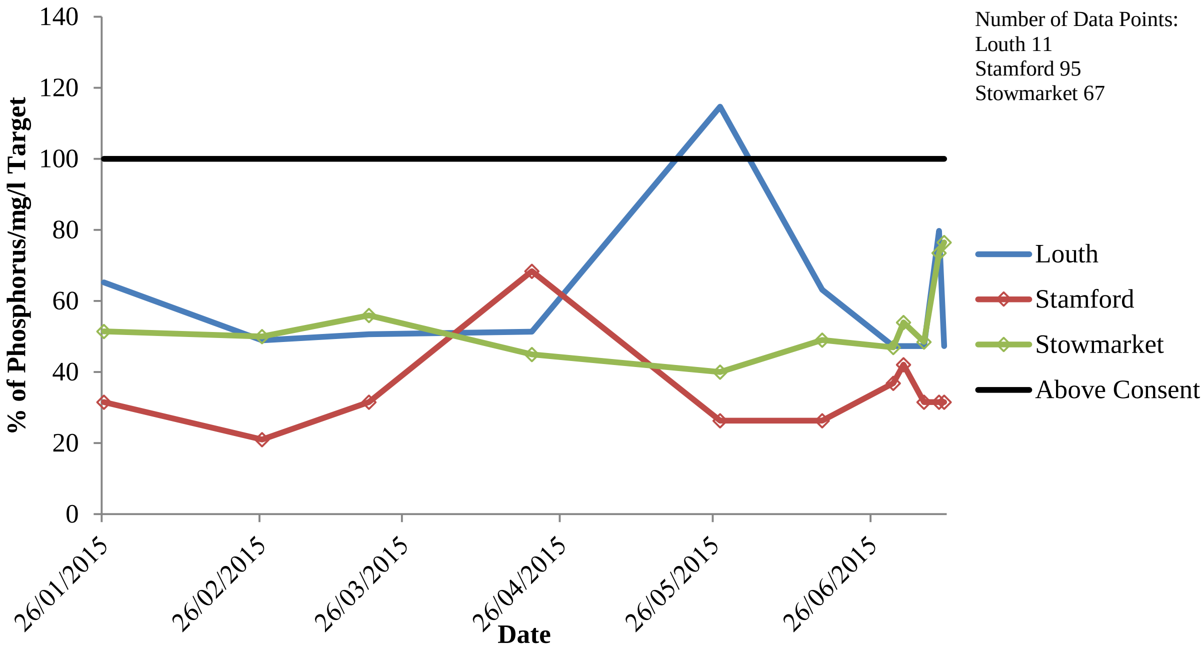

Figure 36 The percentage of Turbidity/TU Target for Louth, Stamford and Stowmarket Sewage Treatment Works

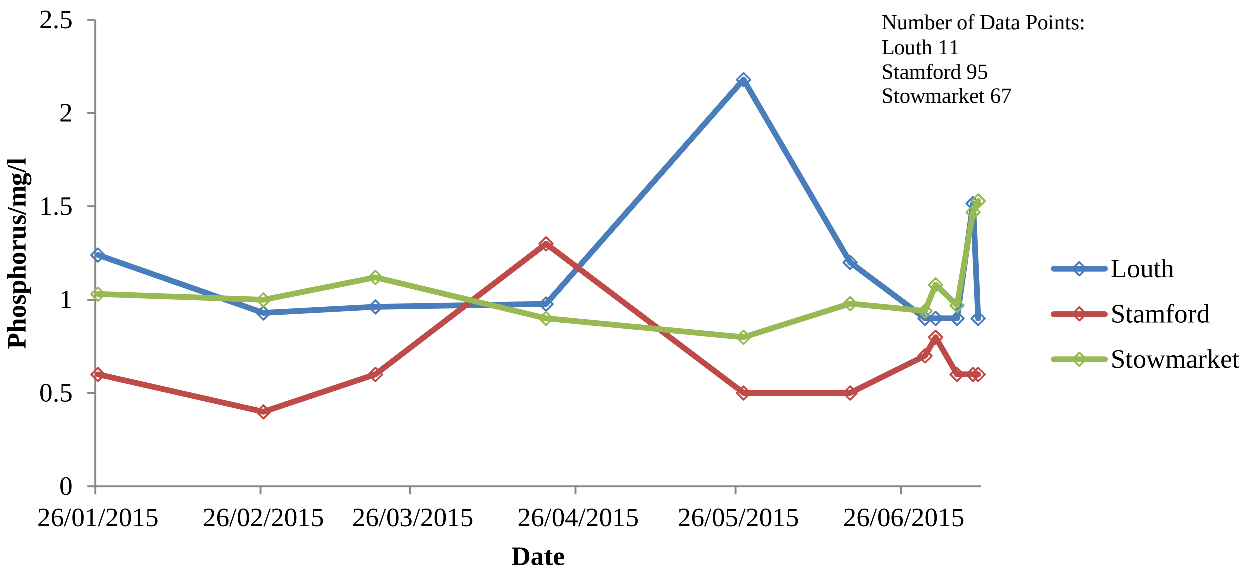

Figure 37 The Phosphorus/mg/l produced at Louth, Stamford and Stowmarket Sewage Treatment Works

9. 4 BOD

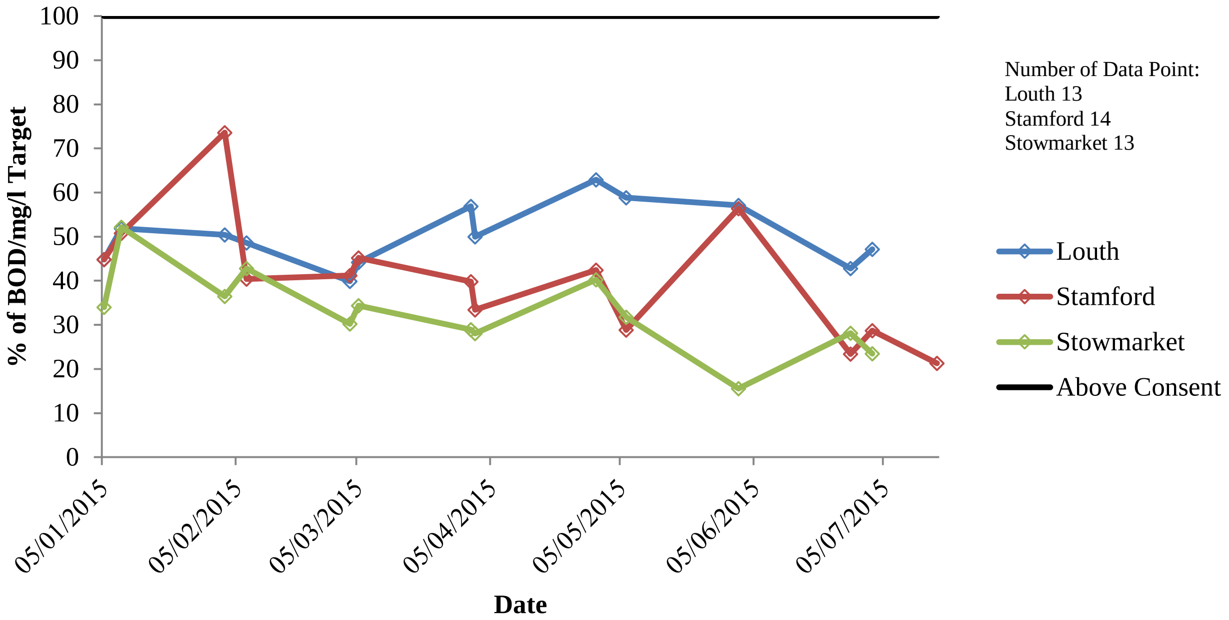

Figure 38 The percentage of BOD/mg/l Target for Louth, Stamford and Stowmarket Sewage Treatment Works

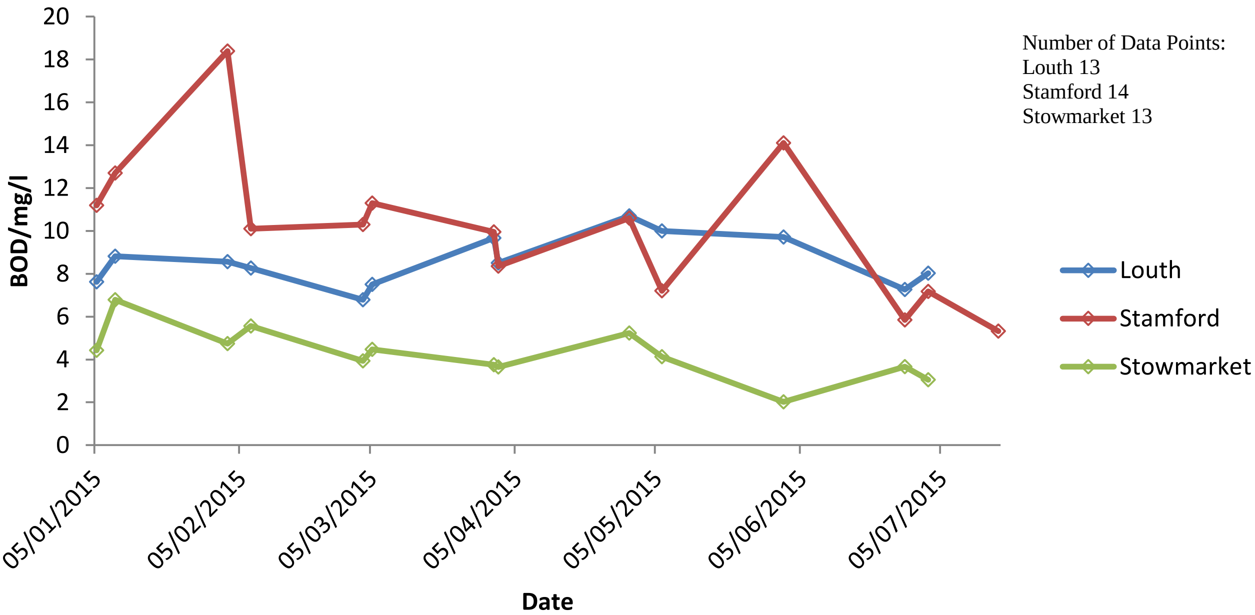

Figure 39 The BOD/mg/l produced at Louth, Stamford and Stowmarket Sewage Treatment Works

9. 5 COD

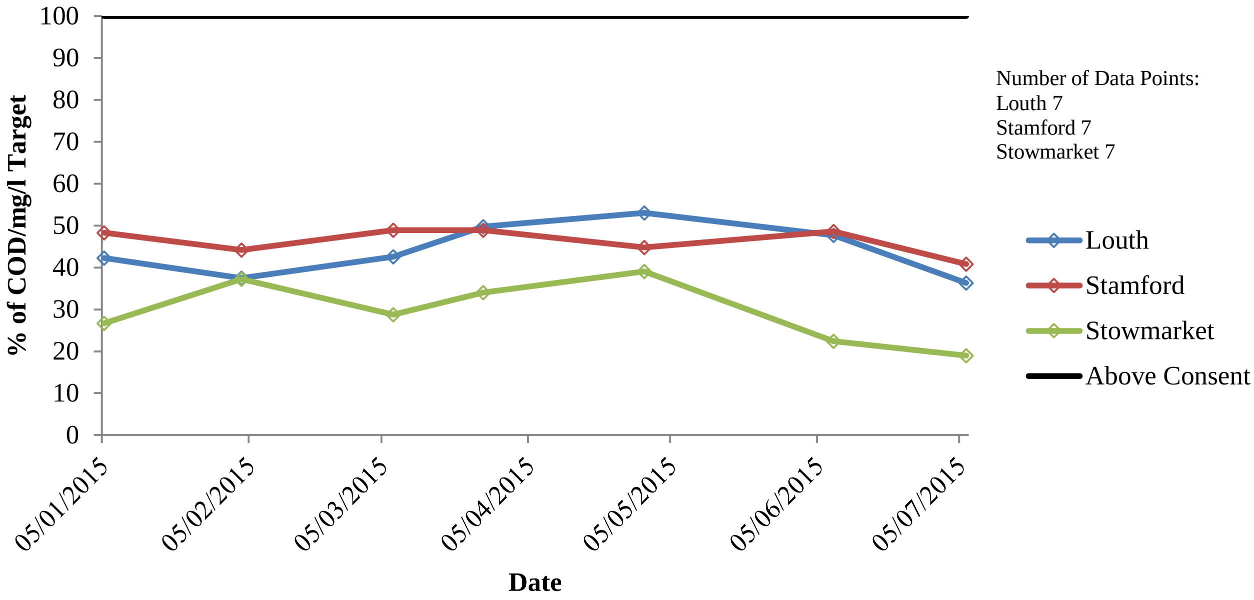

Figure 40 The percentage of COD/mg/l Target for Louth, Stamford and Stowmarket Sewage Treatment Works

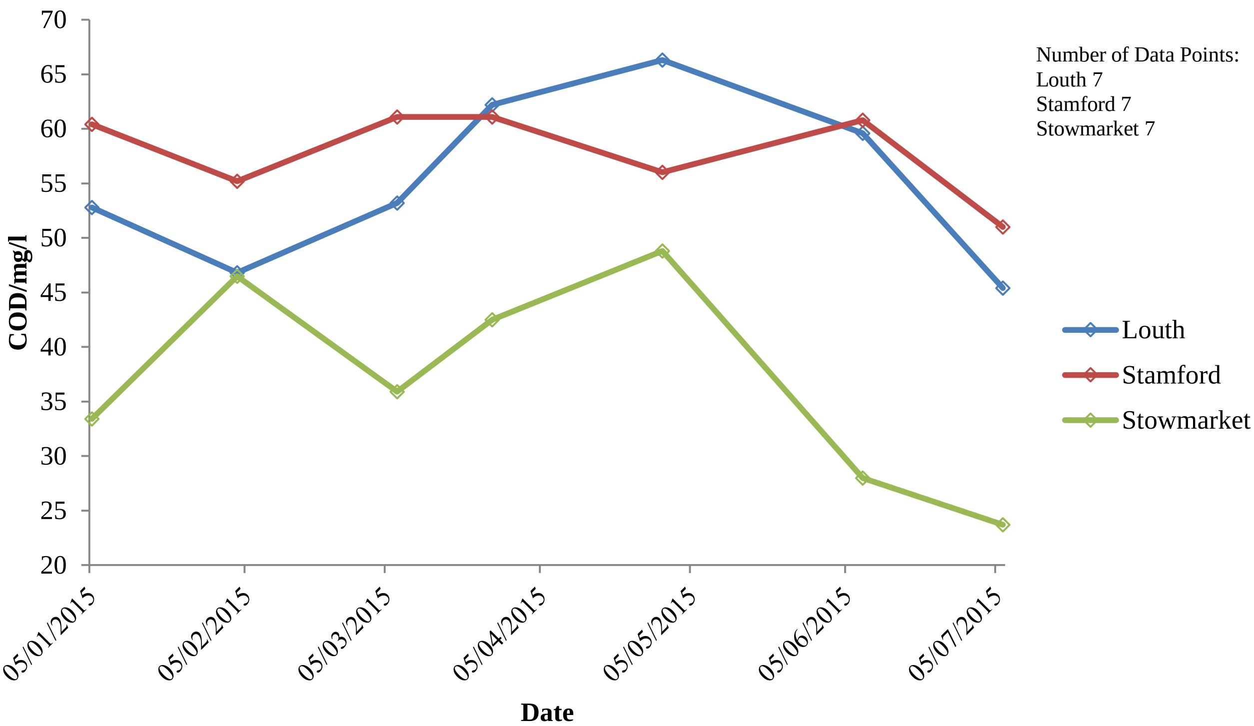

Figure 41 The COD/mg/l produced at Louth, Stamford and Stowmarket Sewage Treatment Works

14. 6 Suspended Solids

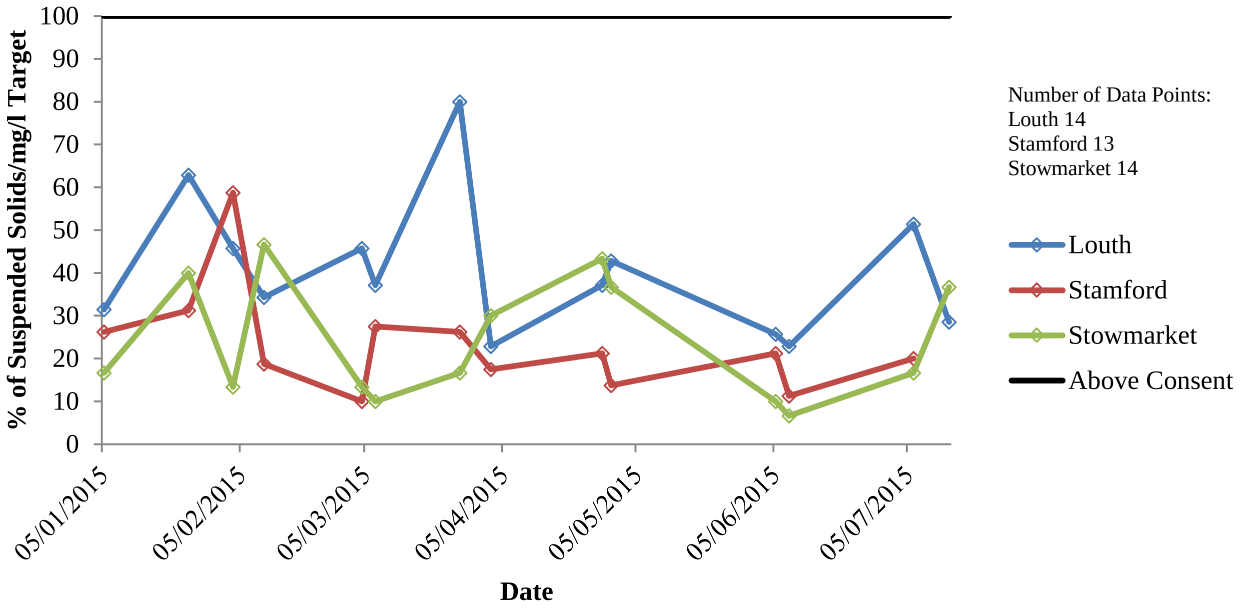

Figure 42 The percentage of Suspended Solids/mg/l Target for Louth, Stamford and Stowmarket Sewage Treatment Works

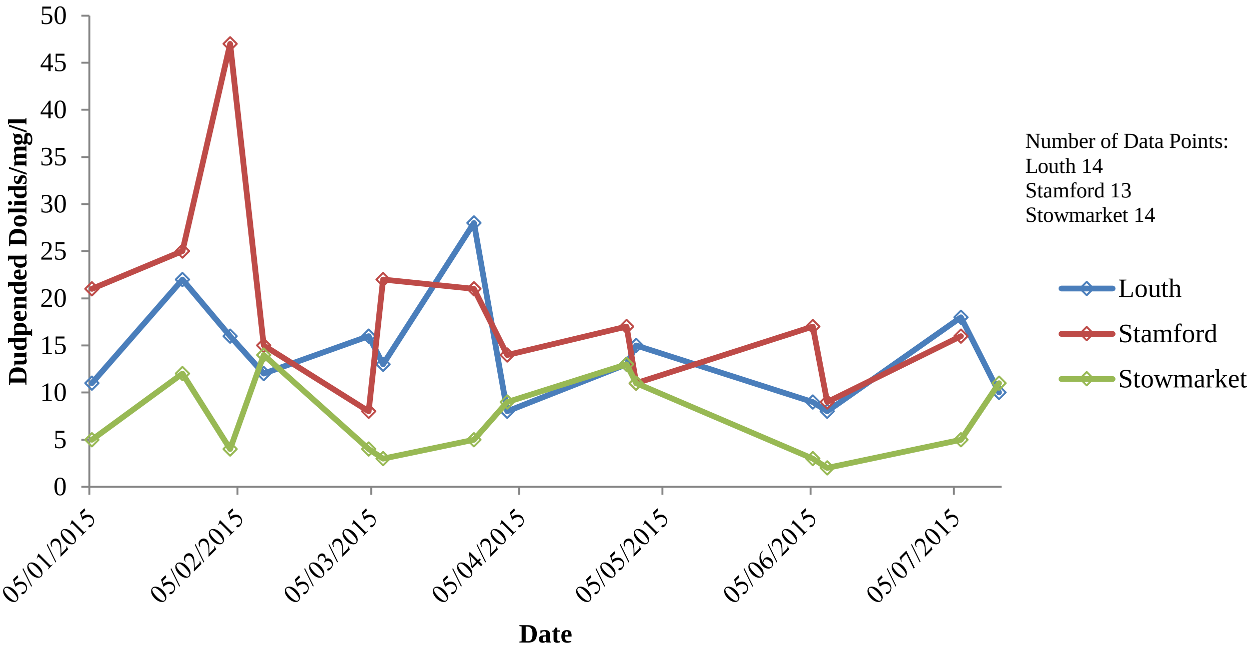

Figure 43 The Suspended Solids/mg/l produced at Louth, Stamford and Stowmarket Sewage Treatment Works