Natural Attenuation of Pathogens and Trace Contaminants in South East Queensland Waterways

Simon Toze[1], Leonie Hodgers[1], Andrew Palmer[1], Jatinder Sidhu[1], Declan Page[1], Mike Williams[1], Rai Kookana[1], Michael Bartkow[2], David Sedlak[3], Helen Stratton[4], Warish Ahmed[1], Sarah Schroeder[4] and Melody Christie[4]

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The Urban Water Security Research Alliance (UWSRA) is a $50 million partnership over five years between the Queensland Government, CSIRO's Water for a Healthy Country Flagship, Griffith University and The University of Queensland. The Alliance has been formed to address South East Queensland's emerging urban water issues with a focus on water security and recycling. The program will bring new research capacity to SEQ tailored to tackling existing and anticipated future issues to inform the implementation of the Water Strategy.

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Enquiries should be addressed to:

The Urban Water Security Research Alliance
PO Box 15087
CITY EAST QLD 4002

Ph: 07-3247 3005
Email: Sharon.Wakem@qwc.qld.gov.au

Project Leader – Simon Toze
CSIRO Land and Water
DUTTON PARK QLD 4102

Ph: 07- 3833 5572
Email: Simon.Toze@csiro.au

Authors: 1 – CSIRO; 2 – Seqwater, 3 - University of California Berkley, USA, 4 - Griffith University

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FOREWORD

Water is fundamental to our quality of life, to economic growth and to the environment. With its booming economy and growing population, Australia's South East Queensland (SEQ) region faces increasing pressure on its water resources. These pressures are compounded by the impact of climate variability and accelerating climate change.

The Urban Water Security Research Alliance, through targeted, multidisciplinary research initiatives, has been formed to address the region's emerging urban water issues.

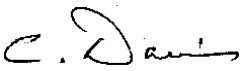
As the largest regionally focused urban water research program in Australia, the Alliance is focused on water security and recycling, but will align research where appropriate with other water research programs such as those of other SEQ water agencies, CSIRO's Water for a Healthy Country National Research Flagship, Water Quality Research Australia, eWater CRC and the Water Services Association of Australia (WSAA).

The Alliance is a partnership between the Queensland Government, CSIRO's Water for a Healthy Country National Research Flagship, The University of Queensland and Griffith University. It brings new research capacity to SEQ, tailored to tackling existing and anticipated future risks, assumptions and uncertainties facing water supply strategy. It is a $50 million partnership over five years.

Alliance research is examining fundamental issues necessary to deliver the region's water needs, including:

- ensuring the reliability and safety of recycled water systems.
- advising on infrastructure and technology for the recycling of wastewater and stormwater.
- building scientific knowledge into the management of health and safety risks in the water supply system.
- increasing community confidence in the future of water supply.

This report is part of a series summarising the output from the Urban Water Security Research Alliance. All reports and additional information about the Alliance can be found at http://www.urbanwateralliance.org.au/about.html.



Chris Davis
Chair, Urban Water Security Research Alliance

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EXECUTIVE SUMMARY

This project was set up to determine the capability of natural processes within reservoirs and water ways in South East Queensland (SEQ) to remove microbial pathogens and chemicals of concern. It is well understood that the SEQ raw drinking water storages are exposed to multiple sources of biological and chemical contaminants. These sources can be diffuse and large scale (e.g. agriculture and recreation) or local and direct (e.g. sewage and stormwater discharges). The treatment of drinking water in SEQ has traditionally relied upon engineered treatment systems as the sole treatment barrier(s), with natural systems such as reservoirs and streams only used as passive barriers. However, if natural treatment processes within SEQ reservoirs could be shown to actively remove pathogens and chemicals from the water, this could relieve some of the burden on the engineered systems. Thus, this project set out to investigate if a level of treatment capacity could be assigned to the reservoirs and associated waterways - the notion of using catchment health as a "*generator of good water quality*".

The aim of the pathogen research was to determine specific decay rates for individual microorganisms under in-situ conditions within SEQ reservoirs and the major environmental factors impacting on this decay. The research on the organic chemicals aimed to determine the potential for natural attenuation through biodegradation and photolysis of a range of chemicals with different physico-chemical properties and considered relevant from a catchment management perspective. At the same time as this Alliance funded research was being conducted, Seqwater and Griffith University had been undertaking work on improving the ability to identify the sources of faecal contamination in reservoirs, using Microbial Source Tracking (MST). With additional funding from the Alliance, this research was combined with the above pathogen and chemical removal experiments to obtain results which could be combined to provide a holistic analysis of the capacity of reservoirs within SEQ to actively remove contaminants.

Research was undertaken investigating the decay of pathogens in Wivenhoe Dam and the mid-Brisbane River and the influence of environmental and physical conditions within the reservoir, specifically sunlight, indigenous reservoir microorganisms and depth. The experiments tested the decay rates of enteric microorganisms including *E. coli, Salmonella enterica, Campylobacter jejuni,* adenovirus, and *Cryptosporidium* oocysts. The January 2011 floods also provided the opportunity to investigate the impact a major flood event had on pathogen decay.

The major findings from the analysis of pathogen decay in Wivenhoe Dam and downstream in the mid-Brisbane River indicate that both systems can have an active role in removing microbial pathogens. The results also showed that: (1) season and climate had minimal impact on decay of the microorganisms tested; (2) sunlight influences decay at the reservoir surface but its effect is impacted by turbidity and most likely dissolved organic matter; (3) decay of *Campylobacter* and adenovirus varies with depth, most likely due to variations in dissolved oxygen concentrations; and (4) the January 2011 floods had an influence on the decay rate of the more resistant microorganisms but the changes in decay rates were still such that these pathogens still had 90% removal times of less than 50 days.

A Quantitative Microbial Risk Assessment (QMRA) undertaken using the accumulated pathogen decay data, determined that an assumed contamination event of each microorganism under pre-flood conditions would take between three and 47 days to reach an acceptable health risk of 10^{-6} DALYs through skin contact exposure (eg, hand washing) and five to 60 days to reach the same acceptable health risk target with exposure through swimming. In this assessment, pathogenic *E. coli* was always the lowest risk and adenovirus posed the highest risk. Using the post-flood decay results, the time required to reach the acceptable health risk target for the hand washing exposure changed to a minimum of two days for *E. coli* up to 109 days for *Cryptosporidium,* and four days (*E. coli*) to 131 days (*Cryptosporidium*) for exposure via swimming. This health risk assessment would be greatly improved by combining with hydrodynamic data of water movement through the reservoir under different conditions along with better information on potential exposure sources and loads via the use of MST.

The experiments investigating the potential for attenuation of organic compounds were conducted under laboratory conditions using natural water sourced from Queensland and South Australia. The organic chemicals selected for testing had either been previously detected in SEQ reservoirs by Seqwater, or were considered as being a potential contaminant. The natural attenuation processes selected for assessment of the organic compounds were biodegradation and photolysis. Biodegradation was very slow under the conditions tested, even after dissolved organic carbon or a microbial inoculum was added to the water, with the exception of three pharmaceuticals (atenolol, methotrexate and trimethoprim) By contrast, photolysis was found to be an important degradation pathway for a number of compounds, particularly propranolol, sulfamethoxazole, diclofenac, triclosan and triclopyr. Photolysis was also found to occur with the generation of reactive species (particularly the OH· species), which was found to increase photolysis rates for a majority of the tested compounds. However, a high degree of attenuation of light intensity was found as depth of the water column increased, which led to a substantial reduction in rates of photolysis. This suggests that photolysis in well-mixed bodies of natural water may be of limited importance as an attenuation process, even when indirect photolysis occurs. Water quality is likely to be another important factor determining the rate of photolysis in water bodies. Other processes, such as sorption to suspended solids within the water column or into the bulk sediment, remain to be assessed to determine their potential effect on the biodegradation and photolysis within the water column.

The final area of research within this project, jointly funded with Seqwater, was on the application of Microbial Source Tracking (MST) tools for distinguishing between human and animal faecal contamination within SEQ reservoirs and on the ability to detect evidence of bovine faecal contamination. The *E. coli* β-Glucuronidase gene was the tool tested to be able to distinguish between human and animal contamination, while bovine-specific *Bacteroides* was tested for detecting evidence of bovine faecal contamination.

The research using the *E. coli* β-Glucuronidase gene was found to be able to discriminate between *E. coli* isolates of human and animal origin with a moderate degree of confidence using phylogenetic analysis. Exploratory ordination statistical methods (PCA and NMDS), however, did not reveal patterns of spatial and temporal variability to sufficiently discriminate between groups of *E. coli* that had a human or animal origin. The phylogenetic analysis consistently grouped 72%-77% of the known human isolates in the human cluster, with false positives falling between 14% and 21%.

The research on bovine markers showed that the bovine bactericides BacCow-UCD and cowM3 markers could be detected in faecal samples from both target and non-target host groups. In comparison, the bovine adenovirus B-AV marker showed absolute host-specificity but had low host-sensitivity. The conclusions from the MST research were that both of the tested MST methods (β-glucuronidase and bovine markers) should be used with caution as single methods, and would be most reliable when used in conjunction with a number of other MST tools.

The overall project conclusions currently indicate that reservoirs in SEQ do have an active treatment capability and are able to remove contaminants, particularly pathogens, under a range of climatic and environmental conditions. It is evident that residence times are important and that there remains to be coordination of the data on the outcomes of contaminant removal with other data, in particular hydrodynamic flow data, including modelling. This will be particularly important for assessing changes in health risk levels under different climatic events and/or exposure scenarios.

1. INTRODUCTION

Research has been undertaken initially in the *Purified Recycled Water* project and continued in the *Pathogens and Chemicals in Dams* project investigating decay rates for a variety of enteric pathogens and degradation of target organic chemicals in South East Queensland (SEQ) reservoirs such as Wivenhoe Dam. The aim of the pathogen research was to determine specific decay rates for individual microorganisms and the major environmental factors driving any decay (or reducing decay). The research on the organic chemicals aimed to determine the impact of biodegradation and photolysis degradation on selected organic chemicals.

At the same time as this Alliance funded research was being conducted, Seqwater and Griffith University had been undertaking work on improving the ability to identify the sources of faecal contamination in the reservoirs using Microbial Source Tracking (MST). It was agreed that, with additional funding through the Alliance, the information from this research would be an important link to the research on pathogen decay and chemical degradation. It was also proposed that this combined research could be potentially incorporated into existing or new Seqwater hydrodynamic models used to predict water flow and conditions in the reservoirs. The ultimate objective of this combined research program was to determine if reservoirs could have a role as an active treatment barrier and if the "health" of the reservoirs could be used as an indicator of its effectiveness in removing biological and chemical contaminants.

1.1. Pathogens in Reservoirs and Fresh Water Sources

Pathogens remain the major critical health risk in freshwater resources. Water-borne pathogens of greatest concern tend to be the enteric pathogens and can enter the water from a range of human related activities around waterways, including sewage discharges, recreational activities and development based activity (Geldrich 1996; Schoen *et al*. 2011). Another set of sources for a number of these pathogens are the zoonotic sources including farm animals and wildlife (Soller *et al*. 2010). Quantitative Microbial Risk Assessment (QMRA) analyses undertaken on the health risks from these pathogens, even relating just to bathing-related gastrointestinal illnesses, indicate that there can be identifiable risks to human health (Soller *et al*. 2010). It can also be assumed that there also health risks linked to exposures for other non-bathing activities (eg, boating, fishing, camping and hiking) in and around waterways.

These health risks can be mitigated by a range of control measures. These have commonly relied upon either control of the pathogen sources, or through removal within a water treatment plant. Source control within reservoirs can be difficult, particularly if a significant source is wildlife and free range farm animals. Treatment plants will always be the predominant method used for controlling pathogens in the final product water, however, those plants are often relied upon to treat water based on hypothetical assumptions on the type and number of pathogens present in the water reaching the treatment plant. Many of these assumptions are based on information generated from the detection of indictor bacteria, not actual pathogens, or on a general estimation of potential risk based on limited data. In addition, these assumptions rarely take into consideration any changes to pathogen numbers based on changing conditions with the reservoirs and waterways.

Data on the persistence of pathogens within the reservoir can be used to help in the control and treatment of drinking water. For example, raw drinking water storages in SEQ are recognised to be potentially exposed to multiple sources of pathogens. These sources can be diffuse and wide spread (eg, agriculture and recreation) or point-source that is local and direct (eg, sewage and stormwater discharges). The treatment of this water from reservoirs for domestic supply has primarily relied upon engineered systems as the sole treatment barrier(s), with natural systems such as reservoirs and streams only used as passive barriers via mechanisms such as restricted public access and, where possible, controlling inputs from upstream communities and farms. This sole reliance on water treatment plants places unnecessary operational requirements on these plants to consistently provide a high quality drinking water. The notion of using catchment health as a "*generator of good water*

quality", in other words, assigning a level of treatment capacity to the reservoirs and streams, would improve the capacity of water treatment operators to produce a consistent quality of water through a better understanding of what contaminants the treatment plants need to cope with and what contaminants will be removed via natural treatment processes in the reservoirs.

To be able to achieve this aim, the most important processes in natural systems for the removal of pathogens need to be documented. In addition, the influence of a range of possible climatic and environmental conditions on these removal processes also needs to be taken into account. This information can then be incorporated into contaminant fate models to assist the development of improved management options that include natural systems as treatment barriers. A better understanding of the natural systems as a treatment barrier can allow the treatment plants to be used as the final barrier, not the sole treatment barrier within the water treatment system. At present however, there is limited scientific information on the level of removal of pathogens that can be expected for the reservoirs, or how the treatment capacity of these water bodies change with changing conditions (eg, seasonal variations and event based changes).

A search of the scientific literature from 1995 onwards has shown that there is little information on pathogen survival in environmental waters. Scientific papers published earlier than 1995 were excluded as experience has shown that much of this information is of limited value due to the less sophisticated analytical techniques for many pathogens used prior to the 1990s and the predominant reliance on faecal indictor bacteria.

The literature reviewed for this study included investigations of the survival of enteric microorganisms in a range of surface waters and groundwater. The published papers, microorganisms studied and water types are given in Table 1. A number of these published studies indicated that certain environmental factors influenced the survival of the microorganisms. These included temperature (Burkhardt *et al.* 2000, Darakas 2002, Espinosa *et al.* 2008, Feng *et al*. 2003, Lund 1996, Ngazoa *et al.* 2008, Skraber *et al*. 2004); pH (Feng *et al*. 2003), sediment (Characklis *et al*. 2005, Karim *et al*. 2004), oxygen concentrations (Rosleve *et al.* 2004), and sunlight (Burkhardt *et al.* 2000, Dan *et al.* 1997, Espinosa *et al.* 2008, Sinton *et al.* 2007).

The results from a number of these studies have potential relevance for the research detailed in this report on the decay of enteric microorganisms in SEQ reservoirs. Temperature has been considered to be an important driver for pathogen decay in environmental waters. All of the papers reviewed showed that changes in temperature influenced the decay rate, with decay rates increasing with increases in temperature (Burkhardt *et al.*2000, Darakas *et al.* 2002, Espinosa *et al.* 2008, Lund 1996, Ngazoa *et al.* 2008, Sinton, *et al.* 2007, Skraber *et al.* 2004). However, most of these studied the changes in decay rates at temperatures less than 10^{o}C to between 15 and 20^{o}C. Being a sub-tropical environment in SEQ, there is relatively little variation in ambient and water temperatures compared to North America and Europe, where most of the above research was undertaken. It is possible, therefore, that, while temperature may be important, it may not be a controlling factor for any variations in decay in the current research.

Likewise, sunlight is commonly referred to as important for pathogen decay in water. The SEQ climate would suggest that sunlight would be an important factor in pathogen decay. Sinton *et al.* (2007), Dan *et al.* (1997), Espinosa *et al.* (2008), and Burkhardt *et al.*(2000) all determined that sunlight had an influence on pathogen survival. All of these researchers noted that different microorganisms responded differently to the influence of sunlight. Dan *et al.* (1997) noted that sunlight impacted the survival of *E. coli* and enterococci but not poliovirus. Burkhardt *et al.* (2000) found that the coliforms were much less resistant than the male specific bacteriophage and *C. perfringens* spores.

The other area mentioned in several of the studies that could be considered important in the current research was the influence of sediment. Both Characklis *et al*. (2005) and Karim *et al*. (2004) found that sediment was important for the partitioning behaviour of pathogens in the water column and into the sediment. In addition, Karim *et al.* (2004) found that the presence of sediment actually decreased

the decay rate of faecal coliforms, *Salmonella, Giardia* and bacteriophage. Sediment can flow into SEQ waterways during rain events, some of which can be large events in a short time period. These large rainfall events can facilitate large amounts of overland flow which can mobilise the transportation of sediment. The impact of suspended sediments on the transport and survival of different pathogens is an area where more research is needed to assist in predicting pathogen movement and decay under different conditions, particularly during events such as storms.

The fate and behaviour of pathogens in SEQ reservoirs and waterways will be important information on the treatment capacity of reservoirs and on the pathogens that are most likely to reach the water treatment plants. This information will assist in the management of Seqwater controlled water bodies and help refine existing and new catchment management practices.

Table 1. Microorganisms and water sources tested for survival of enteric microbes in water.

Authors	Microorganisms Studied	Water Type
Bae and Schwab. (2008)	Indicator viruses	Surface water Groundwater
Burkhardt *et al.* (2000)	faecal coliforms *E. coli* *C. perfringens* spores male specific bacteriophage	Estuarine waters
Characklis *et al.* (2005)	enteric bacteria *C. perfringens* spores Coliphage	Stormwater
Dan *et al.* (1997).	*E. coli* Enterococci Poliovirus	Reservoir
Darakas (2002)	*E. coli*	9% NaCl
Englebert *et al.* (2008)	*E. coli* *Salmonella* *Shigella*	Lake water (algal mats)
Espinosa *et al.* (2008)	Rotavirus Astrovirus	Surface water Groundwater
Feng *et al.* (2003)	Coliphage MS2 Coliphage Q*b*	Sterile pH buffers
Karim *et al.* (2004)	faecal coliforms *Salmonella* *Giardia* Bacteriophage	Constructed wetlands
Lund (1996)	*E. coli* *Campylobacter jejuni* *Yersinia enterocolitica*	lake water
Ngazoa *et al.* (2008)	Norovirus	Mineral water tap water river water sewage effluent
Roslev *et al.* (2004)	*E. coli*	Drinking water
Sinton *et al.* (2007)	*E. coli* *Campylobacter jejuni* *Salmonella enterica*	Stormwater
Skraber *et al.* (2004)	Poliovirus-1 somatic coliphages thermotolerant coliforms	River water
Toze *et al.* (2004)	*E. coli* Poliovirus Coxsackievirus *Salmonella* MS2	Groundwater

1.2. Organic Chemicals in Fresh Water

Attenuation of organic contaminants in freshwater systems has received increasing attention, as knowledge of the presence of a myriad of organic contaminants becomes more apparent through rapid improvements in analytical technologies. It is also becoming apparent that treatment of these organic contaminants cannot be solely undertaken by a single treatment technology and multiple treatment barriers are, therefore, becoming more attractive. For example, while wastewater treatment plants (WWTPs) are highly effective in removing a vast array of potential contaminants prior to discharge, it has been well established that a number of organic contaminants, such as pharmaceuticals, endocrine disrupting chemicals (EDCs) and personal care products, are recalcitrant to removal (Watkinson *et al.* 2007, Sun *et al.* 2008, Muñoz *et al.* 2009). Also, where non-point source contamination occurs, such as pesticides being mobilised into waterways from agricultural areas, it is essential to understand the treatment capacity of the waterways into which they are mobilised. This understanding of the fate of organic contaminants in freshwater systems is critical in being able to formulate the potential ecological risk these contaminants may have. In the case of potable water supplies, understanding of the fate of organic contaminants can also have important implications for human health risk assessment.

From previous studies, the important attenuation processes for organic contaminants include biotic and abiotic processes (OECD 2004, OECD 2008). Abiotic attenuation processes can include degradation through solar radiation (photolysis), reaction with species produced from photolysis (indirect photolysis) and reaction with water (hydrolysis). Biodegradation, where microbial communities use organic contaminants as a substrate, is the principle biotic degradation process. Assessment of contaminants susceptible to abiotic processes, such as photolysis, can be reasonably predictable based on compound-specific factors including chemical structure. However, a number of factors relating to the environment in which the contaminant is present, such as the presence of reactive species produced from photolysis leading to indirect photolysis, can alter predicted degradation rates (Lin and Reinhard 2005, Matamoros *et al.* 2009). Attenuation of organic contaminants through biological degradation is even less well understood in freshwater systems and likely to also be highly dependent on both compound and environment-specific factors that can modify degradation rates (Kagle *et al.* 2009, Yamamoto *et al.* 2009).

The aim of this study was to assess the attenuation of selected organic contaminants in a number of natural water samples, including Wivenhoe Dam, mid-Brisbane River and Salisbury (Adelaide) wetland water, to assess potential attenuation processes that may be important within their respective water columns. The two attenuation processes, photolysis and biodegradation, were simulated under controlled laboratory microcosms. This was undertaken to gain insight into processes that require further experimental assessment in both the laboratory and under field conditions and may subsequently be useful in determining the fate of such compounds if they were present within the Wivenhoe Dam or mid-Brisbane River.

1.3. Microbial Source Tracking

1.3.1. What is Microbial Source Tracking?

Microbial source tracking (MST) is a process of identifying a particular source (such as human, cattle, or bird) of faecal contamination in water, which is generally measured through faecal indicator bacteria, such as *Escherichia coli* (*E. coli*) or enterococci. Ideally, indicators are non-pathogenic, rapidly detected, easily enumerated, have survival characteristics that are similar to those of the pathogens of concern, and can be strongly associated with the presence of pathogenic microorganisms. *E. coli* has long been used as indicator of faecal pollution as it has good characteristics of a faecal indicator, is present at concentrations much higher than the pathogens it predicts, and is not normally pathogenic to humans (Scott *et al.* 2002). There are many limitations associated with using indicator microorganisms as indicators of human faecal contamination as many of them exist in the intestines of humans as well as other warm blooded animals and they are subject to spatial and temporal variation.

E. coli represents over 96.8% of thermotolerant coliforms (Dufour 1977), therefore it is the microorganism that has been chosen as a key indicator of faecal contamination and is a reporting requirement for many guidelines and regulations worldwide. However, the presence of *E. coli* does not provide any information about the source of contamination and therefore is not always an effective indicator of risk to humans (Baggi *et al.* 2001, Allwood *et al.* 2003, Fattouh *et al.* 2004, Jin *et al.* 2004, Stratton *et al*. 2009). The field of microbial source tracking is the subject of many recent reviews (Stoeckel *et al.* 2004, Ahmad *et al.* 2009, Meays, 2004, Field and Samadpour 2007) and the approach is often criticised for the questionable accuracy and interpretation of results, yet the methods continue to be improved and new methods discovered by addressing the many concerns outlined in critical reviews.

The underlying assumption of MST is that certain bacterial strains are unique to a particular host group (ie, host-specific) (Field and Samadpour 2007). Therefore, phenotypic or genotypic patterns of such bacterial strains from host group(s) could be stored in a library for comparison with the patterns of unknown environmental strains to identify their most likely sources. Most MST studies have relied on matching 'fingerprints' from bacterial strains isolated from a water system to those isolated from various hosts. These fingerprints are based on phenotypic traits (eg, antibiotic resistance analysis) or genotypic profiles (eg, rep-PCR, ribotyping) of individual microbial strains.

1.3.2. Traditional and Alternative Indicators of Faecal Pollution in Water

The microbiological quality of water is generally assessed by enumerating traditional faecal indicator bacteria. Faecal indicator bacteria commonly include *E. coli* and enterococci*,* faecal coliforms, and the spores of *Clostridium perfringens* (Field and Samadpour 2007), which are commonly found in the faeces of warm blooded animals (Ahmed *et al*. 2009). However, the reliability of these traditional indicators has been questioned in terms of their ability to predict the likely presence of pathogens (Ahmed *et al*. 2008).

1.3.3. Principles of Microbial Source Tracking

MST aims to identify the origins of faecal pollution in water (Roslev and Bukh 2011). The underlying assumption is that characteristics in, or associated with, faecal pollution can be used to identify the faeces type and source (Field and Samadpour 2007). Therefore, MST not only detects faecal pollution, but provides information about the source. While human and animal faecal contamination of water can pose a serious health risk to public, knowing the source of this contamination can assist in managing the risk more efficiently. In this respect, MST has the potential to be used as an efficient tool by water managers to improve the management of public health (Stratton *et al.* 2009).

Direct monitoring of viable and infective pathogens in water is an attractive option and would provide invaluable information regarding public health risk. However, there are hundreds of different types of pathogens that can be found in water due to faecal pollution. The presence of these pathogens can also be intermittent or in very low numbers in large volumes of water. Therefore, it is not economically, technologically, or practically feasible to routinely monitor the microbiological quality of water for all possible pathogens. As a result, traditional faecal indicators, such as *E. coli,* enterococci, and *Clostridium perfringens* spores have long been used as surrogates for the presence of pathogenic microorganisms and still remain as some of the most viable methods for MST and identification of faecal pollution.

Several microbes, such as Bacteroides and Bifidobacteria, genes from microbes, for example the *nifH* gene of *Methanobreviibacter,* and chemicals have also been considered as potential alternative tracers identifying faecal sources in the environment. However, to date, no one approach has been shown to accurately identify the origins of faecal pollution in environmental samples. The best approach to tracking the source of microbes and faecal pollution is to use multiple methods that employ a combination of phenotypic and genetic markers. It has been recommended that a combination of traditional indicators plus alternative indicators and markers could provide valuable information regarding the extent of faecal pollution, it's origin and possible correlation with pathogens (Ahmed *et al*. 2008, Balleste *et al*. 2010).

1.4. Key Research Question(s)

1. Based on the measured attenuation processes, what trace organic chemicals and pathogens would be of most concern to Seqwater and Queensland Health, and what are the most likely sources of pathogens to cause contamination in water storages?
2. What are the treatment capacities of the studied reservoir(s) to remove target pathogens and trace contaminants under different climatic and seasonal conditions?
3. What are the major influences driving the removal of pathogens and trace contaminants in the water storages and associated waterways? Some of the parameters to be tested include:
   (a) Residence time of the contaminant in the reservoir.
   (b) Water quality (including pH, dissolved oxygen and nutrients).
   (c) Sunlight and temperature.
   (d) Partitioning of contaminants to the sediment and suspended solids.
   (e) Influence of turbidity on sunlight attenuation and photodegradation.
   (f) Water depth and stratification of the water body.
   (g) Indigenous microorganisms.
4. What is the importance of the mid-Brisbane River reach in terms of removal rates and pathways of pathogens and trace contaminants?
5. Which trace contaminants and pathogens are likely to persist in the water column and what impact does this have on downstream treatment processes (eg, will such contaminants reach the water treatment plants)?
6. Can the major sources of microbial faecal contamination be identified?
7. What are the major health risk exposure pathways for microbial pathogens in the reservoirs and what is the best mechanism for the control of these pathways? This will include the estimation of required residence times in the water storages and a determination on what is needed to enable the data on removal rates to be incorporated into appropriate Seqwater models to assist appropriate management and operational decisions?

2. DECAY OF PATHOGENS IN RESERVOIRS

2.1. Methods and Materials

2.1.1. Experimental Sites

Wivenhoe Dam

The pathogen decay experiments undertaken in-situ in Wivenhoe Dam were done at a site established at the mouth of Logan's Inlet (Figure 1). This was a permanent site established within the original Brisbane River riverbed with a fixed anchor that was used through the entire project. Logan's Inlet had originally been chosen as it was the location of the PRW diffuser. After the postponement of PRW being added to Wivenhoe Dam, the site was continued as it was an area of the reservoir near known farms, recreational activity from campers and anglers, and potential inputs from wildlife.

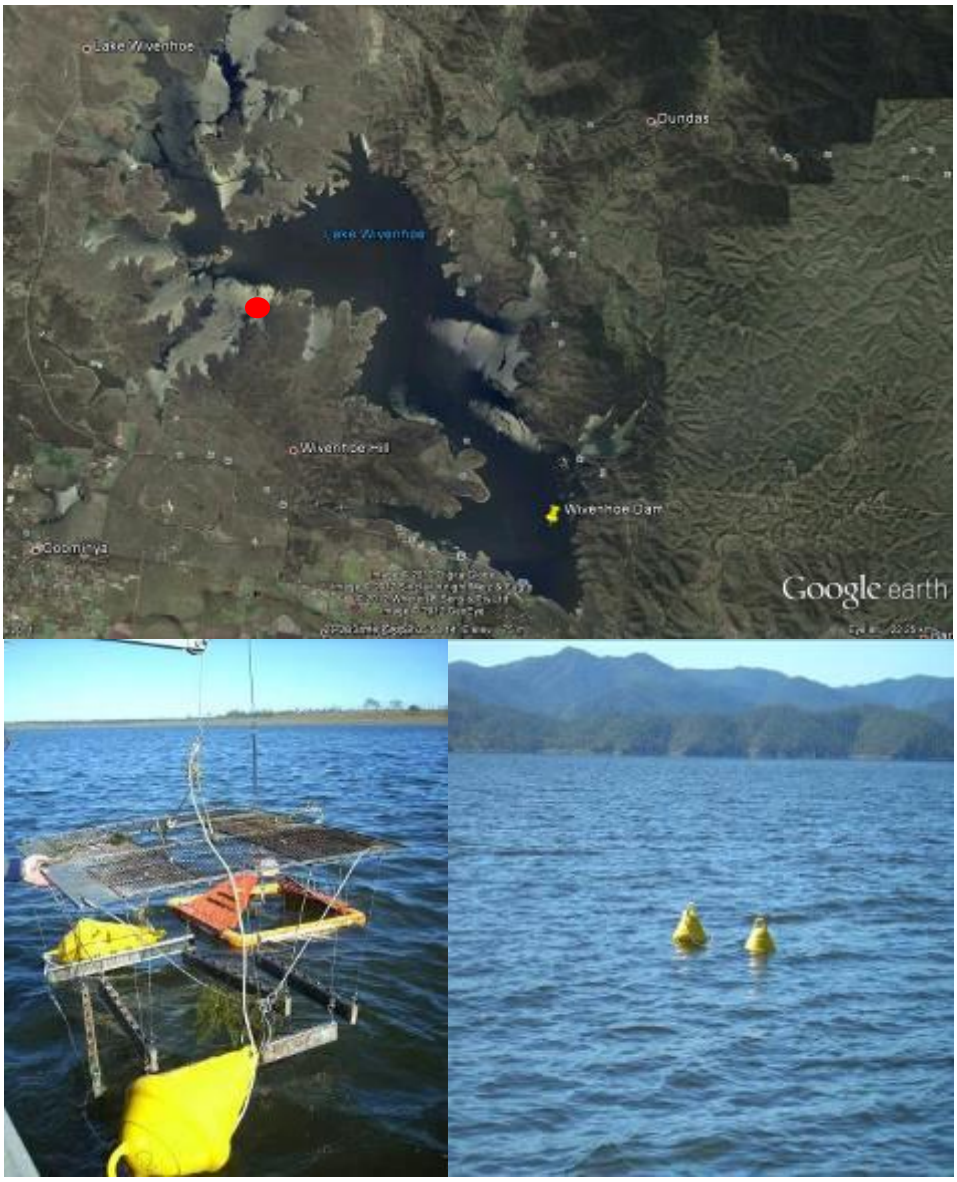


Figure 1. Wivenhoe Dam and In-situ experiment site. Red circle denotes the experimental site at the mouth of Logan's Inlet.

Mid-Brisbane River Site

The mid-Brisbane River site was located in the river approximately 200m downstream of the Pine Mountain crossing (Figure 2). The depth of the river at the experimental site was approximately 3m. The site was chosen to be far enough downstream to be have influences from a wide range of inputs including the Wivenhoe Dam, the Lockyer Creek and other streams, wastewater treatment plant discharge, septic systems and farming activities. The flow rate in the river can vary depending, rain events, releases from Wivenhoe Dam and flows from the streams and creeks. These inputs can also have an influence on the quality of the water reaching the Mt Crosby Drinking Water Treatment plants.

Figure 2. Sample site on the mid-Brisbane River. The red circle denotes the experimental site.

2.1.2. Diffusion Chambers

All of the experiments reported were done *in-situ*. Unless stated otherwise, these *in-situ* experiments were undertaken using diffusion chambers (Figure 3). The chambers are a modification of the chambers used for the study of pathogen decay in groundwater detailed by Toze *et al.* (2009). The chambers and end plates were constructed of Teflon and the size exclusion membranes used were 47 mm diameter Millipore mixed cellulose esters (VSWP) with a pore size of 0.025 µm. This pore size is sufficient to exclude the passage of the smallest viruses (such as the bacteriophage MS2) but still

allow passage of reservoir water across the membrane and through the diffusion chamber (Pavelic *et al.* 1998). Clogging of these membranes has been shown to not be a problem (Sidhu and Toze 2012). The chambers have an internal volume of approximately 12 mL.

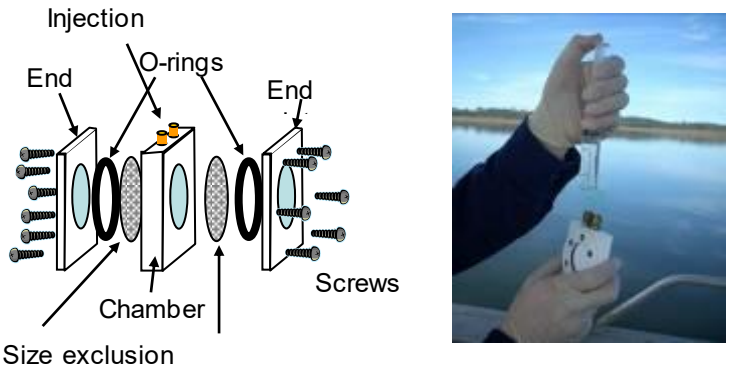


Figure 3. Schematic diagram of pathogen decay chambers and example of a chamber being filled.

To fill the chambers, two volumes of reservoir water (200 – 500 mL depending on the experiment) were collected in sterile polycarbonate jars.

One of these volumes was seeded with the required enteric bacteria suspensions. The other volume of water was seeded with *Cryptosporidium* oocysts and/or virus suspensions to be tested. The microbial suspensions were prepared as discussion in Toze *et al.* (2010). All of the enteric microorganisms were added to the reservoir water to achieve an approximate final number of 4-6 $\log_{10}$ microorganisms mL^{-1} in the seeded reservoir water. The seeded reservoir water volumes were then used to fill the assembled diffusion chambers via the injection ports (Figure 3). An individual chamber contained either the reservoir water seeded with the enteric bacteria or reservoir water seeded with the enteric viruses and *Cryptosporidium* oocysts. Sufficient chambers were used to allow the collection of three replicate chambers containing either bacteria or *Cryptosporidium* and viruses on each of the sampling events.

Where needed, the reservoir water could be treated prior to seeding with the test microorganisms (eg, filtering to remove the indigenous reservoir microorganisms) prior to being put into the chambers. The pathogens tested and the length of the experiment varied depending on the parameters tested and the aims of the individual experiment.

2.1.3. Microorganisms Used and Analytical Methods

The list of all the pathogens tested in all of the experiments and the analytical methods used for each microorganism is given in Table 2.

Table 2. Enteric microorganisms tested in pathogen decay experiments in Wivenhoe Dam.

Enteric Microorganism Group	Enteric Microorganism	Analytical Method
Bacteria	*E. coli*	Spread plate culture on Chromocult Coliform Agar
	Salmonella enterica	Spread plate culture on XLD agar
	Campylobacter jejuni	Spread plate culture on CCDA agar
	Enterococcus faecalis	Spread plate culture on Chromocult Enterococci Agar
Protozoa	*Cryptosporidium* oocysts	Microscopy using vital stains (Campbell *et al.* 1992)
Virus	Adenovirus	Real-time quantitative PCR (Heim *et al.*2003)
	MS2	Culture using double layer method (Havelaar and Hodgeboom 1984)

Individual chambers were collected at different sampling times and destructively sampled. For all of the experiments, the collected diffusion chambers were immediately placed into zip lock plastic bags and then placed on ice in an insulated container. The collected chambers were then transported back to the CSIRO laboratory. In the laboratory the water was collected from the chambers by vigorously shaking the chamber for 30 seconds to resuspend any settled particles, and then using a sterile needle and 20 mL syringe to extract the water from within the chamber. The collected water was transferred to a sterile 15 mL centrifuge tube prior to further processing and analysis. The water that had been seeded with the bacteria and MS2 were plated immediately using the methods described in detail in Appendix 1. The sample for the detection of viable *Cryptosporidium* oocysts was stored at 4°C and the analysis was undertaken within 7 days. The adenovirus samples were frozen at -20°C and analysed all at one time as described in Appendix 1.

2.1.4. Details on Individual Pathogen Decay Experiments

2.1.4.1. Initial Assessment of Pathogen Decay Rates

The aim of this experiment was to obtain an initial understanding of the potential and relative rate of decay of the different enteric microorganisms, both within and across each microbial group (ie, bacteria, viruses and protozoa).

As the preliminary investigation, this experiment tested *E. coli*, *Salmonella*, MS2, adenovirus and *Cryptosporidium* oocysts. The test site was the in-situ site established at the mouth of Logan's Inlet (Figure 1).

The experiment commenced in June 2009. Sampling was undertaken at Time 0, on Day 4 and then every seven days up to Day 28 for the bacteria. MS2 and adenovirus were sampled weekly up to Day 28 and then on Day 42, while *Cryptosporidium* was sampled every seven days up to Day 28, then on Days 42 and 56.

2.1.4.2. Impact of Indigenous Microorganisms on Pathogen Decay

Previous research carried out by CSIRO on pathogen decay in aquifers has demonstrated that the indigenous groundwater microorganisms have a significant influence on the decay rates of microbial pathogens introduced into groundwater (Gordon and Toze 2003, Toze *et al*. 2004). This experiment was undertaken to determine if the indigenous microflora of the reservoir had a similar significant influence. The experiment was undertaken in the same manner as the initial decay experiment described above except that there were two different conditions tested. One set of chambers were set up identically to the chambers in the initial decay experiment. A second set contained reservoir water that had been filtered using a 0.2 μm filter to remove the indigenous microflora before being seeded with the enteric microorganisms.

This experiment was carried out testing *E. coli*, *Salmonella*, *Campylobacter*, MS2 and Adenovirus. *Cryptosporidium* oocysts were not tested in this experiment as there had been problems sourcing samples containing sufficient oocysts at the time of this experiment. The experiment started in October 2009 and progressed for 35 days. As with the initial decay experiment, the chambers containing water seeded with the bacteria were sampled at Time 0, on Days 4, 7, 14, and 21. The chambers containing MS2 and adenovirus were collected at Time 0 and every 7 days.

2.4.1.3. Effect of Sunlight on Pathogen Decay

Sunlight is known to have a germicidal effect (eg, Sinton *et al*. 2002, 2007), but little is known about the influence of sunlight in SEQ waterways and how much this germicidal effect changes with depth.

The first of two sunlight experiments was undertaken at the beginning of April 2010 over a three-day period using *Salmonella*, the bacteriophage MS2, and *Cryptosporidium* oocysts as the target microorganisms. The experiments were undertaken using low-potassium scintillation vials, glass that is transmissive to a much shorter wavelength than borosilicate glass.

The three microorganisms were suspended in reservoir water in the same manner as described in Section 2.1.2 and then added to a series of the scintillation vials. Half of the vials were then wrapped in foil as a negative control. Half of the foil-wrapped and half of the non foil-wrapped vials were then placed in an open tray, which was suspended in the reservoir at 5 m below the surface. The remaining vials were placed in a second tray that was placed so that the vials were within 10 cm of the reservoir surface. The climatic conditions were overcast with a few episodes of clearer sky. Despite the limited sunlight, it was decided to continue with the experiment to determine the effect limited sunlight due to overcast conditions had on pathogen decay.

Triplicate foil-wrapped and non-wrapped vials were collected from both the surface and 5 m trays every hour for 4 hours, then at 22, 24 and 26 hours, and finally at 48 and 72 hours. The samples were placed on ice, transported to the laboratory and processed immediately for *Salmonella* and MS2. The samples were then left at 4ºC for 14 days to allow any germicidal effect on *Cryptosporidium* oocysts to become detectable by the vital staining method used for the detection of viable and non-viable oocysts.

A second sunlight experiment was undertaken in May 2012. This used the same protocol as the first experiment except that only the impact of sunlight at the surface was assessed. Also, the microorganisms tested in the second experiment were *E. coli*, *Enterococcus faecalis*, MS2 and *Cryptosporidium* oocysts. Duplicate foil-wrapped and non-wrapped vials were collected on the first day at Time 0, and hours 1, 2, 4 and 6. On the second day, duplicate vials (foil-wrapped and non-wrapped) were collected at 24, 26, 28 and 30 hours. The weather during the experiment was clear skies with uninterrupted sunlight. Analysis for the microorganisms in this experiment was the same as for the initial sunlight experiment.

2.1.4.4. Pathogen Decay at Different Depths within the Reservoir

In a slow moving water body such as a reservoir, larger microorganisms such as *Cryptosporidium* and microorganisms adhered to solid particles can settle out into the sediment of the reservoir. It is possible that the water quality could vary with depth, particularly parameters such as dissolved oxygen. This experiment was set up, therefore, to determine any impact of depth on pathogen decay.

This experiment was undertaken using the same methodology as the initial decay experiment except that an identical series of chambers were suspended at 1 m and 15 m depths below the reservoir surface. The microorganisms tested in this experiment were *E. coli, Salmonella, Campylobacter*, adenovirus and *Cryptosporidium*. Note that in this experiment, *Cryptosporidium* oocysts were not tested at the 15 m depth due to insufficient oocyst numbers to be able to test decay at both 1 m and 15 m. The experiment commenced in September 2010 and continued over a 70-day period. All microorganisms were tested at Time 0 to gain a baseline number. The bacteria were all then sampled on Day 4, 7, 14, 21 and 28. Adenovirus and *Cryptosporidium* were sampled on Days 7, 14, 21, 35, 42, 56 and 70. All analyses were done as described for the initial decay experiment in Section 2.1.4.1.

2.1.4.5. Impact of Flood Conditions on Pathogen Decay

The January 2011 floods provided an opportunity to test the impact the change in reservoir water conditions caused by the flood had on the decay of the test microorganisms. An experiment was undertaken in April 2011 after access back onto Wivenhoe Dam was permitted. The experiment was undertaken using the same methodology as had been used for the Depth Experiment (Section 2.1.4.4) using the microorganisms *E. coli, Salmonella, Campylobacter,* adenovirus and *Cryptosporidium* oocysts. The bacteria were tested at Time 0 and on days 7, 14 and 21. Adenovirus and *Cryptosporidium* were also sampled on these days as well as on days 35, 49 and 63.

As the water quality within the reservoir improved, a repeat of this experiment was undertaken in November 2011 to determine if the improving water conditions had further influence on the decay rates of the tested pathogens. This second experiment was undertaken testing the same microorganisms under the same conditions and for the same time period as the initial post-flood experiment.

2.1.4.6. Pathogen Decay in the Mid-Brisbane River

While there has been a large amount of research investigating the decay of microbial pathogens in Wivenhoe Dam (described above), the design of the water delivery system from Wivenhoe Dam to the Mt Crosby Water Treatment Plants (WTP) uses the mid-Brisbane River to move the water from the reservoir to the WTP. The mid-Brisbane River also has a range of other inputs apart from the water released from Wivenhoe Dam, including flows from the Lockyer Creek and other smaller tributaries, along with impacts from towns, settlements and agricultural activity along the river between the reservoir and the treatment plants.

The actual site used for the mid-Brisbane experiments can be seen in Figure 2. The microorganisms tested in both experiments undertaken on the mid-Brisbane were *E. coli¸ Salmonella*, adenovirus and *Cryptosporidium*. The first experiment was undertaken using the same methodology as the initial decay experiment in Wivenhoe Dam described in Section 2.1.4.1 to determine what decay times could be expected for these microorganisms in the conditions within the river, and how these times differed from the rates of decay observed in Wivenhoe Dam. This experiment commenced in September 2011 and went for a period of 70 days.

During the first mid-Brisbane experiment, it was observed that there was significant biofouling on the chamber rig compared to the condition of the rig over the same time period in Wivenhoe Dam (Figure 4). This is most likely to the flow of the river bringing more organic material and nutrient past the chamber rig than would occur in the reservoir. It was also considered that this could also be an indicator of the greater potential input sources of nutrients in a smaller body of water. It was hypothesised that this greater availability of nutrients could have an influence on the overall activity of the indigenous microflora in the river. As a result, a second experiment was undertaken testing the influence of the indigenous microflora of the mid-Brisbane river on the decay of these enteric microorganisms using the same methodology as described in Section 2.1.4.2. This experiment started in March 2012 and lasted for 63 days. (Note that, despite this observed fouling on the outside of the chambers, a diffusion test comparing the diffusion of rhodamine across the membrane of a biofouled chamber and a clean unused chamber showed that there was no observable difference in the diffusion of the dye from either chamber [results not shown]).

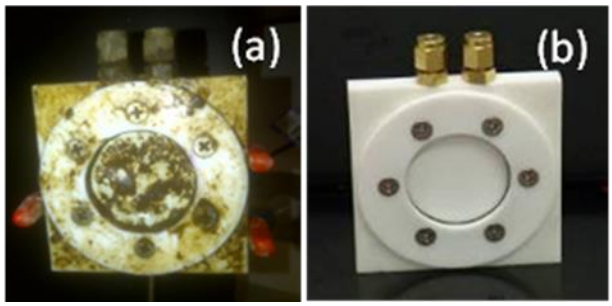


Figure 4. Evidence of biofouling on (a) diffusion chambers left for 14 days in the mid-Brisbane River compared to (b) and unused clean chamber.

2.1.5. Data Analysis

For all of the above experiments, the number of viable or detectable target microorganisms were normalised to the number of organisms per mL of sample at each sampling event. These values were then log transformed and plotted against time. A linear regression was then fitted to each plot and the slope was taken as the decay rate (log day^{-1} for all of the experiments apart from the sunlight experiments which were log $hour^{-1}$). The inverse of these calculated decay rates was then calculated and used as the determination of the time for a 1 log reduction (T90).

2.1.6. Quantitative Microbial Risk Assessment (QMRA)

The Quantitative Microbial Risk Assessment (QMRA) approach has been commonly used to make an assessment on microbial risks associated with recycled and drinking water systems (NRMMC-EPHC-AHMC 2006, NRMMC-EPHC-AHMC 2011).

Assessments using QMRA for evaluating microbial risk focus on estimating the probability of infection to an individual as a result of a single exposure event. These assessments generally assume that multiple or recurring exposures constitute independent events with identical distributions of contamination and that in most cases when this type of model has been employed, it has been implicitly assumed that secondary (person-to-person or person-to-environment-to-person) transmission and immunity are negligible.

For a QMRA to be effective, it requires quantitative input data for pathogen occurrence in the source water and their removal through any treatment barriers such as natural attenuation. When an assessment of pathogen occurrence at the end use is combined with exposure potential and pathogen dose–response relationships, the human disease burden, expressed as disability adjusted life years (DALYs), can be estimated (NRMMC-EPHC-AHMC 2006). DALYs have been previously used extensively by agencies such as the World Health Organisation (WHO) to assess disease burdens (WHO 2011) and is the approach adopted in this study.

For each exposure scenario, the total microbial inactivation credits ($\log_{10}$) were calculated to achieve a tolerable risk, less than 1.0×10^{-6} DALYs per person per year according to the following equation:

$$\text{Log reduction} = \log(\text{concentration in source water} \times \text{exposure (L)} \times N / \text{DALYd})$$

Where N is the number of exposures per year and DALYd is the pathogen dose equivalent to 1×10^{-6} DALYs. A summary of the exposure assumptions and variables used for these calculations for the different end uses are given in Table 3.

Table 3. Parameters used in the calculation of microbial inactivation targets for different end uses.

Parameter	Value	Reference
Adenovirus concentration in source water (n/L)	1	Assumed in this study
Cryptosporidium concentration in source water (n/L)	100	Assumed in this study
E. coli concentration in source water (n/L)	1*	Assumed in this study
Salmonella concentration in source water (n/L)	10	Assumed in this study
Campylobacter concentration in source water (n/L)	10	Assumed in this study
Secondary contact recreation exposure – hand washing and boating(L)	0.001	NRMMC-EPHC-AHMC (2006)
Swimming recreation exposure (L)	0.0034	Stone *et al.* (2008)
Number of swimming exposures (N)	52	Assumed in this study
Number of secondary contact exposures (N)	52	Assumed in this study
DALYd (Adenovirus) (n)	2.5×10^{-3}	NRMMC-EPHC-AHMC (2006)
DALYd (*Cryptosporidium*) (n)	1.6×10^{-2}	NRMMC-EPHC-AHMC (2006)
DALYd (*E. coli*) (n)	7.1×10^{-3}	Calculated from a dose response curve
DALYd (*Campylobacter*) (n)	3.8×10^{-2}	NRMMC-EPHC-AHMC (2006)
DALYd (*Salmonella*) (n)	1.4×10^{-3}	Calculated from a dose response curve

* Pathogenic *E. coli* O157: H7

In this study, site specific decay rates from Wivenhoe Dam were determined for several reference pathogens including: pathogenic strains of *E. coli*; *Salmonella*; *Campylobacter; Cryptosporidium* and adenovirus. These decay rates were coupled with potential residence times of the pathogens in the reservoir prior to exposure to determine the microbial inactivation credits. This paper discusses the use

of QMRA to assess the human health risk from exposure to untreated Wivenhoe Dam water. Exposure to Wivenhoe Dam water was assessed for two scenarios: weekly secondary recreational contact (for example by hand washing or boating) with an exposure of 1 mL and weekly swimming using the mean exposure volumes derived by Stone *et al*. (2008).

2.2. Results of Pathogen Decay Research

2.2.1. Initial General Assessment of Pathogen Decay in Wivenhoe Dam

The decay rates determined as basic decay rates are given in Table 4. They demonstrate that, as previously observed for other aquatic environments, the bacteria have limited survival potential in the reservoir, and that *Cryptosporidium* oocysts have the longest survival of the microorganisms tested. The decay profiles for each of the organisms can be seen in Figures 5 and 6. The decay profiles indicated that the decay of all of microorganisms was linear in nature.

Table 4. Initial assessment of pathogen decay in Wivenhoe Dam.

Microorganism	Kd (log day^{-1})	Std Error	T90 (days)
E. coli	0.2635	0.0441	3.8
Salmonella	0.1987	0.0297	5.0
Campylobacter	0.1049	0.0157	9.5
MS2	0.2185	0.0102	4.6
Cryptosporidium	0.0783	0.0072	12.8

The water quality parameters at the mouth of Logan's Inlet given in Figure 7 showed that temperature was stable starting at approximately 19°C and slowly decreasing to approximately 16°C by the end of the experiment. Other water quality parameters pH, Dissolved Oxygen and Oxidation Reduction Potential (ORP) were less stable. There was a large decrease in turbidity around Day 25 of the experiment with a corresponding decrease in electrical conductivity at the same time. None of these changes in water quality parameters appeared to have any influence on pathogen decay. This indicates that these minor changes in water quality can be expected to have minimal or no influence on pathogen decay in SEQ reservoirs.

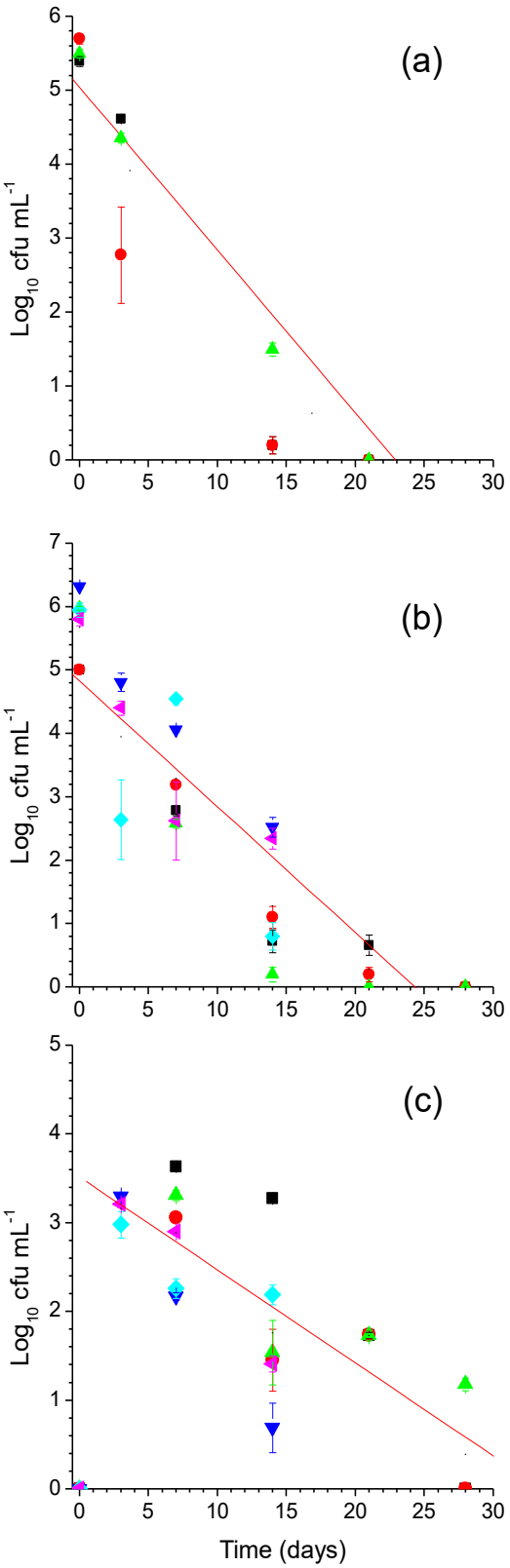


Figure 5. **Decay of (a) *E. coli*, (b) *Salmonella* and (c) *Campylobacter* in Wivenhoe Dam.**

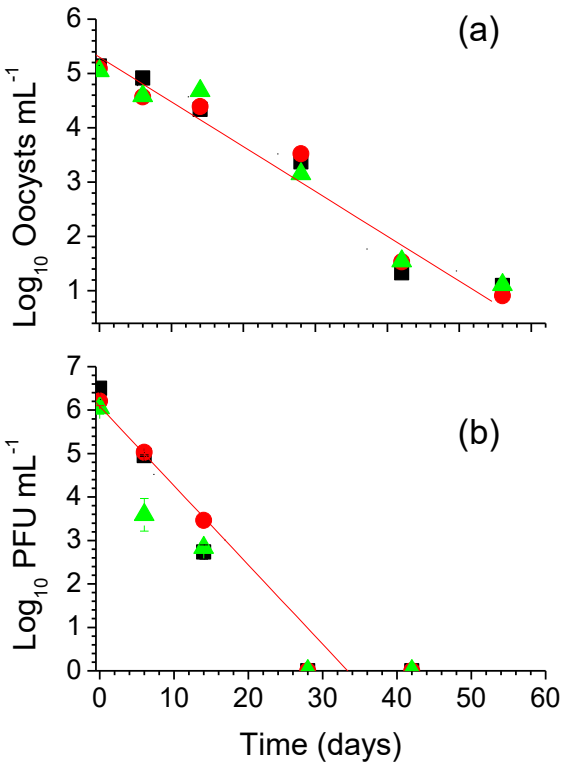


Figure 6. Decay of (a) *Cryptosporidium* oocysts and (b) MS2 in Wivenhoe Dam.

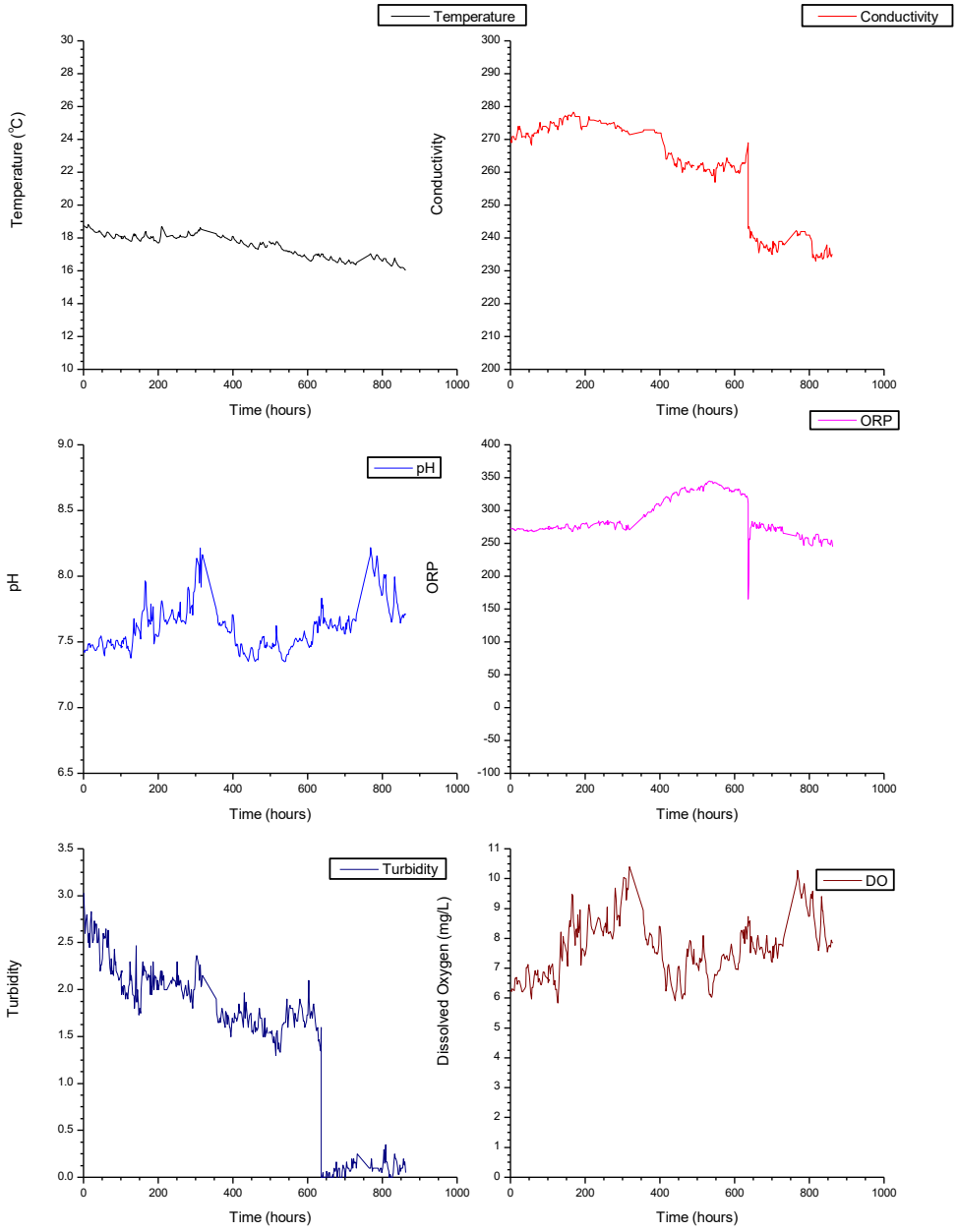


Figure 7. Water quality parameters during the time period of the initial pathogen decay experiment.

2.2.2. Influence of Reservoir Indigenous Microorganisms on the Decay of Enteric Microorganisms in Wivenhoe Dam

The influence of indigenous microorganisms has been previously demonstrated to have a major influence on the decay of pathogens, particularly in groundwater (Gordon and Toze 2003). Thus, this experiment was undertaken to determine the impact the presence of Wivenhoe Dam indigenous microorganisms had on the survival of *E. coli*, the bacteriophage MS2 and adenovirus.

The measured decay rates provided in Table 5 show that the presence or absence of indigenous reservoir microorganisms had little impact on the decay of *E. coli* or MS2. The decay of these two microorganisms in this experiment was similar to the rates measured in the initial decay experiment

(Table 4). This suggests that the decay of these two microorganisms is caused by other non-biological processes or through auto-decay.

Table 5. Influence of indigenous reservoir microorganisms on the decay of enteric microorganisms.

Microorganism	Indigenous Microbes	Kd (log day^{-1})	Std Error	T90 (days)
E. coli	Absent	0.3030	0.1024	3.3
	Present	0.3234	0.0711	3.1
MS2	Absent	0.2411	0.0211	4.1
	Present	0.3057	0.0246	3.3
Adenovirus	Absent	0.0332	0.0242	30.1
	Present	0.0906	0.0236	11.0

In contrast, there was a large difference in the decay of adenovirus in the presence and absence of the indigenous reservoir microbes, with the T90 time in the present of the indigenous reservoir microorganisms being almost a third of the time observed in the absence of the reservoir microorganisms (11 days vs 31 days). The fact that the indigenous reservoir microorganisms only had a measurable impact on adenovirus is probably due to its overall slower decay rate. Adenovirus has previously been recorded to have a much higher environmental resistance compared to other enteric microorganisms (Bofill-Mas *et al.* 2006, Sidhu *et al.* 2010) but the removal of the indigenous microorganisms more than double the T90 time of this virus suggesting that the indigenous have an influence on adenovirus decay.

It should be noted that the use of real time PCR is a measure of the degradation of the virus genome and is not a measure of the loss of infectivity. Thus, the use of the PCR technique will over-estimate survival and may even exaggerate the difference in decay times in the presence and absence of the indigenous reservoir microorganisms.

The decay patterns for all three microorganisms showed that, similar to the results in the initial decay experiment, a linear decay was observed (Figure 8). When this linear decay was matched with changes in the measured water quality parameters given in Figure 9 (notably a decrease in ORP and a corresponding increase in pH and turbidity around Day 25), it showed, as was observed in the initial decay experiment, that the measured parameters had little to no influence on the decay of the enteric microorganisms.

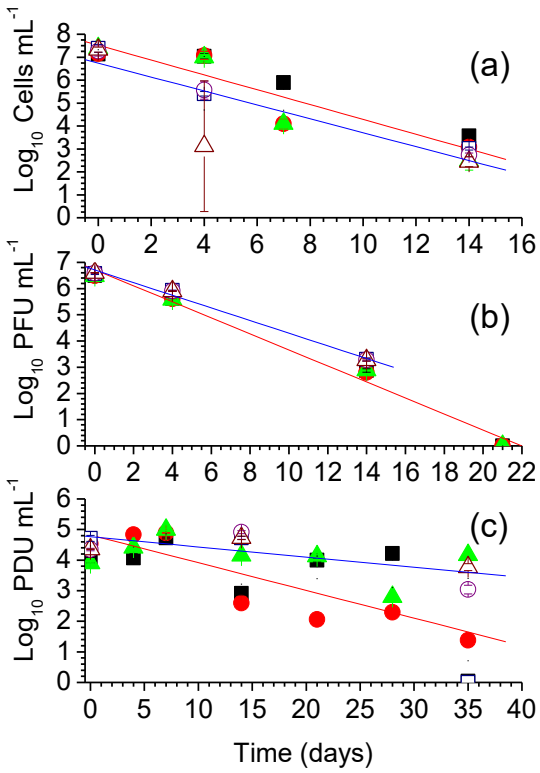


Figure 8. Decay of (a) *E. coli*, (b) MS2 and (c) adenovirus in the presence and absence of indigenous Wivenhoe Dam microflora, where closed symbols and red decay line are samples with the indigenous microorganisms present; and open symbols and blue decay line are samples with the indigenous microorganisms removed.

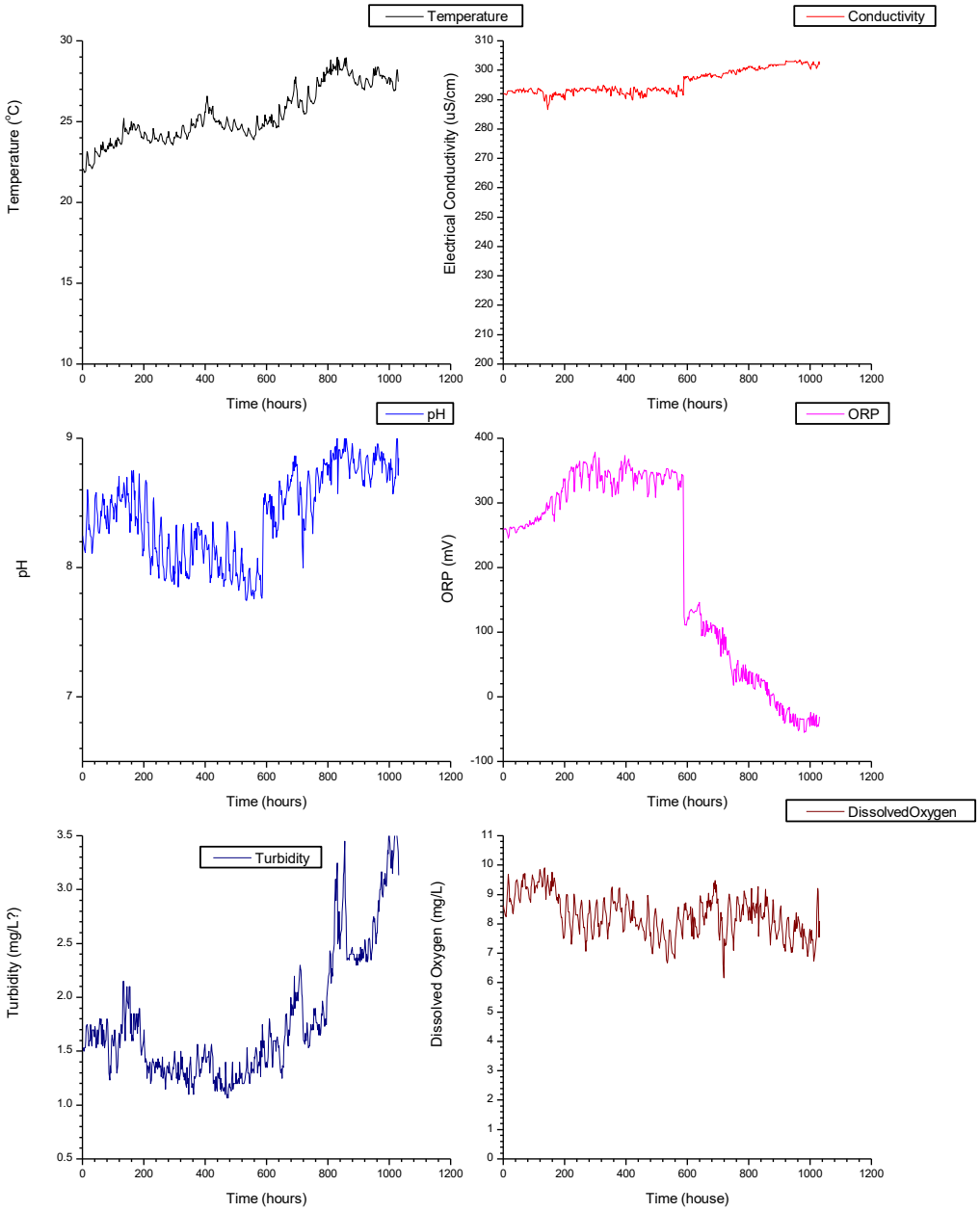


Figure 9. Water quality during experiment on the influence of indigenous reservoir microorganisms on pathogen decay.

2.2.3. Effect of Sunlight on Pathogen Decay

The next set of results reported are experiments undertaken to assess the impact of sunlight on the decay of enteric microorganisms. Sunlight has been previously reported to increase the decay of enteric microorganisms in seawater (Boehm *et al.* 2009, Nasser *et al.* 2007, Sinton *et al.* 1999, 2002, 2007), and in rivers and other fresh water (King *et al.* 2008, Schultz-Fademrecht *et al.* 2008, Sinton *et al.* 2002, 2007). All of these previous studies used laboratory experiments with artificial sunlight generators set at a noon day exposure, or natural sunlight exposing water in tanks containing water that had either natural or dosed enteric microorganisms.

The current research was undertaken directly in the reservoir using low potassium glass scintillation vials. The low potassium glass was selected as it allowed increased transmission of lower wavelengths than common borosilicate glass (Appendix 2) while being considerably cheaper than quartz. Undertaking the experiment in the reservoir instead of replicating conditions in the laboratory or in smaller macrocosms was done to maintain reservoir conditions as close as possible around the vials (eg temperate and turbidity) and to expose the enteric microorganisms within the vials to the full daily range of sunlight intensity. The input of sunlight was done through two experiments, one to study the input of sunlight under overcast conditions, and the other under full sunlight (Figure 10). As the first experiment was undertaken well before the January 2011 floods and the second was undertaken once the water clarity had started to improve after the floods (May 2012), the influence of the conditions caused by the floods was also a variable that could be examined. The first of the experiments also investigated the impact of depth on the influence of sunlight on pathogen survival.

The measured sunlight intensity in Figure 10 showed that there was a large difference in the intensity of sunlight at the surface between experiments 1 and 2, with the first experiment (undertaken in overcast conditions) having a mean sunlight intensity of 7.5 Wm^{-2} and a maximum intensity of 250 Wm^{-2}. The second experiment, undertaken in full sunlight conditions with clear, unclouded skies, had a mean sunlight intensity of 72 Wm^{-2} and a maximum intensity of 339 Wm^{-2}. As evident in Figure 10, the maximum sunlight intensity at the surface for the first experiment was only a transient, short event during a brief break in the cloud layer, while for the second experiment, the maximum sunlight occurred over an extended period around noon for each of the sampling days.

Similarly, there was a notable difference between the sunlight intensity at the surface and at 5 m depth in the initial experiment. The mean sunlight intensity at 5 m was only 0.9 Wm^{-2} compared with 7.5 Wm^{-2} at the surface. (Note that the large spike in intensity in the depth values at 26 hours was caused when the vials set at 5 m were brought to the surface for collection of sample vials during a brief period of clear skies).

The results given in Table 6 show that, despite a large difference in sunlight intensity at the surface for the two experiments, there was a greater impact of sunlight on the decay of the microorganisms in the first experiment where there were overcast conditions. The rates of solar inactivation in the second experiment were up ten times slower for all of the tested microorganism than had been found by Sinton *et al.* (2002) and King *et al.* (2008) apart from *Enterococcus* spp. In comparison, the results obtained in the first experiment were comparable to the results from Sinton (2002, 2007).

The reasons for the differences in solar inactivation rates between the two experiments undertaken in this study were considered to most likely be due to differences in water clarity at the time of the two experiments. The water quality data is not available for the first sunlight experiment as the profiler located at the mouth of Logan's Inlet had been damaged shortly before the experiment, however, the turbidity levels during the first experiment would have been very similar to that shown in Figure 9 for the indigenous microorganisms experiment (average turbidity levels of 1.7 mg L^{-1}) which had been undertaken only two months prior to the first sunlight experiment. Water quality data for the second sunlight experiment undertaken in May 2012 (Figure 11) shows the calculated average turbidity concentrations in this second experiment were 3.1 mg L^{-1}.

Table 6. Effect of Sunlight on the decay of enteric microorganisms.

Microorganism	Experiment	Conditions	Kd (log hour^{-1})	Std Error	T90 (hours)
E. coli	Pre-flood	Surface – Light	ND	ND	ND
		Surface - Dark	ND	ND	ND
		Depth – Light	ND	ND	ND
		Depth - Dark	ND	ND	ND
	Post-Flood	Light	0.0054	0.0015	185
		Dark	0.0036	0.0014	280
Enterococcus spp.	Pre-flood	Surface – Light	ND	ND	ND
		Surface - Dark	ND	ND	ND
		Depth – Light	ND	ND	ND
		Depth - Dark	ND	ND	ND
	Post-Flood	Light	0.126	0.00493	7.9
		Dark	0.00075	0.00197	>1,000
Salmonella	Pre-flood	Surface – Light	0.0527	0.0082	19
		Surface - Dark	0.0360	0.0053	28
		Depth – Light	0.0345	0.0049	29
		Depth - Dark	0.0416	0.0040	24
	Post-Flood	Light	ND	ND	ND
		Dark	ND	ND	ND
MS2	Pre-flood	Surface – Light	0.0364	0.0129	28
		Surface - Dark	0.0082	0.0013	122
		Depth – Light	0.0082	0.0010	121
		Depth - Dark	0.0039	0.0062	253
	Post-Flood	Light	0.0094	0.0129	107
		Dark	0.0069	0.0015	144
Cryptosporidium	Pre-flood	Surface – Light	0.0364	0.0129	91
		Surface - Dark	0.0082	0.0013	287
		Depth – Light	0.0082	0.0010	242
		Depth - Dark	0.0039	0.0062	893
	Post-Flood	Light	0.0094	0.0129	368
		Dark	0.0069	0.0015	532

ND = Not Done

This marginal difference in turbidity makes it difficult to correlate what had caused the differences in the sunlight impacted decay rates between the two experiments (Figures 12 and 13). The sunlight intensity during the first experiment was much less than in the second experiment, yet the decay of the microorganisms was faster and more pronounced in the first experiment than the second. As the turbidity was very similar it would have been expected that the decay rate in the second experiment would have been faster than for the initial experiment where there was a much reduced sunlight intensity due to the overcast conditions. There could be other possible reasons for these unexpected differences in decay rate. The most likely could be a difference in the concentrations of nutrients or natural organic compounds such as humic acids. Dissolved organic matter has been suggested to act as a UV filter reducing photodegradation of some microorganisms (King *et al.* 2008, Romero *et al.* 2011). As these parameters were not measured during either experiment however, the possible impact of natural organic matter on the solar inactivation of pathogens in SEQ reservoirs remains to be confirmed. In addition, a sunlight experiment similar to the ones undertaken by Sinton *et al*. (2002), where the microorganisms were inoculated directly into water held in tanks, would be useful to confirm that the differences in results obtained was not due to some influence caused by the small vials used. It needs to be confirmed if the lack of mixing and water turbulence may have had an influence on the effect of sunlight on microorganisms within the vials.

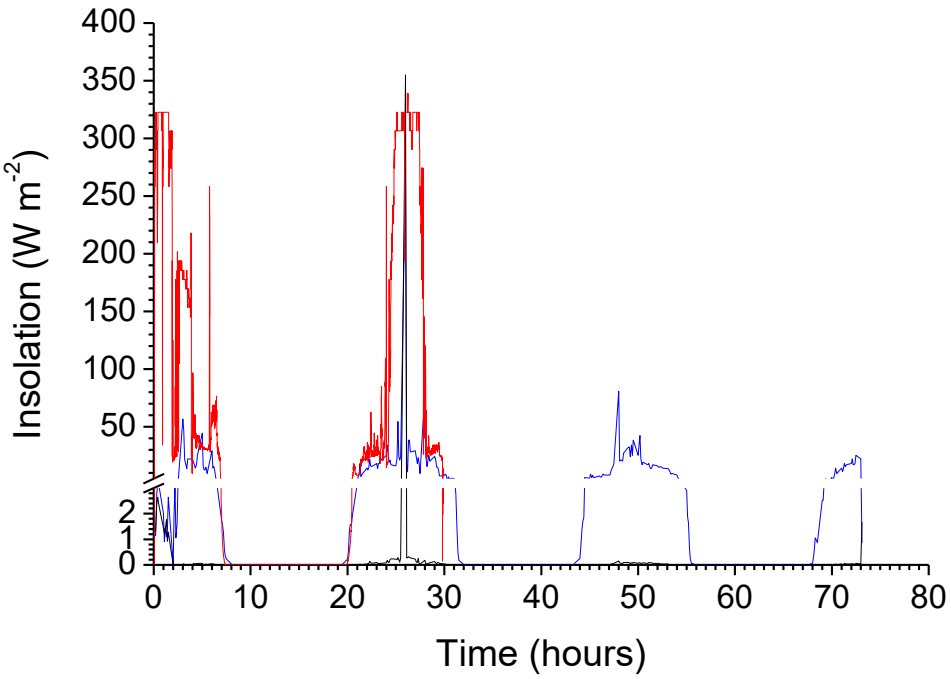


Figure 10. Irradiance values for sunlight experiments 1 and 2. The blue line represents experiment 1 insolation values at the reservoir surface; the black line represents the experiment 1 insolation values at 5 m depth; and the red line represents the experiment 2 insolation values (at the reservoir surface).

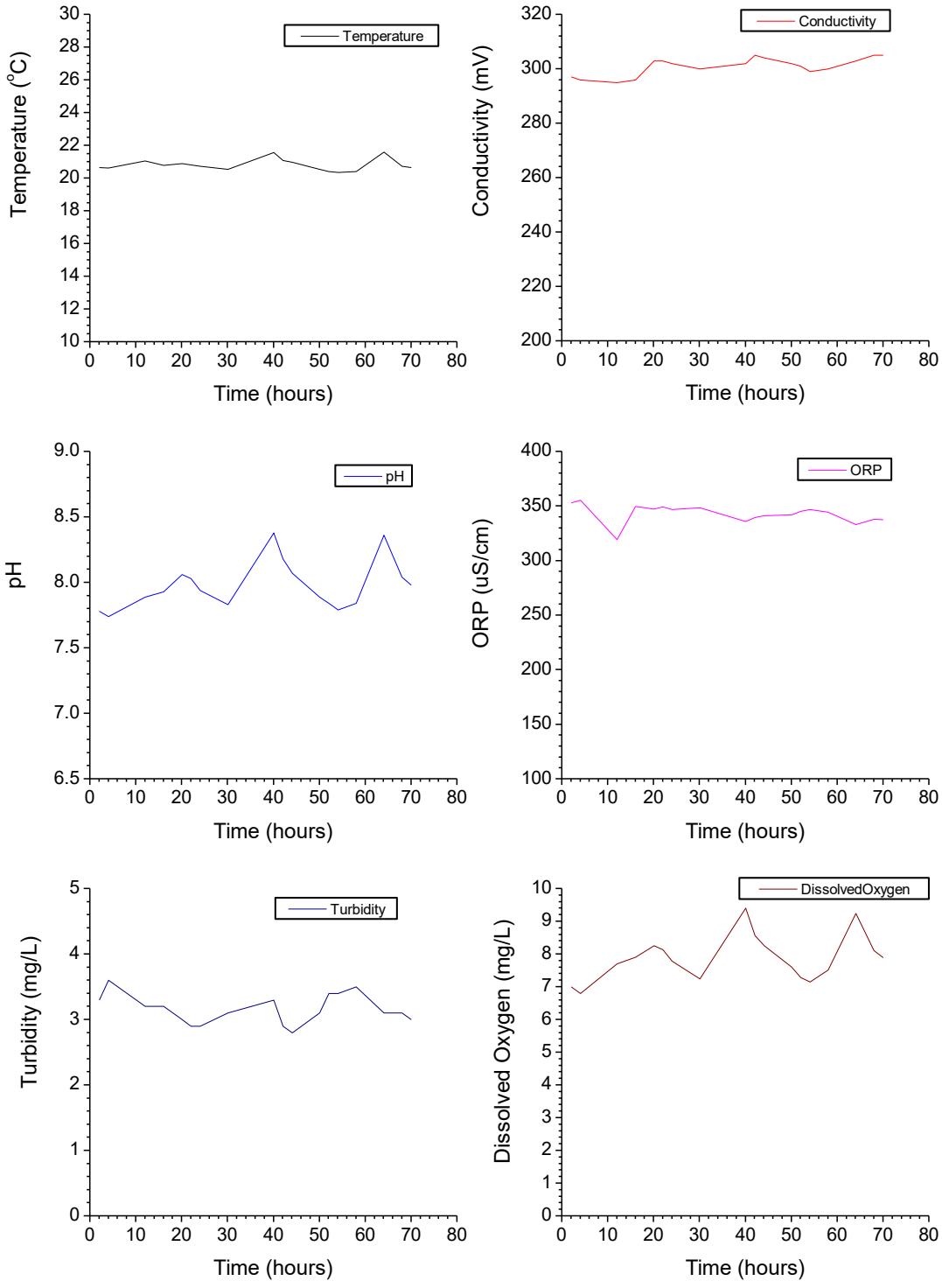


Figure 11. Water quality during the second sunlight experiment undertaken in May 2012.

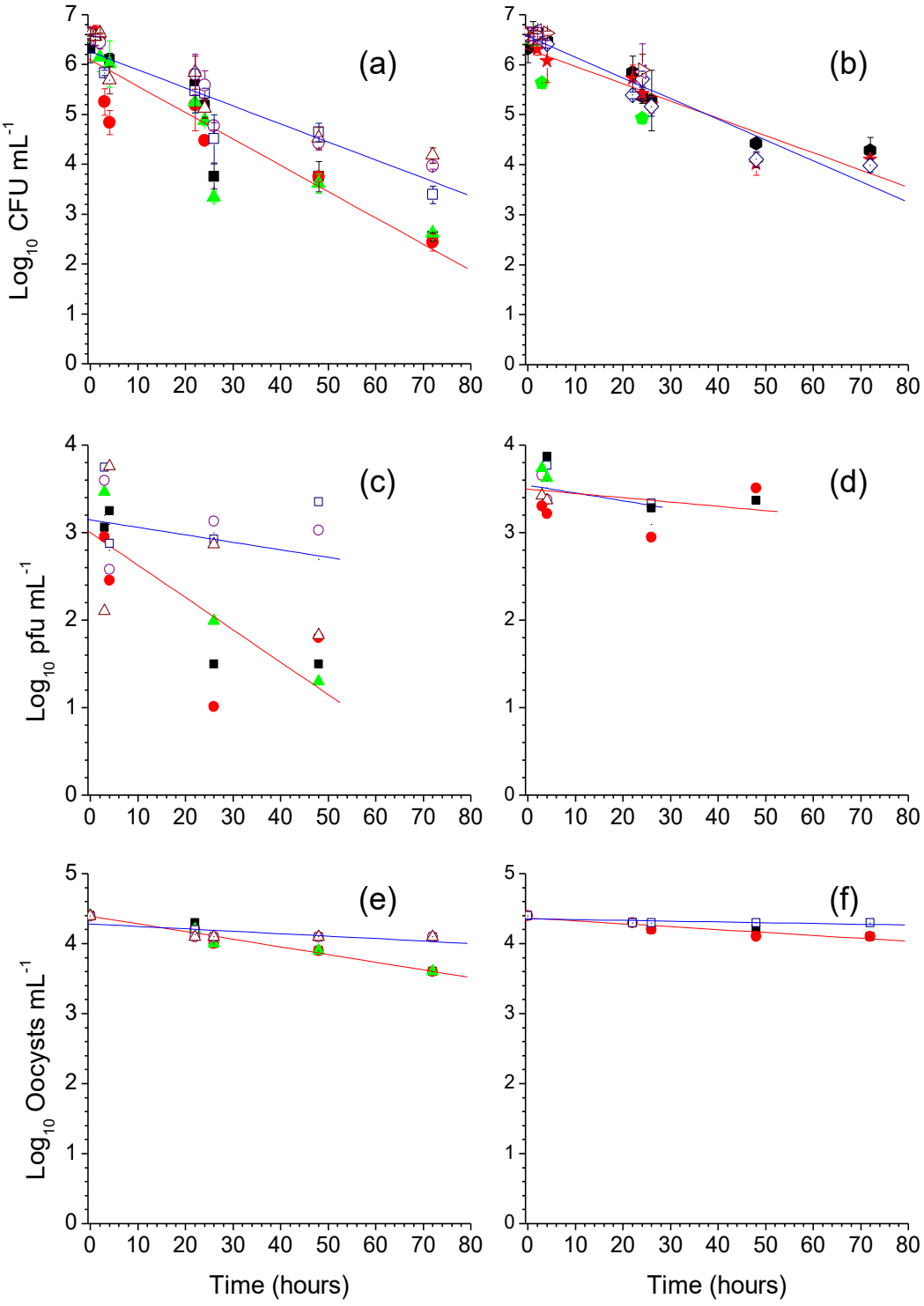


Figure 12. First experiment testing the influence of sunlight on the decay of enteric microorganisms where (a) is *Salmonella* at the surface; (b) is *Salmonella* at 5m depth; (c) is MS2 at the surface; (d) is MS2 at 5m depth; (e) is *Cryptosporidium* at the surface; at the surface; and (f) is *Cryptosporidium* at 5m depth. Enclosed symbols and red decay curve are vials directly exposed to light, while open symbols and blue decay curve are vials shielded from sunlight by wrapping in foil.

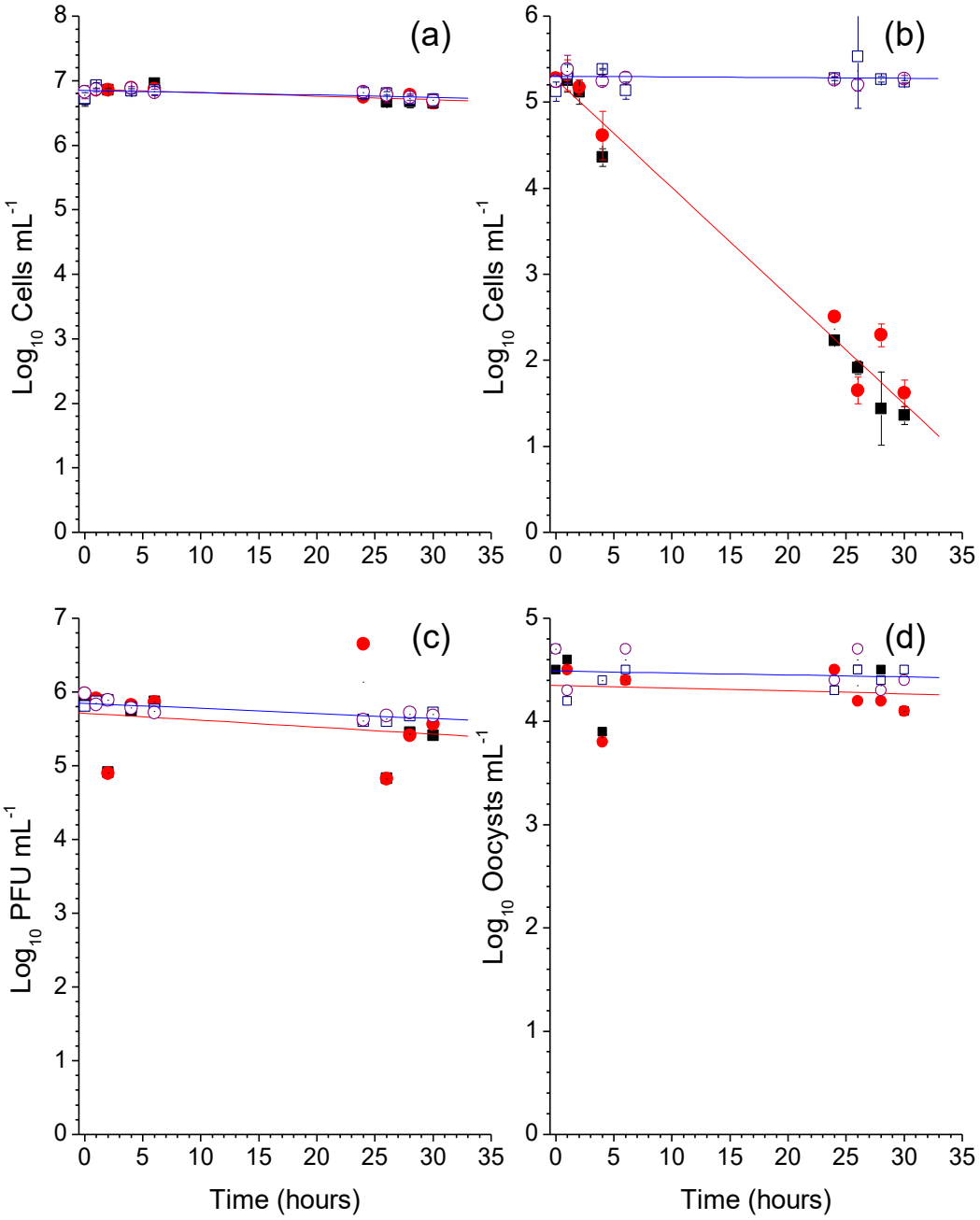


Figure 13. Second experiment testing the influence of sunlight on the decay of enteric microorganisms where (a) is *E. coli*; (b) is *Enterococcus faecalis*; (c) is MS2; and (d) is *Cryptosporidium*. Enclosed symbols and red decay curve are vials directly exposed to light, while open symbols and blue decay curve are vials shielded from sunlight by wrapping in foil.

2.2.4. Influence of Depth on Pathogen Decay in Wivenhoe Dam

All of the earlier research in this project had been undertaken within the first metre of the reservoir surface. The depth of the reservoir can vary, however, with depths up to 30 metres in the original river bed (at 100% storage capacity of the reservoir). There is the potential for the water quality to vary from the surface to the bottom of the reservoir, particularly in these deeper sections. It is probable that any microbial pathogens or faecal indicators would be distributed throughout the water column and, therefore, be as likely to be present at depth as at the surface. In addition, there is the potential for microbial pathogens to settle out to the bottom of the reservoir, either during extended periods of low flow or due to attachment to solids. Because of this, it was hypothesised that any variations in water quality with depth could influence the rate of decay of the microorganisms.

The water quality data presented in Figure 14 shows that there was, in fact, a difference in all the parameters measured apart from ORP over the time period of this experiment at the reservoir surface compared to the bottom of the reservoir. The parameters with the biggest difference were temperature and dissolved oxygen, with lower values for both parameters at the bottom of the reservoir compared to at the surface.

These differences in dissolved oxygen and temperature at the different depths could be the reason for the observed differences in decay for *Campylobacter* and adenovirus at the reservoir surface compared to 15 m depth (Table 7, Figures 15 and 16). *Campylobacter* prefers low oxygen conditions for growth, thus the lower oxygen concentrations at 15 m below the reservoir surface could account for the longer survival at this depth compared to at the surface. Similarly, the lower temperatures and lower oxygen conditions could also account for the longer T90 times for adenovirus at the bottom of the reservoir. It has been previously shown that enteric viruses can survive longer at lower temperatures and oxygen concentrations in groundwater (Cameron and Toze 2003, Sidhu *et al.* 2010). It therefore appears that this is also the case in surface water when the oxygen concentrations become low.

In contrast, the differences in depth had no impact on the decay rate of either *E. coli* or *Salmonella* (Figure 15). Also, while there was no data available for the decay of *Cryptosporidium* oocysts at depth from this experiment, the measured decay rate at the surface was similar to the rate obtained in the initial decay experiment.

Table 7. Decay of enteric microorganisms at the reservoir surface and at 15 m depth.

Microorganism	Conditions	Kd (log day^{-1})	Std Error	T90 (days)
E. coli	Surface	0.3004	0.0053	3.3
	Depth	0.2947	0.0205	3.4
Salmonella	Surface	0.1929	0.0232	5.2
	Depth	0.1992	0.0202	5.0
Campylobacter	Surface	0.1966	0.0316	5.1
	Depth	0.0765	0.0232	13.1
Adenovirus	Surface	0.0507	0.0060	19.7
	Depth	0.0252	0.0089	39.7
Cryptosporidium	Surface	0.0574	0.0049	17.4
	Depth	ND	-	-

ND = Not Done

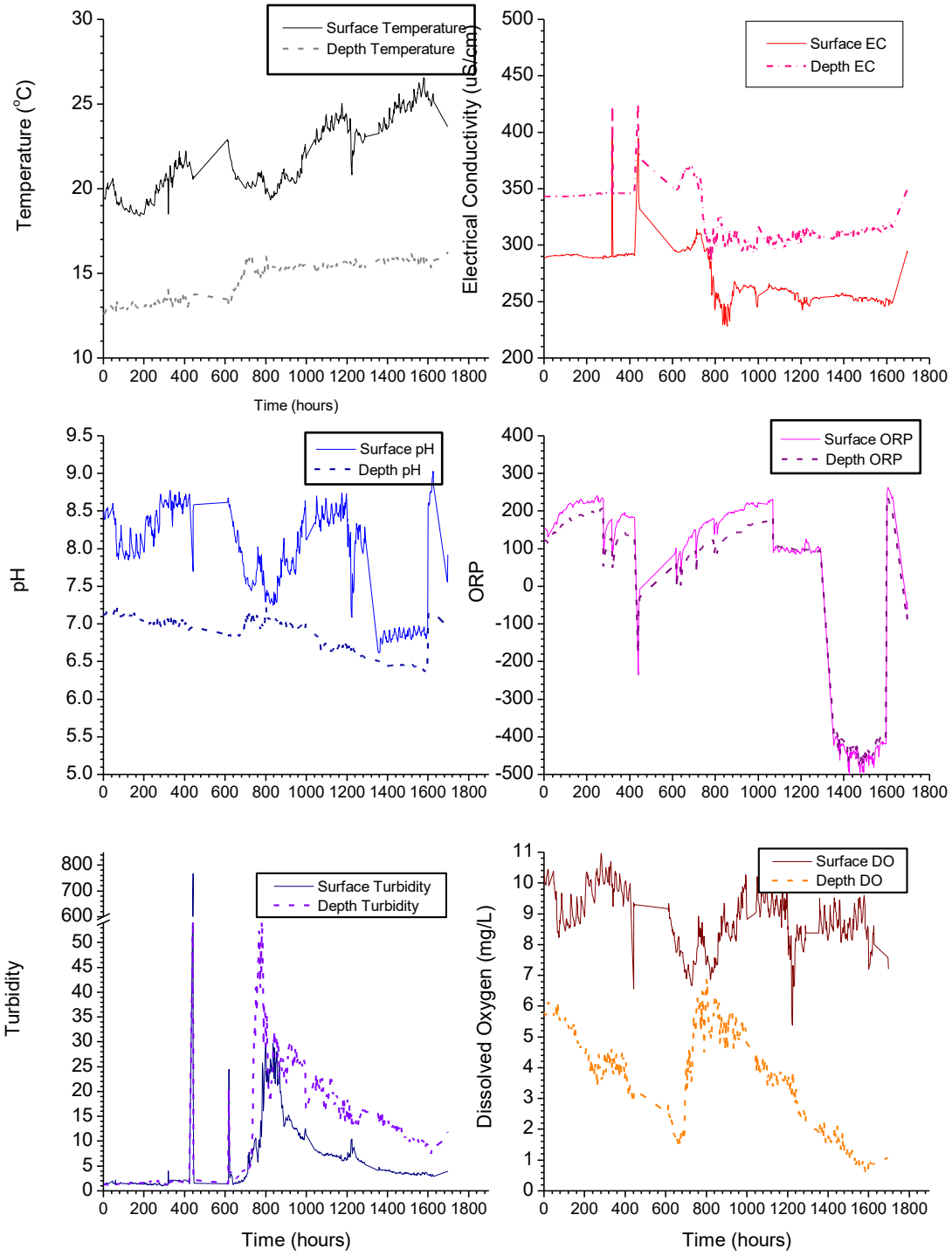


Figure 14. Water quality during the experiment on pathogen decay at different depths.

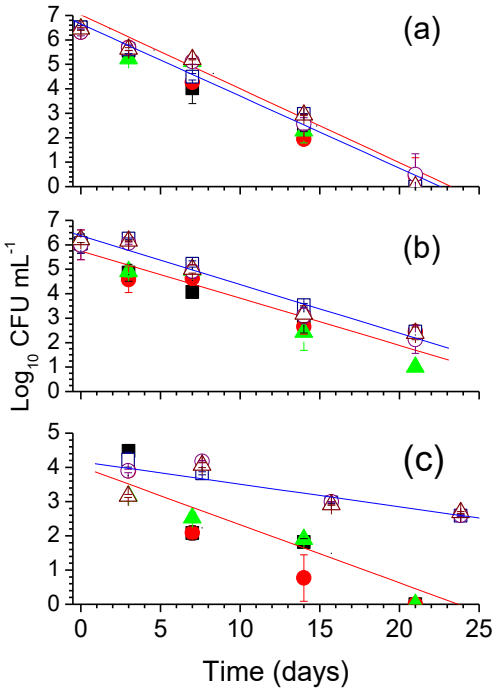


Figure 15. Decay of (a) *E. coli*, (b) *Salmonella*, and (c) *Campylobacter* at different depths in Wivenhoe Dam, where closed symbols and red decay line are chambers set at the surface; and open symbols and blue decay line were chambers set at 15 m depth.

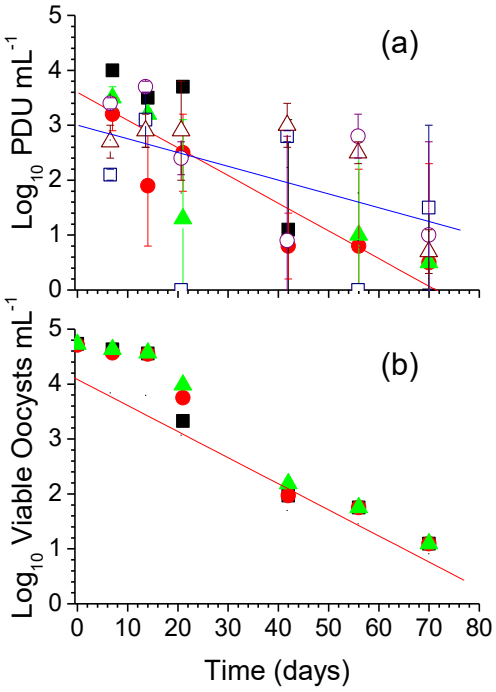


Figure 16. Decay of (a) adenovirus, and (b) *Cryptosporidium* oocysts, at different depths in Wivenhoe Dam, where closed symbols and red decay line are chambers set at the surface; and open symbols and blue decay line were chambers set at 15m depth.

2.2.5. Impact of Post Flood Conditions on Pathogen Decay

The occurrence of the January 2011 floods caused a temporary suspension of research on Wivenhoe Dam. Despite this, the flooding event provided a unique opportunity to investigate if the significant change in water conditions in the dam caused by the floods had any impact on the removal of pathogens by the processes previously observed in the reservoir.

As soon as permission was granted by Seqwater to again have access to the reservoir, an experiment was set up to test the decay of pathogens under the new water quality conditions (beginning of April 2011). This experiment was purposely set up to replicate the last experiment completed in 2010 prior to the floods (the depth experiment). This allowed the best comparison of treatment capacity before and after the floods to be done. Therefore, this initial post-flood experiment was a repeat depth experiment examining the decay of *E. coli*, *Salmonella*, *Campylobacter*, adenovirus and *Cryptosporidium*.

The results for this first post-flood experiment are given in Table 8 and the decay properties seen in Figures 17 and 18. These results demonstrate that pathogen decay was still occurring in the reservoir despite the changed water conditions (experiment 1 in Table 8). The decay rates observed in the post-flood conditions showed that, as had been observed in the pre-flood experiments, the decay of the studied bacteria were still faster than those for adenovirus and *Cryptosporidium* oocysts. In contrast to the decay rates observed in the pre-flood conditions, however, adenovirus now had a faster decay rate than *Cryptosporidium*.

Table 8. Impact of post flood conditions on pathogen decay in Wivenhoe Dam.

Microorganism	Experiment	Conditions	Kd (log day^{-1})	Std Error	T90 (days)
E. coli	1	Surface	0.3923	0.1292	2.5
		Depth	0.3681	0.0992	2.7
	2	Surface	0.3924	0.1335	2.5
		Depth	0.3659	0.1006	2.7
Salmonella	1	Surface	0.2302	0.0547	4.3
		Depth	0.1458	0.0772	6.9
	2	Surface	0.3115	0.0329	3.2
		Depth	0.1451	0.0736	6.9
Campylobacter	1	Surface	0.3538	0.0559	2.8
		Depth	0.3490	0.0016	2.9
	2	Surface	0.3082	0.0077	3.2
		Depth	0.3057	0.0186	3.3
Adenovirus	1	Surface	0.0386	0.0059	25.9
		Depth	0.0533	0.0142	18.8
	2	Surface	0.0612	0.0157	16.4
		Depth	0.0496	0.1479	20.1
Cryptosporidium	1	Surface	0.0345	0.0093	29.0
		Depth	0.0219	0.0087	45.7
	2	Surface	0.0686	0.0060	14.6
		Depth	0.0146	0.0044	68.7

When these results from the first of the post-flood experiments were compared to the results of the pre-flood depth experiment (Table 9), it was shown that there was little difference in the pre- and post-flood decay times for *E. coli* and *Salmonella* at either the surface or at 15 m depth, or for *Campylobacter* at the surface. In contrast, the decay times for *Campylobacter* at 15 m depth, for *Cryptosporidium* at the surface, and for adenovirus at both surface and depth differed between the experiments undertaken in pre- and post-flood conditions (Table 9). There were no decay results available for *Cryptosporidium* at 15 m under pre-flood conditions so no comparison was available at this depth. The T90 decay time for *Campylobacter* at 15 m depth decreased from 13 days under the pre-flood conditions to only 3 days post-flood. It had been hypothesised in the pre-flood depth experiment that the lower concentration of oxygen at depth could be the reason for the longer survival time of *Campylobacter*, a microorganism that grows best under microaerophilic conditions. This hypothesis is strengthened by the decreased T90 time for *Campylobacter* at 15 m after the flood as the concentration of dissolved oxygen had increased to be the same as at the surface (Figure 19). This hypothesis does not necessarily remain valid, however, as the decay rate for *Campylobacter* at both the surface and depth remained the same at approximately 3 days for the second post-flood experiment which was undertaken in November 2011. The water quality results for this experiment (Figure 20) show that the dissolved oxygen concentrations were below 1 mg/L for the period of this experiment, the lowest recorded over the entire life of the project. This suggests that either dissolved oxygen concentrations have less of an influence on *Campylobacter* survival than originally hypothesised, or that there are other factors as well as oxygen that have an impact on the survival of this bacterium. More experiments will be needed to further elucidate exactly what is having the major impact on *Campylobacter* survival.

Table 9. Comparison of pathogen decay times (T90) in Wivenhoe Dam under pre-flood conditions and during the March 2011 post-floods conditions.

Microorganism	Conditions	T90 (days)	
		Pre-flood	Post-flood
E. coli	Surface	3.3	2.5
	Depth	3.4	2.7
Salmonella	Surface	5.2	4.3
	Depth	5.0	6.9
Campylobacter	Surface	5.1	2.8
	Depth	13.1	2.9
Adenovirus	Surface	19.7	25.9
	Depth	39.7	18.8
Cryptosporidium	Surface	17.4	29.0
	Depth	ND	45.7

ND = Not Done

The increase in dissolved oxygen at depth post-floods could also explain the reason for the increased decay of adenovirus at depth (from 40 days pre-flood to 19 days post-flood). As has been discussed earlier, adenovirus is very resistant to decay under anoxic conditions (Sidhu *et al.* 2011). It is therefore very likely that it is dissolved oxygen concentrations that are directly or indirectly controlling the decay rate of adenovirus in the reservoir. More research is needed to further elucidate the actual mechanism dissolved oxygen has on adenovirus survival, as the results of this first post-flood experiment showed that the decay of adenovirus decreased at the surface from 20 days to 26 days (Table 8). The reason for this relatively small amount of slowing of decay at the surface remains unknown at this time as the concentration of dissolved oxygen was the same as at 15 m depth, therefore it indicates that there are more factors than just oxygen involved in controlling the decay of this virus. Despite the fact that the decay of adenovirus did slow down at the reservoir surface, it was not a large enough change, however, to have any significant impact on virus associated human health risks.

As for adenovirus at the surface, the decay rate of *Cryptosporidium* slowed from a T90 of 17 days pre-flood to a T90 of 29 days in the early post-flood conditions. Unfortunately, due to the lack of data for *Cryptosporidium* at depth pre-flood, it could not be determined if the 48 day T90 at depth post-flood had increased or decreased due to the change in the water conditions. Similarly to adenovirus, more research is needed to determine the reason for these differences in decay rates.

As it became apparent that the water quality, in particular turbidity, was slowly recovering back towards pre-flood conditions, (Figure 20), a second post-flood experiment, identical to the first, was undertaken in November 2011. The results of the second experiment (Table 8) showed that the decay of both adenovirus and *Cryptosporidium* had changed since the first post-flood experiment. The decay of adenovirus at the surface increased from a T90 of 26 days to 16 days. At depth, while the decay rate was faster in the March 201 experiment it was now slower than at the surface (20 days). Likewise for *Cryptosporidium,* the decay rate had increased at the surface (T90 of 29 days to 15 days), but the rate of decay at depth continued to slow, with the T90 decreasing from 46 days to 69 days between March and November 2011. The changes in dissolved oxygen concentrations at depth between March (>5 mg/L) and November (<1 mg/L) may be a contributing factor to these differences in decay rates. Additional longer-term assessment of decay rates at depth is still needed, however, particularly for *Cryptosporidium,* to better elucidate the long term behaviour of this microorganism in SEQ reservoirs at depth.

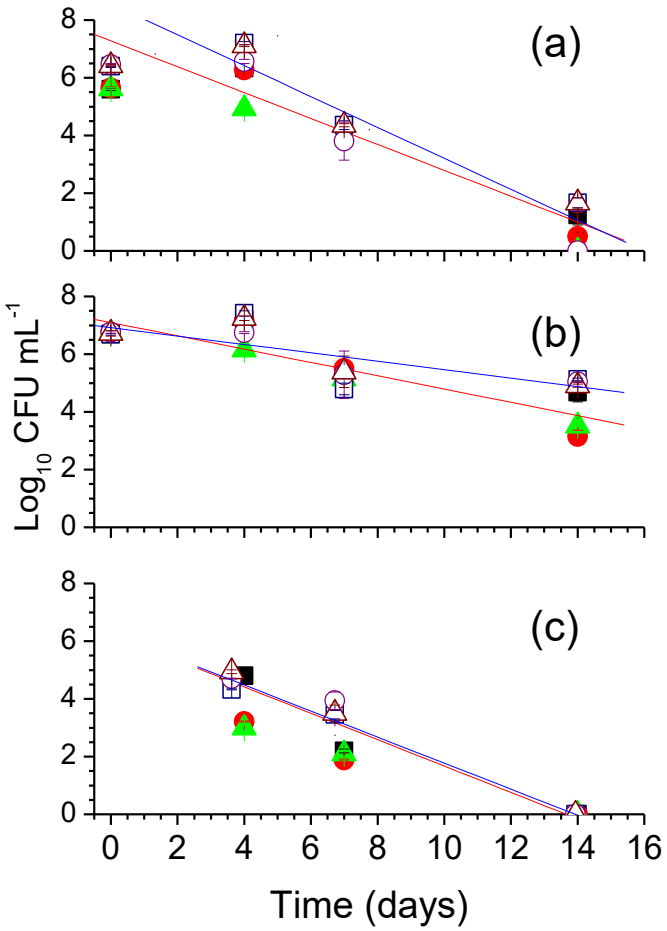


Figure 17. Decay of (a) *E. coli*, (b) *Salmonella*, and (c) *Campylobacter* at different depths in Wivenhoe Dam after the January 2011 floods; where closed symbols and red decay line were chambers set at the surface; and open symbols and blue decay line were chambers set at the surface; and open symbols and blue decay line were chambers set at 15 m depth.

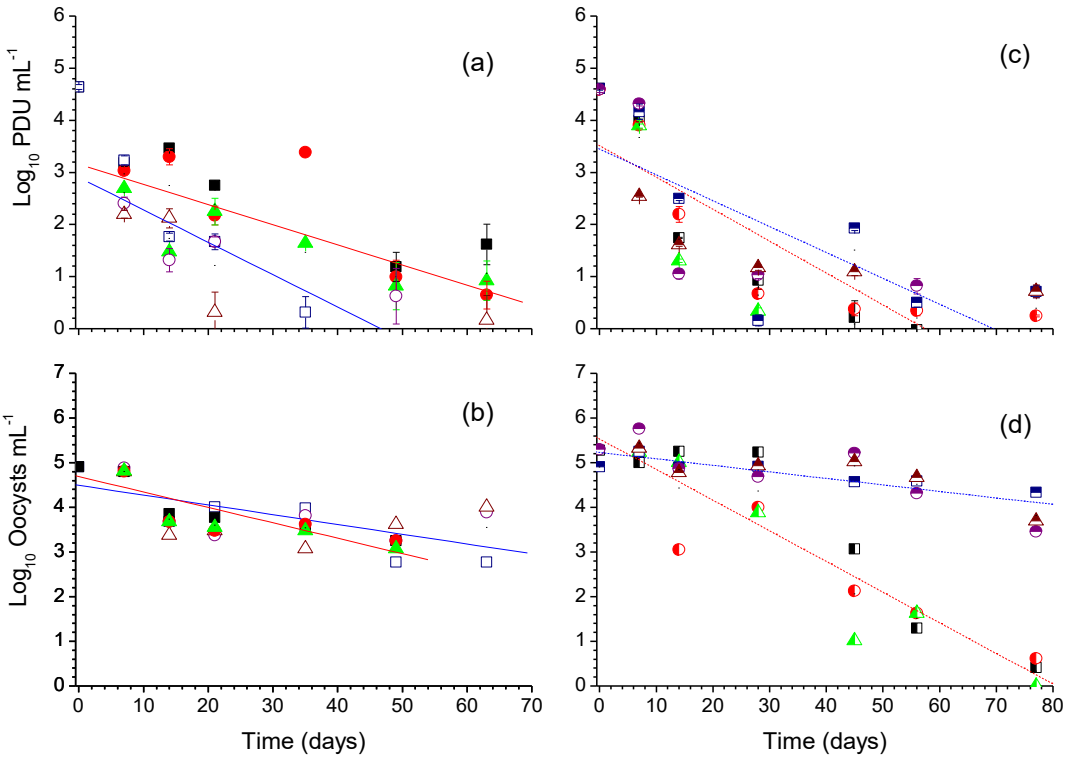


Figure 18. Decay of (a) adenovirus, and (b) *Cryptosporidium* oocysts in the first post-flood experiment; and (c) adenovirus, and (d) *Cryptosporidium* oocysts in the second post-flood experiment at different depths in Wivenhoe Dam after the January 2011 floods; where closed symbols and red decay line were chambers set at the surface; and open symbols and blue decay line were chambers set at 15 m depth.

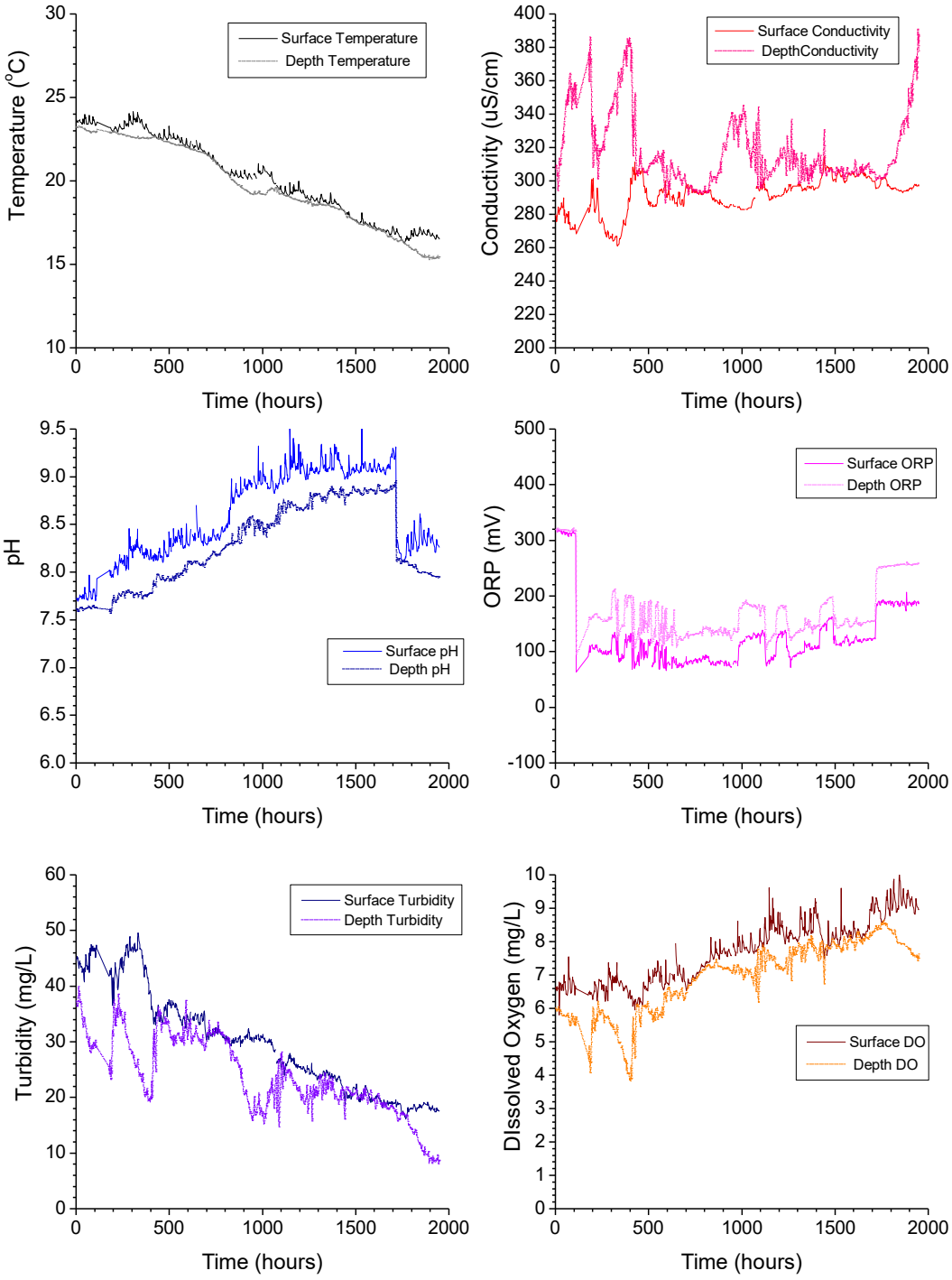


Figure 19. Water quality during first post-flood experiment.

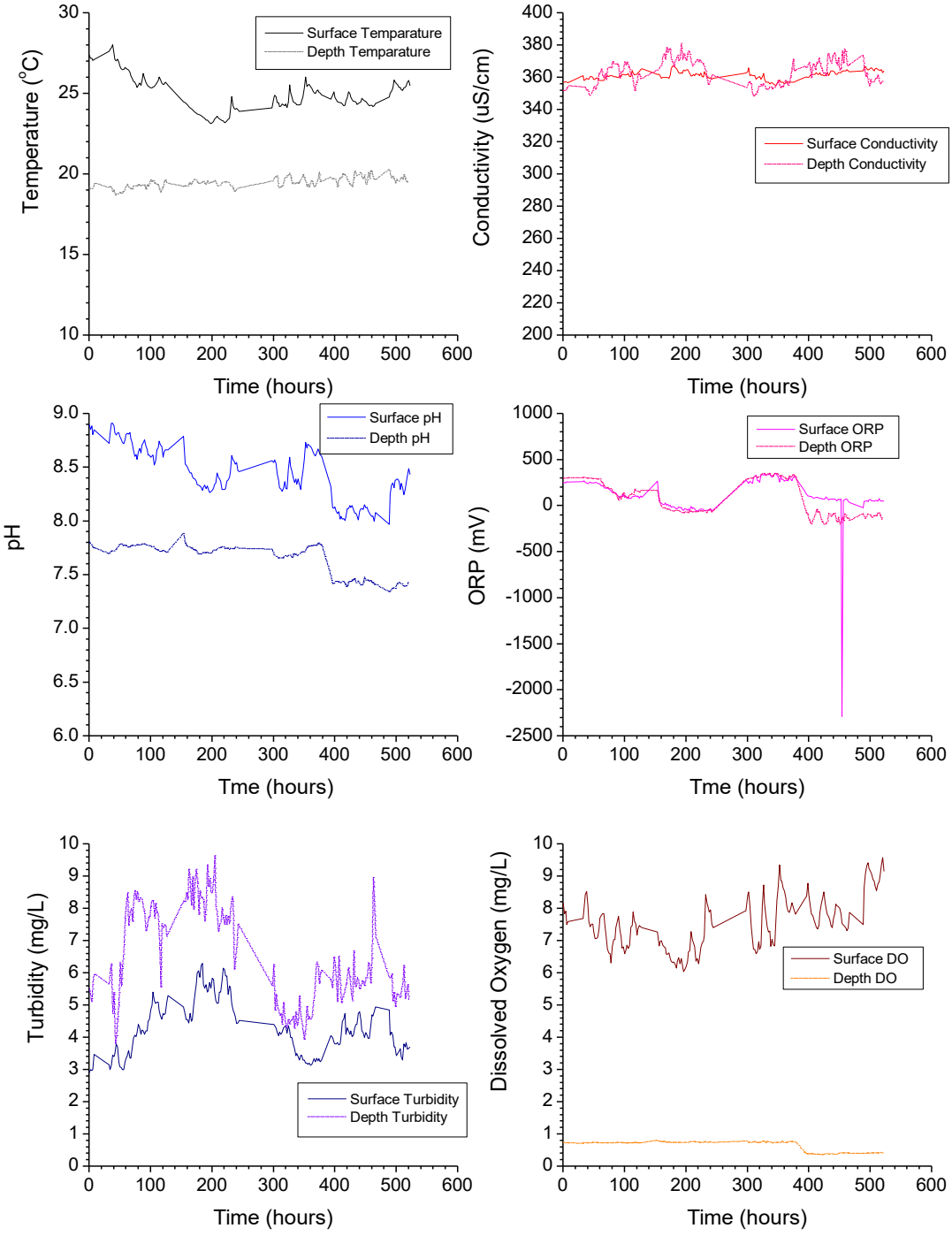


Figure 20. Water quality during second post-flood experiment.

2.2.6. Decay of enteric microorganisms in the mid-Brisbane River

The results of all the experiments described above have demonstrated that a SEQ reservoir such as Wivenhoe Dam has the potential to be used as an active treatment barrier. Unlike other reservoirs in SEQ which take water directly from the reservoir to the local water treatment plant, the ultimate quality of water from Wivenhoe Dam that reaches the Mt Crosby WTP is influenced by the passage of the water from the reservoir through the mid-Brisbane River. Any impacts and influences in this stretch of the river could have a major influence on the potential continuing presence of pathogens and chemicals in the water.

The region of the mid-Brisbane River chosen as the experimental site, approximately one kilometre downstream of the Pine Mountain bridge, was selected as it was considered far enough downstream to have other inputs other then the water from Wivenhoe Dam. The site had a maximum depth of about four metres and was in a quiet pocket out of the main stream flow. While no water quality data was available for this site, visually the water was quite turbid (Figure 21) and the evidence of rapid fouling of the racks and support structures suggested that there were sufficient nutrients in the water to support high microbial activity in the water (Figure 4). Due to the shallow nature of the river and the reasonably fast water flow in the area, it can be assumed that the water temperature would remain high and that the dissolved oxygen concentration would be approaching saturation.

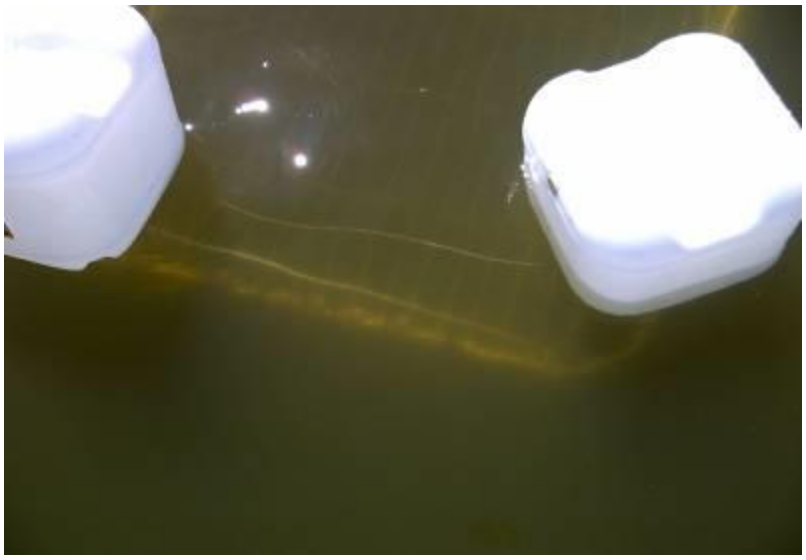


Figure 21. Visual quality of water in the mid-Brisbane River during the first decay experiment.

The first experiment undertaken in the mid-Brisbane River was essentially the same as the first experiment in Wivenhoe Dam (Section 2.2.1), designed to establish a baseline of decay for pathogens and indicators in the river. The results for this first experiment (Table 10) show that the decay rate for *E. coli* was faster than had been observed in Wivenhoe Dam early post-flood but the decay of adenovirus and *Cryptosporidium* was considerably slower than the rate that had been observed in the reservoir under the post-flood condition (Table 8).

Table 10. Decay of enteric microorganisms in the mid-Brisbane River.

Microorganism	Experiment	Conditions	Kd (log day^{-1})	Std Error	T90 (days)
E. coli	1		0.9583	0.0274	1.0
	2	Filtered	0.1901	0.0634	5.3
		Non-Filtered	0.3049	0.1031	3.3
Salmonella	1		ND	-	-
	2	Filtered	0.1860	0.1201	5.4
		Non-Filtered	0.1941	0.1091	5.2
Adenovirus	1		0.0175	0.0070	57
	2	Filtered	0.0014	0.0173	699
		Non-Filtered	0.0251	0.0493	39.8
Cryptosporidium	1		0-0093	0.11169	108
	2	Filtered	0.0041	0.0035	245
		Non-Filtered	0.0310	0.0132	32.2

ND= Not Done

The reason for this slower decay of adenovirus and *Cryptosporidium* is not known but may have links with the level of nutrients in the river. The mechanism for why different concentrations of nutrients influence pathogen decay rates is not obvious, however, is worth further testing as it was noted that as the water quality in the reservoir improved, the decay of *Cryptosporidium* and adenovirus got faster. This could have been due to the concentration of nutrients decreasing as the water quality improved. This also is suggested when comparing the results between the first and second mid-Brisbane experiments, where the rate of decay of both these microorganisms increased in the second experiment which was undertaken more than 12 months after the floods had occurred.

As there had been evidence during the first of the mid-Brisbane experiments of significant biological activity if the river water, it was decided that the second experiment should be designed to test the impact of the indigenous river microorganisms. The experiment design was the same as for the reservoirs in Section 2.2.2. The results of this second experiment showed that, as in the reservoir, the presence of the indigenous river microflora had a major influence on the decay of adenovirus and *Cryptosporidium,* with overall slower decay rates in the absence of the indigenous river microflora. In contrast, the presence or absence of the indigenous microflora had minimal to no effect on the persistence of *E. coli* and *Salmonella,* which have faster overall decay rates (Table 10, Figures 22 and 23).

It should noted, that the experiment undertaken in the mid-Brisbane investigating the impact of the indigenous microflora was undertaken after the floods and during times of high rainfall, with significant inputs from land run-off and inflows from creeks and streams. In comparison, the comparable reservoir experiment had been undertaken early in the project when there had been little inflow into the reservoir for some time, thus the nutrient concentrations would have been much lower in the reservoir at this time which could have had a major influence on the level of metabolic activity of the indigenous microflora. It is acknowledged that at this stage, there is no information on which members of the indigenous microflora present have the greatest influence on the pathogen decay. These results obtained in this second mid-Brisbane experiment have shown that the indigenous microflora can have an influence on the decay of enteric microorganisms in the water, as has been shown elsewhere (Cameron and Toze, 2003, Toze *et al.* 2004), but, as previously shown by Cameron and Toze (2003), this is predicated on the level of overall metabolic activity of these indigenous microbes.

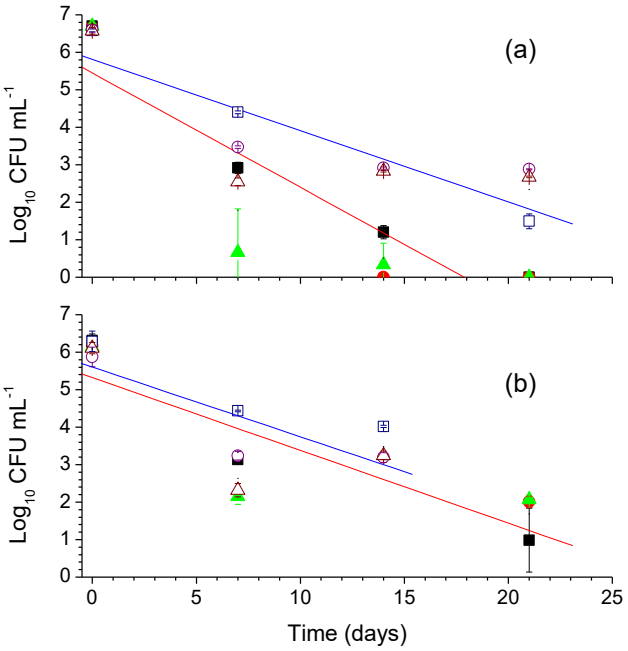


Figure 22. Decay of (a) *E. coli*, and (b) *Salmonella* in the mid-Brisbane River; where closed symbols and red decay line were chambers containing enteric microorganisms seeded into non-filtered mid-Brisbane River water; and open symbols and blue decay line were chambers containing enteric microorganisms seeded into filtered mid-Brisbane River water.

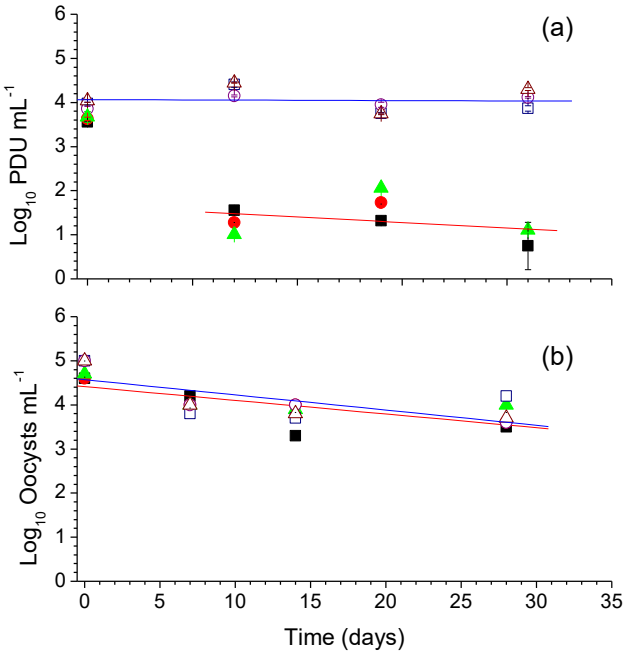


Figure 23. Decay of (a) adenovirus, and (b) *Cryptosporidium* oocysts in the mid-Brisbane River; where closed symbols and red decay line were chambers containing enteric microorganisms seeded into non-filtered mid-Brisbane River water; and open symbols and blue decay line were chambers containing enteric microorganisms seeded into filtered mid-Brisbane River water.

2.2.7. Comparison of Observed Decay Rates

The final analysis undertaken in this project was to compare the decay rates for each of the tested microorganisms across all of the experiments to determine if time of year, season, or reservoir conditions had any significant influence on the ability of the reservoir to act as an active treatment barrier. To allow an accurate comparison to be made, the condition that was most common for all the experiments were used (suspended in unfiltered reservoir water, chambers placed at the reservoir surface, in the absence of sunlight). Note that because of the different conditions tested, the surface dark results from the sunlight experiment were not included in this comparison. Also, because of the small data sets, a statistical analysis was not done and all observations on the amount of change in decay rates are qualitative only.

The results provided in Table 11 show that there was very little variation in decay rates for *E. coli* and *Salmonella* regardless of the year, season or change in quality of the water in the reservoir. This indicates that the presence of these two microorganisms in the reservoir can be considered to be transient only. If the potential impact of sunlight at the near surface of the reservoir is taken into account, then the survival of these microorganisms can be considered to be even less. It also signifies that any detection of either bacterium in the reservoir can be used to indicate either a local source of contamination has occurred within a small area of the reservoir, or that there has been a very large and rapid influx of water. This would also suggest that under most conditions in the reservoir, these two microbes could be considered to pose a limited health risk (considering the *E. coli* as pathogenic strains). Considerations would still need to be made, however, on the most likely contamination sources such as birds and cattle (see section on MST), and on likely exposure potentials (see section on QMRA).

Table 11. Influence of date and season on decay times of enteric microorganisms.

Experiment	Experiment Date	*E. coli*	*Salmonella*	*Campylobacter*	Adenovirus	*Cryptosporidium*
1	June 2009	3.8	5.0	9.5		12.8
2	October 2009	3.1			11.0	
4	September 2010	3.3	5.2	5.1	19.7	17.4
5 (1)	March 2011	2.5	4.3	2.8	25.9	45.7
5 (2)	August 2011	2.5	3.2	3.2	16.4	14.6

The rate of decay for *Campylobacter* increased over time, which correlated with increasing inflows into the reservoir. There is a decrease in the T90 decay times between Experiment 1 undertaken in June 2009 and Experiment 4 undertaken in September 2010. While there is a small seasonal change from Winter to Spring for these two experiments during the time period between June 2009 and September 2010 (with a small increase in water temperature from an average of 17.5 °C for Experiment 1 to 21.5°C for Experiment 4), there had also been significant rain, the breaking of the drought, and an increase in the amount of water held in the reservoir from less than 20% to almost 100% of drinking water storage capacity. This influx of water into the reservoir after such a prolonged drought period would have brought additional nutrients from upstream and via land run-off from agricultural lands into the reservoir. Thus, it could be hypothesised that the increased nutrients could have an influence on the increased decay of *Campylobacter*.

This hypothesis on increased nutrients impacting on the decay of *Campylobacter* is further strengthened by be results from the post-flood experiments. In Section 2.2.5 it was hypothesised that the increased rate of decay of *Campylobacter* at depth was due to an increase in dissolved oxygen concentrations. As dissolved oxygen concentrations varied little pre- and post-flood (average 8.8 mg/L in Exp 4 pre-flood compared to 7.3 mg/L in Exp 5 post-flood) and there was an observed decrease in T90 decay times of *Campylobacter* from 9.5 days prior to the January 2011 floods to 3 days in the post flood conditions, it further indicates that the changing water quality also has an impact on the decay of *Campylobacter*.

Adenovirus and *Cryptosporidium* also changed in decay rate over the time of the project, but unlike *Campylobacter*, the rate of decay of these two microorganisms slowed as the water level of the reservoir increased. This slowing of decay increased significantly immediately after the 2011 floods, adding to the hypothesis that increased nutrients influence the decay rates of the more resistant enteric microorganisms. Again, in comparison to *Campylobacter*, the decay of adenovirus and *Cryptosporidium* increased as the quality of the water in the reservoir slowly changed back toward the pre-flood conditions.

In conclusion, the reservoir maintained the ability to act as an effective treatment barrier despite increasing inflows and a major perturbation of the 2011 floods. Seasonal influences appeared to be minimal, however, changing water quality due to the increased inflows and the flood, most probably increasing nutrients, did influence the decay rates of the more resistant pathogens. These changes in decay rates were minor, however, and all the microorganisms had a measured T90 time of less than 50 days under all conditions tested. In the conditions of normal water flow rates through the reservoir, the observed slowing in T90 times for adenovirus and *Cryptosporidium* would have a minimal impact on the calculated health risks.

2.2.8. Quantitative Microbial Risk Assessment using the Decay Data to Determine Changes in Health Risks

Quantitative Microbial Risk Assessment (QMRA) is a widely adopted tool for investigating, evaluating and managing microbial risks associated with water systems. The process of implementing QMRA requires quantifying pathogen concentration in the source water, removal during environmental attenuation or treatment, through to potential exposure during water use. Analysis of monitoring results in Wivenhoe Dam demonstrated that the faecal indicators *E. coli* and enterococci were frequently detected and by implication faecal material, were consistently present in the water.

Comparison of the annualised probability of infection estimates with the 10^{-6} DALYs benchmark (NHMRC-EPHC-AHMC 2006) indicated that *Salmonella* were the primary bacterial pathogen of concern; for all exposure scenarios, likely due to the order of magnitude higher initial source numbers. *Cryptosporidium* and viruses were the next followed by *Campylobacter* and *E. coli* (Table 12).

Table 12. Required microbial inactivation credits for different end uses to met acceptable health limits.

Exposure	*E. coli*	*Salmonella*	*Campylobacter*	*Cryptosporidium*	Adenovirus
Hand washing	0.9	2.6	1.1	2.5	2.3
Swimming	1.4	3.1	1.7	3.0	2.9

Reduction of pathogen numbers could be achieved by a number of factors including dilution in the reservoir, (not quantified in this study), and natural attenuation. Natural attenuation and dilution are likely to be a key mechanism to reduce exposure to pathogen in the Wivenhoe Dam. The microbial inactivation credits (log10) reduction as a function of time for each pathogen is shown in Figures 24 and 25.

Figure 24 shows that *E. coli* (post-flood) decayed the most rapidly compared to both *E. coli* (post-flood) and *Campylobacter,* and adenovirus and *Cryptosporidium* decayed the slowest.

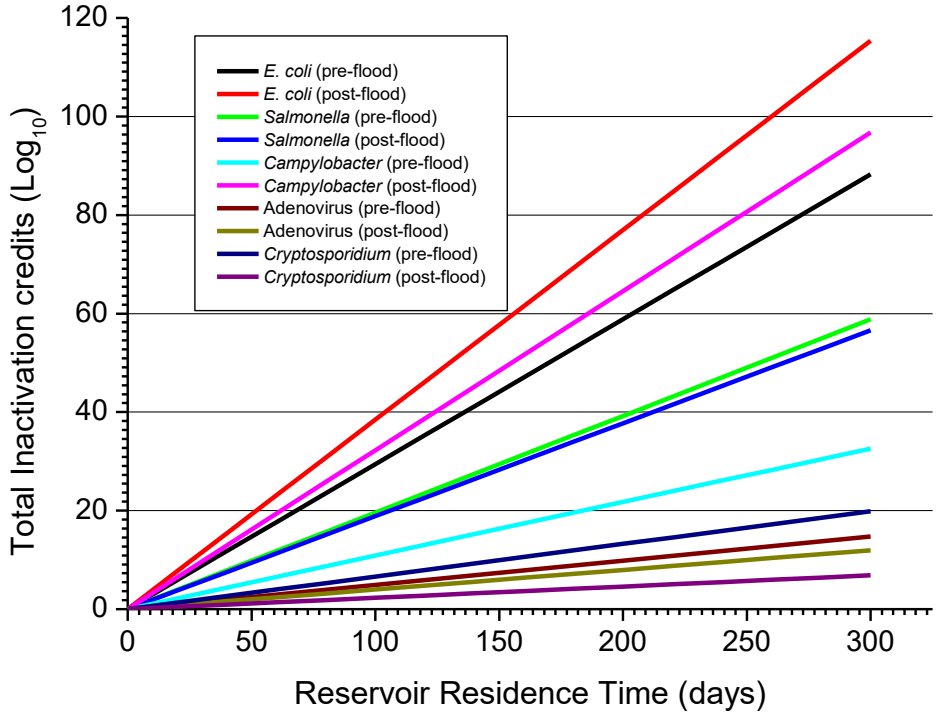


Figure 24. Pathogen inactivation credits as a function of time.

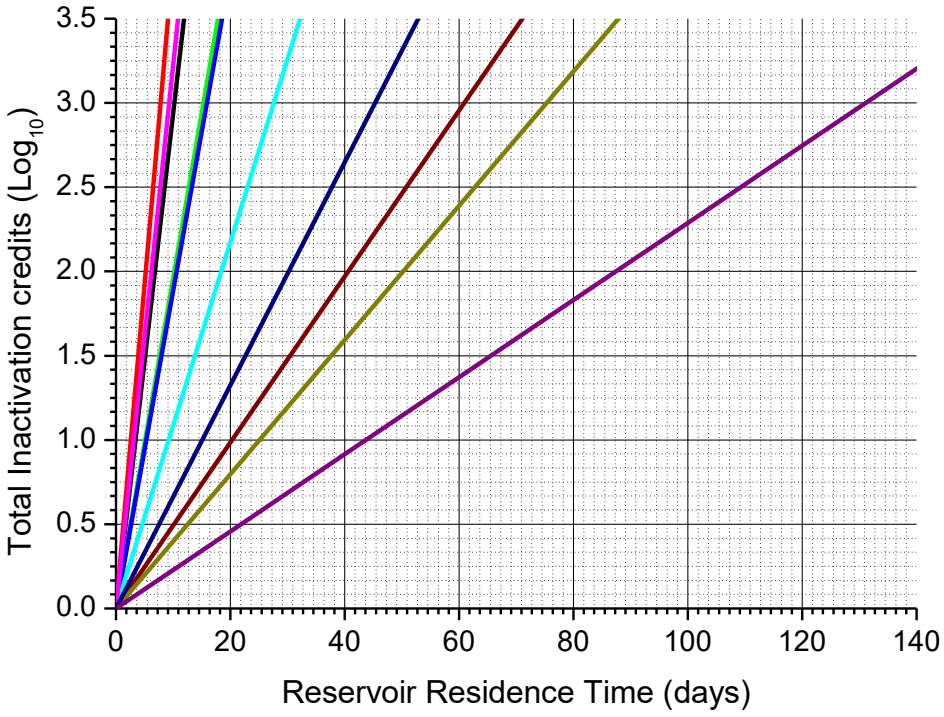


Figure 25. Times for tested microorganisms to reach log reductions required to meet acceptable health risks. (Note: legend is the same as in Figure 24.)

Using the downscaled information presented in Figure 25, a calculation can be made on the number of days that would be required (under pre- and post-flood conditions) for each of the tested microorganisms to reach the acceptable health risk target of 10^{-6} DALYs. The outcomes of this assessment are given below in Table 13.

The results show that all three of the tested bacteria would only pose a short term, localised health risk with pathogenic *E. coli* reaching acceptable health risk limits within 5 days. Both *Salmonella* and *Campylobacter* posed an unacceptable health risk for up to 16 days. Based on expected normal low flow rates within reservoirs such as Wivenhoe Dam, this means that any unacceptable health risk would be localised to a small area around the initial contamination source.

In contrast, adenovirus and *Cryptosporidium* would take between approximately 40 and 130 days to reach an acceptable health risk level (Table 13). This means that these microorganisms could pose a health risk some distance from the initial contamination source depending on local conditions at the time and actual flow rates. These results would need to be combined with flow rate information from a hydrodynamic model to determine the actual distance any health risk would extend from the initial contamination source.

Another observation from the data presented in Table 13 is that the time to achieve an acceptable health risk was impacted more by the floods for some microorganisms than others. For example, the time required for *E. coli and Salmonella* to reach an acceptable health risk varied little between the pre- and post-flood conditions. It can also be observed that these times also were only marginally longer for exposure during swimming than for hand washing. In contrast, the times to reach acceptable health risks limits for *Campylobacter, Cryptosporidium,* and adenovirus were very different between the assessments made for pre- and post-flood conditions (for both exposure scenarios). Also for these pathogens, in particular for adenovirus and *Cryptosporidium,* there was a much larger difference between the two exposure scenarios for the times required to reach an acceptable health risk limit when compared to the bacteria. This is a reflection of both the high level of environmental resistance for these two pathogens, as well as their high levels of infectivity.

Table 13. Time required (days) for pathogens to be reduced to meet acceptable health risk limits for established exposure scenarios in Wivenhoe Dam under pre- and post-flood water quality conditions.

Pathogen	Hand Washing		Swimming	
	Pre-Flood	Post-Flood	Pre-Flood	Post-Flood
E. coli	3.1	2.3	4.8	3.7
Salmonella	13.3	13.8	15.9	16.5
Campylobacter	10.1	3.4	15.7	5.3
Adenovirus	46.7	57.9	59.1	73
Cryptosporidium	37.8	109.3	45.3	131.2

A comparison of the pre- and post-flood differences in the time required to reach acceptable health risk for both exposure scenarios and the differences between the measured decay rates for each of the pathogens (Table 13) indicated that there was good agreement between the results found for both data sets. Where the health risk assessment has greater value, however, is to provide an indication of health risk, which can then be applied to management and operational procedures (eg, identifying potential high risk locations based on close proximity of humans and animals and controlling access of either group to the reservoir).

As with any QMRA, there were several potential sources of uncertainty in this analysis. First, the background data used in the exposure assessment are assumptions. The swimming and boating scenarios are based on best available estimates of the amount of water a person will come in contact with while recreating and remain to be validated. As stated earlier, the current calculations used in this study do not take into account dilution or dispersion. Nor is there any actual information on actual pathogen sources or the numbers that could be present in the reservoir at any one time. The QMRA

was undertaken using estimates based on example data taken from the literature. To obtain more accurate information on pathogen numbers and sources, more work needs to be completed on Microbial Source Tracking and on the location and number of potential sources such as cattle, wild birds and human activities. Care must be taken on which microorganisms are chosen to undertake any such assessment, For example, the relatively rapid attenuation of bacterial faecal indicators such as *E. coli* and enterococci could mask the health risks posed by viruses or *Cryptosporidium* with their much slower decay rates, meaning greater time is required to achieve sufficient inactivation credits for recreational use (Figure 24). Thus, it is important to assess the risk of these slower decaying pathogens using a more appropriate indicator than the faecal indication bacteria (potentially adenovirus itself).

In addition, the levels of exposure to pathogens as they are transported through the reservoir will decline with the passage of time as shown by the results above. This, in turn, could result in lower risks of pathogen-related disease if contact occurred furthest from the source. To be able to determine how far away from the source these lower risks occur, the decay data and QMRA needs to be combined with a hydrodynamic model that uses particle tracking to determine how fast and far particles travel through the reservoir over time under different conditions.

The health risks posed by recreation on Wivenhoe Dam have been recognised through the adoption of risk-based guidelines in the NWQMS. From a QMRA perspective, it is clear that the release of pathogens into the Wivenhoe Dam remains an impediment to achieving a consistent level of water quality. Clearly, faecal inputs such as from grazing around the margins of the water body could be managed to reduce faecal loads, but other sources such as wild birds and feral animals pose a more uncontrollable risk and additional treatments may be required to meet the microbial health based targets.

2.3. Conclusions

The major findings from the analysis of pathogen decay in Wivenhoe Dam and downstream in the mid-Brisbane River indicate that both systems can have an active role in removing microbial pathogens. The results of the different experiments undertaken have shown that:

- Seasonal and climatic changes have minimal impact on the decay of all the microbial pathogens in SEQ;
- Sunlight can have an impact on the survival of enteric microorganisms at the surface, but is limited at depth and is impacted by water quality parameters, most likely to predominantly be turbidity and dissolved natural organic matter;
- Changes in water quality indicated by turbidity appear to have some indirect impacts on the rate of pathogen decay. The changes in turbidity could be used as an indicator for changes in other unmeasured parameters such as nutrient and dissolved oxygen concentrations which may play a role in these impacts on decay rates;
- The potential influence of changing nutrient concentrations on pathogen decay is unknown, however, evidence around changes in decay rates of the tested microorganisms with changing water quality (as indicated by changes in basic water quality parameters) suggest that this is an area that warrants more investigation;
- Changes in water conditions at depth had an observed impact on the rate of decay of adenovirus and *Campylobacter*. Depth may also have an influence on the decay of *Cryptosporidium* oocysts, however, the lack of data from before the floods means that more testing should be undertaken as the water quality in the reservoir continues to improve. Testing the role of depth in other SEQ reservoirs would also assist in testing the impact of depth on these pathogens;
- The major changes in water quality following the January 2011 floods did have an influence on the decay of the more resistant microorganisms, in particular adenovirus and *Cryptosporidium*, however, these changes in decay rates were marginal.
- Decay of pathogens also occurs in the mid-Brisbane River but higher flow rates and additional inputs from a range of further sources are likely to be the reason for the observed variations in the decay rates of these microorganisms compared to their decay in the more static conditions of the reservoir;

- The use of QMRA further enhanced the information on decay by providing information on the level of health risk using the decay rates as an important input parameter. The outputs from the QMRA can be used to determine the residence time required for a pathogen contamination event to drop below acceptable health risk levels. This also requires knowledge of pathogen transport under different conditions using hydrodynamic modelling, and data on potential sources and loads via MST. Both are discussed further in the report but are areas where significant more research is required.

Due to time constraints and the disruption caused by the January 2001 floods, there are important aspects relating to the understanding of pathogen decay that remain to be determined.

- One vital knowledge gap that requires filling is the influence of sediment on pathogen transport and sedimentation through the reservoirs. Pathogens that attach to sediment have the potential to move through the system differently to pathogens that remain free and unattached in the water column. Attachment to sediment can influence how pathogens are transported via overland flow from paddocks to the steams and reservoir; on the rate of transport through the reservoir under different flow regimes; and on the rate that pathogens settle to the bottom of a reservoir into the sediment. There have been suggestions that close association with sediment can also influence the decay rate of pathogens. This research had been intended to be done in the latter stages of this project but the flood event changed the research direction to study the impact of the floods as explained above. Because of this change, there was insufficient time or capacity to do the research on the influence of sediment and this remains to be undertaken.
- All of the research outcomes given in this report are from experiments undertaken in Wivenhoe Dam and the associated regions of the mid-Brisbane River. Wivenhoe Dam was initially chosen as it is the reservoir to receive the Purified Recycled Water (PRW) in times of extended drought. Once the delivery of PRW to the reservoir had been postponed, the research was continued in Wivenhoe Dam as it is the largest drinking water storage in SEQ. It is assumed in this report that the results obtained from this research in Wivenhoe Dam are transferable to the other SEQ reservoirs. Most of the other reservoirs, however, have different inputs from catchments with different characteristics to Wivenhoe Dam, different lithology due to the nature of their construction and supply water to the community via different mechanisms. Thus, it would be warranted to test, at least, the basic decay parameters of the different pathogens (general decay, influence of depth, and impact of water quality parameters, in particular nutrients, sunlight conditions at the reservoir surface and dissolved organic carbon) to verify if the decay rates and behaviour of decay generally match the results determined for Wivenhoe Dam. In addition, more localised hydrodynamic models should be used, along with assessments of the most likely human and animal inputs of pathogens into the local system (based on an MST survey and knowledge of the catchment).

3. FATE OF TRACE CONTAMINANTS IN RESERVOIRS

Similarly to microbial pathogens, there are a range of trace organic compounds that have either been detected in SEQ reservoirs, or are considered a contamination risk. Many of these chemicals can have an impact on water produced by the WTP if present in the raw water, therefore, it would be a benefit to the operation of the WTP to better understand the persistence of these trace chemicals in the reservoirs and on the ability of the reservoirs to actively remove these trace organic compounds. In order to obtain this information a series of experiments were undertake to measure the biodegradation and photodegradation potential of a series of identified problem compounds.

3.1. Methods and Materials

3.1.1. Test Compounds Selected

A number of organic contaminants were selected representing compounds with a variety of physicochemical properties, contamination sources and biological modes of action. They included pharmaceuticals, endocrine disrupting chemicals (EDCs), pesticides, personal care products and antibiotics (Table 14). All the compounds were used in the either photodegradation or biodegradation assessments, but not all compounds were used in every assessment either due to inconsistent analytical or recovery performance.

3.1.2. Collection of Water

Water was collected from Wivenhoe Dam on the 2 March 2011 adjacent to the reservoir outlet wall for the biodegradation experiments and first photolysis experiments and on the 21 November 2011 for the second photolysis experiments. Water was collected from the mid-Brisbane River on the 19 December 2011 and from the outlet drain from the Salisbury wetlands (in Adelaide) on the 30 November 2011. The depth from which the water was sampled was no greater than 1 m, with water quality parameters supplied summarised in Table 15. Water was collected in pre-cleaned 20 L polypropylene containers which excluded light. Water samples were immediately shipped to Adelaide, where they were received within 24 h and immediately placed in the dark at 4°C.

A mean pH of 7.86 was measured in Wivenhoe Dam over a 6-year period (2003-2009), with a range of 6.2 to 9.5 (Hawker *et al.* 2011). Also, the median temperature measured in Wivenhoe Dam over the same period was around 22°C, with a seasonal variation of around 10°C (Hawker *et al.* 2011).

Table 14. Summary of the selected contaminants physicochemical properties and use classification.

Compound	CAS Number	Class	Log K_{ow}	S (mg/L)	pK_a
Pharmaceuticals					
Diclofenac (DCF)	15307-86-5	NSAID	4.51	2.37	4.15
Methotrexate (MTX)	59-05-2	Antineoplastic	-1.85	2,600	4.7
Cyclophosphamide (CPP)	50-18-0	Antineoplastic	0.63	4,000	6
Carbamazepine (CBZ)	298-46-4	Antiepileptic	2.45	112	na
Sertraline (SER)	79617-96-2	SSRI	5.29	3.5	9.5
Venlafaxine (VEN)	93413-69-5	SSNRI	3.28	267	9.4
Trimethoprim (TRM)	738-70-5	Antibiotic	0.91	400	7.1
Sulfamethoxazole (SFM)	723-46-6	Antibiotic	0.89	610	5.7
Metoprolol (MET)	51384-51-1	β-blocker	1.88	4,777	9.7
Propranolol (PRL)	525-66-6	β-blocker	3.48	62	9.42
Atenolol (ATL)	29122-68-7	β-blocker	0.16	1,330	9.6
Personal Care Product					
Triclosan (TCS)	3380-34-5	Antimicrobial	4.76	10	7.8
N,N-diethyl-*m*-toluamide (DEET)	134-62-3	Insect repellent	2.18	912	na
Endocrine Disrupting Chemicals					
Bisphenol A (BPA)	80-05-7	Plasticiser	3.32	120	10.1 acid
4-*t*-octylphenol (OP)	104-40-5	Surfactant by-product	5.76	7	10.5 acid
Pesticides					
Atrazine (ATR)	1912-24-9	Herbicide	2.61	35	1.7
Diuron (DIU)	330-54-1	Herbicide	2.68	42	-
Triclopyr (TCP)	55335-06-3	Herbicide	2.53	440	3.97
2,4-Dichlorophenoxyacetic acid (2,4-D)	94-75-7	Herbicide	2.81	677	2.73 acid
Other					
Benzotriazole (BZT)	95-14-7	Corrosion inhibitor	1.44	1,980	8.37

K_{OW} octanol-water partition coefficient
S water solubility
pK_a acid dissociation constant

Table 15. Water quality parameters of water collected from Wivenhoe Dam, mid-Brisbane River and Salisbury wetlands for the photolysis study.

	pH	EC (µS/cm)	DOC (mg/L)	NO_x (mg/L)	Total N (mg/L)	Total P (mg/L)	Fe (mg/L)
Wivenhoe Dam (Expt 1)	8.55	197	9.4	-[a]	-[a]	-[a]	-[a]
Wivenhoe Dam (Expt 2)	8.85	378	6.6	<0.005	0.3	<0.1	<0.1
Brisbane River	7.37	504	7.7	<0.005	0.7	<0.1	0.14
Salisbury wetland	7.21	1823	7.5	<0.005	0.6	<0.1	<0.1

[a]data not available

3.1.3. Photolysis

The stability of the selected contaminants from exposure to solar radiation was assessed using a Suntest Solar Simulator (Atlas Material Testing Technology) containing a 1500 W xenon lamp, filtered to include the wavelength range of 300-800 nm. The exposure intensity was set with irradiance values ranging from 500 to 750 W/m^2 to correspond with approximate midday summer solar irradiance for Brisbane at 27° latitude (NASA Atmospheric Science Data Center). Experimental temperatures were maintained between 24 and 26°C, using a temperature controlled water bath (Figure 26), which is within the expected temperature range of Wivenhoe Dam (Hawker *et al.* 2011).

Figure 26. Overview of internal set-up of Solar Simulator, with cuvettes submerged and fixed in place within the temperature-controlled water bath.

The photolysis experiments were separated into two components, with one addressing the significance of direct versus indirect photolysis and the other component assessing the effect of depth on the rate of photolysis. For the first component, photolysis experiments were conducted with both water from the Wivenhoe Dam and ultrapure water with resistivity of 18 MΩ.cm (Milli-Q water; Millipore). The pH of ultrapure water was adjusted to pH 8 using H_3BO_3 and NaOH. Comparisons between rates of photolysis (k_{photo}) were used to assess whether indirect photolysis, such as where contaminants react with photosensitised chromophores derived from other components within the water, played an important role in Wivenhoe Dam water. A second experiment was used to determine the most likely species responsible for the observed indirect reactions in Wivenhoe Dam water. A number of experimental manipulations of the natural water was undertaken to exclude common reactive species, include the hydroxyl radical (OH·), singlet oxygen ($^1O_2$) and dissolved organic matter (DOM) in the triplet state. Isopropanol (1% v/v) was used to react with $OH^·$, while instrument grade N_2 (to remove the influence of $^1O_2$ species) and O_2 (to remove the influence of triplet state DOM) were bubbled through respective test solutions for 20 minutes.

The second component involved assessing the k_{photo} of the contaminants in Wivenhoe Dam, mid-Brisbane River and Salisbury wetland water at different water depths. Ultrapure water was again used as a control but due to the ranges of pH values of the natural water samples (Table 15), the pH of ultrapure was adjusted to the appropriate corresponding pH value of the natural water using H_3BO_3 and NaOH. Nominal water light transmission levels of the water samples of 10% and 50% transmission were selected based on their respective absorbance spectra at <330 nm, as this was considered to be the most important wavelength for absorbance of irradiance by the contaminants (Figure 27). The depths for the selected transmission values in the water samples ranged from 1 cm

(50% transmission in Brisbane River water) to 10 cm (10% transmission in Wivenhoe Dam water). The rates of photolysis were also assessed without any experimental water overlaying the glass tubes, although a minimal depth of reverse osmosis water was required to maintain a constant experimental temperature.

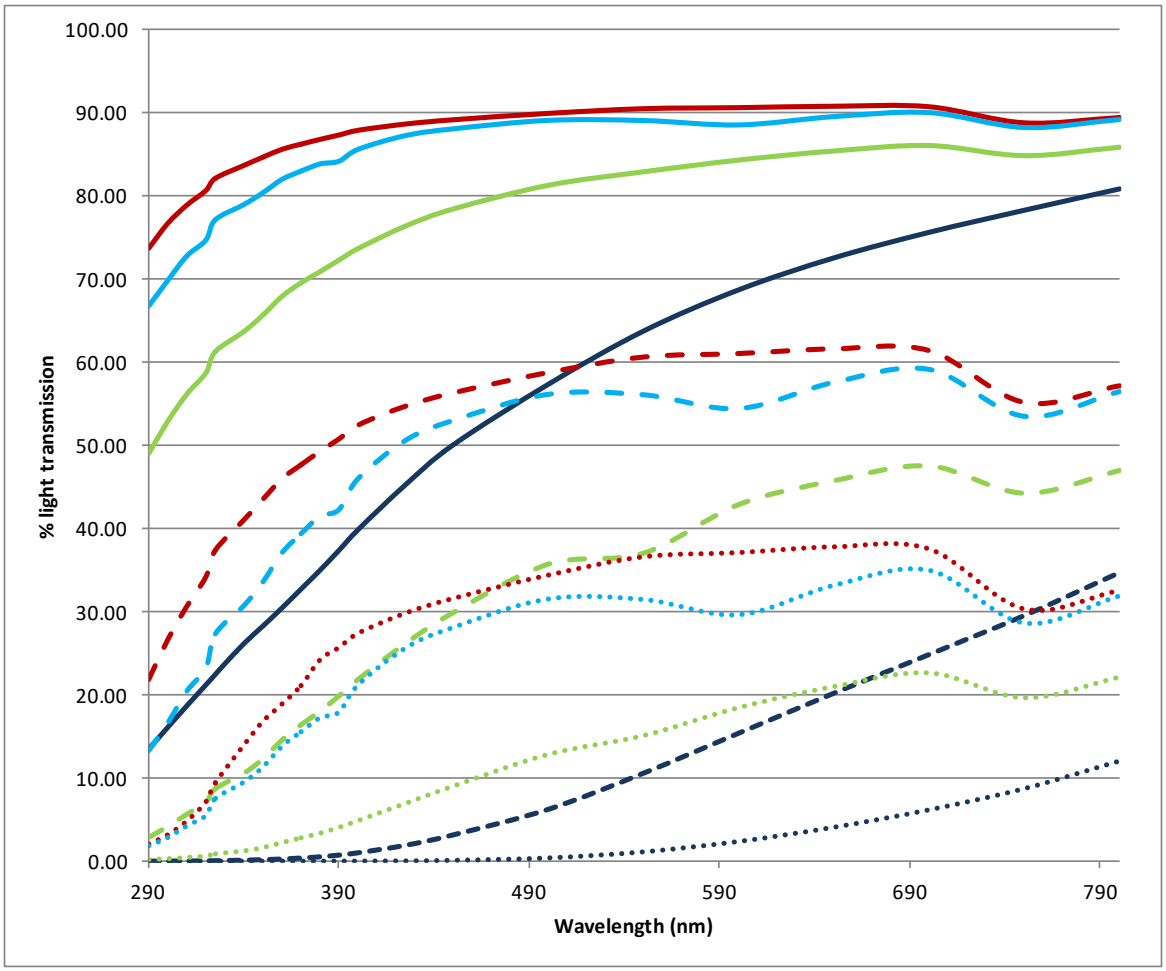


Figure 27. Percentage of light transmitted through Wivenhoe Dam first photolysis experiment (dark blue lines), Wivenhoe Dam second photolysis experiment (red lines), mid-Brisbane River (green lines) and Salisbury wetlands (light blue lines) at 1 cm (solid line), 5 cm (dashed line) and 10 cm (dotted line) depths for wavelengths ranging from 290-800 nm.

Nominal concentrations of the mixed contaminants were 100 µg/L and were prepared by adding 100 µL of 1 g/L individual stock solutions (in methanol) to a 1 L volumetric flask. Methanol was evaporated under N_2, experimental water was added to volume and the volumetric flask was sonicated for 1 h.

Experimental containers varied according to the experiment. The first component of the assessment of direct and indirect photolysis rates were conducted in 50 mL borosilicate glass beakers containing 40 mL spiked solutions. Beakers were wrapped in aluminium foil to ensure exposure to light was only due to that coming directly overhead the beakers. The second component of the direct and indirect photolysis rates was undertaken in 15 mL borosilicate glass tubes (with minimal headspace) to prevent evaporation of isopropanol during the experiment. For the assessment of depth, experimental solutions were also placed in borosilicate glass tubes, with minimal headspace (Figure 26). Preliminary experiments determined that the rate of photolysis was increased by a factor of approximately 1.5 using borosilicate glass, compared with quartz, with quartz a preferable medium as it would give a more realistic value of photolysis rates. With the number of treatments used in the experimental, however, the use of expensive quartz was precluded. All treatments were undertaken in triplicate.

All experimental containers were placed within an externally controlled water bath to maintain a constant temperature during the experimental period. The water bath level was maintained at the same depth as the experimental solutions within the beakers or at a minimal level above the borosilicate tubes. For experiments assessing the effect of depth of water on photolysis, the final depth used was measured from the surface of the water to the glass of the experimental tubes. Reverse osmosis water was used to fill the water bath for temperature control, except for depth experiments where the corresponding natural water was used to fill the water bath. Dark controls were completely covered in aluminium foil. Exposures within the Solar Simulator were maintained for up to 24 hours in all experiments to simulate surface conditions and up to 48 hours for the depth assessments. Preliminary assessments found that the compounds susceptible to degradation would have at least reached their half-lives during this period. This was balanced against having the more susceptible compounds being degraded below their instrumental limits of quantification prior to the completion of the experiment. Furthermore, a 24 hour period under simulated conditions is equivalent to a considerably longer exposure period under environmental conditions due to diurnal variations in solar irradiance. A HOBO light and temperature meter (Onset, USA) were immersed in the water bath to monitor water temperature during the exposure period. An overview of the absorbance spectra of each contaminant is given in Figure 46 (Appendix 3).

3.1.4. Biodegradation

The biodegradability of the selected contaminants spiked to Wivenhoe Dam and Salisbury Wetland water was determined in conjunction with levels of biodegradable dissolved organic carbon (BDOC). This was to assess the influence of BDOC on the biodegradation potential of the contaminants. Experiments were initially undertaken on a limited number of contaminants in Wivenhoe Dam water, including ATL, ATR, CBZ, DEET and PRL, with the addition of a number of extra compounds during assessment within Salisbury Wetland water.

Water was used for the biodegradation experiments within 4 days of receipt, including a 24 h pre-experimental equilibration period at the experimental temperature. Water was filtered through a Whatman GF/C 1.2 µm glass fibre filter, to remove particulate matter, prior to placing 500 mL into 1 L clean amber glass jars. Water samples were equilibrated within a constant temperature room (22.2±1.6°C) close to the long term median temperature (approximately 22°C) previously measured in Wivenhoe Dam (Hawker *et al.* 2011). Jars were sealed with a screw cap and were placed on a horizontally rotating flat-bed shaker, rotating at 100 rpm for the duration of the experiment.

In the preliminary experiment, the biodegradability of the selected contaminants spiked to Wivenhoe Dam water was found to be minimal, along with the levels of biodegradable dissolved organic carbon (BDOC) of the water samples over. To assess whether biodegradation could be achieved through the manipulation of BDOC levels and the quantity and quality of microbial populations, an experiment was undertaken in Salisbury Wetland water. For this experiment, the solution was amended with an additional source of DOC and an additional microbial inoculum, respectively, to assess whether these amendments would facilitate the degradation process. Additional contaminants were included to try and broaden the range of potentially degradable analytes. For the DOC amendment, wetland reeds (*Phragmites australis*) were collected and finely ground (~1 mm) using a kitchen blender for two minutes at ambient temperature and the water soluble dissolved organic carbon extracted into high purity Milli-Q water (1 L). The slurry was sequentially filtered using GF/F (Whatman) glass and 0.45 µm nitrocellulose filters (Millipore, Australia). The DOC leachate was diluted into the microcosms to give a final concentration of DOC in the range of 10–30 mg/L.

An additional microbial inoculum was prepared from the Glenelg activated sludge wastewater treatment plant (WWTP). Activated sludge, collected post-DAFF, was maintained under aerobic conditions in the dark and transported immediately to the laboratory. The sludge had coarse particles (< 2mm) removed, followed by centrifugation at 1,100 g for 10 min and the supernatant was discarded. The remaining sludge was then washed with a synthetic mineral media and centrifuged again 1,100 g for 10 min. The pellets were split into ~0.625 g portions and a 50:50 mixture of glycerol:water was added to each ampoule prior to being stored and frozen at -80°C. When required

the inoculum was removed overnight to -20°C and then maintain at 4°C prior to reconstitution. For reconstitution, an ampoule was added to 125 mL mineral and aerated for 7 days and finally 5 mL was added directly each microcosms.

Microcosms were prepared as per the initial assessment in Wivenhoe Dam water, with contaminants spiked at a concentration of 100 ng/L into the microcosms with Salisbury Wetland water (unamended), Salisbury Wetland water + DOC and Salisbury + microbial inoculums. All treatments also had parallel sterile controls that were autoclaved prior to spiking.

3.1.5. Chemical Analysis

The microcosms assessed for contaminants were spiked with stable isotopes, including ATL d7, BPA d16, CBZ d10, DCF d4, EE2 $^{13}C_2$, IBU $^{13}C_3$, PRL d7 and TCS $^{13}C_{13}$, to account for recovery and analytical interferences. To account for recoveries of analytes that did not have an analogous stable isotope, standard addition recoveries were also checked. The 500 mL sample was then passed through a 6 mL, 200 mg Oasis HLB solid phase extraction (SPE) cartridge to extract the contaminants. Following extraction, cartridges were rinsed with 2 x 5 mL aliquots of 10% methanol before the contaminants were eluted from the SPE cartridges using 2 x 3 mL aliquots of methanol and 1 x 3 mL of dichloromethane. The collected solvents were then blow down to dryness under a stream of N_2 and reconstituted in 10% methanol for analysis by a Finnigan TSQ Quantum Discovery Max (Thermo Electron Corporation) LC-MS/MS.

Aniline was analysed by directly injecting a 1 mL aliquot removed from the microcosm into an Agilent 1100 HPLC-UV detector (Agilent Technologies), using a detection wavelength of 235 nm.

DOC samples were filtered through 0.45 µm syringe filters and analysed with a Thermalox TOC analyser (Analytical Sciences) for total carbon (TC) and total inorganic carbon (TIC), with the difference giving the total organic carbon (TOC). For the filtered samples, this was equivalent to DOC.

The water quality parameters were monitored with a WTW pH-Electrode Sentix 41 (pH), a WTW Tetrafon® 325 EC probe (electrical conductivity) and a WTW OxiCal®-SL CellOx 325 DO probe (dissolved oxygen) attached to a WTW Multiline P4 universal meter (ITT Analytics).

3.2. Results and Discussion

3.2.1. Photolysis

3.2.1.1. Direct versus Indirect

Direct photolysis was only found to be important for a number of compounds, namely PRL, SFM, TCS, DCF and TCP (Figure 28). Rates of photolysis, however, were generally found to be more rapid in Wivenhoe Dam water, relative to ultrapure water, suggesting indirect photolysis was an important process. Exceptions to this were noted for SFM and TCS, where a slower rate of photolysis was noted in Wivenhoe Dam water. Also, some compounds, such as CPP, ATR and DIU, which underwent minimal photolytic degradation in ultrapure water had similarly low rates of photolysis in Wivenhoe Dam water (Figure 28). Rates of photolysis, k_{photo}, were generally less than 0.5 d^{-1}, corresponding with a half-life ($t_{0.5}$) in Wivenhoe Dam water of >1 d. However, for some compounds the rates were even more rapid, including for PRL ($t_{0.5}$=0.3 d), SFM ($t_{0.5}$=0.8 d), TCS ($t_{0.5}$=0.07 d), DCF ($t_{0.5}$=0.2 d) and TCP ($t_{0.5}$=0.22 d) had relatively rapid degradation rates under the exposure conditions. Previous studies have also found PRL (Boreen *et al.* 2003; Yamamoto *et al.* 2009), SFM (Andreozzi *et al.* 2003; Trovó *et al.* 2009), TCS (Boreen *et al.* 2003), DCF (Andreozzi *et al.* 2003; Boreen *et al.* 2003) and TCP (Woodburn *et al.* 1993) to be photolabile.

Addition of isopropanol reduced the rate of photolysis for a number of compounds to that found in ultrapure water, suggesting the hydroxyl radical (OH·) was the most important reactive species where indirect photolysis was noted (Figure 29). The reduction of the rate of photolysis for PRL and VEN after solutions were saturated with O_2 suggests that dissolved organic matter in its triplet state was partly responsible for their indirect photolysis. A previous study has also determined that triplet state DOM, along with OH·, is an important pathway for the photolysis of PRL (Chen *et al.* 2009). Data is not available for this component of the experiment for DCF, although previous work suggests direct photolysis is the predominant pathway for its photodegradation (Packer *et al.* 2003).

This demonstrates that, relative to ultrapure water, photolysis rates can be modified in the Wivenhoe Dam water, although this modification can be either an enhancement or reduction of the photolytic process. Based on the contaminants selected for this assessment, this modification of photolysis rates is likely to be contaminant-specific. Therefore, based on the difference in photolysis rates between ultrapure and Wivenhoe Dam water, factors indirectly affecting rates of photolysis, such as reactive chemical species that can enhance photolysis or species quenching radiation and reducing photolysis rates (Atkinson *et al.* 2011; Razavi *et al.* 2011), should be investigated further.

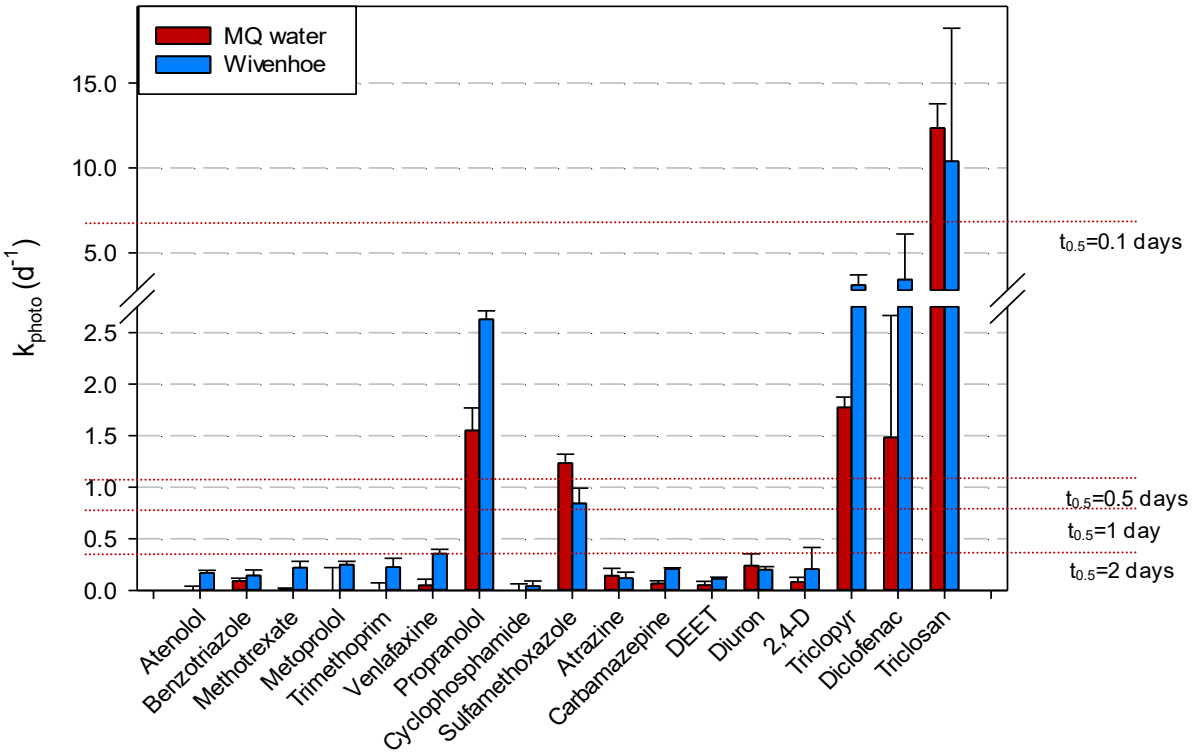


Figure 28. Photolysis rate constants (k_{photo}) determined for the contaminants in ultrapure (Milli-Q) water and water collected from Wivenhoe Dam for the first of the photolysis experiments; the lower plot has had the y-axis scale adjusted. Corresponding half lives (t0.5) are indicated by red dotted lines. Error bars represent one standard deviation of the mean.

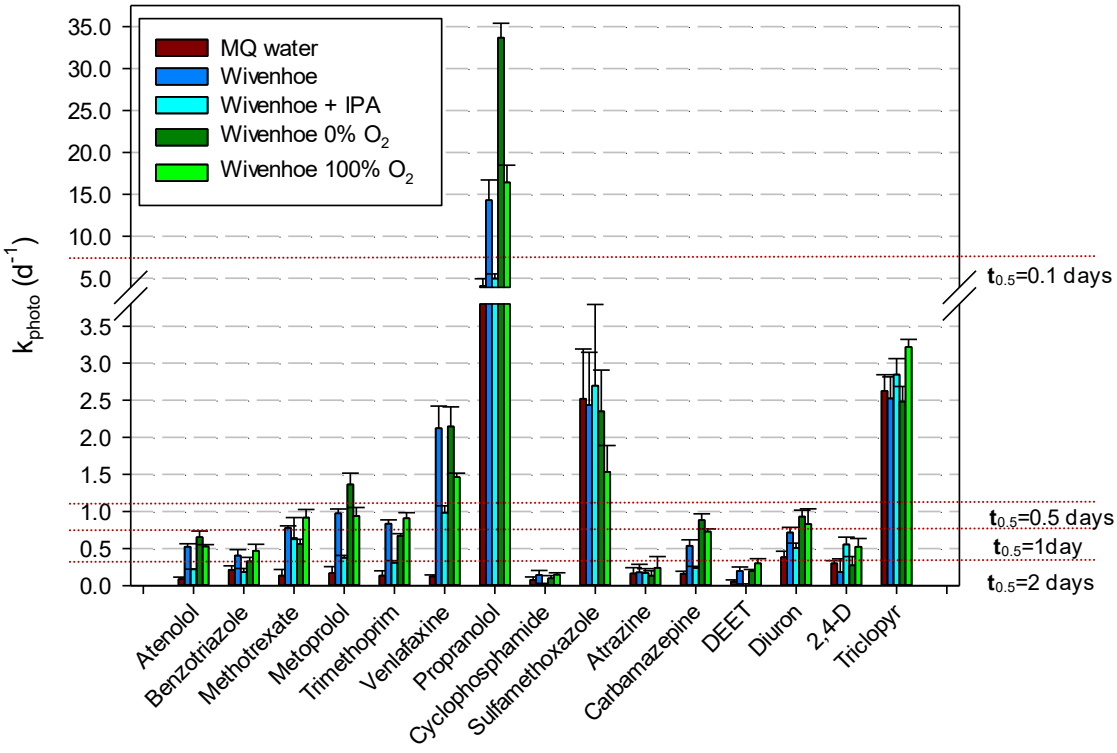


Figure 29. Assessment of photolysis rate constants (k_{photo}) for indirect photolysis processes in water collected from Wivenhoe Dam water collected for the first experiment. Treatments include ultrapure water (Mq), unamended Wivenhoe water (Wiv 20% O2), Wivenhoe water + 1% isopropanol (Wiv IPA), Wivenhoe water sparged with N2 (Wiv 0% O2) and Wivenhoe water sparged with O2 (Wiv 100% O2). The lower plot has had the y-axis scale adjusted. Corresponding half lives (t0.5) are indicated by red dotted lines. Error bars represent one standard deviation of the mean.

The second series of experiments is summarised in Figures 30 and 31 and Table 28 and 29 in Appendix 4. The major difference with the first component of photolysis experiments, was that the water collected from Wivenhoe Dam was around 6 months apart, with slight variations in water quality (see Table 15), and the irradiance was greater in the second component (nominally 750 W/m^2 compared with 500 W/m^2). Once again, the majority of the selected contaminants were resistant to photolysis with k_{photo} values less than 0.75 d^{-1} ($t_{0.5}$ >0.9 d) (Figure 30).

In general, the rates of photolysis in the natural water samples were unchanged relative to ultrapure water, or increased relative to ultrapure. Photolysis was again found to be important for PRL, SFM, TCP and DCF (no data was available for TCS in the second series of experiments). The values of k_{photo} was greater for DCF and PRL in natural water, compared with ultrapure water, as well as for ATL, MTX, VEN, ATR and DIU (Figure 30). This indicates that indirect photolysis was an important contributing factor to the photodegradation of these compounds, although the rates of photolysis were still relatively low for ATL, MTX, VEN, ATR and DIU (Figure 30). Also, the k_{photo} values for the contaminants were generally similar between natural water samples, although relatively slower rates were noted in Salisbury Wetland water samples for MTX, VEN and ATR. Conversely, k_{photo} was substantially reduced for SFM in the natural water samples. The k_{photo} value for TCP and 2,4-D, CBZ did not change in natural water relative to ultrapure water, while photolysis was not found to occur in the case of CPP under any conditions.

Based on the higher irradiance levels during the second photolysis experiments, it would be expected that photolysis rates would increase by a factor of 1.5 in the second component in the ultrapure water. However, the rates were found to be considerably higher in the second experiment than what would be predicted from the rates determined in the first experiment (Table 28, Appendix 4). In particular, the relative difference in the k_{photo} values in ultrapure water could not be reconciled by the nominal difference in the intensity of the experimental irradiance. This disparity may be partially related to the significance of the fit of the degradation plots used to derive the k_{photo} values for these contaminants.

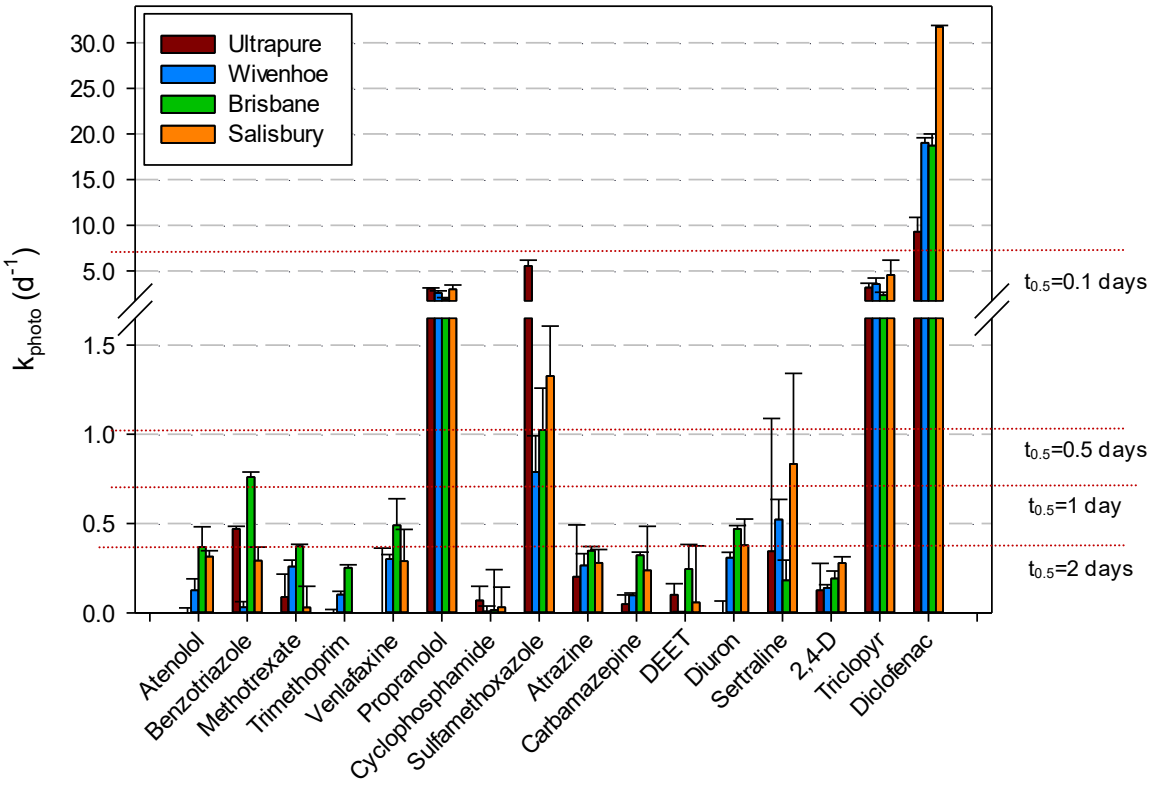


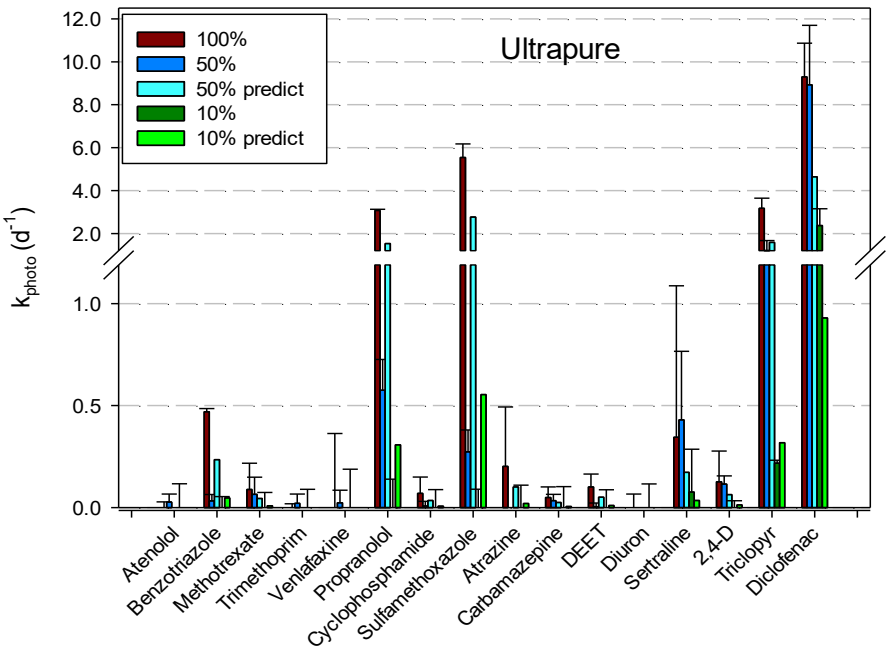
Figure 30. Photolysis rate constants (k_{photo}) determined for the contaminants in ultrapure (Milli-Q) water and water collected from Wivenhoe Dam, mid-Brisbane River and Salisbury wetlands for the second photolysis experiments; the lower plot has had the y-axis scale adjusted. Corresponding half lives ($t_{0.5}$) are indicated by red dotted lines. Error bars represent one standard deviation of the mean.

3.2.2. Effects of Water Depth on k_{photo}

Photolysis experiments were also conducted at two different water depths, corresponding with light attenuation in water of 50% and 90% (light transmission of 50% and 10%, respectively) based on absorbance readings of Wivenhoe and Brisbane river water (Figure 31). The experiment assessing light transmission of 50% was conducted at a depth of 3 cm and 1 cm for Wivenhoe and Brisbane River water, while the light transmission was reduced by 90% at a depth of 10 cm for Wivenhoe water. To allow for reduced rates of photolysis, experiments were run for 28 h (50% light transmission) and 48 h (10% light transmission). Results from the depth experiments are summarised in Table 29, Appendix 4.

Overall, there was good agreement between the rates of photolysis that were predicted (Figure 31) from a decrease in light transmission through interpolation of absorbance values of water measured on a spectrophotometer. That is, there was a corresponding decrease in k_{photo} values at the various experimental water depths, as would be expected from the predicted decrease in irradiance levels at that depth. Notable exceptions to this were seen for the photolabile contaminants, namely SFM, TCP, PRL and DCF, in Wivenhoe Dam water. For SFM k_{photo} were greatly overestimated, while k_{photo} values for PRL and DCF were underestimated (Figure 31). The k_{photo} values were the same in both 50% and 10% light transmission treatments for TCP (Figure 31), meaning there was both an under-estimation and over-estimation of k_{photo}. Photolysis rates for all other contaminants, which were generally resistant to photodegradation, were also overestimated in the mid-Brisbane River water treatments. Due to their resistance to photolysis, this would effectively mean the predicted minor importance of photolysis in their degradation would be even less important.

These results demonstrate that attenuation of light transmission would play a critical role in the relative importance of photolysis in the removal of the selected contaminants in natural water bodies. As was predicted from initial absorbance experiments of the natural water samples (Appendix 3, Figure 46), attenuation of solar irradiance occurs rapidly as the water depth increases and this has a corresponding effect on the k_{photo} of the selected analytes.



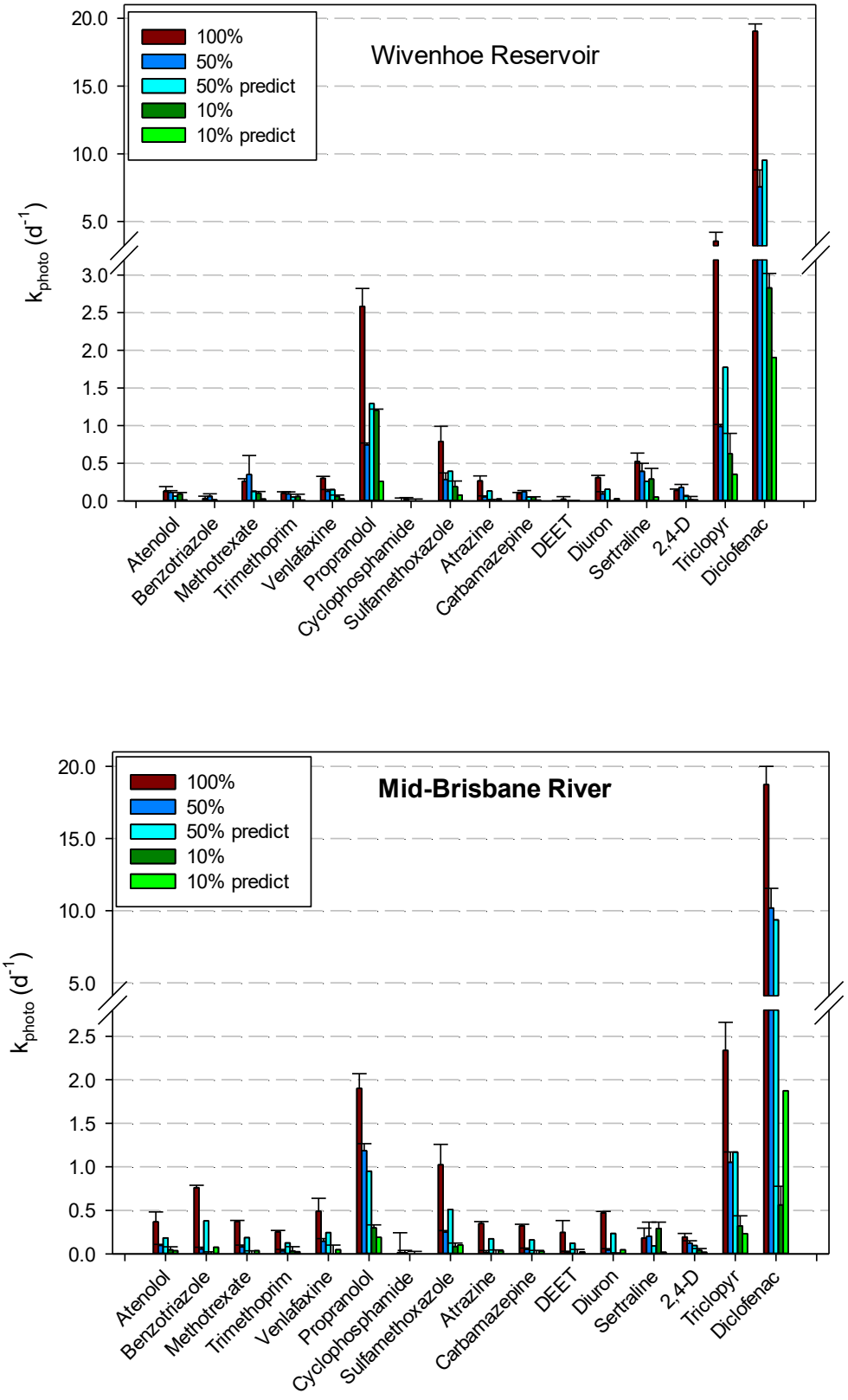


Figure 31. Photolysis rate constant (k_{photo}) values for selected contaminants at the surface and at light transmission levels of 50% and 10%, relative to surface levels, in ultrapure, Wivenhoe Dam and mid-Brisbane River water. Error bars represent one standard deviation of the mean value of triplicate samples.

3.2.3. Implications of Effects of Depth on Photolysis

The DOC present in the Wivenhoe Dam has the potential to form reactive species that can then react with the organic contaminants. However, the DOC can also be an important factor in the quenching of radicals generated through photolysis, thereby reducing the rate of indirect photolysis (Schwarzenbach *et al.* 2003). The comparison between photolysis rates in ultrapure water and Wivenhoe Dam water demonstrated, however, that photolysis rates in Wivenhoe Dam water were usually greater. Where photolysis was found to be greater in Wivenhoe Dam water, the reaction with OH· was likely to be the most likely contributor to the photodegradation of the trace organic contaminants. This was evident when addition of isopropanol reduced the rate of photolysis to that evident in ultrapure water, where only direct photolysis was occurring. For ATL, BZT, MET, TRM, CBZ and DIU, reaction with OH· was likely to have been the main pathway for enhanced photolysis rates in Wivenhoe Dam water, while OH· was likely to have at least partly contributed to enhanced photolysis rates of VEN and PRL. The decreased photolysis rate of VEN in Wivenhoe Dam water sparged with O_2, suggests that reaction of VEN with triplet state DOM was as important as reacting with OH. Conversely, the rates of photolysis of both SFM and TCP were unaffected by different treatments suggesting that direct photolysis was the most likely mechanism of their photolysis and, also, that the presence of DOM did not competitively reduce their respective rates of photolysis in Wivenhoe Dam water.

The estimated half-life ($t_{0.5}$) values (Table 28, Appendix 4) for a number of photosensitive compounds suggest that photolysis should be an important attenuation process, especially for PRL, SFM, TCP, DCF and TCS. The slight loss of the majority of the other compounds in the natural water samples also indicates that the generation of reactive species can contribute to their overall attenuation under environmental conditions. The attenuation of insolation in the natural water samples (see Figure 27), however, demonstrates that photolytic reactions are only going to be of minor importance at depths greater than 10 cm in any of the collected natural water samples. Furthermore, the photolysis rates decreased proportionally with light transmission levels, which decreased rapidly within this 10 cm zone in the Wivenhoe Dam water (Figure 31). Since the water samples were collected from sites where the majority of the water bodies are at a depth considerably greater than 10 cm, a rapid depth-dependent decline of the photolysis rates needs to be taken into account. Although Wivenhoe Dam water had the highest light transmission levels, a bulk water depth of 5 m (500 cm), for example, would reduce the effect of photolysis by a factor of 50 in a well mixed system. This effect would be expected to be more marked in the mid-Brisbane River water due its greater ability to attenuate light. However, these estimates do not take into account the temporal variation of WQ in the water bodies. Figure 32 summarises the monthly averaged diurnal variation in irradiance at four times over a year.

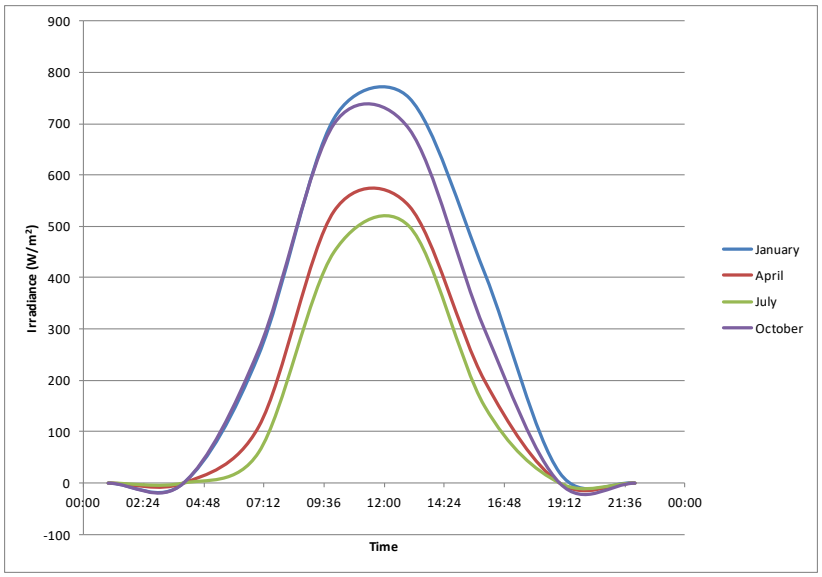


Figure 32. Diurnal trend of monthly averaged solar irradiance levels on a horizontal surface at Brisbane's latitude (27°) during spring (October), summer (January), autumn (April) and winter (July).

Figure 33 summarises a scenario for the photolabile compound DCF where depth effects (a factor of 50) and diurnal (factor of 5) and seasonal (factor of 1.2) variation of solar irradiance would be expected to increase its half-lives under environmental conditions. Table 30, Appendix 4 summarises the expected effect that these reasonably predictable environmental factors would have in decreasing the influence of photolysis in water bodies for all tested compounds. This table suggests that for the selected contaminants, only the photolabile DCF would not be considered to be very persistent (vP) in water columns, which is defined as having a half-life >60 days (European Parliament and European Council 2006). Even so, application of these additional environmental factors would be expected to increase the half-life of DCF from a few hours to more than one week (Figure 33), principally due to dilution of photolysis through depth effects.

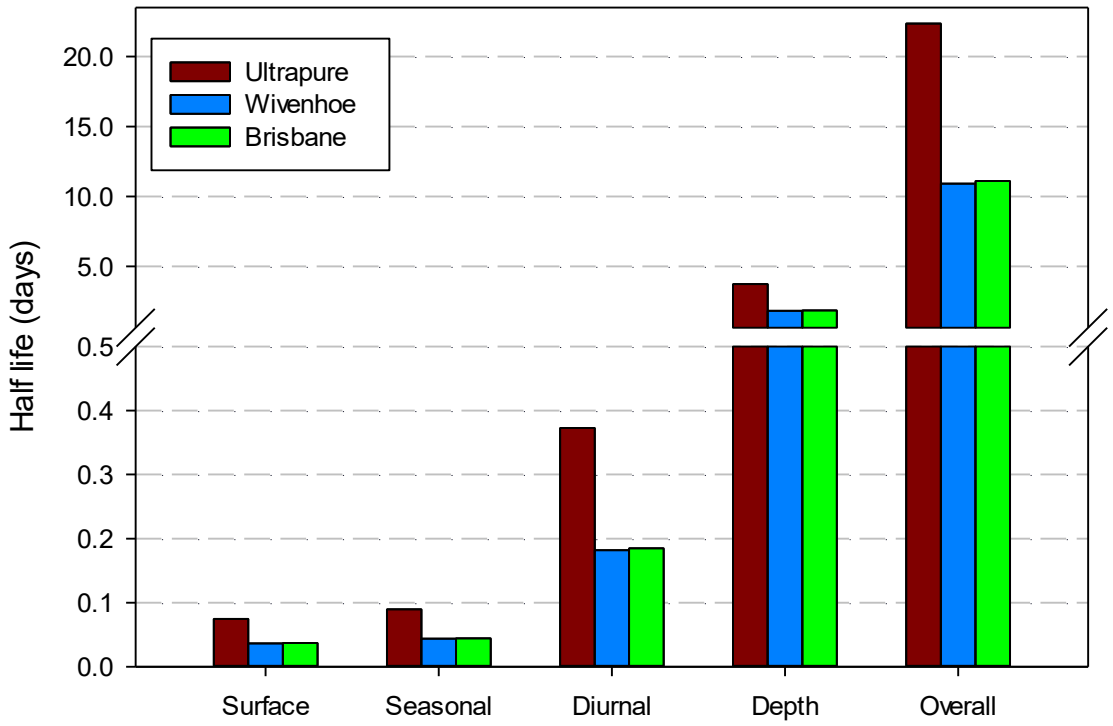


Figure 33. An example on the effects of environmental factors in the increase of the half life of diclofenac (DCF) at the surface of ultrapure (MQ), Wivenhoe reservoir and mid-Brisbane River, relative to half lives derived under laboratory conditions (surface). A decrease in irradiance during winter months (seasonal) would increase half-lives by a factor of 1.2, diurnal variation (diurnal) in irradiance would increase half-lives by a factor of 5 and a well mixed water body of depth 5 metres where photolysis occurred in the top 0.1 metres would increase half-lives by a factor of 50. The combination of these factors would be expected to increase overall half-lives by a factor of 300.

While it was demonstrated that the majority of indirect photolysis was probably due to the reaction of the selected contaminants with OH·, the effect of depth on the rate of indirect photolysis was not assessed in this study. It is known, however, that indirect photolysis of a range of organic compounds, including contaminants and dissolved organic matter, through reactions with OH· are generally rapid and tend to approach the rate of a diffusion-limited reaction (Schwarzenbach *et al.* 2003). That is, OH· is likely to react rapidly after its formation in the very upper surface of the water bodies before it can be transported to greater depths through processes such as diffusion. The extent of this rapid reaction in the upper layers is especially dependent on the levels of DOM that can react with OH·, effectively acting as a quenching agent. The importance of OH· as a reactive species is therefore likely to be limited to a depth similar to that predicted for direct photolysis. In a large, deep body of water, such as Wivenhoe Dam, the extent of mixing and, therefore, dilution would be expected to be greatest in winter where greatest mixing of the epilimnion and hypolimnion can occur.

Further mitigating the effectiveness of photolysis is the seasonal reduction in intensity of insolation, which is approximately 1.5 times less in mid-winter (Figure 32). This seasonal variation at the latitude of Brisbane was accounted for in the experimental set-up with the irradiance levels set on the solar simulator within the seasonal range. Along with variation in seasonal intensity of sunlight, diurnal variation needs to take into account the experimental conditions, where photolysis rates were undertaken at constant irradiance levels. That is, the estimates of k_{photo} and corresponding half lives are under these constant exposure conditions. Taking this into account, the maximum irradiance values used for the solar simulator will occur for approximately 3 hours around midday, with >80% of this maximal value also occurring for around 3 hours per day and >50% of this maximal value occurring for around 2 hours a day throughout the year. This approximates to maximal solar irradiance occurring for only 5 hours per day (or one fifth of a day) under cloudless conditions. Under environmental conditions, the estimated $t_{0.5}$ values would therefore be at least five times slower at the surface of a water body, relative to the $t_{0.5}$ values estimated from k_{photo} determined under laboratory conditions (Table 28 and 29, Appendix 4).

3.2.4. Use of Water Quality Measurements to Predict Photolysis Rates under Field Conditions

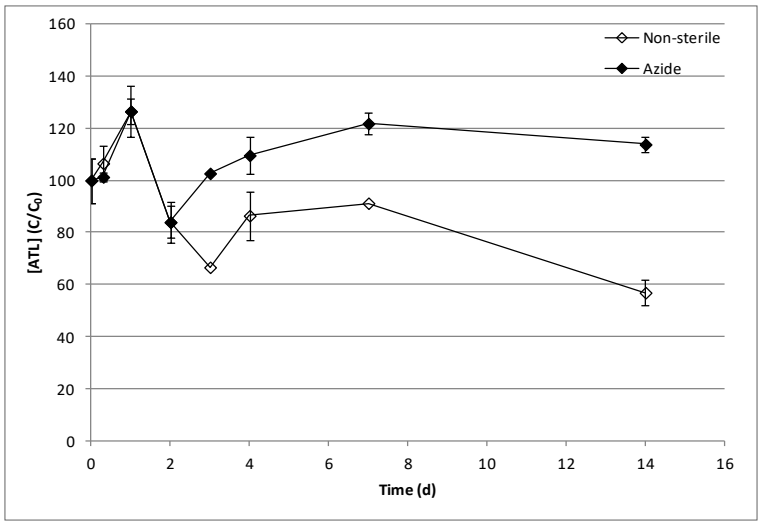
Although photolysis, both directly and indirectly, has been determined in laboratory exposures to be important for a number of contaminants, mitigation of photolysis through increasing depth is likely to reduce this degradation pathway substantially. The reduction in effectiveness in the extent of photolysis at depth, based on relative rate constants, was well predicted from the relative absorbance of water samples, where an increase in absorbance led to a decrease in photolysis rates with increasing depth. This effect of absorbance at depth was seen despite the differences in water quality parameters of Wivenhoe Dam and mid-Brisbane River water (see Table 15). Measuring the absorbance of field collected water with a UV-Visible spectrophotometer offers a cheap and rapid means of estimating the extent of light attenuation in a water body and, therefore, the extent of "dilution" of photolysis. Although measuring absorbance does not provide a means of estimating whether a contaminant will undergo photodegradation, this study demonstrates that understanding the light-mitigation potential of water can give a factor by which photodegradation (if it occurs) can be reduced in a body of water. For example, water collected from the Wivenhoe Dam was estimated to reduce effectiveness of photolysis by a factor of 50, with a water depth of 5 metres. Monitoring of temperature at various depths would also provide a rapid means of determining the extent of stratification, or layering of water due to temperature variations, which can prevent mixing of the bulk water. For example, stratification of lake water can reduce mixing, reducing the dilution of photolysis of photolabile drugs and making photolysis more important for pharmaceuticals present in the upper layer, or epilimnion (Poiger *et al.* 2001). These factors could then be combined with the seasonal variation in irradiance, which gives a more realistic estimate of exposure periods and intensity, to give an estimate of the likely extent of reduction in the importance of photolysis as a process. This knowledge can then be applied to photodegradation rate constants derived from laboratory exposures or from literature to provide a more realistic rate constant.

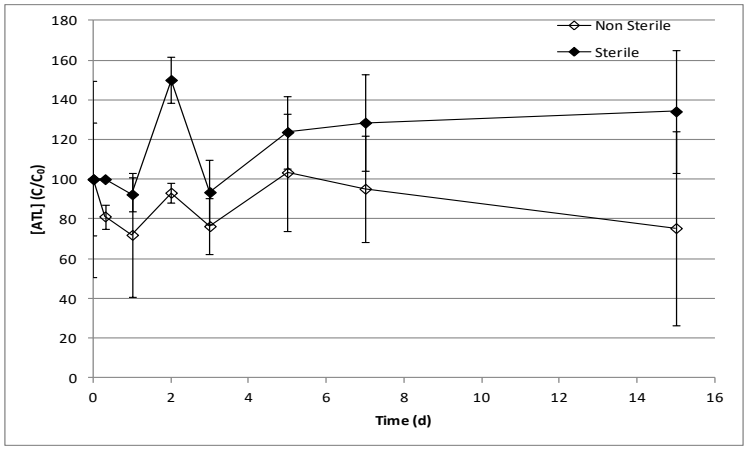
A number of water quality parameters are also known to have an influence on indirect photolysis, such as NO_3^-/NO_2^- and Fe^{2+} ions, and DOM, and direct photolysis, such as pH, (Schwarzenbach *et al.* 2003) and it is therefore important to monitor their concentrations in solution if predictions of photolysis rates are to be transferred from laboratory exposures to field conditions. However, these measurements can be more difficult to obtain rapidly, relative to absorbance and temperature, in terms of time and costs. Monitoring more laborious water quality parameters may be more important when the fate of specific contaminants is required and the influence of these parameters on the specific contaminants has been previously demonstrated in the laboratory or in available literature. Caution should be employed when using literature obtained rate constant values, as baseline rates of photolysis were found to vary between water samples due to the influence of indirect photolysis processes.

3.3. Biodegradation

Changes in the relative concentrations over time are given in Figures 47,48 and 49 in Appendix 5 for all contaminants assessed in the biodegradation study. Generally, there was little degradation apparent for the selected contaminants during the experimental period. One exception to this was ATL, which was around 50% of its original value after 14 days within the non-sterile Wivenhoe Dam and the amended Salisbury Wetland water microcosm (Figure 34). This is in contrast with the plots typically seen for most compounds, for example ATR (Figure 35). Two of the selected contaminants, TRM and MTX, were not assessed in Wivenhoe Dam water but were found to degrade in the Salisbury Wetland water treatments (Figures 36 and 37). It is also possible that some degree of degradation of TCP and TCS had occurred at day 21 of the amended Salisbury Wetland water samples, although there was a large degree of variability associated with these samples. MTX degraded rapidly in unamended ($t_{0.5}$ = 1 day) and amended treatments ($t_{0.5}$ = 0.4 days DOC and $t_{0.5}$ = 1.7 days for the inoculum) while TRM only degraded appreciably in both amended Salisbury treatments ($t_{0.5}$ = 9 days for the inoculum and $t_{0.5}$ = 6 days). There was at least a 20% loss in the TRM control samples, while more than 30% of MTX was degraded in the unamended Salisbury water samples suggesting sterility was also not maintained following autoclaving. No data was available for MTX in the control samples at day 21 in the amended Salisbury experiments, due to poor recovery, and further loss at this final time point in the control sample cannot be excluded. The degradation of MTX in the non-sterilised treatments, however, was quite rapid relative to controls, indicating biodegradation is likely to be an important loss pathway for MTX. It is worth noting that the Salisbury Wetland water treatments had a large degree of variability in both the sterile and non-sterile treatments, particularly at day 14, making clear distinctions between control and treatments difficult. This was also noted for DEET, 2,4-D and DCF in all treatments (Figure 48, Appendix 5).

The positive control, aniline, was found to degrade rapidly between day 4 and 7 (Figure 49, Appendix 5), which demonstrated that there was sufficient microbial activity within the system to degrade susceptible compounds. Although the rapid rate of degradation noted for aniline may have been due to its relatively high concentration in solution, it demonstrated that there was sufficient microbial activity within the system to degrade this biologically labile compound. However, aniline was also degraded within a similar time-frame for the NaN_3 treatment, suggesting that 100 mg/L of NaN_3 within the Wivenhoe Dam water was insufficient to suppress microbial activity. Despite this, the difference in levels of ATL in the NaN_3 treated water and untreated water were apparently sufficient to prevent biodegradation of the contaminants at a lower concentration during the experimental period. Other sterilisation techniques, such as autoclaving, have potential advantages over NaN_3 although a comparison of NaN_3 and autoclaving demonstrated that neither could effectively prevent degradation of β-blockers in a water/sediment system (Ramil *et al.* 2010). Despite this, autoclaving was employed for sterilisation of the Salisbury wetland microcosm control samples primarily due to the issues relating to the handling and disposal of NaN_3. Also, the electrical conductivity was significantly higher in the NaN_3-amended treatment due to the relatively high ionic strength of the NaN_3. Other water quality parameters, including pH and dissolved oxygen concentrations, measured in the biodegradation experiments show little observable change occurred in all treatments (Appendix 4). Dissolved oxygen was not found to change during the experimental period in Wivenhoe Dam and the amended Salisbury Wetland water treatments, while it decreased slightly in the unamended Salisbury wetland water after 15 days incubation.





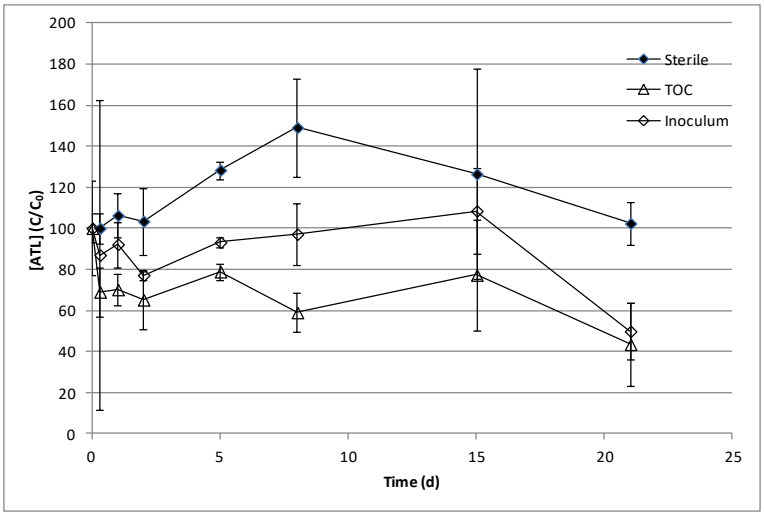
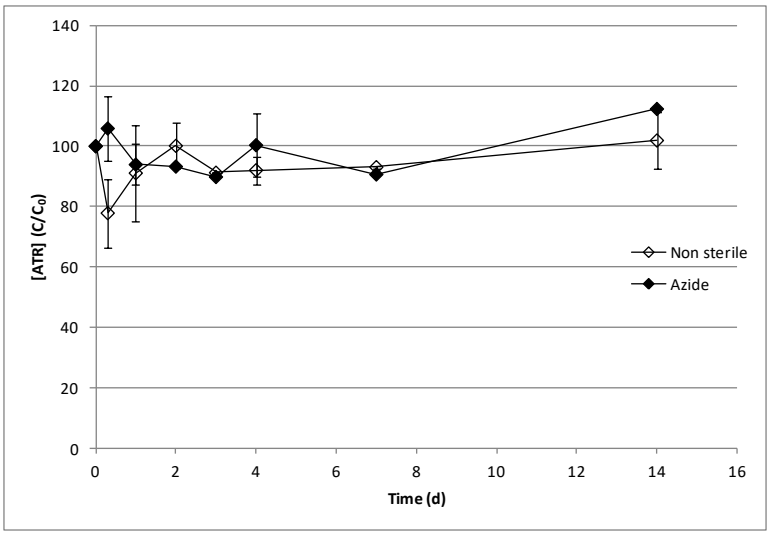
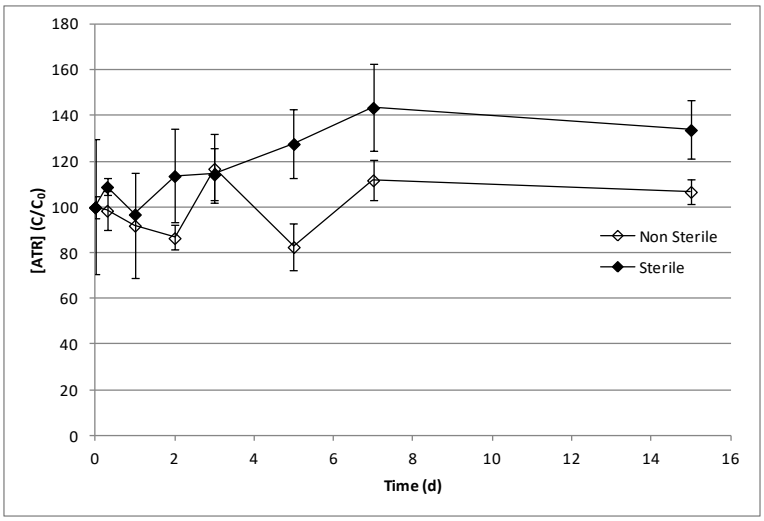


Figure 34. Concentrations relative to initial concentrations (C/C_0) of atenolol (ATL) in Wivenhoe Reservoir, Salisbury Wetland and amended Salisbury Wetland water. In Wivenhoe Reservoir water, azide ions were used to inhibit microbial activity, while autoclaving was used for sterilisation (ST) of controls. Other amendments to Salisbury wetland water included addition of extra organic carbon (TOC) or a microbial inoculum (INO). Error bars represent one standard deviation of the mean.





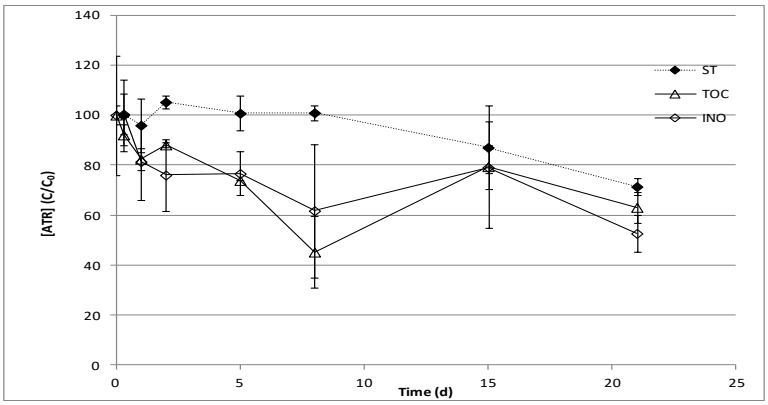


Figure 35. Concentrations relative to initial concentrations (C/C_0) of atrazine (ATR) in Wivenhoe Reservoir, Salisbury wetland and amended Salisbury wetland water. In Wivenhoe Reservoir water, azide ions were used to inhibit microbial activity, while autoclaving was used for sterilisation (ST) of controls. Other amendments to Salisbury wetland water included addition of extra organic carbon (TOC) or a microbial inoculum (INO).

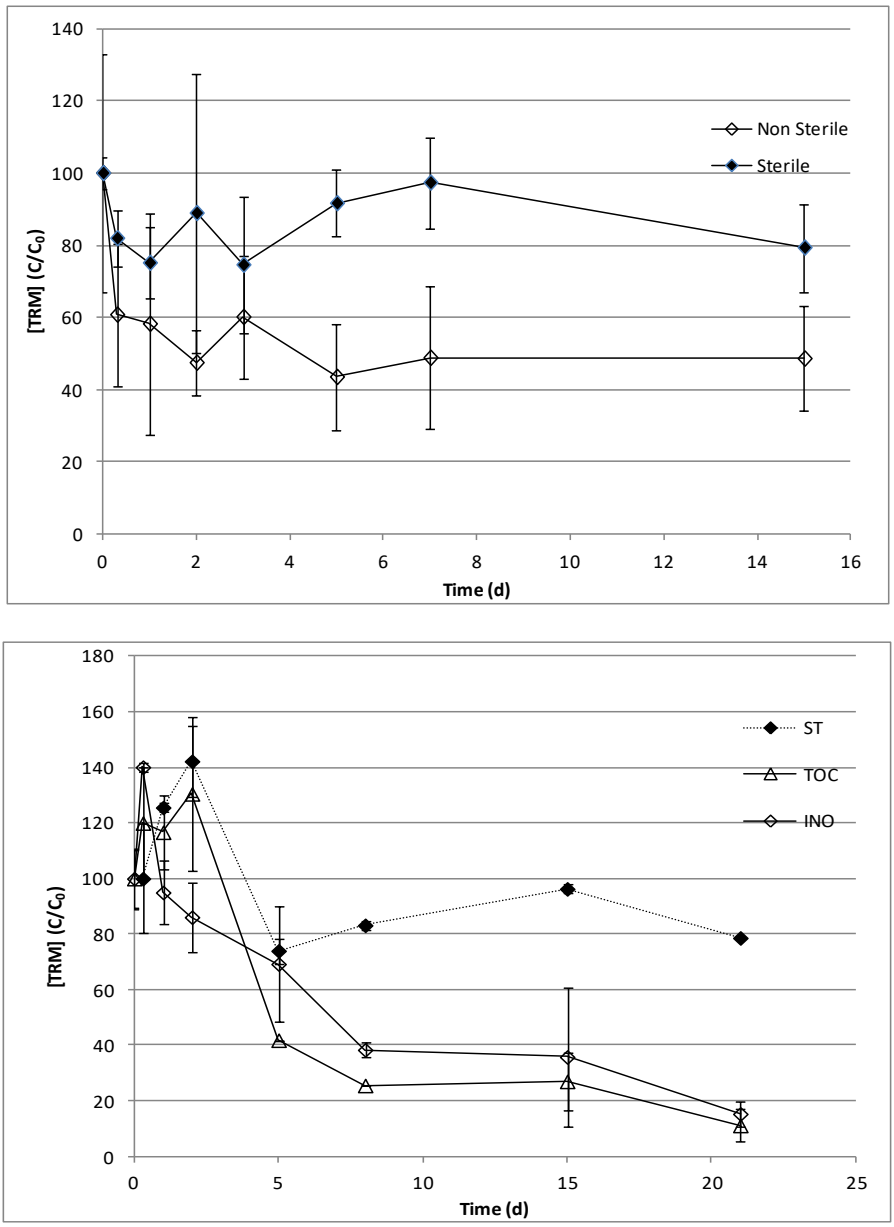


Figure 36. Concentrations relative to initial concentrations (C/C_0) of trimethoprim (TRM) in Salisbury Wetland and amended Salisbury Wetland water. Autoclaving was used for sterilisation (ST) of controls, while other amendments to Salisbury Wetland water included addition of extra organic carbon (TOC) or a microbial inoculum (INO). Error bars represent one standard deviation of the mean.

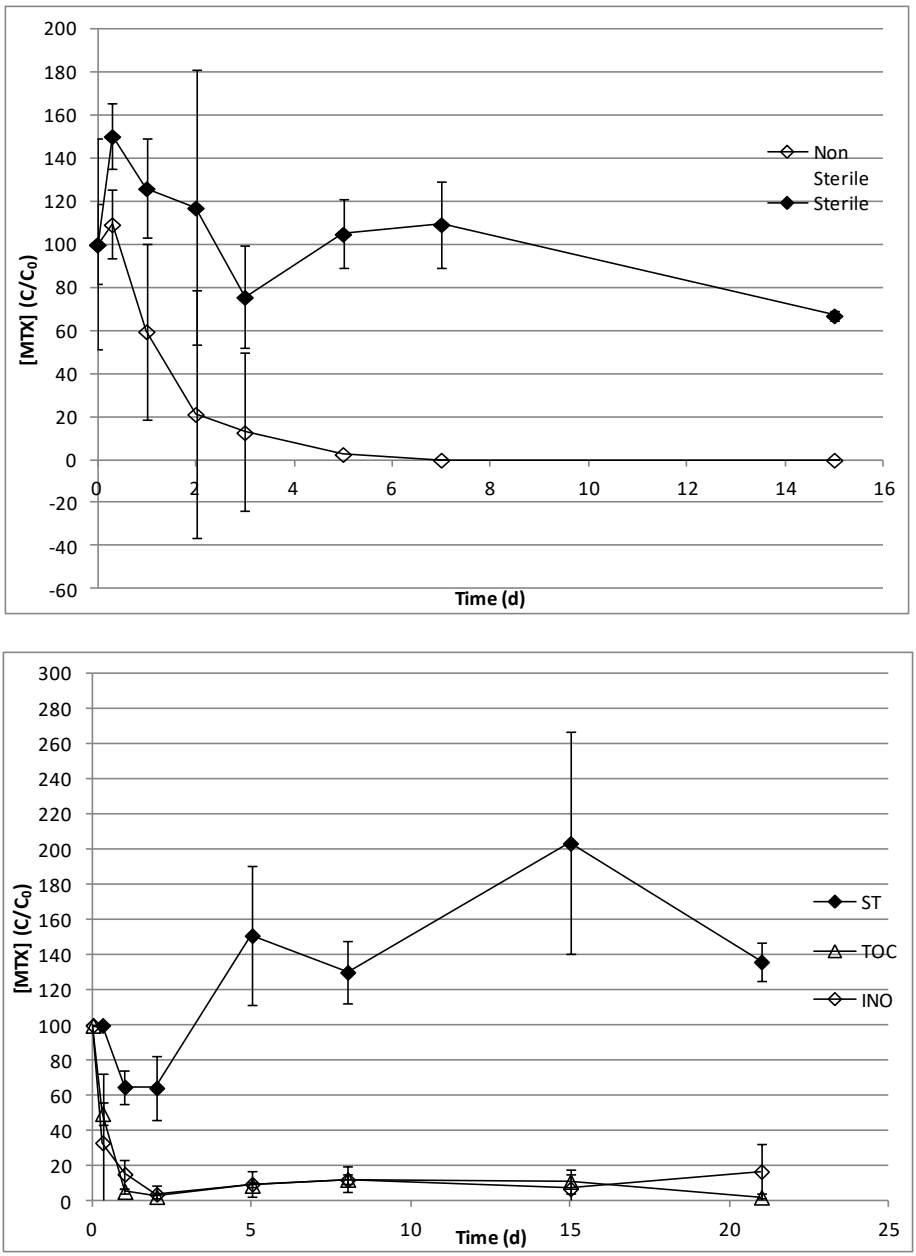


Figure 37. Concentrations relative to initial concentrations (C/C_0) of methotrexate (MTX) in Salisbury Wetland and amended Salisbury Wetland water. Autoclaving was used for sterilisation (ST) of controls, while other amendments to Salisbury Wetland water included addition of extra organic carbon (TOC) or a microbial inoculum (INO). Error bars represent one standard deviation of the mean.

The stability of a number of these contaminants was expected with CBZ, ATR and DEET, which have been previously found to be persistent to microbial degradation in freshwater systems (Kolpin and Kalkhoff 1993; Lam *et al.* 2004; Kagle *et al.* 2009). PRL, another β-blocker assessed in this study, has been found to be both more susceptible to degradation than ATL (Yamamoto *et al.* 2009) and less susceptible to degradation than ATL (Ramil *et al.* 2010). Also, it has been found to be sensitive to biodegradation in wetland water relatively rapidly, with a degradation rate constant of 0.08 d^{-1} giving a half-life of around 8 days (Fono and Sedlak 2005) PRL would therefore have been expected to have degraded to some extent within this system. The loss of PRL relative to control samples in all systems, however, was apparently minimal (Figures 47 and 48, Appendix 5), although there was a high degree of variability associated with the Salisbury wetland water samples making differentiation between

treatments difficult. The biodegradability of antineoplastic agents, such as CPP and MTX, are considered to be resistant to biodegradation (Kummerer 2004). An assessment of biodegradability of CPP and MTX under simulated wastewater treatment plant conditions, at low mg/L concentrations demonstrated that CPP is resistant to biodegradation while all of MTX was degraded under these conditions (Kiffmeyer *et al.* 1998). While the exposure conditions of this study are unrealistic in the context of environmental biodegradation in surface waters, it indicates that CPP would be highly unlikely to degrade under realistic environmental conditions, MTX could be susceptible under ideal conditions. TRM has also been determined to be susceptible to degradation within a microcosm system (Lam *et al.* 2004), although this is likely to be system-specific since it has also been found to be highly resistant in seawater microcosms (Benotti and Brownawell 2009).

A total of, at most, 17 contaminants were spiked to the microcosms at around 100 ng/L, indicating the relative levels of carbon contributed by the contaminants was only a fraction of the measured BDOC. The concentration of DOC measured both in-house and independently (Australian Water Quality Centre, SA Water) showed concentration of biodegradable DOC (BDOC) of the Wivenhoe Dam water was low relative to the total DOC. Initial DOC measured in-house was 9.4±1.1 mg/L and a final value of 9.3 mg/L, giving an experimental BDOC of 0.1 mg/L (Figure 38). An independent analysis gave an initial DOC value of 7 mg/L and a final DOC of 5.6 mg/L, giving an experimental BDOC of 1.4 mg/L. Similarly, BDOC measured in-house for unamended Salisbury Wetland water was low (1.1 mg/L) and confirmed through independent analysis (1.3 mg/L). Although the levels of BDOC were relatively low, a study using diluted effluents giving a BDOC of 0.7 mg/L over a one week period showed, for example, rapid degradation of ATL (Lim *et al.* 2008). However, this study also demonstrated that the quality of BDOC, as well as the amount of BDOC, is important in determining degradation rates of contaminants. This is supported by the levels of BDOC in the amended Salisbury Wetland water treatments, where there was 7.8±0.7 mg/L of BDOC present in the DOC-amended and 3.4±0.9 mg/L in the microbial inoculum-amended treatments (Figure 38). Despite the higher levels of BDOC within the system, there was little observable degradation for the majority of the selected contaminants

While Salisbury wetland water is not necessarily representative of the water samples collected from Queensland, it does highlight that even with a DOC at the upper limits of the normal freshwater range of DOC or addition with an inoculum with high diversity and numbers of microbes, biodegradation could not be induced over a two week exposure period. It is, therefore, considered unlikely that a similar amendment within the water collected for the second photolysis experiment from the Wivenhoe Dam and mid-Brisbane River is likely to occur. Alternatively, if biodegradation was able to occur in amended treatments, it is considered unlikely to occur in a manner that would be predictable based on previous experiments or reports in literature. In other words, biodegradation is unlikely to be an important short term attenuation pathway for the selected compounds or, where biodegradation did occur, it would not be a predictable process between different freshwater systems.

A previous modelling study assessing the fate of trace organic contaminants in Wivenhoe Dam predicted that the residence time of these contaminants in the water column would be 21 days or greater, when biodegradation was the most likely means of attenuation (Hawker *et al.* 2011). That is, the microcosms in this study are not likely to be representative of field conditions due to the length of exposure being limited to a maximum of 21 days. Within the microcosms, the BDOC was found to be largely used up within this experimental time-frame, suggesting that increasing the length of exposure in the microcosms would not have effectively reflected environmental conditions where nutrients are continually replenished. Considerably larger microcosms/mesocosms have been previously used to assess the fate of organic contaminants, including pharmaceuticals (Lam *et al.* 2004; Sanderson *et al.* 2007) and are less likely to be compromised by changing system conditions that can be observed in smaller systems, such as BDOC (Lim *et al.* 2008). Furthermore, to assess a specific system, such as the Wivenhoe Dam, larger scale microcosms would need to be sited close to the source water, to minimise changes in water quality due to transport, or, ideally, to conduct *in situ* microcosm within the system itself. Although located within the system, these microcosms would need to be isolated from the bulk water system so that movement of water into the microcosms or movement of contaminants out of the microcosms would not occur.

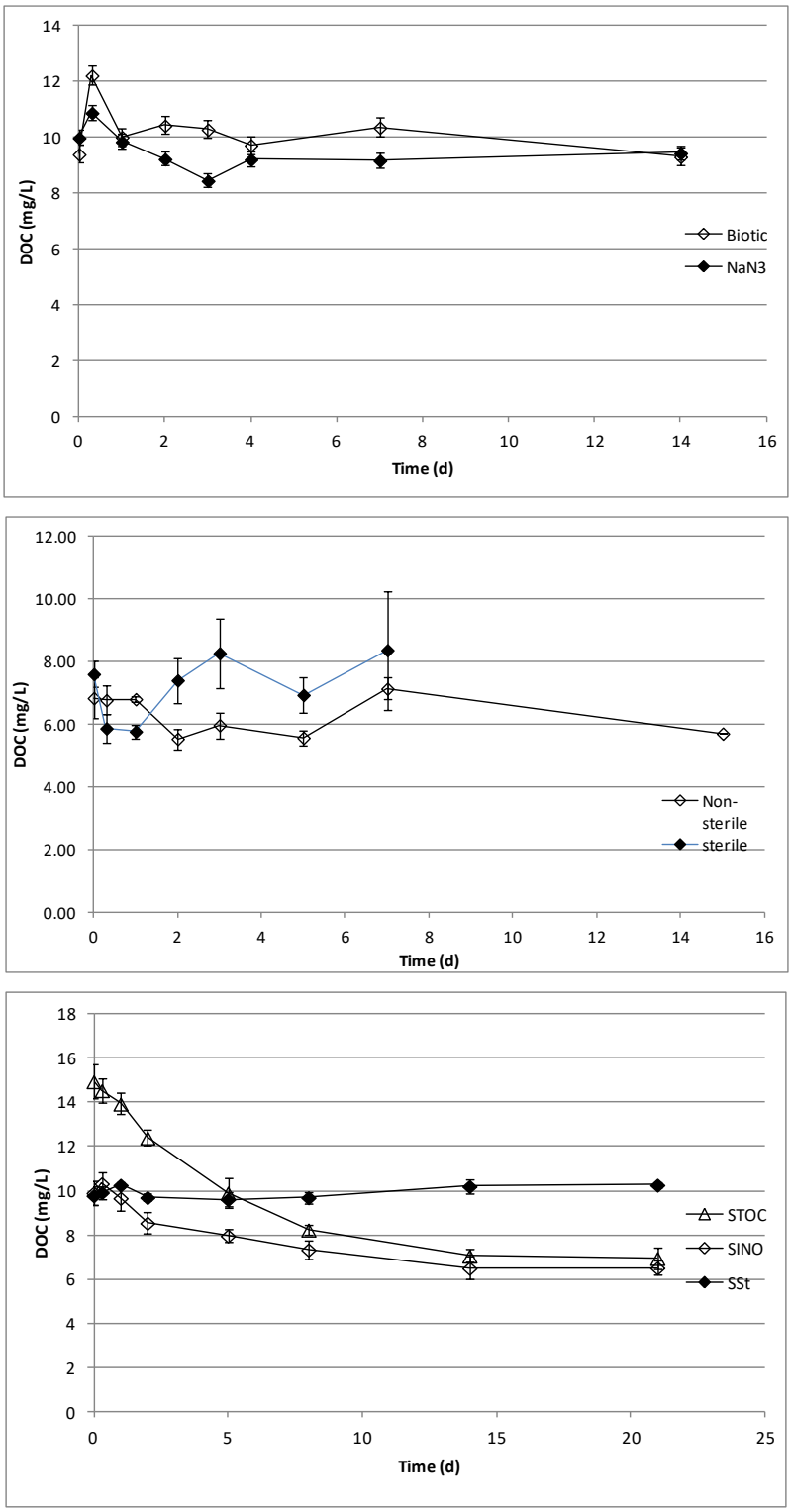


Figure 38. Concentrations of biologically available dissolved organic carbon (BDOC) measured within triplicate samples of Wivenhoe Reservoir (A), Salisbury Wetland (B) and amended Salisbury wetland (C) water microcosms during the biodegradation experiment. Error bars represent one standard deviation of the mean.

3.4. Conclusions

Two potential attenuation pathways, photolysis and biodegradation, were assessed for a range of trace organic contaminants. Biodegradation was found to be of minor importance for Salisbury Wetland water, even where an additional quantity of BDOC or a microbial inoculum was added, with the exception of the pharmaceuticals methotrexate (MTX) and trimethoprim (TRM). In contrast, photolysis was found to be an important degradation pathway for a number of compounds, particularly propranolol (PRL), sulfamethoxazole (SFM), diclofenac (DCF), triclosan (TCS) and triclopyr (TCP). Indirect photolysis, most likely due to the reaction with the OH· species, also led to the degradation of the majority of the test micropollutants, with half lives ($t_{0.5}$) ranging from a few days to a few hours. The high degree of light attenuation in all the natural waters, however, is likely to limit the importance that solar irradiance has on micropollutants that exist within the tested water systems. The degree of photolysis will be highly dependent on a number of potentially transient water quality parameters, such as turbidity (reducing effectiveness of photolysis) or concentrations of precursors of reactive species (such as OH·, $^1O_2$ or triplet DOM) and agents that can quench reactive species (such as DOM quenching OH·). Consideration of the relationship between light attenuation and water depth should be taken into account when estimating the importance of photolysis of organic contaminants in large bodies of water. The other important factor to account for is the predictable effect of diurnal variation of insolation on baseline rates of photolysis measured in the laboratory. Also, based on the physicochemical properties of a number of these compounds (such as high K_{ow} values or presence of ionisable functional groups) other potential attenuation processes, such as sorption, and the factors which can influence their importance as an overall process should also be considered when assessing the loss of trace organic contaminants within these water systems.

In summary:

- Photolysis could be important for the removal of contaminants within the Wivenhoe Dam.
- A number of factors may reduce the rate of photolysis, such as reduction in intensity of incident solar radiation; for example, increasing depth would be expected to contribute to this reduction, as well as other processes such as sorption to organic matter.
- Indirect processes apparently enhanced the rate of photolysis for a majority of the tested contaminants, although this contaminant-specific variation in photolysis rate requires further assessment, particularly relating to the relationship depth might have on the extent of its mitigation.
- Biodegradation was of minor importance despite the addition of extra BDOC and a microbial inoculum.

Other processes such as sorption to suspended solids within the water column or into the bulk sediment should be assessed to determine their potential effect on the biodegradation and photolysis within the water column. Furthermore, scaling up of the study presented here, through spiking closed microcosms within the respective water bodies would provide additional confirmation of the findings presented here.

4. MICROBIAL SOURCE TRACKING

This component of the project was divided into two sections. The first involved the study into the potential use of the *E. coli gus*A gene for the source tracking of a number of *E. coli* isolates from a two Seqwater catchments (Baroon Pocket and Somerset). The second focused on testing the specificity and sensitivity of three bovine markers using Wivenhoe Dam as the study site.

4.1. Methods

4.1.1. *E. coli* β-Glucronidase Gene

The potential origin of 442 *E. coli* isolates from Somerset, Baroon Pocket, and Wivenhoe Dams were estimated using the sequence analysis of the β-glucuronidase gene. A fragment of the gene 518 base pairs (bp) in length consisting of 59 variable points was employed here to distinguish differences between isolates. The feasibility and biological likelihood of using this particular segment of the gene was previously demonstrated in studies by Stratton *et al.* (2008) in which human and animal sources were identified with an acceptable margin of error supporting further investigation into the method. Since then the library has been extended to include a total of 196 isolates from known sources, including 103 from human faecal and urinary samples and 96 from local wild and domestic animals. Sequence alignment of the targeted 518 bp fragment in isolates derived from the faeces of the same animal or human source showed very little, if any, variation. As identical sequences from the same faecal sample are presumed to be clones, these sequences were discarded from the library to avoid any inaccurate statistical bias (USEPA, 2005). From those groups of identical sequences, only one sequence was used as a reference in the final analysis, thus reducing the size of the library of known isolates from 393 to 196. Although this library represents a significant number of known sources, other studies attempting library dependant sequence analysis for source tracking use much larger library sizes. Ram *et al.* (2004), who pioneered the use of the β-glucuronidase gene for source tracking, successfully discriminates between *E. coli* derived from different animals used between 8 and 63 isolates from each source host. The size of a library necessary to successfully identify host sources is yet to be determined and we are continuing to build the database of known isolates to improve the reliability of the data presented here.

4.1.1.1. Sampling

Unknown Isolates

All water sampling from the Baroon Pocket and Somerset Dams was carried out by Seqwater. Several sites were selected during each sampling event. Allconnex were contracted to process the samples and confirm *E. coli* isolates from the samples. Up to five isolates of *E. coli* isolates were selected from each site, depending on how many were able to isolated. For the Baroon Pocket and Somerset Dam samples, confirmed isolates of *E. coli* on MFC plates were transported on ice to the laboratory at Griffith University, Nathan Campus. The MFC plates were checked for contamination and processed within 48 - 72 hours. The Wivenhoe Dam samples were processed at CSIRO and copies of the stored isolates were transported to the same laboratory at Griffith University. Isolates that were confirmed to be *E. coli* were sub-cultured into nutrient broth and incubated over night at 37°C. One ml of broth culture was then centrifuged at 6,000 RPM for 10 min and resuspended in fresh nutrient broth containing 30% v/v glycerol and immediately stored at -80°C until processed.

The Wivenhoe Dam isolates were part of a research project designed to correlate faecal indicators and potential zoonotic pathogens with bovine-associated markers. Water samples were collected by CSIRO on four separate events between November 2011 and April 2012 from nine different sites on Wivenhoe Dam (WD1-WD9) (Figure 39). Of the *E. coli* isolates cultured from these 36 samples, a total of 83 were positive for β-glucuronidase activity and therefore included in this study.

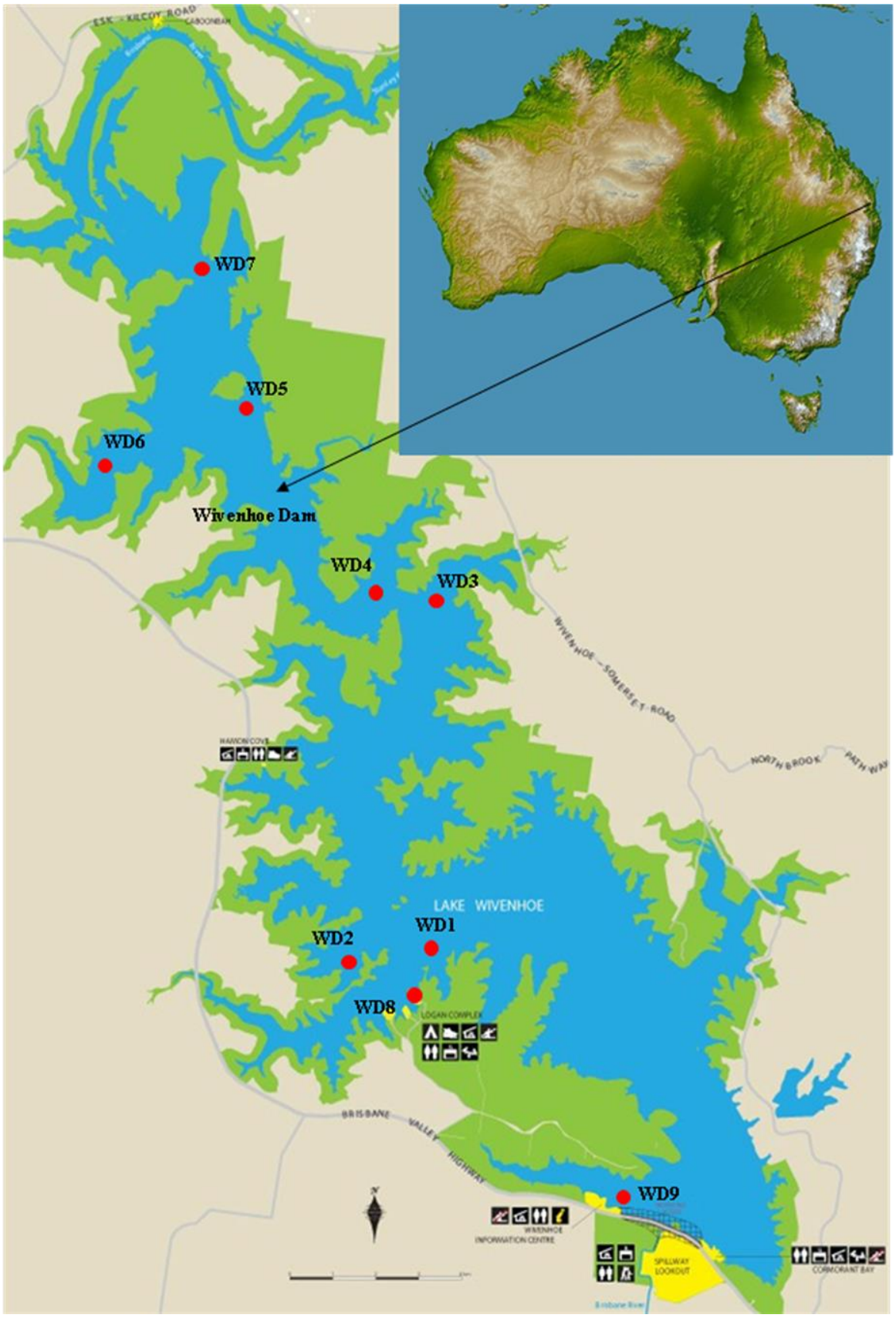


Figure 39. MST sampling points on Wivenhoe Dam.

Known Isolates

A database of 196 isolates from humans and animals were included in the sequencing of the β-glucuronidase gene 518 bp fragment and subsequent analysis. The human data from a previous study (Stratton *et al.* 2008) were included, as were all of the animals (see Figure 53, Appendix 6). Extra animal isolates from cattle, kangaroo, possum and bird faeces collected from the catchments were also included in the reference library. The additional animal isolates were processed at CSIRO and subcultured at Griffith University. Human faecal isolates were also added to the library database. Fresh faecal samples were collected from individual volunteers aseptically using eSwab collection system (Copan, Italia S.P.A). The faecal swabs were mixed in the buffer provided in the collection tube and 10 ul inoculations were applied to MFC agar plates. Ten isolates from each sample were acquired and processed at the Griffith University laboratory following the same protocol as isolates from the water samples outlined above.

4.1.1.2. PCR and Sequencing

The polymerase chain reaction (PCR) protocol for the β-glucuronidase gene assay was performed using whole bacterial cells. DNA extractions were not performed to reduce the time and expense of the research. To compensate for this an initial denaturation step of 98 °C for 10 min was added to the PCR protocol. This, coupled with freeze thawing, provided sufficient genomic DNA for a successful amplification of the 968 bp fragment of the β-glucuronidase gene. The primers used for amplification, sequencing and temperature cycles for each reaction of the β-glucuronidase gene have been previously described (Matthews *et al.* 2008). GoTaq Green Master Mix from Promega was used for PCR amplification. The DNA templates were loaded into a 1% Agarose gel for electrophoresis and the DNA products were purified using QIAquick Gel Extraction Kit from Qiagen. Primers were designed using the web-based resource Primer3 and all primers used throughout the study were obtained from Proligo and Invitrogen.

Sequence reactions were prepared in the laboratory at Griffith University using the purified DNA products and the forward and reverse primers. The sequence reactions were then transported to the Australian Genome Research Facility Ltd (AGRF) at the University of Queensland for routine sequencing on purified DNA using an AB3730x/ 96-capillary sequencer. All sequence files were edited using the BioEdit software package. The forward sequence and reverse complement of the reverse sequence were aligned for each sample to confirm the fidelity of the sequence. Multiple sequence alignments were performed using the ClustalX software package. Gap-only columns in the multiple sequence alignment were removed and final editing of the alignment was performed in BioEdit to yield an aligned 518 base pair fragment. Phylogenetic analysis was conducted using the MEGA software package. The neighbour-joining algorithm with maximum composite likelihood method was used to generate phylogenetic trees for both the known database as well as the knowns with isolates from each of the three catchments. Statistical analysis of each phylogenetic tree was undertaken using a bootstrap analysis with 1,000 replicates (re-sampling of the data).

4.1.1.3. β-glucuronidase Gene Genetic Analyses

The forward sequence and reverse complement of the reverse sequence were aligned for each sample to confirm the fidelity of the sequence. Multiple sequence alignments were performed using ClustalX (Larkin *et al.* 2007). Gap-only columns in the multiple sequence alignment were removed and final editing of the alignment was performed in BioEdit to yield an aligned 518 base pair fragment. Phylogenetic analysis involved the construction of phylogenetic trees using the Neighbour-Joining (NJ, Saitou and Nei 1987) method. The NJ tree was generated using MEGA version 5 (Tamura *et al.* 2007), employing the Maximum Composite Likelihood model/method and 1,000 bootstrap replicates. Principal Component Analysis was also performed on the final library database as a simple way to demonstrate any distinct clustering of the known isolates into animal or human groupings.

4.1.2. Bovine-Associated Microbial Source Tracking Markers and their Correlations with Faecal Indicators and Potential Zoonotic Pathogens

The primary objective of this study was to evaluate the host-specificity and -sensitivity of the bovine-associated bacterial (i.e., BacCan-UCD and cowM3) and viral (i.e., B-AVs) markers in faecal samples collected from various target and non-target host-groups in Brisbane, Australia. Environmental water samples were also collected from the Wivenhoe dam (WD) in Brisbane potentially affected by bovine faecal pollution. The WD water samples were also tested for the presence of BacCan-UCD, cowM3, and B-AVs markers using real-time PCR assays. In addition, water samples were also tested for the Faecal Indicator Bacteria (FIB) (i.e., *E. coli* and *Enterococcus* spp.) using culture-based methods and potential zoonotic bacterial pathogens (ie, *Campylobacter* spp., *E. coli* O157:H7, and *Salmonella* spp.) using real-time PCR assays. The occurrence of bovine-associated markers, FIB and bacterial zoonotic pathogens were used to identify the source(s) of faecal pollution and as well as to obtain information on the correlation among FIB, bovine-associated MST markers and zoonotic pathogens in Wivenhoe Dam.

4.1.2.1. Wivenhoe Dam (WD) Water Sampling Sites

Water samples were collected between November 2011 and April 2012 from nine different sites (ie, WD1-WD9) (see Figure 39). From each site, four samples were collected on four separate events giving a total number of 36 samples. Approximately 10 L water sample was collected from each site in 10-L sterile carboy containers (Nalgene Labware, Rochester, N.Y., USA) at 30 cm below the water surface. The water samples were transported on ice to the laboratory and processed within 6-8 h.

4.1.2.2. Primers, Probes and Positive Controls for Real-Time PCR Assays of Bovine-Associated Pathogens

For the real-time PCR detection of the bovine-associated markers and zoonotic bacterial pathogens, previously published primers and probes were used (Table 16). For BacCow-UCD, cowM3 and B-AVs real-time PCR assays, positive controls were isolated from cattle wastewater. Production of the positive controls is described in Appendix 7.

Table 16. Real-time PCR assays used in this study including the sequences for primers and probes.

Real-Time PCR Assays	Targets	Primer and Probe Sequences (5´-3´)	Amplicon Size (bp)	References
BacCow-UCD	16S rRNA	F: CCA ACY TTC CCG WTA CTC R: GGA CCG TGT CTC AGT TCC AGTG P: FAM-TAG GGG TTC TGA GAG GAA GGT CCC CC-TAMRA	177	Bernhard and Field 2000; Kildare *et al.*, 2007
cowM3	Sialic acid-specific 9-*0*-acetylesterase secretory protein homolog	F: CCT CTA ATG GAA AAT GGA TGG TAT CT R: CCA TAC TTC GCC TGC TAA TAC CTT P: FAM-TTA TGC ATT GAG CAT CGA GGCC-TAMRA	122	Shanks *et al.*, 2008
Bovine adenoviruses	Hexon	F: GRT GGT CIY TRG ATR TRA TGGA R: AAG YCT RTC ATC YCC DGG CCA F: ATT CAR GTW CCW CAR AAR TTT TTT GC R: CCW GAA TAH RIA AAR TTK GG ATC	641 430	Maluquer de Motes *et al.*, 2004
Bacteroides HF183	16S rRNA	F: ATC ATG AGT TCA CAT GTC CCG R: TAC CCC GCC TAC TAT CTA ATG	82	Bernhard and Field, 2000; Seurinck *et al.*, 2005
Campylobacter spp.	16S rRNA	F : CAC GTG CTA CAA TGG CAT AT R: GGC TTC ATG CTC TCG AGTT P: FAM-CAG AGAA CAA TCC GAA CTG GGA CA-BHQ1	108	Lund *et al.*, 2004
E. coli O157	*rfbE*	F: GCAGATAAACTCATCGAAACAAGG R: CGATAGGCTGGGGAAACTAGG P: TET-TCCACGCCAACCAAGATCCTCAGC-TAMRA	141	Nguyen *et al.*, 2004
Salmonella spp.	*invA*	F: ACA GTG CTC GTT TAC GAC CTG AAT R: AGA CGA CTG GTA CTG ATC GAT AAT	244	Chiu and Ou, 1996

4.1.2.3. Target and Non-Target Host-Groups Sampling

To determine the host-specificity and -sensitivity of the bovine-associated markers, faecal and wastewater samples (n = 130) were collected from 10 target and non-target host-groups. Individual cattle faecal samples (n = 20) were collected from various cattle farms, whereas composite cattle wastewater samples (n = 20) were collected from an abattoir located outskirts of Brisbane. Individual cattle faecal samples were not included in composite cattle wastewater samples. Bird faecal samples (n = 10) were collected from the City Botanical Garden adjacent to Queensland University of Technology in Brisbane, and Currumbin Wildlife Sanctuary Hospital in Gold Coast. The bird species include plover, crow, ibis, seagull, wood duckling, noisy miner, fantail cuckoo, rainbow lorikeet, crested tern, and topknot pigeon. Chicken faecal samples (n = 10) were collected from the backyard of a household and a chicken-processing farm in Brisbane. Dog faecal samples (n = 10) were collected from a dog park in the Sunshine Coast. Duck faecal samples (n = 10) were collected from areas adjacent to ponds and lakes in the Sunshine Coast region. Kangaroo faecal samples (n = 10) were collected from the University of the Sunshine Coast located in the Sunshine Coast. Pig faecal samples (n = 10) were collected from two pig farms in Brisbane. Possum faecal samples (n = 10) were collected from the rooftops of various households within Brisbane. Horse faecal samples (n = 10) were collected from a horse racecourse in the Sunshine Coast. Human wastewater samples (n = 10) were collected from the primary influent of two sewage treatment plants (STPs) in Brisbane. A fresh animal faecal sample was collected from the fresh defecation of individual animal. All samples were transported on ice to the laboratory, stored at 4ºC and processed within 6 h. DNA extract was undertaken as described in Appendix 7.

4.1.2.4. Isolation and Enumeration of Faecal Indicator Bacteria (FIB)

The membrane filtration method was used for the isolation and enumeration of FIB. Water sample serial dilutions were made and filtered through 0.45-mm pore size (47-mm diameter) nitrocellulose membranes (Millipore, Tokyo, Japan), and placed on modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC agar) (Difco, Detroit, MI, USA) and membrane- *Enterococcus* indoxyl-D-glucoside (mEI) agar (Difco) for the isolation of *E. coli* and *Enterococcus* spp. Modified mTEC agar plates were incubated at 35ºC for 2 hours to recover stressed cells, followed by incubation at 44ºC for 22 hours (US EPA, 1997) while the mEI agar plates were incubated at 41ºC for 48 hours (US EPA, 2002).

4.1.2.5. Sample Concentration and DNA Extraction

The composite human wastewater and cattle wastewater samples were concentrated and desalted with Amicon® Ultra centrifugal filters (Ultracel - 50K) (Millipore, Billerica, MA) as previously described (Ahmed *et al.* 2010). DNA extract was undertaken as described in Appendix 7.

Approximately, 9 L of water sample from each sample site on Wivenhoe Dam was concentrated by hollow-fiber ultrafiltration system (HFUS), using Hemoflow HF80S dialysis filters (Fresenius Medical Care, Lexington, MA, USA) as previously described (Hill *et al.* 2005). The method is described in detail in Appendix 7.

4.1.2.6. Quantitative Real-time PCR Assays

All of the bovine MST markers and pathogens were detected using real-time PCR. This included rigorous quality control to ensure that false positives through contamination or non-specific detections occurred; the evaluation of the potential inhibition of the PCR process which could impact on the detection or reduce the accuracy of quantitation of the PCR results resulting in a false negative or underestimation of the numbers of microorganisms present. Finally, an assessment of the limit of PCR detection (PLOD) for each of the pathogens was assessed to ensure accurate quantitation was obtained.

The detailed methodologies for all real-time PCR, quality control, PLOD and inhibition tests are given in Appendix 7.

4.1.2.7. Data Analysis

The host-specificity and -sensitivity of the BacCow-UCD, cowM3 and B-AVs markers were determined as: sensitivity = a/(a + c) and specificity= d/(b + d); where 'a' is true positive (samples were positive for the marker of its own species), 'b' is false positive (samples positive for the PCR marker of another species), 'c' is false negative (samples were negative for the marker of its own species),'d' is true negative (samples were negative for the PCR marker of another species) (Gawler *et al.* 2007). Pearson's correlation (r_p) was used to test the relationship between *E. coli* and *Enterococcus* spp. numbers in water samples.

Bayes' Theorem was used to calculate the conditional probability that the detection of BacCow-UCD and cowM3 markers in the WD water samples originated from cattle faeces rather than faeces from non-target host-groups. The following formula has been used to calculate the conditional probability (Kildare *et al.* 2007; Weidhaas *et al.* 2011).

$$P(H \setminus T) = \frac{P(T\setminus H)P(H)}{P(T \setminus H)P(H) + P(T \setminus H')P(H')}$$

Where:

P(H\T) is the probability (*P*) of cattle faecal pollution (*H*) in a water sample given a positive test result (*T*) for the sample.

P(T\H) is the true positive.

P(H) is the background probability of detecting a bovine-associated marker in a water sample.

P(T\H') is the false positive.

P(H') is the background probability that a bovine-associated marker was not detected in a water sample. The value of *P(H')* is 1 – *P(H)*.

A binary logistic regression analysis was also performed to obtain correlations between the presence of bovine-associated markers and FIB numbers (Minitab version 16, Minitab Inc., State College, PA). Binary logistic regression is a technique commonly used to model the binary (presence/absence) responses from water samples. The presence/absence of bovine-associated markers and zoonotic bacterial pathogens was treated as the dependent variable (i.e., a binary variable). When a marker/pathogen was present, it was assigned the value 1, and when a marker/pathogen was absent, it was assigned the value 0. Relationships were considered significant when the *P* value for the model chi-square was < 0.05 and the confidence interval for the odds ratio did not include 1.0. Greater odd ratios indicate a higher probability of change in the dependent variable with a change in the independent variable.

4.2. Results and Discussion

4.2.1. *E. coli* β-Glucuronidase Gene Analysis

Using the library of β-glucuronidase sequences from *E. coli* isolates of known sources, it was possible to distinguish between isolates derived from human and animal origins with reasonable certainty. Once identical sequences in the library were identified and reference sequences were established, the differences between the 196 sequences in the library of known source isolates were demonstrated using phylogenetic analysis. Although the sequences were very similar causing bootstrap values to be considerably low, the phylogenetic tree generated in MEGA using the library database consistently groups the same sequences together after multiple testing. The same false positive sequences from animal sources consistently group with the human clade and include isolates from possums, birds, kangaroo and koala. This pattern is consistent throughout each phylogenetic analysis of the environmental water isolates.

Table 17 summarises the phylogenetic analysis applied to the database of known isolates (196 sequences) and the 442 isolates from the Wivenhoe, Somerset, and Baroon Pocket dams. The sequence alignment of the *E. coli* isolates from known sources generated in BioEdit shows 59 variable points along the 518 bp fragment of the β-glucuronidase gene. The phylogenetic tree constructed using the 196 sequences from known sources grouped the *E. coli* isolates into two main groups, one belonging to the humans and the other to the animals (Figure 53, Appendix 6). Although the two main groups are obvious, some isolates from both animal and human sources (indicated with arrow in Figure 53, Appendix 6) do not correlate strongly with either group. These ambiguous sequences are more like the majority of known animal isolates and for the purpose of this report are grouped in to that clade. These results support the view that a more rigorous investigation of the 59 variable points would be necessary to identify which of those points discriminate between the two major groups and which point mutations could be leading to background 'noise'. If certain discriminating points can in fact be identified this could lead to a quicker and more precise assay for source tracking with the β-glucuronidase gene. In addition to this there are significant numbers of sequences from both human and animals that are analogous and align together. Based on the clustering of the sequences in the NJ tree generated in MEGA a point was chosen as the cut-off between the human and animal clades. This line is shown in red in Appendix 6.

Table 17. Summary of analysis of the β-glucuronidase gene of *E. coli* isolates from known and unknown sources.

Known Isolates	Align with Animals	Align with Humans	Total Isolates
Animal	79 (85%)	14 (15%)	93
Human	25 (24%)	78 (76%)	103
Unknown Isolates	**Align with Animals**	**Align with Humans**	**Total Isolates**
Wivenhoe Dam	73 (88%)	10 (12%)	83
Somerset Dam	65 (78%)	18 (22%)	83
Baroon Pocket Dam	206 (75%)	70 (25%)	276

The accuracy of the phylogenetic human grouping, as measured by the percentage of known human isolates grouping in the human clade, was quite high at 76% (Figure 40a). Additionally the false positives, as measured by the percentage of the isolates of known animal origin grouping in the human clade, were at acceptable levels at 15% (Figure 40b). This is comparable to other studies using sequencing methods for source tracking of *E. coli* using both β-glucuronidase and other genes for phylogenetic analysis (Guan *et al.* 2002, Ram *et al.* 2004, Ivanetich *et al.* 2006).

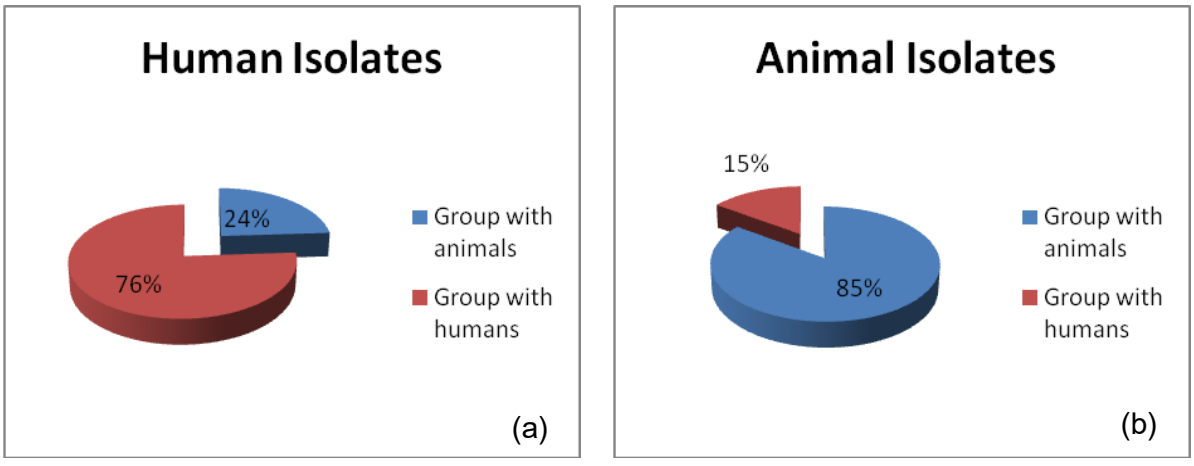


Figure 40. The accuracy of the phylogenetic analysis on β-glucuronidase gene of *E. coli* isolates from known sources summarised as percentage of isolates grouping in human or animal clades.

4.2.1.1. Sequence Processing and Analysis for Ordination Plots

Using the current library of β-glucuronidase sequences from isolates of known sources it is possible to distinguish between isolates derived from human and animal origins with a reasonable level of certainty using phylogenetic analysis. However, when the isolates are compared using ordination plots the sequences do not separate as expected. Sequence data were processed and analysed using MOTHUR (Schloss 2009). To begin, a matrix of uncorrected pair wise distances between each pair of sequences was generated. These distances were then used to conduct both principal coordinate and nonmetric multidimensional scaling analyses to investigate sequence similarity. The first and second axes of each analysis were then plotted with the sequences grouped as "human" or "animal" to look for discrete separation between the two source categories. From these plots it is evident that the human and animal sequences are too alike. There needs to be sufficient variability to create enough 'room' to form distinct groups.

Figure 41 demonstrates the lack of variability along the β-glucuronidase gene using two different ordination plots (a principal components analysis plot and a nonmetric multidimensional scaling plot). Both of these statistical techniques can represent multivariate data in relatively few dimensions. Although the underlying assumptions and objectives of nonmetric multidimensional scaling (NMDS) and principal components analysis (PCA) are different, if there is a natural similarity among profiles in a data set it is usually detected by either approach. The plots shown in Figure 41 demonstrate the mixing of human and animal isolates and show that there is no real distinction between the two groups as a whole. Based on this type of analysis it is evident that the sequences are too similar to plot separately. If an unknown isolate were to be added to this data there is not enough distinction between the isolates from known sources to decide which group it would belong to. If only point mutations that discriminate between the main animal and human groups were considered, perhaps the accuracy of this gene to distinguish between these two groups would be improved. Informative regions of the gene may exist outside the 518bp fragment chosen for this study which could improve the specificity of the method. Comparative analysis with other genes in *E. coli* as well as other methods such as antibiotic resistance, biochemical fingerprinting, phylogenetic grouping, and virulence gene analysis would help to establish ability of the β-glucuronidase gene to correctly classify samples and increase confidence in the library database.

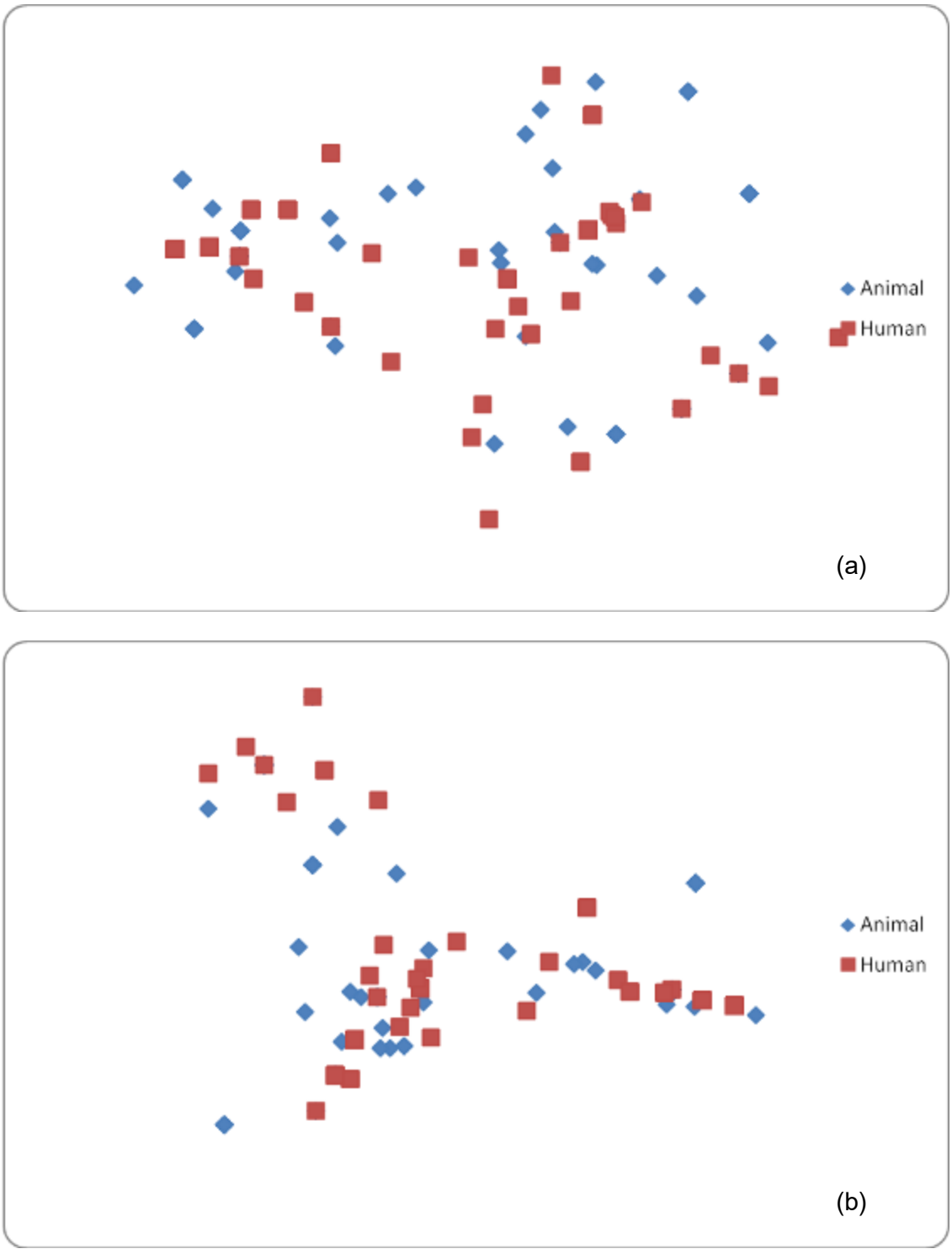


Figure 41. Ordination plots (a) Principal Components of Analysis (PCoA) and (b) nonmetric multidimensional scaling (NMDS) based on the β-glucuronidase gene sequence from *E. coli* isolates of animal and human origin.

4.2.1.2. Source Tracking of *E. coli* Isolates from Environmental Waters

Phylogenetic analysis was performed separately on each of the three catchments using the set of β-glucuronidase sequences generated for each location. The results showed that the majority of isolates from each site appeared to be of animal origin; the highest was Wivenhoe Dam at 88% animal source and the lowest Baroon Pocket Dam at 75% of isolates from animal sources (Figure 42).

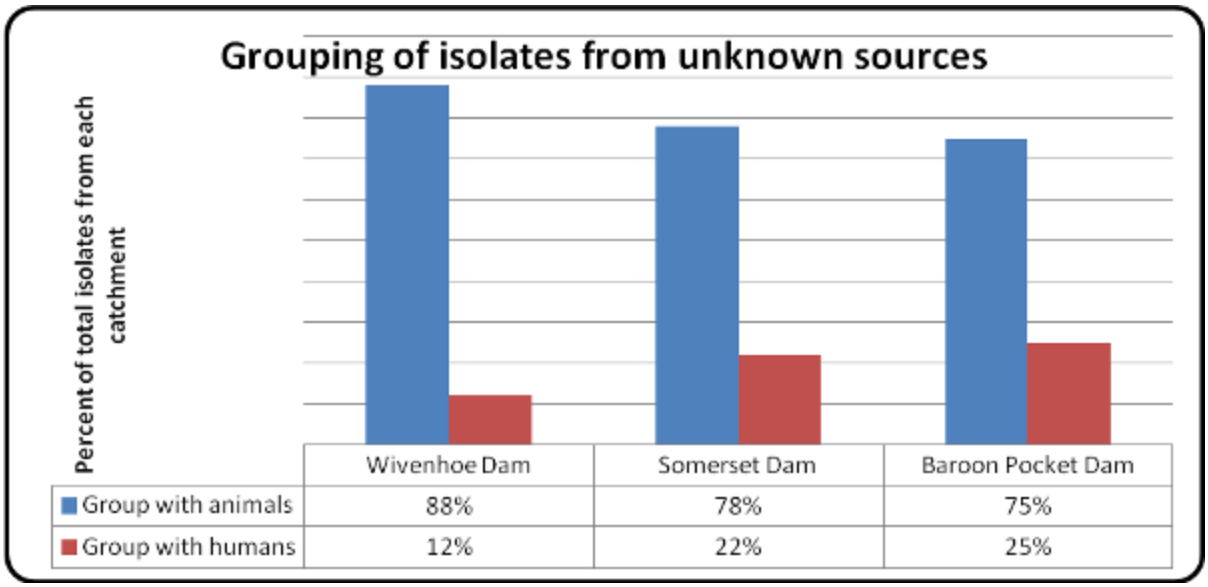


Figure 42. Summary of phylogenetic analysis using the β-glucuronidase gene in *E. coli* isolates from the Wivenhoe Dam, Baroon Pocket Dam and Somerset Dam, Southeast QLD.

4.2.1.3. Source tracking of *E. coli* isolates from Wivenhoe Dam

Water samples were collected from the Wivenhoe Dam by CSIRO from nine sites over a period of six months. From the *E. coli* isolates derived from the sampling, 83 isolates were sequenced using the β-glucuronidase gene. The 83 sequences from the dam were aligned with the 196 sequences from the library (Figure 54, Appendix 6). The phylogenetic analysis applied to the alignment suggests that 12% (10 isolates) were of human origin, while the majority (88%; 73 isolates) were from animal origin (Table 18). The accuracy of the human grouping was quite high at 77% while the level of false positives was measured at 21%.

Table 18. Summary of phylogenetic analysis of the β-glucuronidase gene in *E. coli* isolates from Wivenhoe Dam (unknown source) and from known sources (animal and human).

Isolate Source	Align with Animals	% in Animal Clade	Align with Humans	% in Human Clade	Total Isolates
Unknown	73	88%	10	12%	83
Animal	73	79%	20	21%	93
Human	24	23%	79	77%	103

4.2.1.4. Source Tracking of *E. coli* Isolates from Somerset Dam

Water samples were collected from the Somerset Dam at several sites by Seqwater. From the *E. coli* isolates derived from the sampling 83 isolates were sequenced using the β-glucuronidase gene. The 83 sequences from the dam were aligned with the 196 sequences from the library (Figure 55, Appendix 6). The phylogenetic analysis applied to the alignment suggests that 22% (18 isolates) were

of human origin, while the majority (78%; 65 isolates) were from animal origin (Table 19). The accuracy of the human grouping was moderate at 72% (74 of the 103 human isolates clustered together) while the level of false positives, as measured by the percentage of known animal isolates grouping with the human clade was relatively low at 14%.

Table 19. Summary of phylogenetic analysis of the β-glucuronidase gene in *E. coli* isolates from Somerset Dam (unknown source) and from known sources (animal and human).

Isolate Source	Align with Animals	% in Animal Clade	Align with Humans	% in Human Clade	Total Isolates
Unknown	65	78%	18	22%	83
Animal	80	86%	13	14%	93
Human	29	28%	74	72%	103

4.2.1.5. Source Tracking of *E. coli* Isolates from Baroon Pocket Dam

Water samples were collected from the Baroon Pocket Dam at several sites by Seqwater. From the *E. coli* isolates derived from the sampling, 276 isolates were sequenced using the β-glucuronidase gene. The 276 sequences from the dam water isolates were aligned with the 196 sequences from the library (Figure 56, Appendix 6). The phylogenetic analysis applied to the alignment suggests that 25% (70 isolates) were of human origin, while 75% (206 isolates) were from animal origin (Table 20). This is the highest occurrence of human classification of *E. coli* of the three sample regions according to this analysis. The accuracy of the human grouping was quite high at 77% (79 of the 103 human isolates clustered together) while the level of false positives, as measured by the percentage of known animal isolates grouping with the human clade was 16%.

Table 20. Summary of phylogenetic analysis of the β-glucuronidase gene in *E. coli* isolates from Baroon Pocket Dam (unknown source) and from known sources (animal and human).

Isolate Source	Align with Animals	% in Animal Clade	Align with Humans	% in Human Clade	Total Isolates
Unknown	206	75%	70	25%	276
Animal	78	84%	15	16%	93
Human	24	23%	79	77%	103

4.2.2. Bovine Markers

4.2.2.1. PCR Inhibition for Composite Wastewater and Individual Faecal DNA Samples

All composite wastewater and individual faecal DNA samples ($n = 130$) isolated from target and non-target host-groups were checked for the potential presence of PCR inhibitors. For the HF183 spiked distilled water, the C_T values ranged from 26.6 to 27.1. For the HF183 spiked undiluted cattle wastewater and individual cattle, chicken, dog, duck, kangaroo, pig and possum faecal DNA samples, the C_T values ranged from 26.8 to 28.0. (Table 21). Student's paired t test was performed to determine the differences between the mean C_T values. No significant difference ($P > 0.01$) was observed between the mean C_T values for spiked distilled water and undiluted DNA suggested the cattle wastewater and individual cattle, chicken, dog, duck, kangaroo and possum faecal DNA samples were potentially free of PCR inhibitors. Undiluted DNA samples were used for the PCR assays for the above host-groups.

For the HF183 spiked undiluted individual bird and horse faecal DNA samples, the C_T values ranged from 27.5 to 38.1 and 27.6 to 31.9, respectively (Table 21). Certain bird ($n = 4$) and horse ($n = 3$) faecal DNA samples had higher C_T values suggested the presence of PCR inhibitors in undiluted faecal DNA samples. For the HF183 spiked 10-fold diluted bird and horse faecal DNA samples, the C_T values, however, ranged from 27.3 to 28.1 and 27.1 to 27.8, respectively. No significant difference

($P > 0.03$) was observed between the mean C_T values for spiked distilled water and 10-fold diluted DNA suggested the bird and horse faecal DNA samples were potentially free of PCR inhibitors.

For the cowM3 spiked distilled water, the C_T values ranged from 24.2 to 25.1. For the cowM3 spiked undiluted human wastewater DNA samples, the C_T values ranged from 24.6 to 25.8 (Table 21). Student's paired t-test was performed to determine the differences between the mean C_T values obtained for distilled water and for human wastewater DNA samples. No significant difference ($P > 0.01$) was observed between the mean C_T values for spiked distilled water and undiluted DNA suggested the human wastewater DNA samples were potentially free of PCR inhibitors.

4.2.2.2. Host-Specificity and -Sensitivity of the Bovine-Associated Markers

Among the 90 faecal DNA samples tested from non-target host groups, 31 (34%) and 11 (12%) were PCR positive for the BacCow-UCD and cowM3 markers, respectively (Table 22). The BacCow-UCD marker was detected in chicken, dog, duck, kangaroo, pig, possum, horse and human wastewater DNA faecal samples. The cowM3 marker, however, detected in dog, duck and possum faecal DNA samples. The B-AVs could not be detected in DNA faecal samples of any non-target host-groups like the BacCow-UCD and cowM3 markers. The overall host-specificity values of the BacCow-UCD, cowM3 and B-AVs markers to differentiate between bovine and other non-target host-groups were 0.66, 0.88 and 1.00 (maximum value of 1.00).

Among the 40 target host-groups faecal samples tested, 37 (93%), 36 (90%) and 24 (60%) were PCR positive for the BacCow-UCD, cowM3, and B-AVs markers, respectively. The prevalence of B-AVs in individual cattle faecal DNA samples was low compared to BacCow-UCD and cowM3 markers. The prevalence of all three bovine-associated markers in composite cattle wastewater DNA samples was high. The overall sensitivity values of the BacCow-UCD, cowM3 and B-AVs markers in composite cattle wastewater and individual cattle faecal DNA samples were 0.93, 0.90 and 0.60 (maximum value of 1.00).

Table 21. Evaluation of PCR inhibition in faecal and wastewater DNA samples from target and non-target host-groups.

Wastewater and Faecal DNA Samples	No of Samples	Volumes used for DNA Extraction	C_T Value (range) of Real-Time PCR	
			Undiluted DNA	10-Fold Dilution
Cattle[a]	20	180-220 mg	27.1-27.9	-
Cattle wastewater[b]	20	10 mL	27.3-27.6	-
Birds	10	50-150 mg	27.5-38.1	27.3-28.1
Chickens	10	150-200 mg	26.8-27.1	-
Dogs	10	180-220 mg	27.1-27.6	-
Ducks	10	100-200 mg	27.2-28.0	-
Kangaroos	10	180-220 mg	26.9-27.4	-
Pigs	10	180-220 mg	27.3-27.9	-
Possums	10	180-220 mg	27.3-27.6	-
Horses	10	180-220 mg	27.6-31.9	27.1-27.8
Human wastewater[b]	10	10 mL	24.6-25.8	-

[a]: individual faecal samples; [b]: composite samples; C_T: threshold cycle.

Table 22. Host-specificity and -sensitivity of bovine-specific bacterial and viral markers.

Wastewater and Faecal DNA Samples	No. of Samples	Real-Time PCR Positive Results/Numbers of Samples Tested		
		BacCow-UCD	cowM3	B-AVs
Cattle[a]	20	17/20	16/20	6/20
Cattle wastewater[b]	20	20/20	20/20	18/20
Birds	10	0/10	0/10	0/10
Chickens	10	4/10	0/10	0/10
Dogs	10	9/10	5/10	0/10
Ducks	10	3/10	4/10	0/10
Kangaroos	10	5/10	0/10	0/10
Pigs	10	1/10	0/10	0/10
Possums	10	2/10	2/10	0/10
Horses	10	4/10	0/10	0/10
Human wastewater[b]	10	3/10	0/10	0/10

a: individual faecal samples; b: composite samples

4.2.2.3. Numbers of Faecal Indicator Bacteria (FIB)

Among the 36 samples from Wivenhoe Dam, 16 (44%) and 27 (75%) samples yielded culturable *E. coli* and *Enterococcus* spp., respectively. The numbers of *E. coli* in the water samples ranged from 0.3 to 2.31 $\log_{10}$ colony forming units (CFU) per 100 mL of water. The numbers of *Enterococcus* spp. ranged from 0.7 to 3.40 $\log_{10}$ CFU per 100 mL of water (Figure 43). Pearson's correlation was used to test the relationship between *E. coli* and *Enterococcus* spp. numbers. The numbers of *E. coli* did not correlate with the numbers of *Enterococcus* spp. ($r_p = 0.009$; $P < 0.956$).

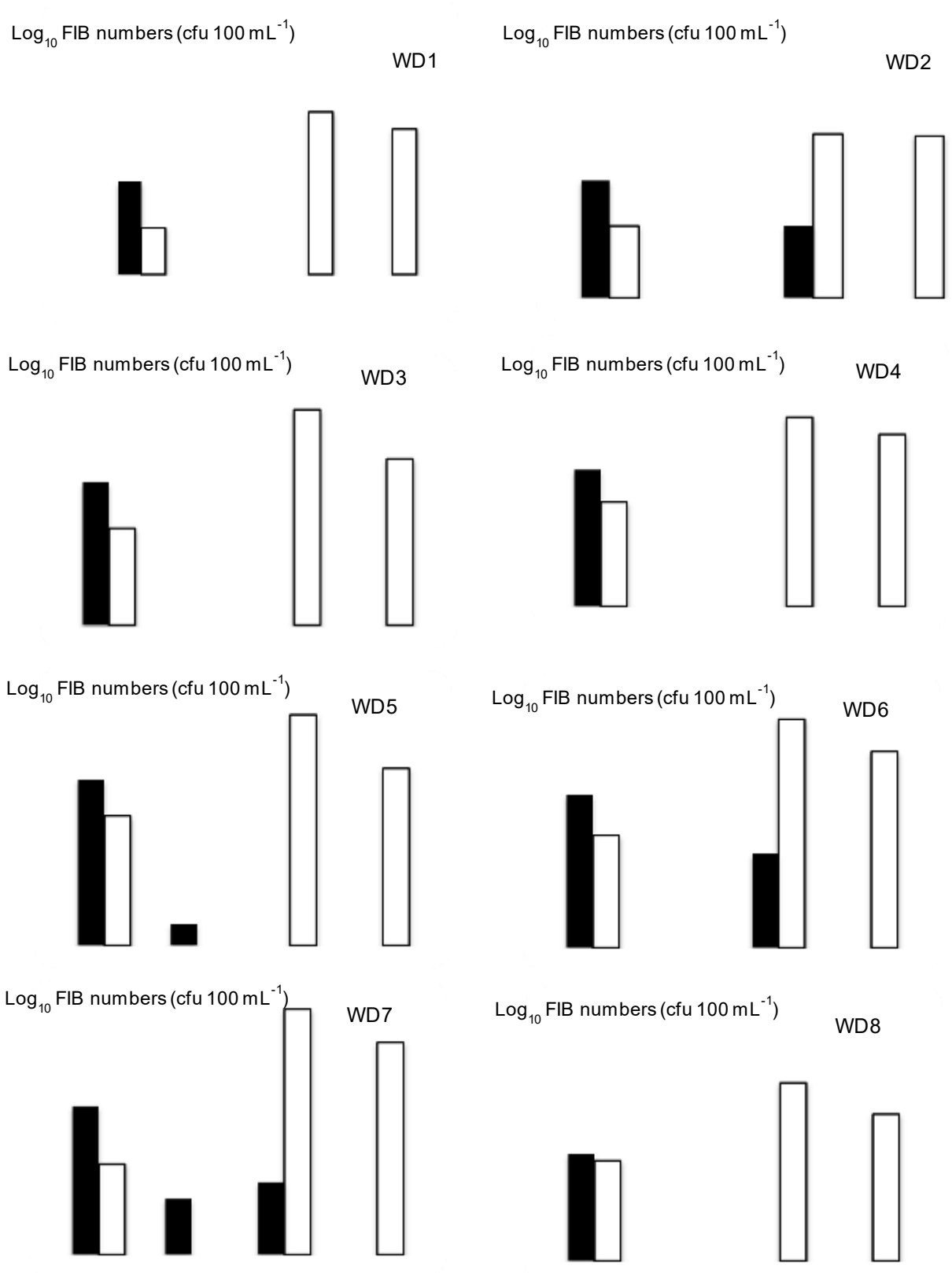


Figure 43. Number of faecal indicator bacteria detected at each sampling site in Wivenhoe Dam.

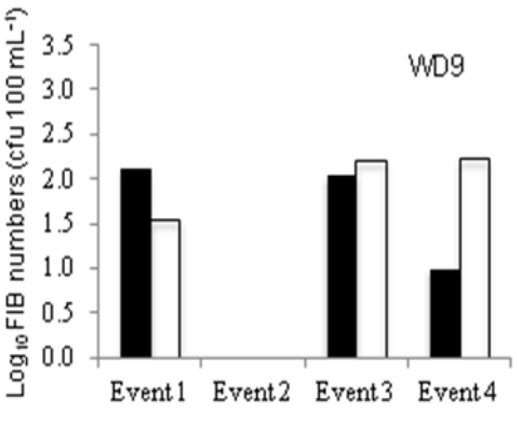


Figure 43. Number of faecal indicator bacteria detected at each sampling site in Wivenhoe Dam *(continued)*.

4.2.2.4. PCR Inhibition for Wivenhoe Dam water Samples

All DNA extracted from the Wivenhoe Dam water samples were checked for the potential presence of PCR inhibitors. For the HF183 spiked distilled water, the C_T values ranged from 25.1 to 26.4 (Table 23). For the HF183 spiked undiluted DNA extracted from water samples, the C_T values ranged from 26.0 to 28.7. Student's paired t test was performed to determine the differences between the mean C_T values obtained for distilled water and for DNA extracted from water samples. No significant difference ($P > 0.03$) were observed between the mean C_T values for spiked distilled water and undiluted DNA suggested the WD water samples were potentially free of PCR inhibitors.

Table 23. Evaluation of PCR inhibition in DNA isolated from Wivenhoe Dam water samples.

Wivenhoe Dam Water DNA Samples	No of Samples	Volumes used for DNA Extraction	C_T Value (range) of Real-Time PCR	
			Undiluted DNA	10-Fold Dilution
WD1	4	10 L	26.3-28.7	25.5-27.1
WD2	4	10 L	26.0-28.1	25.8-27.3
WD3	4	10 L	26.4-28.1	26.1-27.2
WD4	4	10 L	26.3-27.6	25.9-27.4
WD5	4	10 L	26.6-28.0	26.2-27.2
WD6	4	10 L	25.9-27.1	-
WD7	4	10 L	26.1-28.3	25.7-27.2
WD8	4	10 L	26.5-27.5	-
WD9	4	10 L	26.5-27.0	-

C_T: threshold cycle.

4.2.2.5. PCR Limit of Detection (PLOD)

PCR Limit of Detection (PLOD) assays were performed using plasmid DNA and genomic DNA. To determine the reproducibility of the assays, several replicates ($n = 9$) were tested. The PLOD limits were as low as 5 gene copies for the target genes.

4.2.2.6. Prevalence of Bovine-Associated Markers and Zoonotic Bacterial Pathogens

Among the three bovine-associated markers tested, BacCow-UCD was more prevalent than cowM3 and B-AVs markers (Figure 44). Of the 36 samples tested, 20 (56%), 8 (22%) and 2 (6%) samples were PCR positive for the BacCow-UCD, cowM3 and B-AVs markers. Twenty (56%) samples were

positive for at least one marker, 8 (22%) were positive for at least two markers, two (6%) was positive for all three markers tested in this study. Of the 36 samples tested, 18 (50%) and 5 (14%) samples were PCR positive for the *Campylobacter* spp. 16S rRNA and *E. coli* O157 *rfbE* genes, respectively. Eighteen (50%) samples were positive for at least one marker and five (14%) samples were positive for both the *Campylobacter* spp. 16S rRNA and *E. coli* O157 *rfbE* genes. *Salmonella* spp. *invA* gene could not be detected in any of the samples tested.

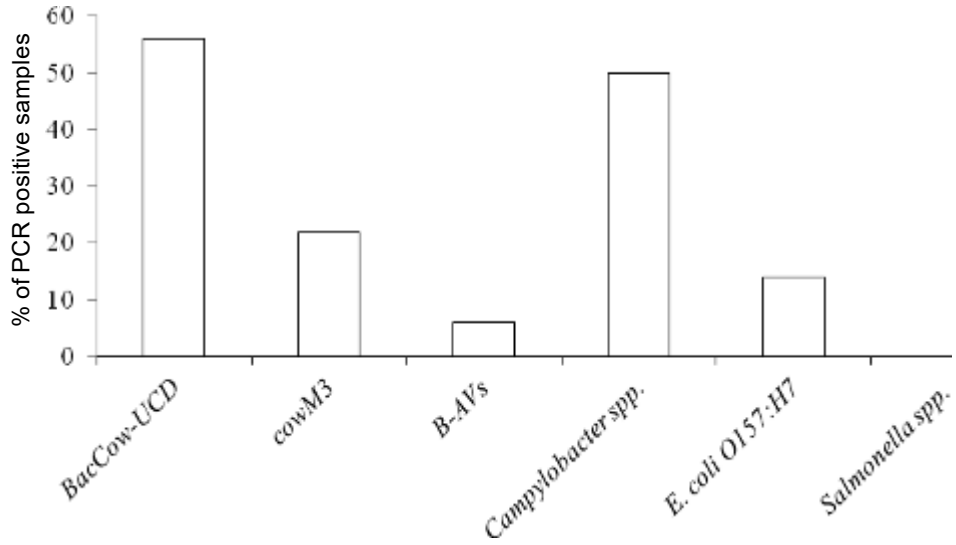


Figure 44. Detection of zoonotic pathogens in Wivenhoe Dam.

4.2.2.7 Application of Baye's Theorem to Estimate the Conditional Probability of Accurately Detecting Cattle Faecal Pollution in the Wivenhoe Dam Water Samples

Baye's Theorem was used to estimate the conditional probability of accurately detecting cattle faecal pollution in the Wivenhoe Dam water samples as these markers were detected in non-target host-groups. BacCow-UCD and cowM3 markers were detected in 56% (20 of 36 samples) and 22% (8 of 36 samples) of the Wivenhoe Dam water samples, respectively by real-time PCR assays. The background probabilities, $P(H)$, of detecting the BacCow-UCD and cowM3 markers in the Wivenhoe Dam water samples, therefore, were 0.56 and 0.22. The background probability that these markers were not detected in the Wivenhoe Dam water samples were $1 - P(H)$, or 0.44 (for the BacCow-UCD marker) and 0.78 (for the cow M3 marker). $P(T\backslash H)$ is the true-positive rate of the assay and the values were calculated from the host-sensitivity assays as reported in this study. The values were 0.93 and 0.90 for the BacCow-UCD and cowM3 markers, respectively. $P(T\backslash H')$ is the false-positive rate of the assay, and the values were calculated from the host-specificity assays in this study. The values were 0.34 and 0.12 for the BacCow-UCD and cowM3 markers, respectively.

Based on the detection and non-detection results of the BacCow-UCD marker in the Wivenhoe Dam water samples and faecal samples from target and non-target host-groups, there was a 78% probability that the detection of the BacCow-UCD marker in a Wivenhoe Dam water sample was due to the true cattle faecal pollution and not due to faecal pollution from non-target hosts such as chicken, dog, duck, kangaroo, pig, possum, horse and human wastewater. Similarly, there was a 68% probability that the detection of the cowM3 marker in a Wivenhoe Dam sample was due to the true cattle faecal pollution and not due to faecal pollution from non-target hosts such as dog, duck and possum.

4.2.2.8. Agreement and Correlations between Faecal Indicator Bacteria (FIB) and Bovine-Associated Markers and Zoonotic Bacterial Pathogens

The occurrence of FIB, bovine-associated markers and zoonotic bacterial pathogens were compared pair wise to estimate the percentage of co-detection and non co-detection for the Wivenhoe Dam water samples (Table 24). The highest (i.e., 22%) mean co-detection was found for *Enterococcus* spp. with other microorganisms. Similarly, the lowest (i.e., 0%) mean co-detection was found for *Salmonella* spp. with other microorganisms.

In contrast, the highest (i.e., 62%) mean non co-detection was found for *Salmonella* spp. with other microorganisms. The lowest (i.e., 20%) mean non co-detection was found for *Enterococcus* spp. with other microorganisms. The cowM3 marker had the highest (i.e., 65%) mean agreement and *Enterococcus* spp. had the lowest (i.e., 42%) agreement with other microorganism when co-detection and non co-detection data were pooled for each microorganism. The Overall mean pair wise co-detection value was relatively smaller than non co-detection value. The overall mean pair wise agreement value was 58%. Discrepancies were observed in terms of the occurrence of faecal indicators, bovine-associated markers and zoonotic bacterial pathogens (see Table 24 column 5). Binary logistic regressions were used to identify whether any correlation existed between the numbers FIB and the presence/ absence results for bovine-associated markers and zoonotic bacterial pathogens (Table 25). The PCR results for Salmonella spp. invA gene was not included in the analysis as Salmonella spp. invA gene could not be detected in any water samples tested. The presence/absence of the bovine-associated markers and zoonotic bacterial pathogens did not correlate with FIB numbers.

Table 24. Agreement on the co-detection and non co-detection among faecal indicators, bovine-associated markers and zoonotic pathogens in water samples collected from the Wivenhoe Dam.

Pairwise Comparison	%			
	Co-Detection	Non Co-Detection	Total Agreement	Disagreement
E. coli vs. *Enterococcus* spp.	39	19	58	42
E. coli vs. BacCow-UCD	22	22	44	56
E. coli vs. cowM3	8	42	50	50
E. coli vs. B-AVs	3	53	56	44
E. coli vs. *Campylobacter* spp. 16S rRNA	22	28	50	50
E. coli vs. *E. coli* O157 *rfbE*	3	44	47	53
E. coli vs. *Salmonella* spp. *invA*	0	56	56	44
Enterococcus spp. vs. BacCow-UCD	42	14	56	44
Enterococcus spp. vs. cowM3	17	19	36	64
Enterococcus spp. vs. B-AVs	6	25	31	69
Enterococcus spp. vs. *Campylobacter* spp. 16S rRNA	39	14	53	47
Enterococcus spp. vs. *E. coli* O157 *rfbE*	11	22	33	67
Enterococcus spp. vs. *Salmonella* spp. *invA*	0	25	25	75
BacCow-UCD vs. cowM3	22	44	66	44
BacCow-UCD vs. B-AVs	6	44	50	50
BacCow-UCD vs. *Campylobacter* spp. 16S rRNA	47	42	89	11
BacCow-UCD vs. *E. coli* O157 *rfbE*	11	42	53	47
BacCow-UCD vs. *Salmonella* spp. *invA*	0	44	44	56
cowM3 vs. B-AVs	6	78	84	16
cowM3 vs. *Campylobacter* spp. 16S rRNA	17	44	61	39
cowM3 vs. *E. coli* O157 *rfbE*	6	72	78	22
cowM3 vs. *Salmonella* spp. *invA*	0	78	78	22
B-AVs vs. *Campylobacter* spp.	3	47	50	50
B-AVs vs. *E. coli* O157 *rfbE*	0	81	81	19
B-AVs vs. *Salmonella* spp. *invA*	0	94	94	6
Campylobacter spp. 16S rRNA vs. *E. coli* O157 *rfbE*	14	50	64	36
Campylobacter spp. 16S rRNA vs. *Salmonella* spp. *invA*	0	50	50	50
E. coli O157 *rfbE* vs. *Salmonella* spp. *invA*	0	86	86	14

Table 25. Correlations among faecal indicator bacteria with bovine-associated markers and zoonotic bacterial pathogens using binary logistic regression analysis.

Comparison	Concordance (%)	Odds ratios	*P* value[a]
E. coli vs. BacCow-UCD	55.8	0.99	0.140
E. coli vs. cowM3	44.3	0.99	0.459
E. coli vs. B-AVs	41.7	0.98	0.378
E. coli vs. *Campylobacter* spp. 16S rRNA	38.9	1.00	0.752
E. coli vs. *E. coli* O157 *rfbE*	45.5	0.99	0.614
Enterococcus spp. vs. BacCow-UCD	64.6	1.00	0.131
Enterococcus spp. vs. cowM3	46.4	1.00	0.883
Enterococcus spp. vs. B-AVs	88.3	1.00	0.163
Enterococcus spp. vs. *Campylobacter* spp. 16S rRNA	43.7	1.00	0.709
Enterococcus spp. vs. *E. coli* O157 *rfbE*	45.5	1.00	0.835

[a]*P* value for the model chi-square was < 0.05, and the confidence interval for the odds ratio did not include 1.0.

4.3. Conclusions

This section of the project involved the study of potential microbial source tracking (MST) tools that could distinguish between human and animal faecal contamination and specifically identify bovine faecal contamination of water. The underlying assumption of MST is that certain bacterial strains are unique to a particular host group (i.e. host-specific) but the current MST tools are either limited in their range of detection (host-sensitivity) or in their host-specificity. Most MST tools are also limited to distinguishing between human and animal faecal sources and more tools are needed to distinguish between the different animal sources of concern.

The research using the *E. coli* β-Glucuronidase gene was able to discriminate between the β-glucuronidase gene in *E. coli* from isolates of human and animal origin with a moderate degree of confidence using phylogenetic analysis. However exploratory ordination statistical methods (PCA and NMDS) did not reveal patterns of spatial and temporal variability to sufficiently discriminate between groups of human or animal origin. The phylogenetic analysis consistently grouped 72%-77% of the known human isolates in the human cluster, with false positives falling between 14% and 21%. This information suggests that the outcomes of the analysis and use of the β-glucuronidase gene for MST should be interpreted with caution.

The results demonstrated that Baroon Pocket Dam had the highest number of isolates (276), of which 70 aligned with the human cluster, making this site the highest occurrence of human isolate source at 25%. Wivenhoe and Somerset Dams had equal isolates each totalling 83 and Wivenhoe Dam had the lowest number of isolates clustering with the human clade at 12%.

The β-glucuronidase gene in *E. coli* may be very useful in determining if faecal contamination in water is of human or animal origin. However this work is preliminary and the data should be viewed with caution. The lack of variability within the gene is a limiting factor for strong discrimination between the two main groups (human and animal). The results of the phylogenetic analysis revealed this lack of variability by a region of the constructed tree with ambiguous sequences that did not correlate strongly to either group. The similarity of many human and animal sequences was evident from the lack of distinct grouping in ordination plots. It is envisaged that by further investigation into the variable points responsible for sequence differences, expanding on the genes that could be used as markers, and comparative analysis with other methods this process will eventually enable the use of the β- glucuronidase gene in *E. coli* as a feasible monitoring tool for microbial source tracking.

For the work done on the bovine markers, the bovine bactericides BacCow-UCD and cowM3 markers were detected in faecal samples from both target and non-target host groups in SEQ. In comparison, the bovine adenovirus B-AV marker showed absolute host-specificity but there was low host-sensitivity as their prevalence was low in bovine faeces and wastewater. The results indicate that the application of a single Bovine Faecal (BFA) marker may not be sensitive enough to provide the definite evidence of bovine faecal pollution, and therefore, it is recommended that multiple (BFA) markers should be used if possible.

Nonetheless, the prevalence of BFA markers in water samples collected from Wivenhoe Dam suggests that the quality of water may be affected by bovine faecal pollution. The presence of the multiple BFA markers also indicates that there is potential for zoonotic pathogens to be in the reservoir from these cattle sources. This is further supported by the evidence of bacterial zoonotic pathogens such as *Campylobacter* spp. and *E. coli* O157 through the detection of their 16S rRNA and *rfbE* genes respectively in reservoir water samples. No correlations could be found between the numbers of the faecal indicator bacteria (FIB) *E. coli* and enterococci and the BFA markers or bacterial zoonotic pathogens, thus indicating that FIB could not be relied upon alone to obtain information on the microbiological quality of the reservoir water.

An important area for further research is to understand the decay rates of these bovine markers in environmental water samples in relation to FIB and zoonotic pathogens. Additionally, quantitative PCR data would be required to assess the magnitude of fecal pollution and associated public health risks.

5. OVERALL PROJECT CONCLUSIONS

This project was set up to determine the capability of natural processes within reservoirs and water ways in SEQ to remove microbial pathogens and organic chemicals of concern. It is well understood that these SEQ raw drinking water storages are exposed to multiple sources of biological and chemical contaminants. These sources can be diffuse and large scale (e.g. agriculture and recreation) or local and direct (e.g. sewage and stormwater discharges).

The treatment of this water for domestic supply has primarily relied upon engineered treatment systems as the sole treatment barrier(s) with natural systems such as reservoirs and streams only used as passive barriers via mechanisms such as restricted public access and, where possible, controlling inputs from communities and farms upstream of the reservoirs. It was considered that this sole reliance on water treatment plants places unnecessary operational requirements on these plants to consistently provide a high quality drinking water. In contrast, if natural treatment processes within SEQ reservoirs could be shown to actively remove pathogens and chemicals from the water then this could relieve some of the burden on the engineered systems. Thus, this project set out to investigate the notion of using catchment health as a "*generator of good water quality*", that is, to investigate if a level of treatment capacity could be assigned to the reservoirs and associated waterways. An understanding of what and how biological and chemical contaminants can be removed via natural treatment processes in the reservoirs would: (1) improve management processes and operational procedures for the reservoirs; (2) enable better and more accurate assessment of the health risks associated with pathogens and chemicals in the reservoirs; and (3) eventually, potentially improve the capacity of water treatment operators to produce a consistent quality of water through a better understanding of what contaminants the treatment plants need to cope under different environmental conditions.

At the same time as the Alliance funded research was being conducted, Seqwater and Griffith University had been undertaking work on improving the ability to identify the sources of faecal contamination in the reservoirs using MST. Understanding the source of faecal pollution can provide valuable information on the type and potential load of microbial pathogens in a reservoir. If the MST tools indicate that there is human or agricultural animal input, these results could even be extrapolated to predict what types of organic chemicals could also be in the water. Knowing what types of pathogens (and chemicals) could be in the water and the source(s) is vitally important to be combined with the information on pathogen and chemical removal to enable an accurate health risk assessment to be undertaken. This in turn would assist in improved predictions of risk to be made under different climatic and environmental conditions. This all has the potential to lead to more efficient management of reservoirs and water treatment plants.

Microbial pathogens are the most acute health risk reservoir managers and water treatment plant operators have to deal with. The research presented in this report demonstrated that the natural processes within Wivenhoe Dam have the capacity to remove pathogens from the water and that this removal is not greatly influenced by seasonal variations or other gradual changes in reservoir conditions. The impact of a major flooding event was also shown to have some influence on the decay rate of the different pathogens, however, the observed changes in decay rates were not large and were observed to revert back towards the pre-flood rates as the water conditions in Wivenhoe Dam improved.

More information is still needed on the impact of sediment and reservoir residence times, and on the transferability of the results to other SEQ reservoirs. The results demonstrate, however, that under all but the most severe of conditions, all pathogen types are removed by natural processes and that there is a reduction in health risks within the reservoir.

The research on MST showed there could be discrimination of the sources of faecal inputs using the *E. coli* β-glucuronidase gene pointing to the level of human and animal contamination of the different reservoirs sampled. The work testing the Bovine markers indicated that bovine faeces were most likely to be predominantly from bovine sources. The research concluded that both tested MST methods

(β-glucuronidase and bovine markers) should be used with caution as single methods, and would be most feasible when used in conjunction with a number of other microbial source tracking tools.

The assessment of the attenuation potential of organic chemicals found that biodegradation was a minor mechanism for removal for the majority of compounds under the experimental conditions. This was the case even when an additional quantity of BDOC or additional microbial inoculum was added. The potential for longer-term biodegradation still needs to be considered, however, particularly in the context of expected hydraulic residence times in Wivenhoe Dam. In contrast, direct and indirect photolysis led to the degradation of the majority of the tested contaminants, with complete degradation of a few of these contaminants expected to occur within hours of exposure to sunlight. A high degree of light absorbance in all the natural waters tested, however, is likely to limit the importance that solar irradiance would have for organic micropollutants that exist within the respective water systems. More work would be required to assess if other processes such as sorption within the water column should be assessed to determine their potential effect on the biodegradation and photolysis within the water column. Furthermore, up-scaling of the study presented here, through spiking closed microcosms within the respective water bodies would provide additional *in situ* confirmation of the findings presented in this report.

The overall results currently indicate that reservoirs in SEQ do have a treatment capability and are able to remove contaminants, particularly pathogens, under a range of climatic and environmental conditions. It is evident that residence times are important and that there remains to be coordination of the data on the removal of these contaminants with hydrodynamic flow data, including modelling. For example, the results presented in the QMRA assessing the health risks show the time taken for a hypothetical contamination event to reach an acceptable health risk target (based on the decay rate data). If information from hydrodynamic modelling was available to provide details on the travel time for particles to move through the reservoir under different conditions, then this could be used in conjunction with the QMRA outputs and pathogen decay data to provide a more accurate assessment for pathogens demonstrating how far from a contamination event the health risk remained "unacceptable". This will be particularly important for assessing changes in health risk levels under different climatic events and/or exposure scenarios.

If the information derived from the combination of pathogen decay assessment/health risk assessment and hydrodynamic modelling is then pooled with data on sources and loads provided by a MST assessment then the ability to manage the reservoir could be significantly enhanced. A similar potential exists for managing the risks from organic chemicals, however, more details are needed on removal mechanisms and on how to assess the health risk potential than is currently available for pathogens.

It is recommended that this additional research combining the different discipline areas (pathogens and chemical decay, MST, and hydrodynamic modelling) be undertaken at the nearest opportunity. The outcomes of this additional research could then be utilised to update and enhance existing reservoir and water treatment plant operational procedures or to develop newer procedures that take into account the more accurate health risk assessment outcomes. This will be particularly important to complete prior to any addition of recycled water to the reservoirs to promote public confidence in water management in SEQ.

APPENDIX 1: Detection Methods for Microorganisms

1. Analytical Methods for Bacteria

For the detection of the number of *E. coli*, and enterococci in both the primary and secondary treated effluent, samples were homogenised to achieve a uniform suspension and then serially diluted in sterile deionised water. 50 µL replicates of each dilution were then spread plated onto the appropriate isolation media (Table 26) which was then incubated aerobically overnight at 44°C for *E. coli* and 37°C for enterococci.

Detection of *Campylobacter* spp. involved plating 100 µL samples onto modified CCDA agar. These inoculated plates were incubated at 37°C for up to 48 hours in gar jars using Oxoid Campy gas sachets to create a microaerophilic environment.

The detection of *Salmonella* spp. involved an enrichment step. This involved enriching 5mL samples of primary or secondary effluent in Buffered peptone water overnight at 37°C. After this enrichment 10 mL, 1 mL and 100µL samples were incubated at 37°C overnight in 90 mL, 9 mL and 0.9 mL respectively of RV Broth as an MPN test. Samples that showed positive growth in any of the MPN dilutions were then spread plated onto XLD agar for confirmation of the presence of *Salmonella* spp. cells.

After incubation all inoculated media plates with filters were visually inspected for the presence of colonies that had characteristics representative of the target microorganisms (Table 26) growing on the surface of the media. The number of bacterial colonies displaying the characteristics of the target bacterial strain was counted as presumptive positive results. Representative presumptive positive colonies on each plate were subcultured and further tested to confirm that they were isolates of the target bacterium. The recorded results for numbers of detected bacterial strains was then amended if necessary based on the outcomes of the confirmation tests.

Table 26. Isolation media used to detect enteric bacteria in raw and treated sewage effluent.

Enteric Bacteria	Isolation Medium	Characteristics of Representative Bacterial Colonies
E. coli	Chromocult® coliform Agar (Merck)	Violet colonies (Chromocult)
Enterococcus spp.	Chromocult® enterococci Agar (Merck))	Pink Colonies (Chromocult)
Salmonella spp.	XLD Agar (Difco) for confirmation	Black colonies surrounded by yellowing of the media
Campylobacter spp.	CCDA + selective supplement (Oxoid)	Cream coloured colonies

2. Detection Method for *Cryptosporidium* oocysts

Detection of *Cryptosporidium* oocysts in both primary and secondary effluent was achieved using Vital Dye staining followed by counting of Cryptosporidium oocysts by fluorescent microscopy.

Staining was undertaken by adding a 2mg/mL solution of the nucleic acid stain DAPI (at 20 µL/mL of sample) to a predetermined volume of collected sample. The volume of sample was determined by an initial test to determine the largest volume that could be analysed before background microorganisms and other aquatic materials (sediment etc) obscured the view of any oocysts present. This stained sample was then incubator at 37°C for 2 hours. Of sample) Following this incubation, the sample was further strain with a 1mg/mL solution of the dye Propridium Iodide (at 20µL/mL) and incubated at room temperature for a further 5 minutes.

The stained sample was the filtered through a 25 mm diameter 02.2µm pore sized black polycarbonate filter using a low vacuum to reduce edge effects. The filter was then rinsed will two sent volumes of deionised water, air dried and mounted on glass slide using immersion oil between the filter and a coverslip.

The filter was then viewed under a fluorescent microscope using x100 magnification. A total of 20 fields of view were examined for the presence of stained *Cryptosporidium* oocysts using filter blocks to detect oocysts stained with DAPI or Propridium Iodide. Oocysts that were only stained blue with DAPI were classed as viable, while those that stained blue with DAPI and red with Propridium Iodide were classed as nonviable. Any oocysts that contained no visible trophozoites were ignored. The number of detected oocysts in the 20 fields of view were then averaged and used to calculate the number of oocysts per litre in the original sample.

3. Detection Methods for Human Adenovirus

The detection method specifically involved extracting adenovirus DNA from a 200 µL portion of collected sample. Adenovirus DNA was extracted from the water concentrate using the Blood and Tissue DNA Extraction Kit (Qiagen) using the manufacturer's instructions.

The extracted adenovirus DNA was then amplified using quantitative PCR using adenovirus specific primers to determine the number of these viruses in the collected samples. The primers used for the detection of adenovirus were the primer set Heim I and Heim II (Heim *et al.* 2003). The sequence of all primer sets and probes is given in Table 27. Detection and quantification of the amplification products was achieved using the DNA-binding dye SYBR Green.

Quantitative PCR reactions were performed on Bio-Rad iQ5 (Bio-Rad Laboratories, California, USA), using iQ Supermix (Bio-Rad). Each 25µL PCR reaction mixture contained 12.5 µL of SuperMix, 120 nM of each primer, and 3 µL of template DNA. Bovine serum was added to each reaction mixture to a final concentration of 0.2 µg μL^{-1} to relieve PCR inhibition (Kreader 1996). For each PCR experiment, corresponding positive (i.e., target DNA) and negative (sterile water) controls were included.

Table 27. PCR primers and probe used for the detection of adenovirus.

Primer	Adenovirus
Forward	5´- GCC ACG GTG GGG TTT CTA AAC TT - 3´
Reverse	5´- GCC CCA GTG GTC TTA CAT GCA - 3´
Probe	5'-(FAM)-TGC ACC AGA CCC GGG CTC AGG AGG TAC TCC GA -(BHQ1)-3'

Thermal cycling conditions for adenovirus were as outlined in Sidhu *et al.*, (2010). A melt curve analysis was performed after the PCR run to differentiate between actual products and primer dimmers, and to eliminate the possibility of false-positive results. The melt curve was generated using 80 cycles of 10 s each starting at 55°C and increasing in 0.5°C intervals to a final temperature of 95°C.

Thermocycling conditions for polyomavirus were an initial denaturation at 95ºC for 5 min, followed by 40 cycles at 95ºC for 20 s, 55ºC for 15 s, 60ºC for 60 s. As detection of the amplicon was achieved through the use of a Taqman probe no melt curve was used. As for adenovirus, the Tm for each amplicon was determined using the iQ5 software.

APPENDIX 2: Wavelength Passage through Low Potassium Glass

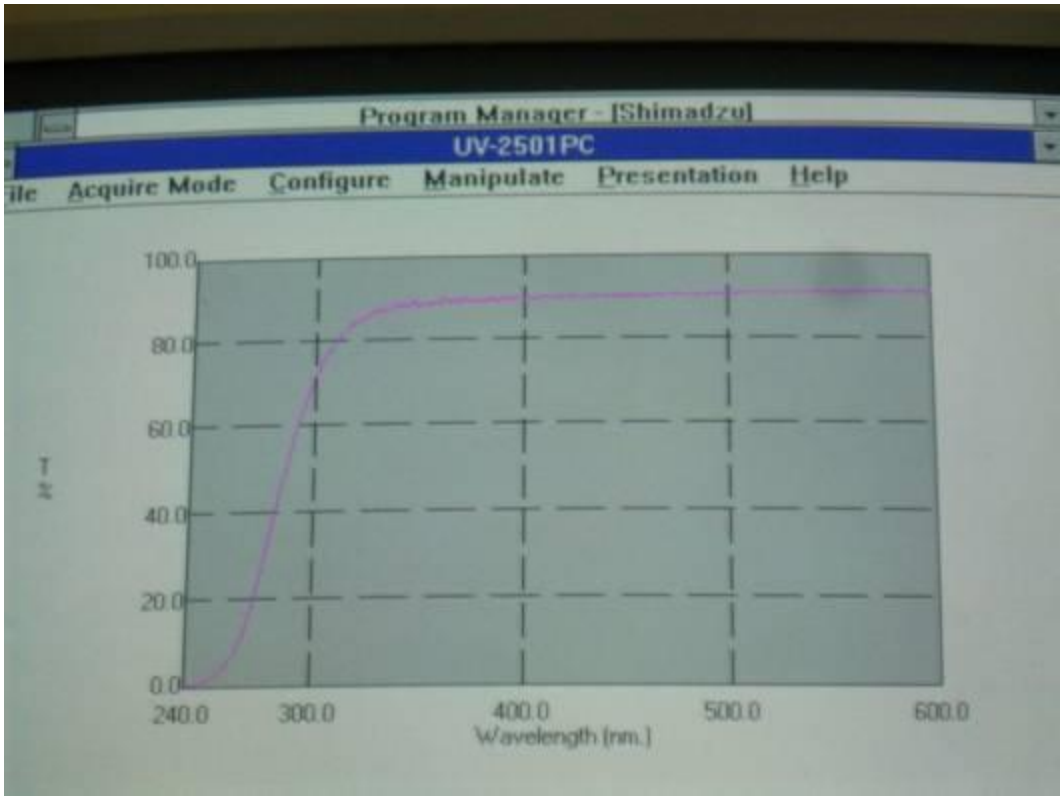


Figure 45. Percent transmittance of low potassium glass.

APPENDIX 3: Absorbance Spectra of Selected Contaminants

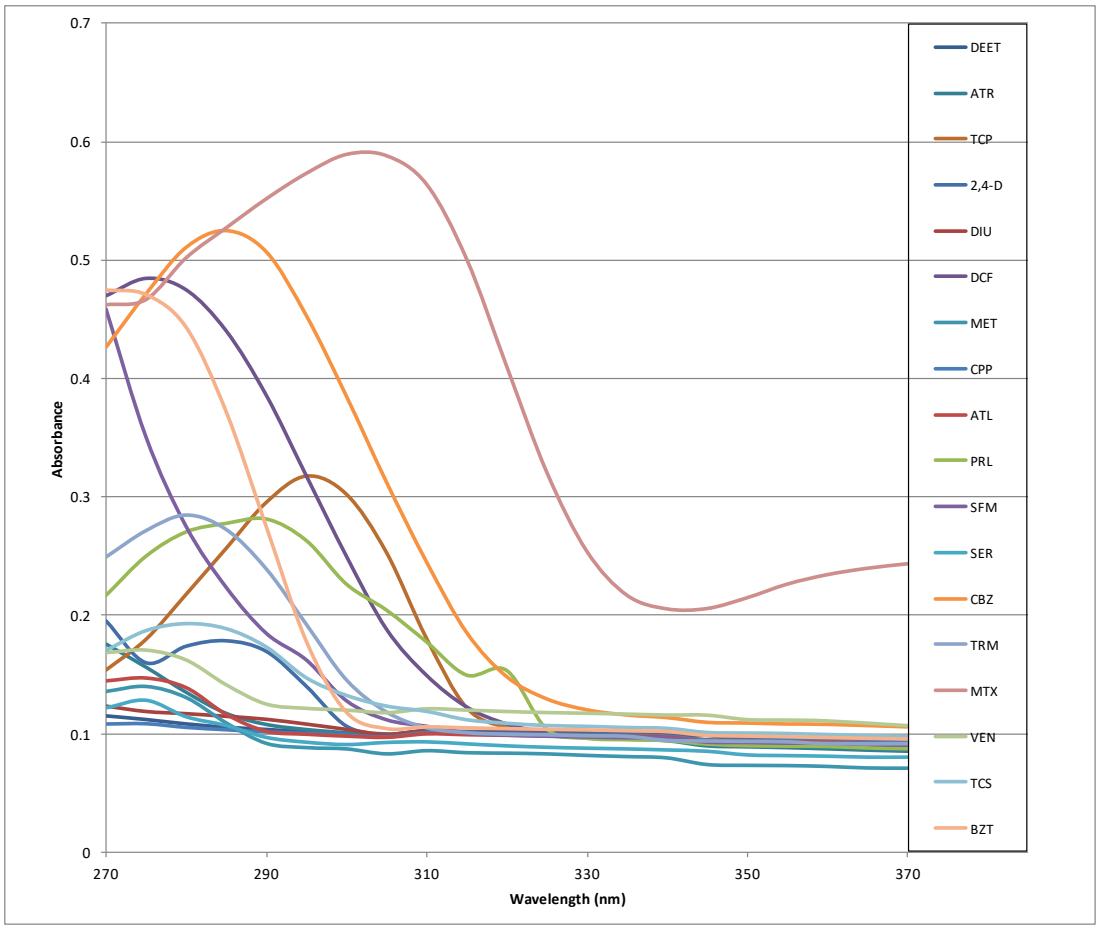


Figure 46. Absorbance spectra from 270-370 nm of selected compounds in ultrapure (Milli-Q) water buffered at pH 7.5 (H_3BO_3/NaOH) at a nominal concentration of 10 mg/L in a quartz cuvette of 1 cm pathlength. The wavelength selected corresponded with the region that was most important for absorbance with the majority of selected contaminants.

APPENDIX 4: Photolysis Rates of Selected Contaminants

Table 28. Summary of first-order rates of photolysis (k_{photo}; d-1) under experimental conditions and corresponding half-lives ($t_{0.5}$) (in parentheses).

Compound	Milli-Q Expt 1	Wivenhoe Expt 1	Milli-Q Expt 2	Wivenhoe Expt 2	Brisbane River	Salisbury
Atenolol (ATL)	-0.01±.04 (na)	0.17±0.03 (4.1 days)	-0.04±0.02 (na)	0.13±0.06 (5.5 days)	0.37±0.1 (1.9 days)	0.21±0.02 (3.3 days)
Benzotriazole (BZT)	0.09±0.1 (7.5 days)	0.15±0.05 (4.8 days)	0.48±0.02 (1.4 days)	0.03±0.03 (22 days)	0.76±0.03 (0.9 days)	0.21±0.04 (3.2 days)
Methotrexate (MTX)	0.01±0.01 (>30 days)	0.22±0.06 (3.1 days)	0.09±0.1 (7.8 days)	0.26±0.03 (2.7 days)	0.37±0.01 (1.9 days)	0.02±0.06 (34 days)
Metoprolol (MET)	-0.1±0.2 (na)	0.25±0.03 (3.1 days)	nd	nd	nd	nd
Trimethoprim (TRM)	0.01±0.06 (>30 days)	0.22±0.09 (3.1 days)	-0.1±0.01 (na)	0.1±0.02 (6.8 days)	0.25±0.02 (2.7 days)	nd
Venlafaxine (VEN)	0.05±0.06 (14 days)	0.4±0.04 (1.9 days)	-0.4±0.1 (na)	0.3±0.03 (2.3 days)	0.49±0.15 (1.4 days)	0.19±0.04 (3.6 days)
Propranolol (PRL)	1.55±0.2 (0.4 days)	2.6±0.1 (0.3 days)	3.08±0.06 (0.22 days)	2.6±0.2 (0.27 days)	1.9±0.2 (0.36 days)	2±0.2 (0.35 days)
Cyclophosphamide (CPP)	-0.03±0.1 (na)	0.04±0.04 (16 days)	0.07±0.08 (10 days)	0.01±0.03 (>30 days)	0.02±0.2 (>30 days)	0.02±0.02 (>30 days)
Sulfamethoxazole (SFM)	1.2±0.1 (0.6 days)	0.84±0.1 (0.82 days)	5.5±0.6 (0.12 days)	0.79±0.2 (0.88 days)	1.02±0.2 (0.68 days)	0.88±0.3 (0.78 days)
Atrazine (ATR)	0.14±0.07 (4.8 days)	0.12±0.05 (5.8 days)	0.2±0.3 (3.4 days)	0.27±0.06 (2.6 days)	0.35±0.02 (2 days)	0.19±0.07 (3.7 days)
Carbamazepine (CBZ)	0.07±0.03 (10 days)	0.2±0.01 (3.3 days)	0.05±0.05 (14 days)	0.1±0.01 (7 days)	0.32±0.02 (2.1days)	0.16±0.01 (4.4 days)
Diethyltoluamide (DEET)	0.05±0.04 (13.7 days)	0.11±0.01 (6.1 days)	0.1±0.06 (6.8 days)	0±0.0 (na)	0.25±0.13 (2.8 days)	0.04±0.01 (17.7 days)
Diuron (DIU)	0.24±0.11 (2.9 days)	0.11±0.01 (3.4 days)	-0.1±0.01 (na)	0.31±0.03 (2.2 days)	0.47±0.02 (1.5 days)	0.25±0.06 (2.7 days)
Sertraline (SER)	nd	nd	0.34±0.7 (2 days)	0.52±0.11 (1.3 days)	0.18±0.11 (3.8 days)	0.56±0.04 (1.2 days)
2,4-dichlorophenoxy acetic acid (2,4-D)	0.08±0.04 (8.7 days)	0.21±0.21 (3.3 days)	0.13±0.15 (5.5 days)	0.14±0.02 (4.9 days)	0.19±0.04 (3.6 days)	0.19±0.05 (3.7 days)
Triclopyr (TCP)	1.77±0.1 (0.39 days)	3.1±0.6 (0.22 days)	3.18±0.5 (0.22 days)	3.55±0.7 (0.19 days)	2.34±0.3 (0.29 days)	3±0.3 (0.23 days)
Diclofenac (DCF)	1.48±1.2 (0.47 days)	3.42±2.7 (0.2 days)	9.3±1.6 (0.07 days)	19±0.5 (0.03 days)	19±1.3 (0.04 days)	21±0.7 (0.03 days)

na not applicable
nd not done

Table 29. Summary of photolysis rate constants (k_{photo}; d-1) and associated half lives in parentheses (days) under surface conditions and at a water depth corresponding with a comparable light transmission of either 50% or 10% of that experienced at the surface.

Compound	Ultrapure Water[a]	Wivenhoe Reservoir	Brisbane River
Atenolol (ATL)	-0.04±0.02 (na)	0.13±0.06 (5.5 days)	0.37±0.1 (1.9 days)
50%	0.22±0.12 (3.1 days)	0.17±0.03 (4.1 days)	0.15±0.02 (4.7 days)
10%	-0.2±0.13 (na)	0.09±0.02 (7.4 days)	0.05±0.04 (4.1 days)
Benzotriazole (BZT)	0.48±0.02 (1.4 days)	0.03±0.03 (22 days)	0.76±0.03 (0.9 days)
50%	0.03±0.0.03 (21 days)	0.06±0.04 (12 days)	0.06±0.02 (12 days)
10%	0.12±0.04 (5.8 days)	0 (na)	-0.2±0.02 (na)
Methotrexate (MTX)	0.09±0.1 (7.8 days)	0.26±0.03 (2.7 days)	0.37±0.01 (1.9 days)
50%	0.06±0.08 (11 days)	0.35±0.25 (2 days)	0.08±0.02 (8.7 days)
10%	0±0.02 (na)	0.1±0.02 (6.7o days)	-0.04±0.03 (na)
Trimethoprim (TRM)	-0.1±0.01 (na)	0.1±0.02 (6.8 days)	0.25±0.02 (2.7 days)
50%	0.02±0.05 (>30 days)	0.09±0.03 (7.5 days)	0.04±0.02 (20 days)
10%	0±0.1 (na)	0.06±0.03 (11.7 days)	0.03±0.05 (21 days)
Venlafaxine (VEN)	-0.4±0.1 (na)	0.3±0.03 (2.3 days)	0.49±0.15 (1.4 days)
50%	0.02±0.06 (29 days)	0.13±0.02 (5.3 days)	0.15±0.03 (4.8 days)
10%	-0.7±0.5 (na)	0.06±0.02 (11 days)	-0.1±0.1 (na)
Propranolol (PRL)	3.08±0.06 (0.22 days)	2.6±0.2 (0.27 days)	1.9±0.2 (0.36 days)
50%	0.57±0.15 (1.2 days)	0.74±0.02 (0.9 days)	1.19±0.08 (0.6 days)
10%	0.62±0.06 (1.1 days)	1.2±0.02 (0.6 days)	0.3±0.03 (2.3 days)
Cyclophosphamide (CPP)	0.07±0.08 (10 days)	0.01±0.03 (>30 days)	0.02±0.2 (>30 days)
50%	0.01±0.02 (>30 days)	0.03±0.02 (27 days)	0.01±0.03 (>30 days)
10%	-0.05±0.1 (na)	-0.01±0.03 (na)	-0.05±0.03 (na)
Sulfamethoxazole (SFM)	5.5±0.6 (0.12 days)	0.79±0.2 (0.88 days)	1.02±0.2 (0.68 days)
50%	0.27±0.1 (2.5 days)	0.29±0.09 (2.4 days)	0.25±0.02 (2.8 days)
10%	-0.01±0.1 (na)	0.19±0.08 (3.7 days)	0.8±0.04 (8.2 days)

Compound	Ultrapure Water[a]	Wivenhoe Reservoir	Brisbane River
Atrazine (ATR)	0.2±0.3 (3.4 days)	0.27±0.06 (2.6 days)	0.35±0.02 (2 days)
50%	-0.02±0.02 (na)	0.06±0.01 (12 days)	0.02±0.03 (>30days)
10%	-0.06±0.1 (na)	-0.01±0.03 (na)	-0.02±0.03 (na)
Carbamazepine (CBZ)	0.05±0.05 (14 days)	0.1±0.01 (7 days)	0.32±0.02 (2.1days)
50%	0.03±0.03 (20 days)	0.12±0.02 (5.7 days)	0.05±0.01 (13 days)
10%	0.05±0 (14 days)	0.05±0.01 (13 days)	-0.02±0.03 (na)
Diethyltoluamide (DEET)	0.1±0.06 (6.8 days)	0±0.0 (na)	0.25±0.13 (2.8 days)
50%	0±0.02 (na)	0.02±0.04 (>30 days)	0.01±0.01 (>30 days)
10%	-0.4±0.1 (na)	-0.02±0.01 (na)	-0.13±0.05 (na)
Diuron (DIU)	-0.1±0.01 (na)	0.31±0.03 (2.2 days)	0.47±0.02 (1.5 days)
50%	0±0.06 (na)	0.09±0.03 (7.4 days)	0.04±0.01 (15 days)
10%	-0.8±0.1 (na)	-0.03±0.01 (na)	-0.18±0.01 (na)
Sertraline (SER)	0.34±0.7 (2 days)	0.52±0.11 (1.3 days)	0.18±0.11 (3.8 days)
50%	0.43±0.3 (1.6 days)	0.39±0.11 (1.8 days)	0.2±0.16 (3.4 days)
10%	0.08±0.21 (9.1 days)	0.29±0.21 (2.4 days)	0.29±0.07 (2.4 days)
2,4-dichlorophenoxyacetic acid (2,4-D)	0.13±0.15 (5.5 days)	0.14±0.02 (4.9 days)	0.19±0.04 (3.6 days)
50%	0.12±0.04 (6 days)	0.18±0.04 (3.9 days)	0.12±0.03 (5.6 days)
10%	-0.19±0.03 (na)	0.02±0.04 (>30 days)	0.04±0.02 (17 days)
Triclopyr (TCP)	3.18±0.5 (0.22 days)	3.55±0.7 (0.19 days)	2.34±0.3 (0.29 days)
50%	1.21±0.5 (0.57 days)	0.99±0.03 (0.7 days)	1.05±0.12 (0.66 days)
10%	0.22±0.01 (3.2 days)	0.63±0.27 (1.1 days)	0.32±0.12 (2.2 days)
Diclofenac (DCF)	9.3±1.6 (0.07 days)	19±0.5 (0.03 days)	19±1.3 (0.04 days)
50%	8.9±2.8 (0.08 days)	7.6±1.2 (0.09 days)	10.2±1.4 (0.07 days)
10%	2.37±0.8 (0.29 days)	2.83±0.19 (0.24 days)	0.56±0.2 (1.2 days)

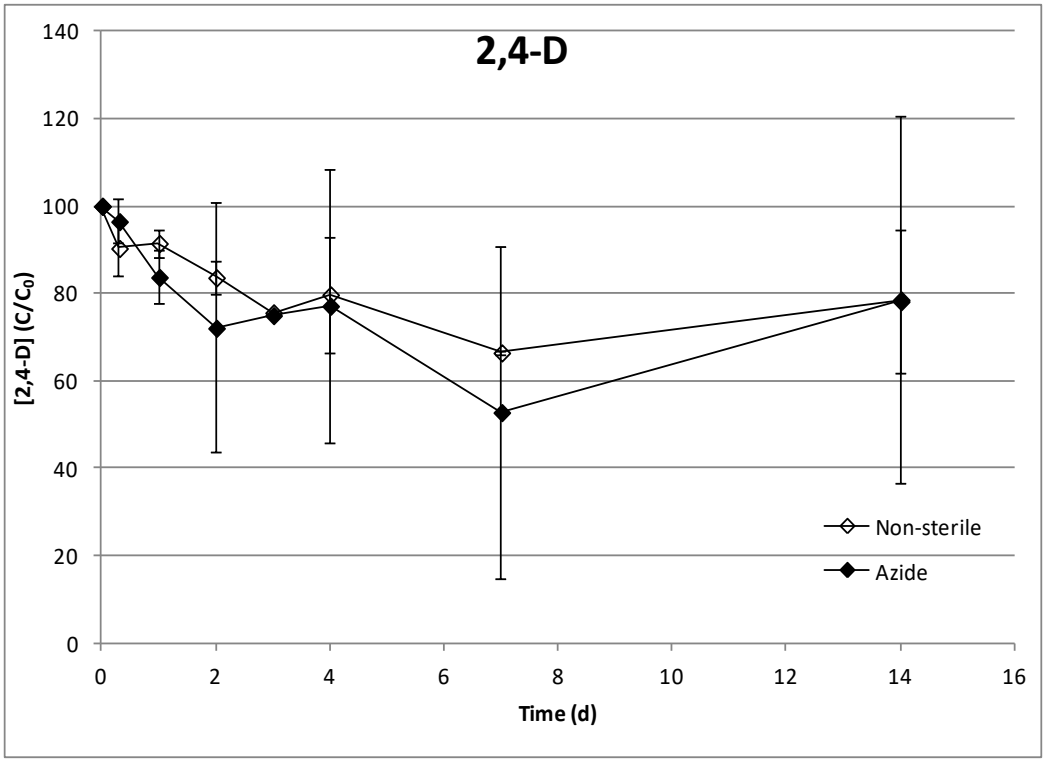
[a]ultrapure water values given for Wivenhoe pH buffered controlled
na not applicable

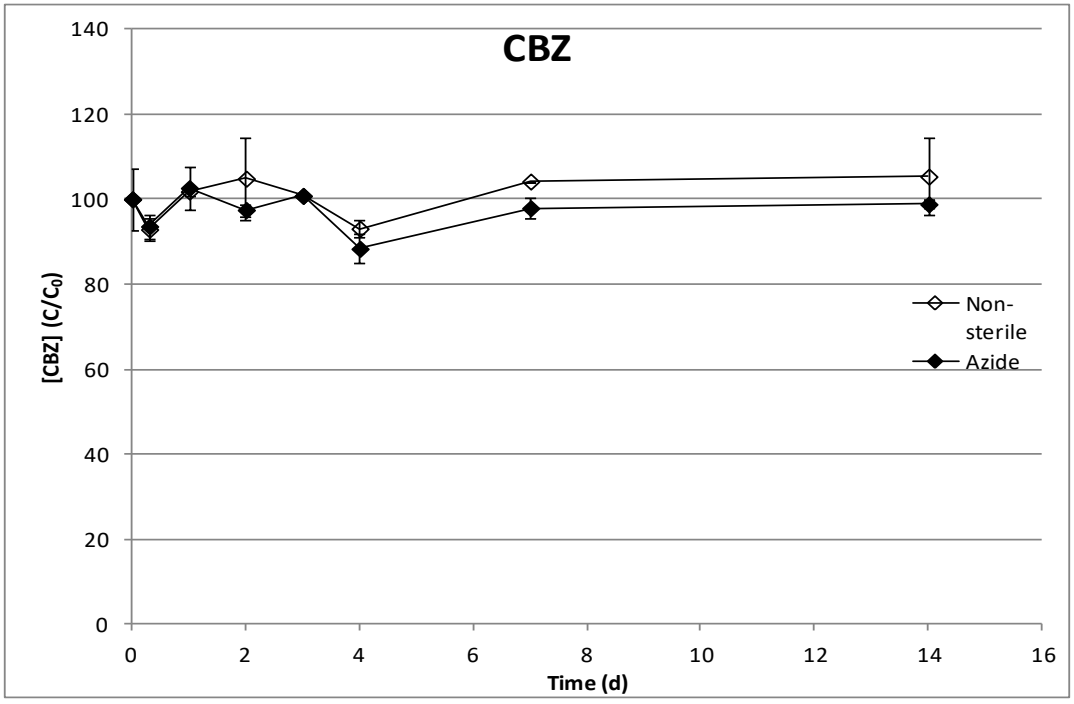
Table 30. Summary of rates of photolysis (k_{photo}; d-1), derived from Table B1 experiment 2, adjusted for environmental factors that would decrease photolysis rates and correspondingly increase estimates of half-lives (in parentheses). Environmental factors include diurnal (factor of 5) and seasonal (factor of 1.2) variation in solar irradiance and assuming a well-mixed system of depth 5 metres where photolysis only occurs in the top 0.1 metres of the water body (factor of 50).

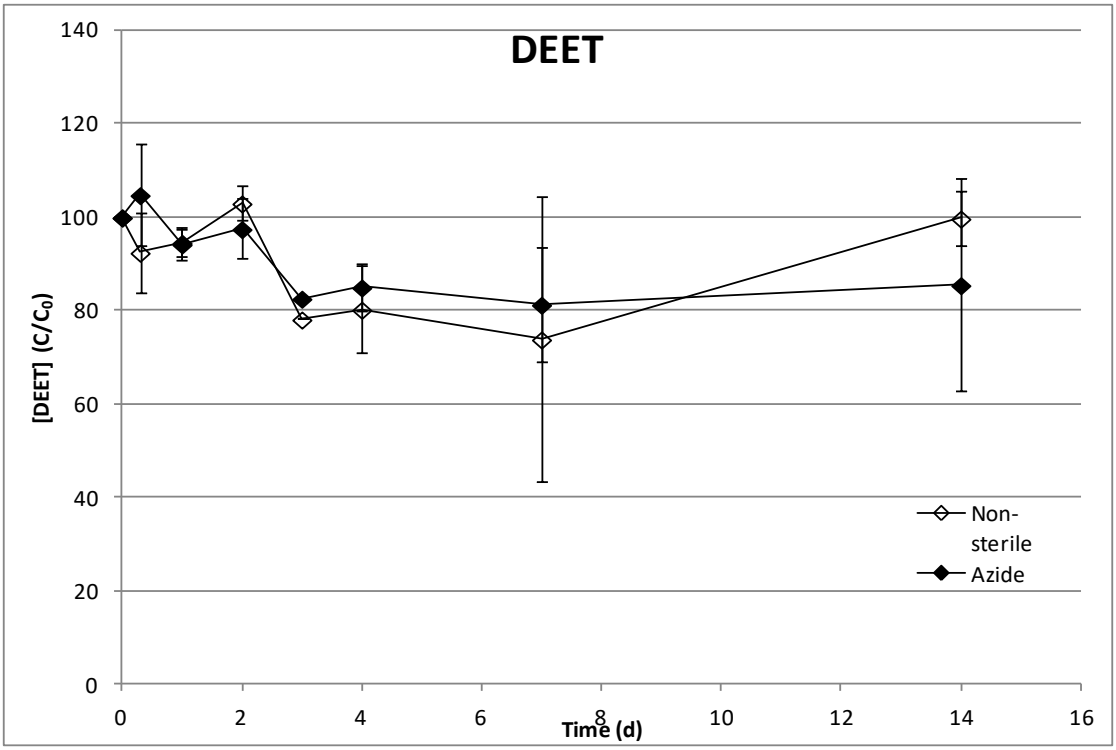
Compound	Milli-Q	Wivenhoe	Brisbane River	Salisbury
Atenolol (ATL)	0 (na)	0.0004 (>60 days)[a]	0.00123 (>60 days)	0.0007 (>60 days)
Benzotriazole (BZT)	0.002 (>60 days)	0 (na)	0 (na)	0 (na)
Methotrexate (MTX)	0.0003 (>60 days)	0.00086 (>60 days)	0.001 (>60 days)	0.0001 (>60 days)
Trimethoprim (TRM)	0 (na)	0.0003 (>60 days)	0.001 (>60 days)	0.003 (>60 days)
Venlafaxine (VEN)	0 (na)	0.001 (>60 days)	0.002 (>60 days)	0.0006 (>60 days)
Propranolol (PRL)	0.01 (>60 days)	0.009 (>60 days)	0.006 (>60 days)	0.007 (>60 days)
Cyclophosphamide (CPP)	0.0002 (>60 days)	0 (na)	0 (na)	0.0001 (>60 days)
Sulfamethoxazole (SFM)	0.019 (37 days)	0.003 (>60 days)	0.003 (>60 days)	0.003 (>60 days)
Atrazine (ATR)	0.0007 (>60 days)	0.0009 (>60 days)	0.001 (>60 days)	0.0006 (>60 days)
Carbamazepine (CBZ)	0.0002 (>60 days)	0.0003 (>60 days)	0.001 (>60 days)	0.0005 (>60 days)
Diethyltoluamide (DEET)	0.0003 (>60 days)	0 (na)	0.0008 (>60 days)	0.0001 (>60 days)
Diuron (DIU)	0 (na)	0.001 (>60 days)	0.002 (>60 days)	0.0008 (>60 days)
Sertraline (SER)	0.001 (>60 days)	0.002 (>60 days)	0.0006 (>60 days)	0.002 (>60 days)
2,4-dichlorophenoxy acetic acid (2,4-D)	0.0004 (>60 days)	0.0005 (>60 days)	0.0006 (>60 days)	0.0006 (>60 days)
Triclopyr (TCP)	0.01 (>60 days)	0.012 (59 days)	0.008 (>60 days)	0.01 (>60 days)
Diclofenac (DCF)	0.03 (22 days)	0.06 (11 days)	0.06 (11 days)	0.07 (9.8 days)

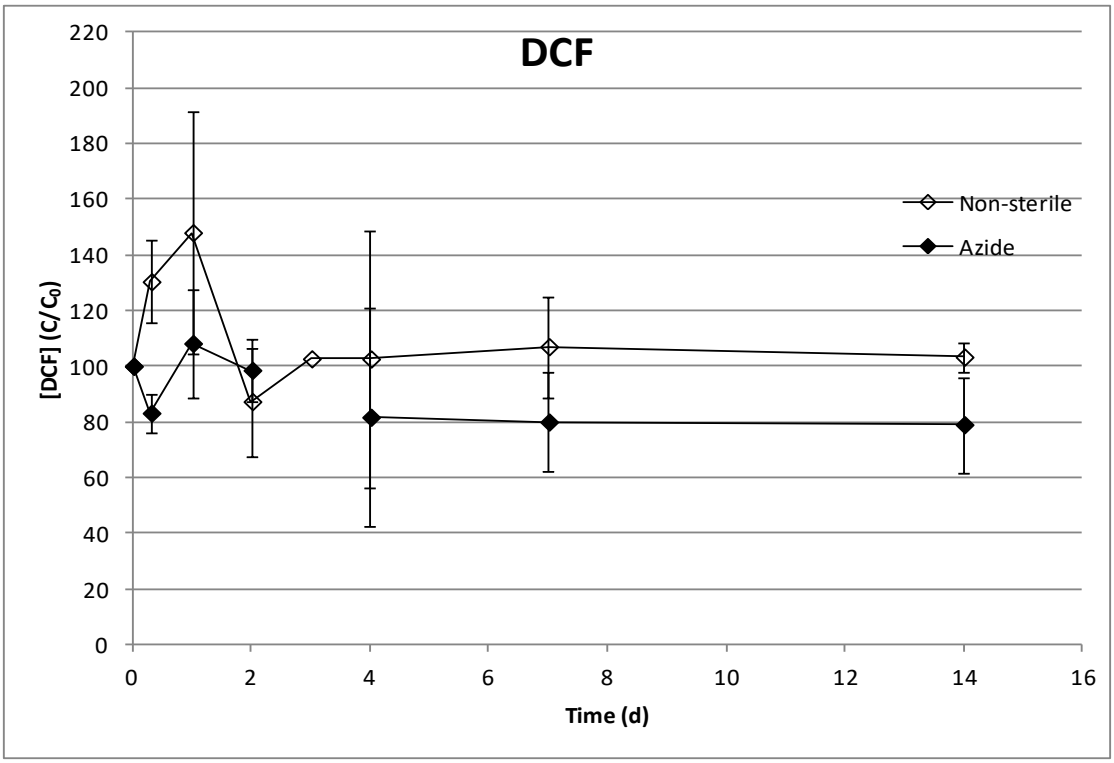
na not applicable
[a] definition of very persistent chemical in water (European Parliament and European Council 2006)

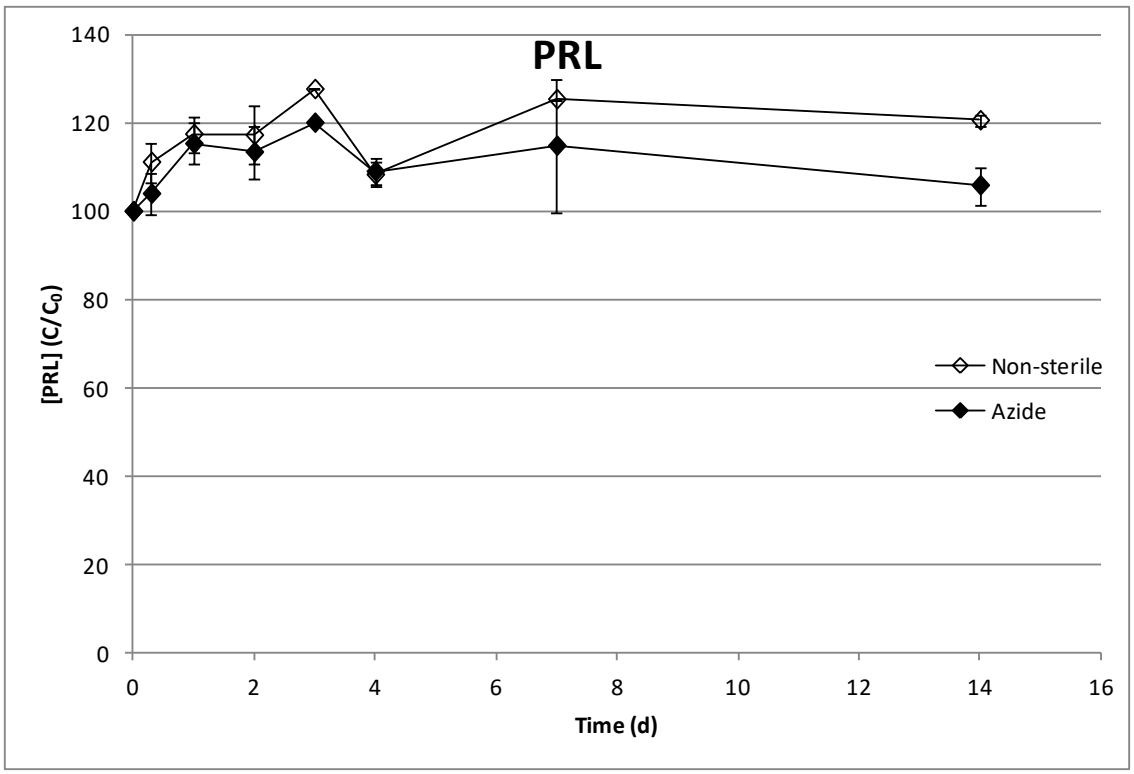
APPENDIX 5: Plots of Relative Concentration and Time for Biodegradation Microcosms in Wivenhoe Reservoir and Salisbury Wetland Water

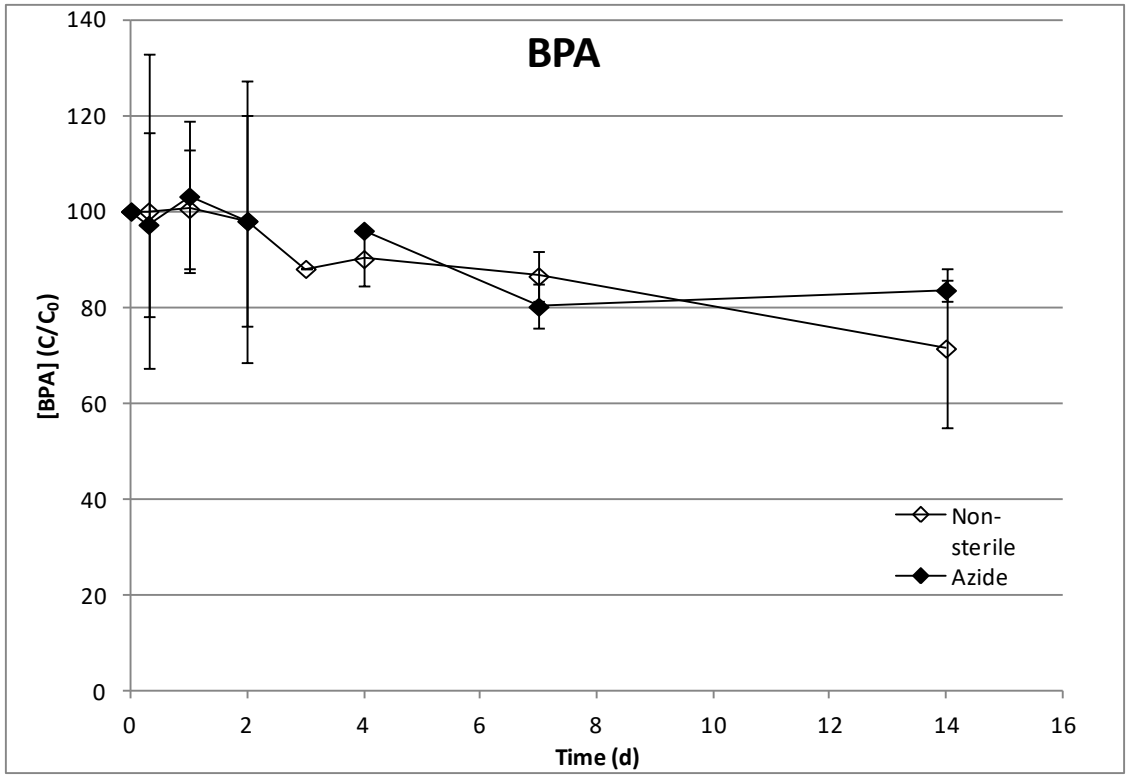





DEET
[DEET] (C/C0)
Time (d)
Non-sterile
Azide


DCF
[DCF] (C/C0)
Time (d)
Non-sterile
Azide


PRL
[PRL] (C/C0)
Time (d)
Non-sterile
Azide
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
16


BPA
[BPA] (C/C0)
Time (d)
Non-sterile
Azide
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
16

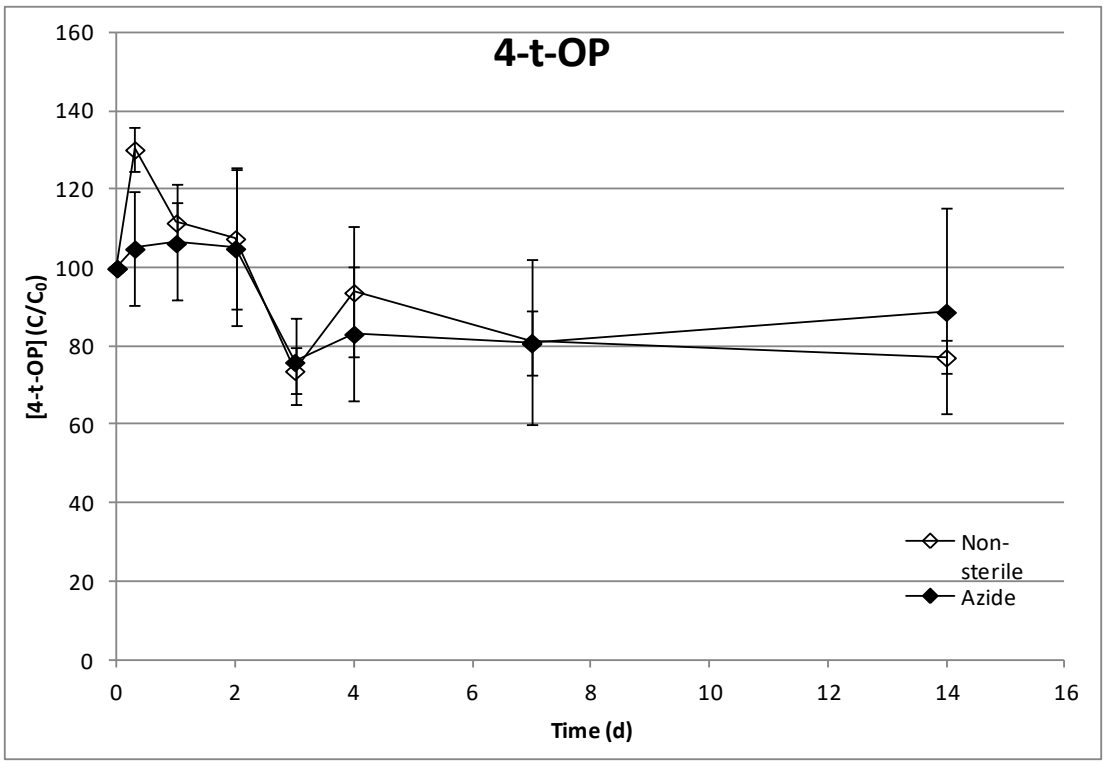
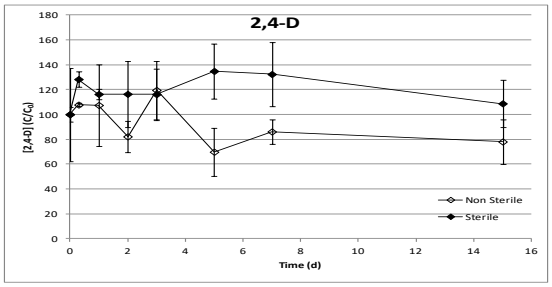
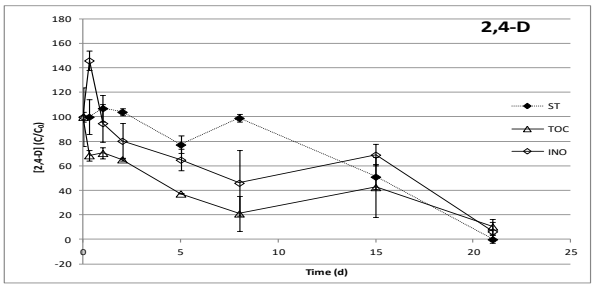
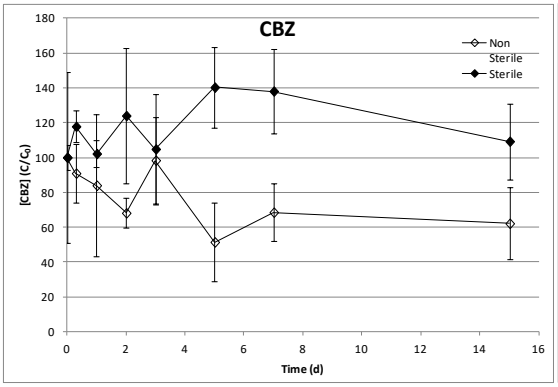
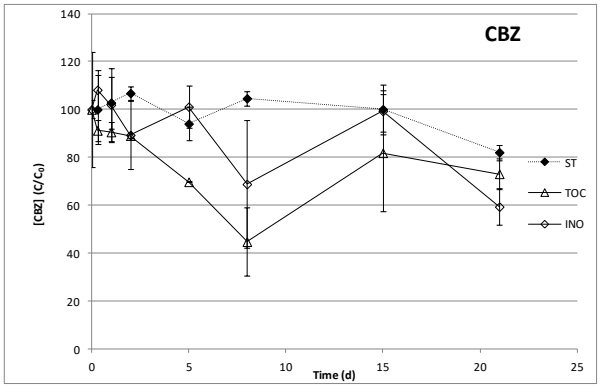


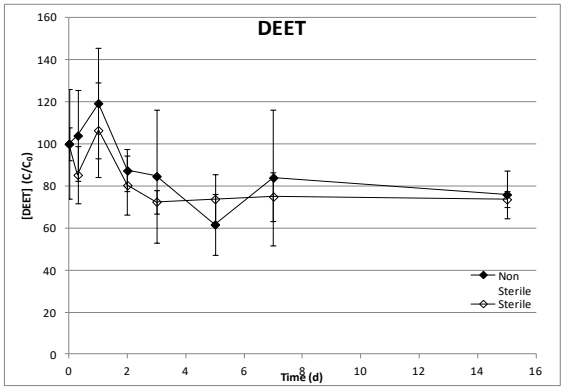
Figure 47. Concentrations relative to initial concentrations (C/C_0) of the selected contaminants in Wivenhoe Reservoir water over 14 days for selected compounds.

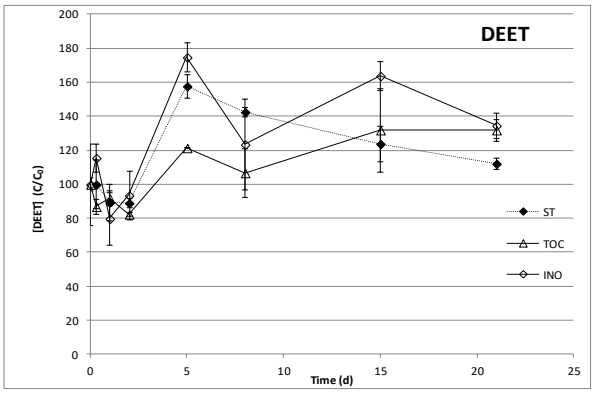

2,4-D
[2,4-D] (C/C0)
Time (d)
Non Sterile
Sterile

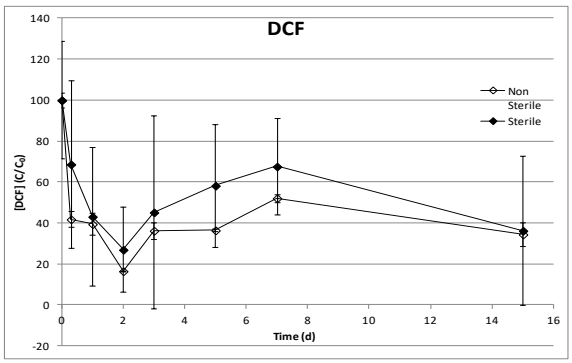

2,4-D
[2,4-D] (C/C0)
Time (d)
ST
TOC
INO

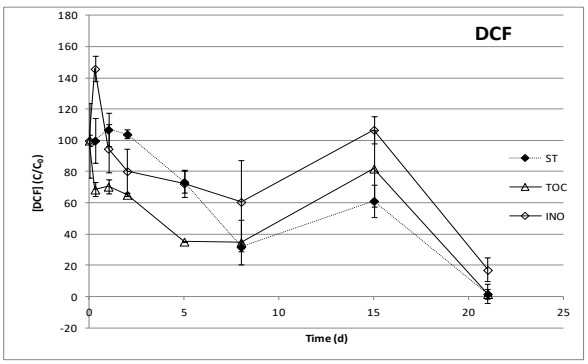

CBZ
[CBZ] (C/C0)
Time (d)
Non Sterile
Sterile

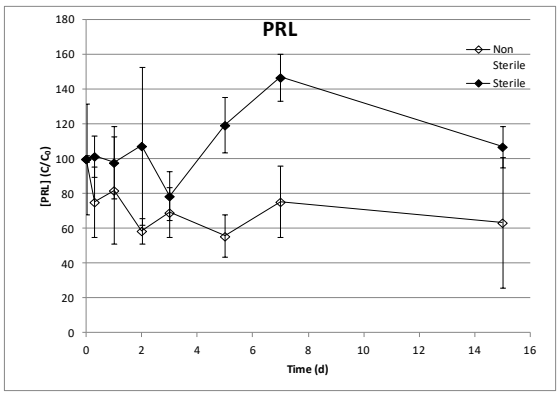

CBZ
[CBZ] (C/C0)
Time (d)
ST
TOC
INO

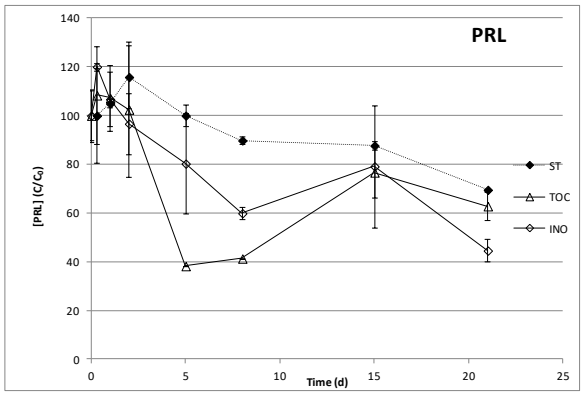

DEET
[DEET] (C/C0)
Time (d)
Non Sterile
Sterile

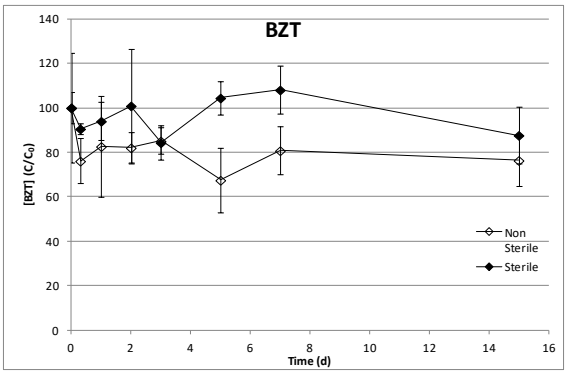

DEET
[DEET] (C/C0)
Time (d)
ST
TOC
INO

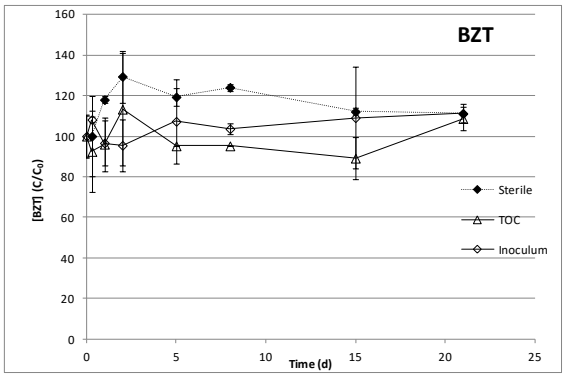

DCF
[DCF] (C/C0)
Time (d)
Non Sterile
Sterile

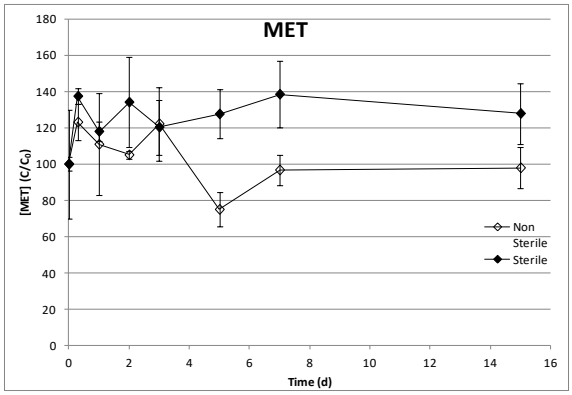

DCF
[DCF] (C/C0)
Time (d)
ST
TOC
INO

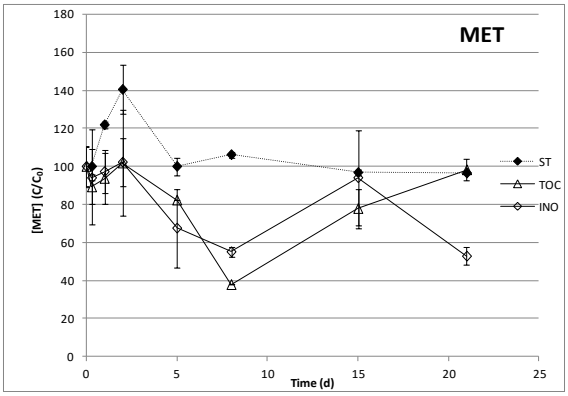

PRL
Non Sterile
Sterile
[PRL] (C/C0)
Time (d)

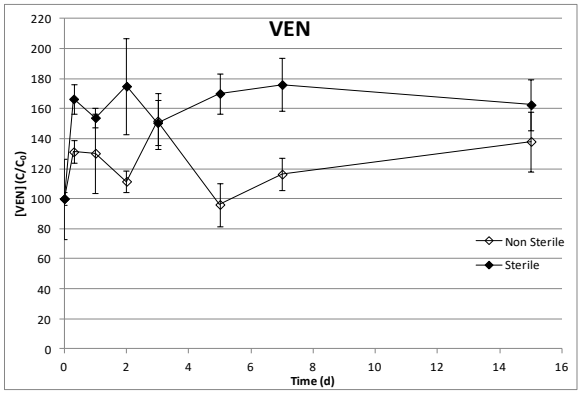

PRL
ST
TOC
INO
[PRL] (C/C0)
Time (d)

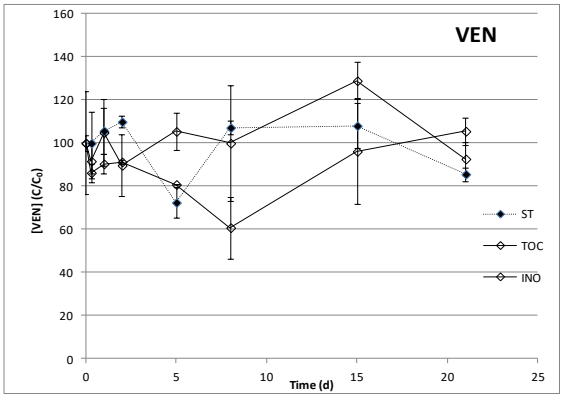

BZT
Non Sterile
Sterile
[BZT] (C/C0)
Time (d)


BZT
Sterile
TOC
Inoculum
[BZT] (C/C0)
Time (d)


MET
Non Sterile
Sterile
[MET] (C/C0)
Time (d)


MET
ST
TOC
INO
[MET] (C/C0)
Time (d)


VEN
Non Sterile
Sterile
[VEN] (C/C0)
Time (d)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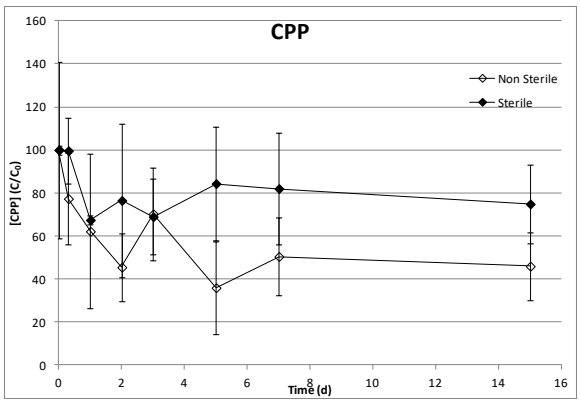
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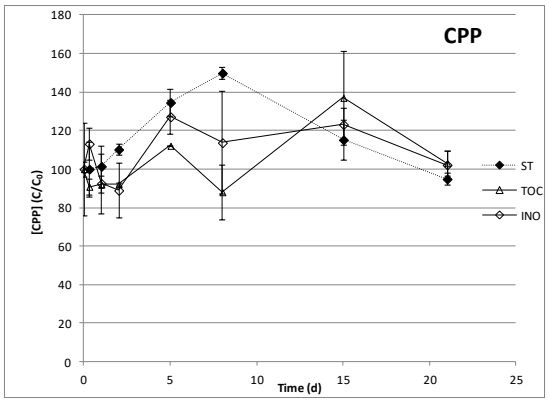
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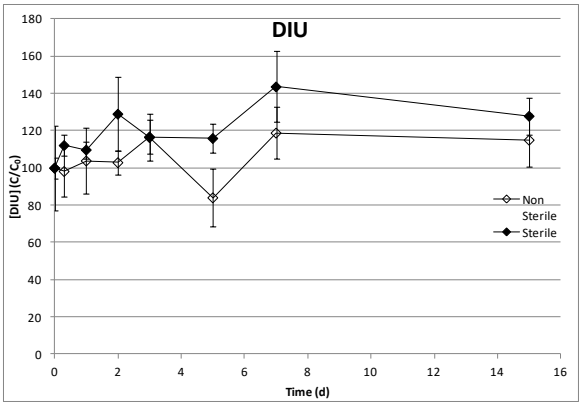
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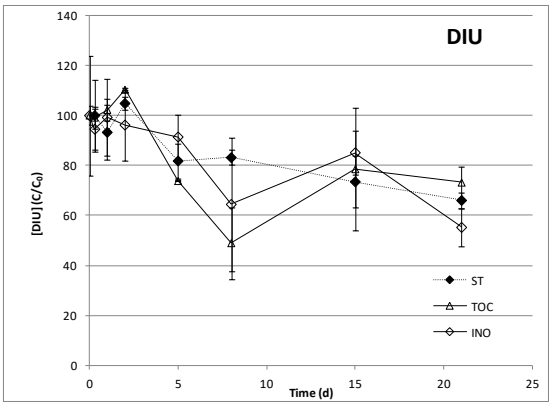
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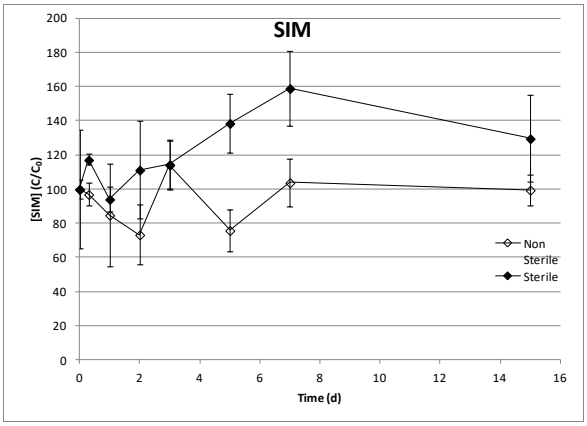
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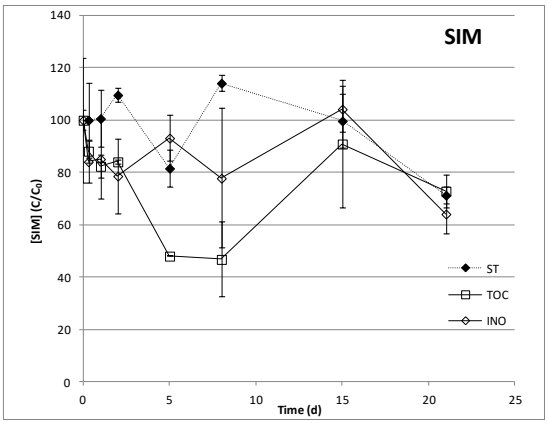
Sterile
[SIM] (C/C0)
Time (d)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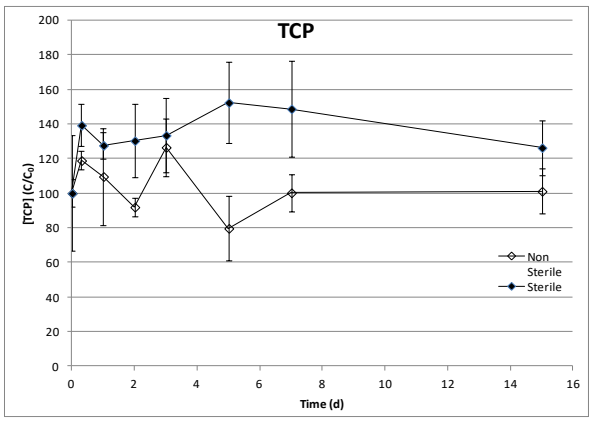
[SIM] (C/C0)
Time (d)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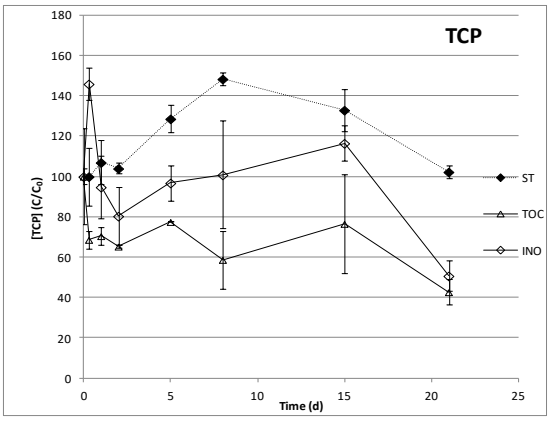
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Time (d)

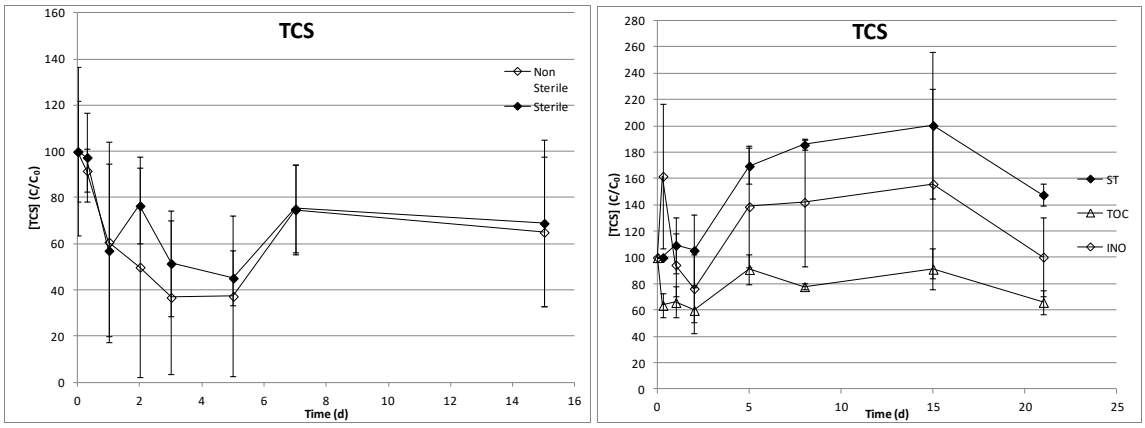

Figure 48. Concentrations relative to initial concentrations (C/C_0) of the selected contaminants in unamended Salisbury Wetland water (over a 14 day exposure period) and in Salisbury wetland water amended with BDOC (TOC)or microbial inoculum (INO), with a sterile control (ST) for selected compounds (over a 21 days exposure period).

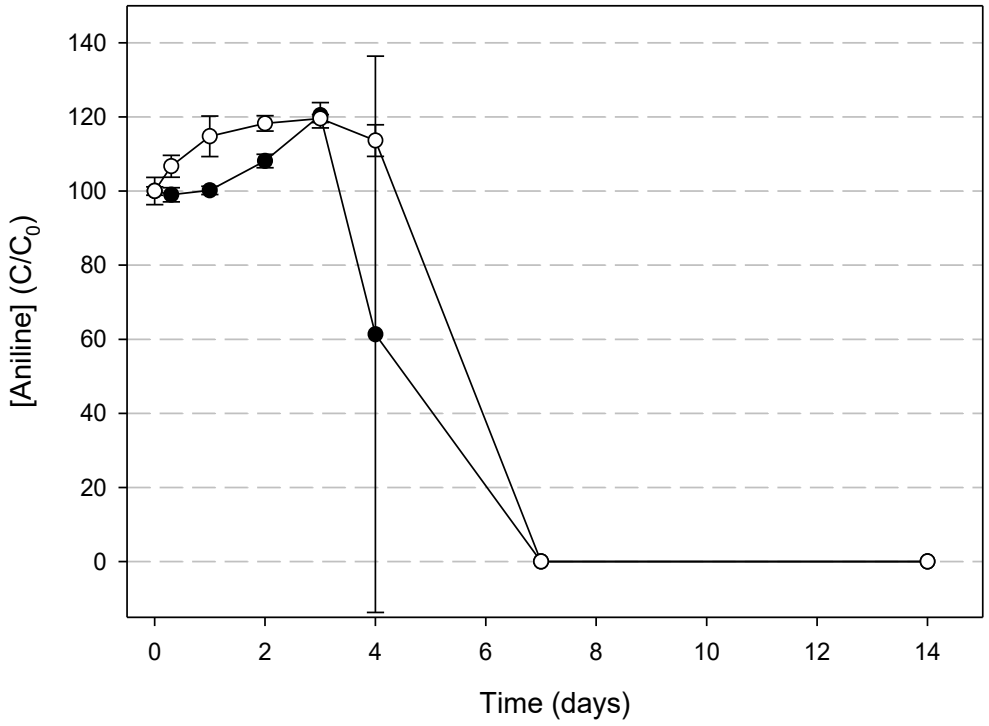

Figure 49. Concentrations of aniline relative to initial concentrations (C/C_0) during the Wivenhoe Reservoir degradation experiments. Open circles represent NaN_3 amended treatments while closed circles represent unamended water samples.

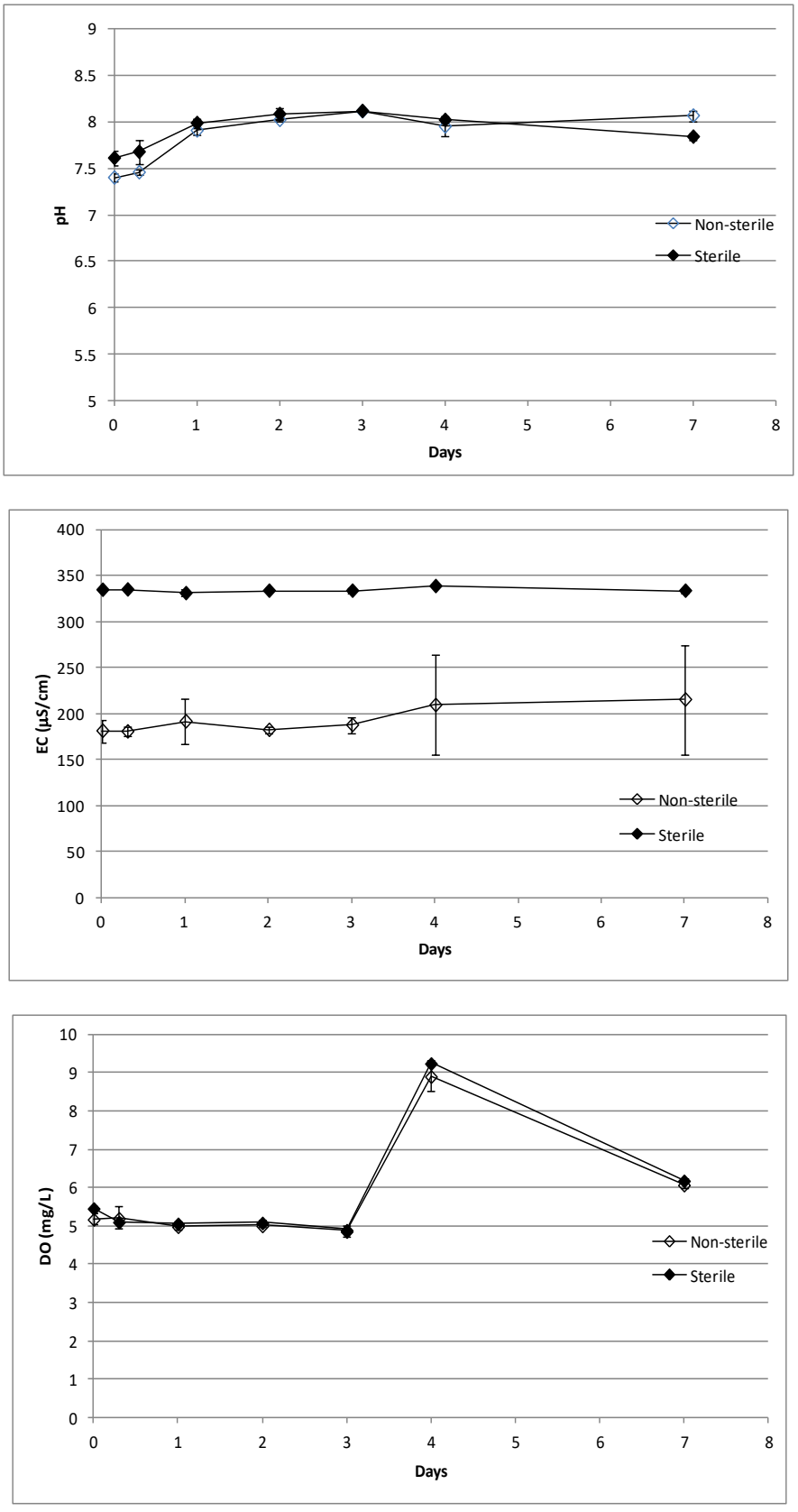

Figure 50. Water quality parameters monitored during the biodegradation assessment in Wivenhoe Reservoir water.

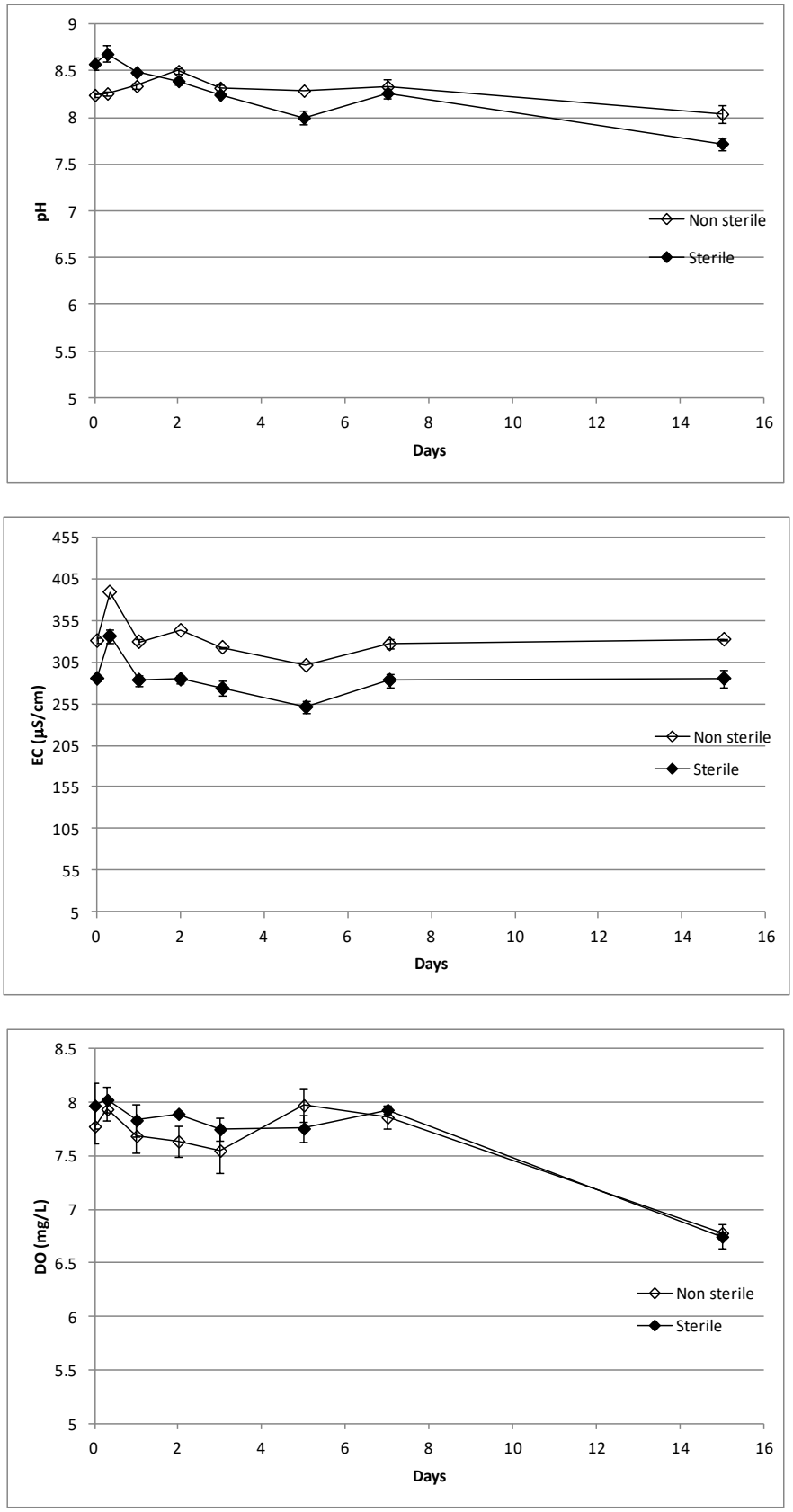

Figure 51. Water quality parameters monitored during the biodegradation assessment in Salisbury Wetland water.

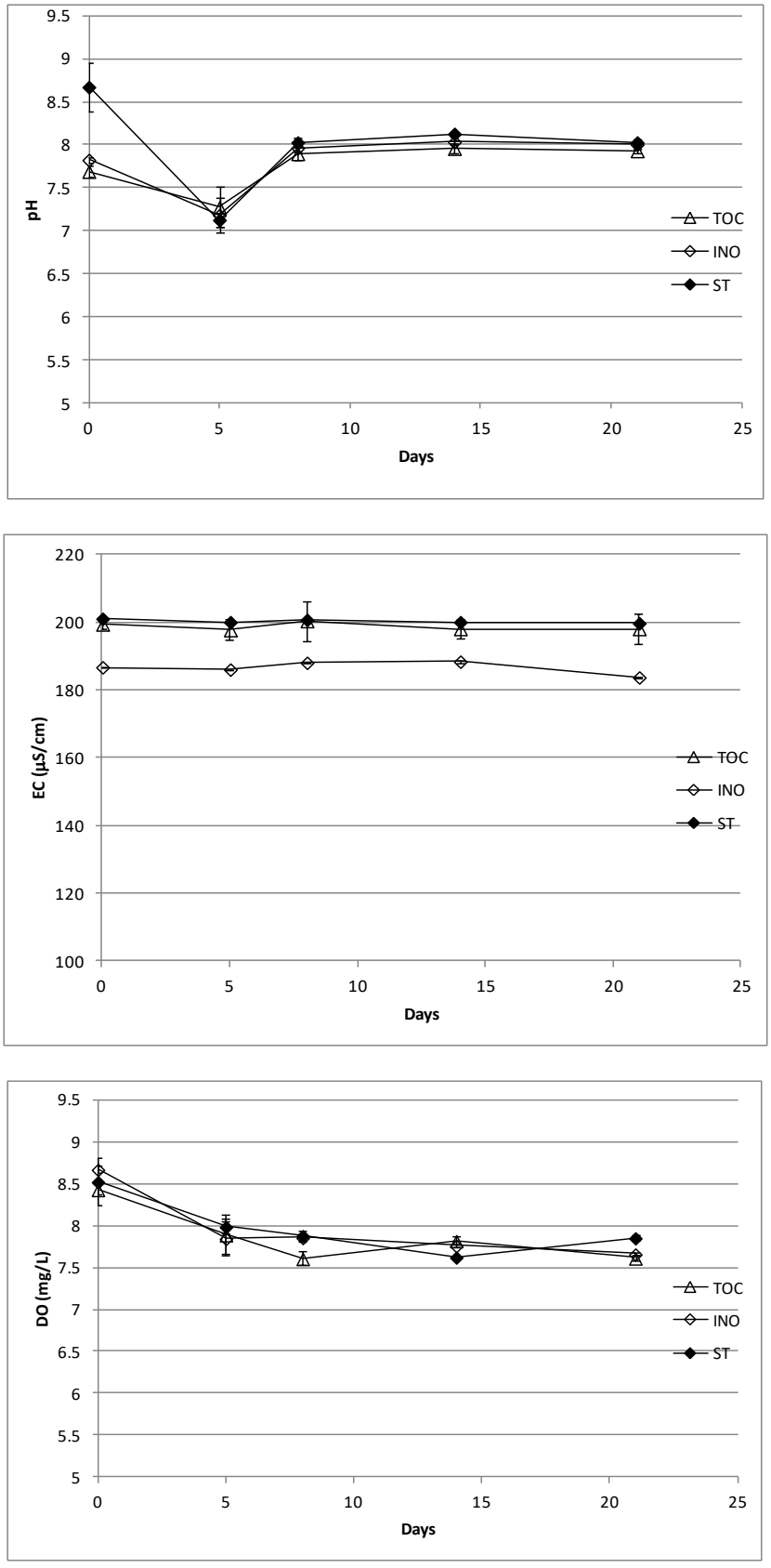

Figure 52. Water quality parameters monitored during the biodegradation assessment in amended Salisbury Wetland water, including enhanced dissolved organic carbon (TOC) and a microbial inoculum (INO).

APPENDIX 6: Microbial Source Tracking

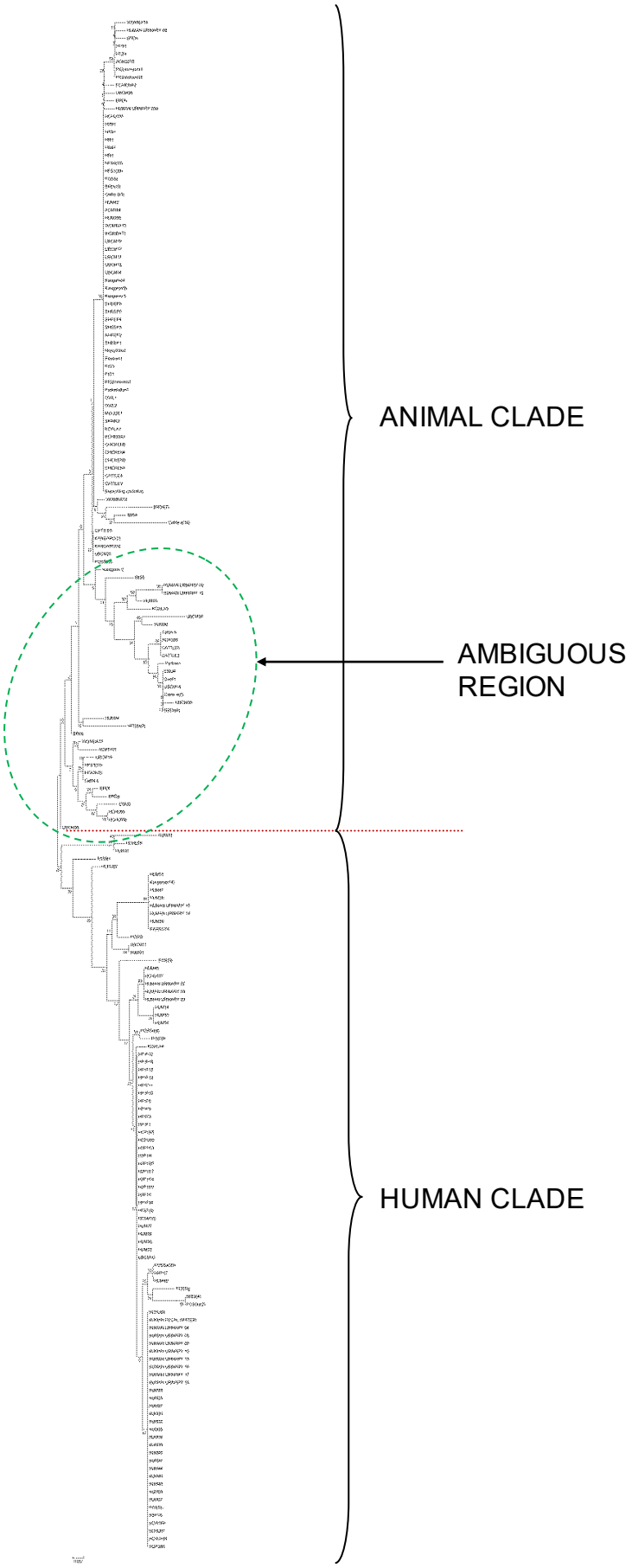

Figure 53. Phylogenetic tree of β-glucuronidase sequences from known human and animal sources. Total number of sequences is 196 (93 animal; 103 human).

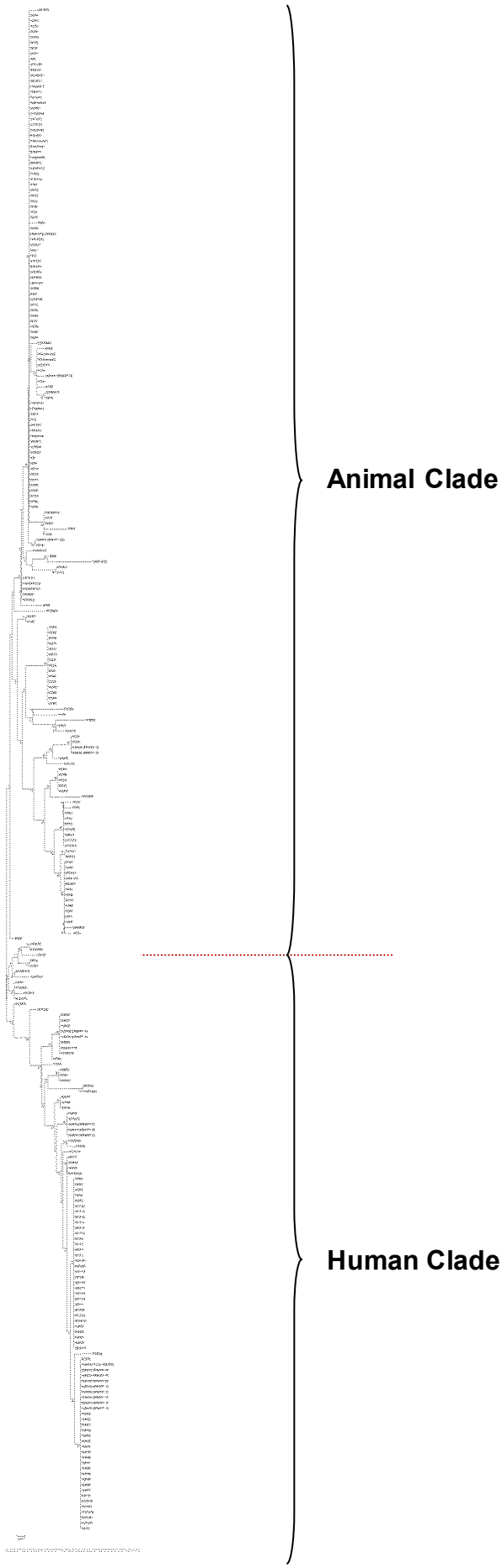

Figure 54. Phylogenetic tree of β-glucuronidase sequences from known sources and the unknown Wivenhoe Dam isolates.

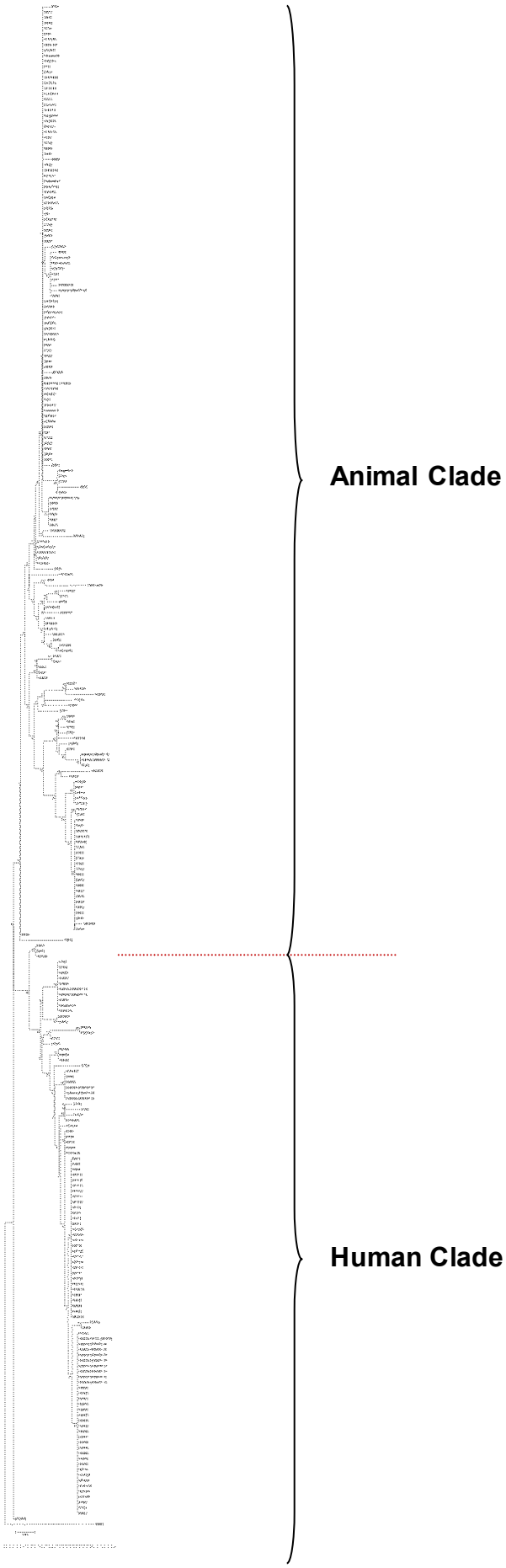

Figure 55. Phylogenetic tree of β-glucuronidase sequences from known sources and the unknown Somerset Dam isolates.

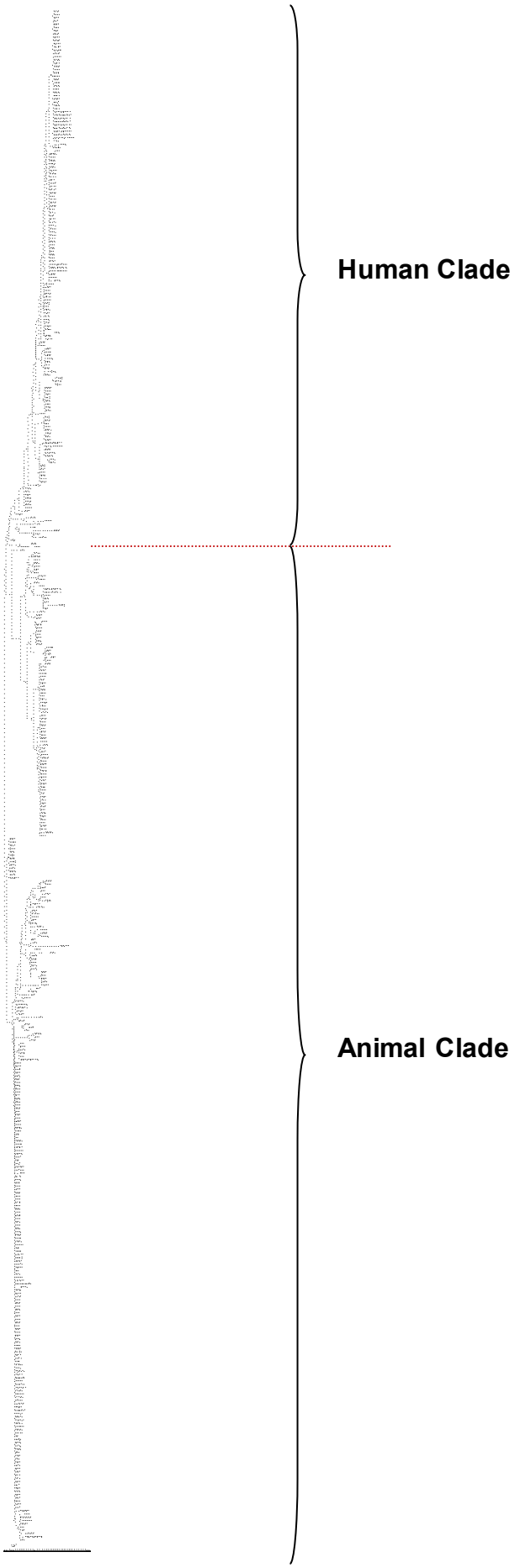

Figure 56. Phylogenetic tree of β-glucuronidase sequences from known sources and the unknown Baroon Pocket Dam isolates.

APPENDIX 7: Methods Used in the Testing of Bovine Microbial Source Tracking Markers

1. Concentration of Water Samples

Approximately, 9 L of water sample from each sample site on Wivenhoe Dam was concentrated by hollow-fiber ultrafiltration system (HFUS), using Hemoflow HF80S dialysis filters (Fresenius Medical Care, Lexington, MA, USA) as previously described (Hill *et al.*, 2005). Briefly, each water sample was pumped with a peristaltic pump (Masterflex: Cole-Parmer Instrument Co., USA) in a closed loop with high-performance, platinum-cured L/S 36 silicone tubing (Masterflex; Cole-Parmer Instrument Co.). Tubing was sterilised by soaking in 10% bleach, washed with sterile distilled water and autoclaved at 121ºC for 15 mins. At the end of the sample concentration process, pressurised air was passed through the filter cartridge from the top to recover as much samples as possible. A new filter cartridge was used for each sample. The samples were concentrated to approximately 100-150 mL depending on the turbidity of the water. Each sample was further centrifuged at 3,000 g for 30 mins at 4ºC to obtain a pellet. The supernatant was discarded, and the pellet was resuspended in 5 mL of sterile distilled water.

2. Extraction of DNA from Faecal Samples

In summary, the PCR amplified products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA), and cloned into the pGEM®-T Easy Vector System (Promega, Madison, WI, USA), transferred into *E. coli* JM109 competent cells, and plated on Luria Bertani (LB) agar plates containing ampicillin, IPTG (isopropyl-β-D-thiogalactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl-β- D galactopyranoside) as recommended by the manufacturer. Plasmid DNA was extracted using plasmid mini kit (Qiagen). DNA sequencing was carried out at the Australian Genome Research Facility (St. Lucia, Queensland, Australia). For *Campylobacter* spp., *Salmonella* spp. and *E. coli* O157:H7 real-time PCR assays, genomic DNA was isolated from *C. jejuni* NCTC 11168, *E. coli* O157:H7 ATCC 35150, and *S. enteric* var Typhimurium ATCC 14028, respectively.

3. Extraction of DNA from faecal and wastewater samples

DNA was extracted from the concentrated primary influent and cattle wastewater samples using DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). QIAmp Stool DNA Kit was used to extract DNA from 50-120 mg of fresh feces from each individual animal. DNA was extracted from the concentrated environmental water samples using the Power Soil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA).

DNA was extracted from the 1.5 ml of the 5 mL concentrated water samples using the Power Soil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA). All DNA samples extracted from target host-groups, non-target host-groups and water samples were quantified using a NanoDrop spectrophotometer (ND-1000, NanoDrop Technology, Wilmington, DE). For further confirmation on the DNA extraction process, each DNA sample was amplified using a universal bacterial PCR assay (Boon *et al.*, 2003).

4. Evaluation of PCR Inhibition

An experiment was conducted to determine the potential presence of PCR inhibitors in composite wastewater and individual faecal DNA samples extracted from target and non-target host-groups. All DNA samples were 10-fold diluted with sterile water. Undiluted and 10-fold diluted animal composite wastewater and faecal DNA samples were spiked with 10^3 gene copies of sewage-associated *Bacteroides* HF183 marker (Seurinck *et al.*, 2005). On the other hand, human wastewater DNA samples were spiked with 10^3 gene copies of the bovine-associated cowM3 marker (Shanks *et al.,* 2008). An experiment was also conducted to determine the potential presence of PCR inhibitors in the WD water samples. Water samples were spiked with 10^3 gene copies of the HF183 marker. The C_T

values of the spiked undiluted and 10-fold diluted DNA samples were compared to those of the distilled water spiked with the same gene copies of the HF183 and cowM3 markers (Ahmed *et al.,* 2010).

5. Real-Time PCR Assays

The BacCow-UCD 16S rRNA, cowM3, *Campylobacter* spp. 16S rRNA, *E. coli* O157 *rfbE*, and *Salmonella* spp. *invA* real-time PCR assays were performed in 25-µl reaction mixtures using iQ Supermix (Bio-Rad Laboratories, CA, USA). The PCR mixture contained 12.5 µl of Supermix, 400 nM each primer and 80 nM probe (for BacCow-UCD 16 S rRNA assay), 800 nM each primer and 80 nM probe (for cowM3 assay), 500 nM each primer and 400 nM probe (for *Campylobacter* spp. 16 S rRNA assay) and 300 nM each primer and probe (for *E. coli* O157 *rfbE* assay) and 5 µl of template DNA. The real-time PCR conditions were as follows: 10 min at 95ºC, 40 cycles of 15 s at 95ºC and 1min at 60ºC (for BacCow-UCD and cowM3 assays), 10 min at 95°C, 45 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C (for *Campylobacter* spp. assay), 2 min at 95ºC, 35 cycles of 15 s at 95ºC, 45 s at 57ºC (for *E. coli* O157 assay).

The HF183 16S rRNA (for PCR inhibition assay), *Salmonella* spp. *invA* and B-AVs hexon gene amplifications were performed in 20-µl reaction mixtures using Sso Fast™ EvaGreen® Supermix (Bio-Rad Laboratories). The PCR mixture for all three assays contained 10 µl of Supermix, 300 nM each primer and 5 µl of template DNA. The HF183 real-time PCR consisted of 10 min at 95ºC followed by 45 cycles of 30 s at 95ºC, 1 min at 53ºC, and 1 min at 60ºC. The *Salmonella* spp. *invA* real-time PCR consisted of 5 min at 94ºC followed by 35 cycles of 30 s at 94ºC, 35 s at 59ºC, and 2 min at 72ºC. For the detection of B-AVs marker two rounds real-time PCR protocol was used. Both rounds of B-AVs PCR consisted of 4 min at 94º, 30 cycles of 60 s at 92ºC, 30 s at 52ºC and 75 s at 72ºC. The two rounds real-time PCR was performed using the same conditions as in the first-round of PCR, except that 1 µl of the PCR products from the first round was added to a new 10 µl PCR mixture, and 30 amplification cycles were performed. For each real-time PCR assay, a positive control (e.g. corresponding plasmid DNA or genomic DNA) and a negative control (e.g. sterile water) were included. The real-time PCR assays were performed using the Bio-Rad iQ5 real-time PCR detection system (Bio-Rad Laboratories).

6. Real-Time PCR Limit of Detection (PLOD)

To determine the real-time PCR limit of detection (PLOD), plasmid DNA (BacCow-UCD, cowM3 and B-AVs) and genomic DNA (*C. jejuni* NCTC 11168, *E. coli* O157:H7 ATCC 35150, and *S. enterica* var Typhimurium ATCC 14028) were quantified using a spectrophotometer. Ten-fold serial dilutions were made and tested with the real-time PCR assays.

7. Quality Control Assessment

To minimise PCR contamination, DNA extraction and PCR setup were performed in separate laboratories. To prevent false positive results for water samples, a method blank was included for each batch of water samples. A reagent blank was included to prevent cross contamination of samples during DNA extraction. To separate the specific product from non-specific products, DNA melting curve analysis was performed for HF183, *Salmonella* spp. *invA* and B-AVs PCR assays. During melting curve analysis, the temperature was increased from 57ºC to 95ºC at approximately 2ºC per min. Samples were considered to be positive when the sample had the same melting temperature as the positive control for each PCR assay.

REFERENCES

Pathogens

Bae, J., and Schwab, K.J. 2008. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. *Applied and Environmental Microbiology*, **74**(2):477-484.

Blattner, F.R. 1999, *Hot papers - Genetics - The complete genome sequence of Escherichia coli K-12 by F.R. Blattner, G. Plunkett, C.A. Bloch, N.T. Perna, V. Burland, M. Riley, J. Collado-Vides, J.D. Glasner, C.K. Rode, G.F. Mayhew, J. Gregor, N.W. Davis, H.A. Kirkpatrick, M.A. Goeden, D.J. Rose, B. Mau, Y. Shao - Comments.* Scientist, **13**:17-17.

Bofill-Mas, S., Albinana-Gimenez, N., Clemente-Casares, P., Hundesa, A., Rodriguez-Manzano, J., Allard, A., Calvo, M., and Girones, R. 2006, Quantification and stability of human adenoviruses and polyomavirus JCPyV in wastewater matrices. *Applied and Environmental Microbiology*, **73**: 7894-7896.

Burkhardt Iii, W., Calci, K.R., Watkins, W.D., Rippey, S.R., and Chirtel, S.J. 2000, Inactivation of indicator microorganisms in estuarine waters. *Water Research*, **34**(8):2207-2214.

Campbell, A.T., Robertson, L.J., and Smith, H.V. 1992, Viability of *Cryptosporidium parvum* oocysts: correlation of in vitro excystation with inclusion or exclusion of fluorogenic vital dyes. *Applied and Environmental Microbiology*, **58:**3488-3493.

Chang, T.C., and Huang, S.H. 1997, A modified immuno-polymerase chain reaction for the detection of [beta]-glucuronidase from *Escherichia coli*. *Journal of Immunological Methods*, **208**:35-42.

Characklis, G.W., Dilts, M.J., Simmons, O.D., Likirdopulos, C.A., Krometis, L.A.H., and Sobsey, M.D. 2005, Microbial partitioning to settleable particles in stormwater. *Water Research*, **39**(9):1773-1782.

Dan, T.B.B., Wynne, D., and Manor, Y. 1997, Survival of enteric bacteria and viruses in Lake Kinneret, Israel. *Water Research*, **31**:2755-2760.

Darakas, E. 2002, *E-coli* kinetics - Effect of temperature on the maintenance and respectively the decay phase. *Environmental Monitoring and Assessment*, **78**:101-110.

Dontorou, A., Papadopoulou, C., Filioussis, G., Apostolou, I., Economou, V., Kansouzidou, A., and Levidiotou, S. 2004, Isolation of a rare *Escherichia coli O157:H7* strain from farm animals in Greece. *Comparative Immunology, Microbiology and Infectious Diseases*, **27**:201-207.

Englebert, E.T., McDermott, C., and Kleinheinz, G.T. 2008, Impact of the alga *Cladophora* on the survival of *E-coli, Salmonella,* and *Shigella* in laboratory microcosm. *Journal of Great Lakes Research*, **34**:377-382.

Espinosa, A.C., Mazari-Hiriart, M., Espinosa, R., Maruri-Avidal, L., Méndez, E., and Arias, C.F. 2008, Infectivity and genome persistence of rotavirus and astrovirus in groundwater and surface water. *Water Research*, **42**:2618-2628.

Feng, Y.Y., Ong, S.L., Hu, J.Y., Tan, X.L. and Ng, W.J. 2003, Effects of pH and temperature on the survival of coliphages MS2 and Qbeta. *Journal of Industrial Microbiology and Biotechnology*, **30**:549-552.

Field, K.G., and Samadpour, M. 2007, Faecal source tracking, the indicator paradigm, and managing water quality. *Water Research*, **41**:3517-3538.

García-Sánchez, A., Sánchez, S., Rubio, R., Pereira, G., Alonso, J.M., Hermoso de Mendoza, J., and Rey, J. 2007, Presence of Shiga toxin-producing *E. coli O157:H7* in a survey of wild artiodactyls. *Veterinary Microbiology*, **121**:373-377.

Geldreich, E.E. 1996, Pathogenic agents in freshwater resources. *Hydrological Processes*, **10**:315-333.

Gordon, C. and Toze, S. 2003, Influence of groundwater characteristics on the survival of enteric viruses. *Journal of Applied Microbiology*, **95**:536-544.

Green, D. H., Lewis, G.D., Rodtong, S., and Loutit, M.W. 1991, Detection of faecal pollution in water by an Escherichia coli uidA gene probe. *Journal of Microbiological Methods*, **13**:207-214.

Hall, T.A. 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**:95-98.

Havelaar, A.H., and Hogeboom, W.M. 1984, A method for the enumeration of male specific bacteriophages in sewage. *Journal of Applied Microbiology* **56:**439-447.

Heim, A., Ebnet, C., Harste, G., Pring-Akerblom, P. 2003, Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *Journal of Medical Virology*, **70:**228-239.

Karim, M.R., Manshadi, F.D., Karpiscak, M.M., and Gerba, C.P. 2004, The persistence and removal of enteric pathogens in constructed wetlands. *Water Research*, **38**:1831-1837.

King, B.J., Hoefel, D., Daminato, D.P., Fanok, S., Morris, P.T. 2008, Solar UV reduces Cryptosporidium parvum oocysts infectivity in environmental waters. *Journal of Applied Microbiology*, **104**:1311-1323.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., and Higgins, D.G. 2007, ClustalW and ClustalX version 2. *Bioinformatics*, **23**:2947-2948.

Lund, V. 1996, Evaluation of *E. coli* as an indicator for the presence of C*ampylobacter jejuni* and *Yersinia enterocolitica* in chlorinated and untreated oligotrophic lake water. *Water Research*, **30**:1528-1534.

Martins, M.T., Rivera, I.G., Clark, D., Stewart, I.M.H., Wolfe, R.L., and Olson, B.H. 1993, Distribution of uidA gene sequences in *Escherichia coli* isolates in water sources and comparison with the expression of beta-glucuronidase activity in 4-methylbellileryl-beta-glucuronide media. *Applied and Environmental Microbiology*, **59**:2271-2276.

Matthews, B, Stratton, H.M., and Higgins, J. 2008, *Determining the source of E. coli contamination in effluent ponds.* CRC Water Quality and Treatment Report.

Meays, C.L., Broersma, K., Nordin, R., and Mazumder, A. 2004, Source tracking faecal bacteria in water: a critical review of current methods. *Journal of Environmental Management*, **73**:71-79.

Monday, S.R., Whittam, T.S., and Feng P.C.H. 2001, Genetic and evolutionary analysis of mutations in the gusA gene that cause the absence of beta-glucuronidase activity in *Escherichia coli O157:H7. Journal of Infectious Diseases*, **184**:918-921.

Ngazoa, E.S., Fliss, I., and Jean, J. 2008, Quantitative study of persistence of human norovirus genome in water using TaqMan real-time RT-PCR. *Journal of Applied Microbiology*, **104**:707-715.

NHMCR-ARMCANZ 1996, *Australian Drinking Water Guidelines*, Commonwealth of Australia.

NRMMC-EPHC-AHMC. 2006, *Australian Guidelines for Water Recycling: Phase 1.* Commonwealth of Australia.

NRMMC-EPHC-AHMC. 2011, *Australian Drinking Water Guidelines.* Commonwealth of Australia.

Pavelic, P, Ragusa, S.R., Flower, R.L., Rink-Pfeiffer, S.M., and Dillon, P.J. 1998, Diffusion chamber method for in situ measurement of pathogen inactivation in groundwater. *Water Research*, **31:**1144-1150.

Ram, J. L., Ritchie, R.P., Fang, J., Gonzales, F., and Selegean, J.P. 2004, *S*equence-based source tracking of *Escherichia coli* based on genetic diversity of beta-glucuronidase. *Journal of Environmental Quality*, **33**:1024-1033.

Ram, J.L., Thompson, B., Turner, C., Nechvatal, J.M., Sheehan, H., and Bobrin, J. 2007, Identification of pets and raccoons as sources of bacterial contamination of urban storm sewers using a sequence-based bacterial source tracking method. *Water Research*, **41**:3605-3614.

Romero, O.C., Straub, A.P., Kohn, T., and Nguyen, T.H. 2011, Role of temperature and Suwannee River natural organic matter on inactivation kinetics of rotavirus and bacteriophage MS3 by solar radiation. *Environmental Science and Technology.* 45:10385-10393.

Roslev, P., Bjergbæk, L.A., and Hesselsoe, M. 2004, Effect of oxygen on survival of faecal pollution indicators in drinking water. *Journal of Applied Microbiology*, **96**:938-945.

Rzezutka, A., and Cook, N. 2004, Survival of human enteric viruses in the environment and food. *FEMS Microbiology Reviews*, **28**:441-453.

Sandhya, S., Chen, W., and Mulchandani, A. 2008, Molecular beacons: A real-time polymerase chain reaction assay for detecting *Escherichia coli* from fresh produce and water. *Analytica Chimica Acta*, **614**:208-212.

Saitou N., and Nei, M. 1987, The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**:406–425.

Schoen, M.E., Soller, J.A., and Ashbolt, N.J. 2011, *Evaluating the importance of faecal sources in human-impacted waters.* Water Research **45**:2670-2680.

Sidhu, J.P.S., and Toze, S. Assessment of pathogen survival potential during managed aquifer recharge with diffusion chambers. *Journal of Applied Microbiology.***113**:693-700.

Sidhu, J.P.S., Toze, S., Hodgers, L., Shackleton, M., Barry, K., Page, D., and Dillon, P. 2010, *Pathogen inactivation during passage of stormwater through a constructed reedbed and aquifer transfer, storage and recovery*. Water Science and Technology, **62**(5):1190-1197.

Sinton, L.W., Finlay, R.K., Lynch, P.A. 1999, Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Applied and Environmental Microbiology.* **65**:3605-3613.

Sinton, L., Hall, C., and Braithwaite, R. 2007, Sunlight inactivation of *Campylobacter jejuni* and *Salmonella enterica*, compared with *Escherichia coli*, in seawater and river water. *Journal of Water and Health*, **5**(3):357-365.

Sinton, L., Hall, C., Lynch, P.A., and Davies-Colley, R.J. 2002. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilisation pond effluent in fresh and saline waters. *Applied and Environmental Microbiology*. 68:1122-1131.

Skraber, S., Gassiloud, B., Schwatzbrod, L., and Gantzer, C. 2004, Survival of infectious Poliovirus-1 in river water compared to the persistence of somatic coliphages, thermotolerant coliforms and Poliovirus-1 genome. *Water Research*, **38:**2927-2933.

Soller, J.A., Schoen, M.E., Bartrand, T., Ravenscroft, J.E., and Ashbolt, N.J. 2010, Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Research*, 44:4674-4691.

Stoeckel, D.M., Mathes, M.V., Hyer, K.E., Hagedorn, C., Kator, H., Lukasik, J., O'Brien, T.L., Fenger, T. W., Smamdpour, M., Strickler, K.M., and Wiggins, B.A. 2004, Comparison of seven protocols to identify faecal contamination sources using *Escherichia coli*. *Environmental Science and Technology*, **38**:6109.

Stone, D.L., Harding, A.K., Hope, B.K., and Slaughter-Mason, S. 2008, Exposure assessment and risk of gastrointestinal illness among surfers. *Journal of Toxicology and Environmental Health, Part A*. 71:1603-1615.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011, MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28:** 2731-2739.

Toze, S. 2004, *Pathogen survival in groundwater during artificial recharge*, in *Wastewater Re-Use and Groundwater Quality*, J. Steenvoorden and T. Endreny, Editors. International Association of Hydrological Sciences: Wallingford. pg. 70-84.

Toze, S., Hanna, J., Smith, T., Edmonds, L., and McCrow, A. 2004, *Determination of water quality improvements due to the artificial recharge of treated effluent*, in *Wastewater Re-Use and Groundwater Quality*, J. Steenvoorden and T. Endreny, Editors. 2004, International Association of Hydrological Sciences: Wallingford. pg. 53-60.

Chemicals

Andreozzi, R., Rafafaele, M., and Nicklas, P. 2003, Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. *Chemosphere*, **50:**1319-1330.

Atkinson, S.K., Marlatt, V.L., Kimpe, L. E., Lean, D.R.S., Trudeau, V.L., and Blais, J.M. 2011, Environmental Factors Affecting Ultraviolet Photodegradation Rates and Estrogenicity of Estrone and Ethinylestradiol in Natural Waters. *Archives of Environmental Contamination and Toxicolology*, **60**:1-7.

Benotti, M.J., and Brownawell, B.J. 2009, Microbial degradation of pharmaceuticals in estuarine and coastal seawater. *Environmental Pollution*, **157** (3) 994-1002.

Boreen, A.L., Arnold, W.A., and McNeill, K. 2003, Photodegradation of pharmaceuticals in the aquatic environment: A review. *Aquatic Sciences - Research Across Boundaries*, **65:**320-341.

Chen, Y., Hu, C., Hu, X.X., and Qu, J.H. 2009, Indirect Photodegradation of Amine Drugs in Aqueous Solution under Simulated Sunlight. *Environmental Science and Technology*, **43**(8):2760-2765.

European Parliament, European Council, 2006, Regulation (EC) No 1907/2006 reach, criteria for the identification of persistent, bioaccumulative and toxic substances, and very persistent and very bioaccumulative substances. *Official Journal of the European Communities* ANNEX XIII.

Fono, L.J., and Sedlak, D.L. 2005, Use of the chiral pharmaceutical propranolol to identify sewage discharges into surface waters. *Environmental Science and Technology*, **39**(23):9244-9252.

Hawker, D.W., Cumming, J.L., Neale, P.A., Bartkow, M.E., and Escher, B.I. 2011, A screening level fate model of organic contaminants from advanced water treatment in a potable water supply reservoir. *Water Research*, **45:**768-780.

Kagle, J., Porter, A.W., Murdoch, R.W., Rivera-Cancel, G., and Hay, A.G. 2009, Biodegradation of Pharmaceutical and Personal Care Products. *Advances in Applied Microbiology*, **67:**65-108.

Kiffmeyer, T., Gotze, H.J., Jursch, M., and Luders, U. 1998, Trace enrichment, chromatographic separation and biodegradation of cytostatic compounds in surface water. *Fresenius Journal of Analytical Chemistry*, **361:**185-191.

Kolpin, D.W., and Kalkhoff, S.J. 1993, Atrazine degradation in a small stream in Iowa. *Environmental Science and Technology*, **27**(1):134-139.

Kummerer, K. 2004, *Emissions from medical care units. Pharmaceuticals in the Environment; sources, fate, effects and risks.* K. Kummerer. Berlin, Springer-Verlag**:** 27-44.

Lam, M.W., Young, C.J., Brain, R.A., Johnson, D.J., Hanson, M.A., Wilson, C.J., Richards, S.M., Solomon, K.R., and Mabury, S.A. 2004, Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environmental Toxicology and Chemistry*, **23:**1431-1440.

Lim, M.H., Snyder, S.A., and Sedlak, D.L. 2008, Use of biodegradable dissolved organic carbon (BDOC) to assess the potential for transformation of wastewater-derived contaminants in surface waters. *Water Research*, **42:**2943-2952.

Lin, A.Y.-C., and Reinhard, M. 2005, Photodegradation of common environmental pharmaceuticals and estrogens in river water. *Environmental Toxicology and Chemistry*, **24:**1303-1309.

Matamoros, V., Duhec, A., Albaiges, J., and Bayona, J.M. 2009, Photodegradation of Carbamazepine, Ibuprofen, Ketoprofen and 17 alpha-Ethinylestradiol in Fresh and Seawater. *Water Air and Soil Pollution*, **196:**161-168.

Muñoz, I., Gómez-Ramos, M.J., Agüera, A., Fernández-Alba, A.R., García-Reyes, J.F., and Molina-Díaz, A. 2009, Chemical evaluation of contaminants in wastewater effluents and the environmental risk of reusing effluents in agriculture. *Trends in Analytical Chemistry*, **28:**676-694.

OECD 2004, *OECD Guideline 309: Aerobic mineralisation in surface water - simulation biodegradation test. OECD guidelines for the testing of chemicals.* OECD. Paris.

OECD 2008, *OECD Guideline 316: Phototransformation of chemicals in water – direct photolysis.* OECD guidelines for the testing of chemicals. OECD. Paris.

Packer, J.L., Werner, J.J., Latch, D.E., McNeill, K., and Arnold, W.A. 2003, Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. *Aquatic Sciences - Research Across Boundaries*, **65:**342-351.

Poiger, T., Buser, H.R., and Mueller, M.D. 2001, Photodegradation of the pharmaceutical drug diclofenac in a lake: Pathway, field measurements, and mathematical modeling. *Environmental Toxicology and Chemistry*, **20:**256-263.

Ramil, M., El Aref, T., Fink, G., Scheurer, M., and Ternes, T.A. 2010, Fate of Beta Blockers in Aquatic-Sediment Systems: Sorption and Biotransformation. *Environmental Science and Technolology*, **44**:962-970.

Razavi, B., Ben Abdelmelek, S., Song, W.H., O'Shea, K.E., and Cooper, W.J. 2011, Photochemical fate of atorvastatin (lipitor) in simulated natural waters. *Water Research*, **45:**625-631.

Sanderson, H., Laird, B., Pope, L., Brain, R., Wilson, C., Johnson, D., Bryning, G., Peregrine, A.S., Boxall, A., and Solomon, K. 2007, Assessment of the environmental fate and effects of ivermectin in aquatic mesocosms. *Aquatic Toxicology*, **85:**229-240.

Schwarzenbach, R.P., Gschwend, P.M., and Imboden, D.M. 2003, *Environmental Organic Chemistry*. New Jersey, USA, John Wiley & Sons, Inc.

Sun, Q.F., Deng, S.B., Huang, J., Shen, G., and Yu, G. 2008, Contributors to estrogenic activity in wastewater from a large wastewater treatment plant in Beijing, China. *Environmental Toxicology and Pharmacology*, **25:**20-26.

Trovó, A.G., Nogueira, R.F.P., Agüera, A., Sirtori, C., and Fernández-Alba, A.R. 2009, Photodegradation of sulfamethoxazole in various aqueous media: Persistence, toxicity and photoproducts assessment. *Chemosphere*, **77:**1292-1298.

Watkinson, A.J., Murby, E.J., Costanzo, S.D. 2007, Removal of antibiotics in conventional and advanced wastewater treatment: Implications for environmental discharge and wastewater recycling. *Water Research*, **41:**4164-4176.

Woodburn, K.B., Batzer, F.R., White, F.H., and Schultz, M.R. 1993, The aqueous photolysis of triclopyr. *Environmental Toxicology and Chemistry*, **12:**43-55.

Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y., Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., and Sekizawa, J. 2009, Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. *Water Research*, **43:**351-362.

Microbial Source Tracking

Ahmed, W., Powell, D., Goonetilleke, A., and Gardner, T. 2008, Detection and Source Identification of Faecal Pollution in Non-sewered Catchment by Means of Host-Specific Molecular Markers. *Water Science and Technology*, **58**:579-586.

Ahmed, W., Goonetilleke, A., Powell, D., Chauhan, K., and Gardner. 2008, Alternative Indicators for Detection and Quantification of Faecal Pollution. *Journal of the Australian Water Association*, pg, 39-45.

Ahmed, W., A. Goonetilleke, A., Powell, D., Chauhan, K., and Gardner, T. 2009, Comparison of molecular markers to detect fresh sewage in environmental waters. *Water Research*, **43**:1-10.

Ahmed, W., Goonetilleke, A., Powell, D., and Gardner, T. 2009, Evaluation of multiple sewage-associated Bacteroides PCR markers for sewage pollution tracking. *Water Research*, **43**:1-6.

Ahmed, W.M., Hargreaves, M., Goontilleke, A., and Katouli, M. 2008, Population similarity analysis of indicator bacteria for source prediction of faecal pollution in a coastal lake. *Marine Pollution Bulletin*, **56**:1469-1475.

Ahmed, W., and Katouli, M. 2008, Phenotypic variations of enterococci in surface waters: analysis of biochemical fingerprinting data from multi-catchments. *Journal of Applied Microbiology*, **105:**452-458.

Ahmed, W., Kirs, M. Golpin, B. 2011, *Source Tracking in Australia and New Zealand: Case Studies*. Microbial Source Tracking: Methods, Applications, and Case Studies. (ed) Hagedorn, C., Blanch, A.R., and Harwood, V.J. Springer, London. pg. 485-514.

Ahmed, W., Powell, D., Goonetilleke, A., and Gardner, T. 2008, Detection and source identification of faecal pollution in non-sewered catchment by means of host-specific molecular markers. *Water Science and Technology*, **58(3):**579-586.

Ahmed, W., Sidhu, J.P.S., and Toze, S. 2012, Evaluation of the nifH Gene Marker of Methanobrevibacter smithii for the Detection of Sewage Pollution in Environmental Waters in Southeast Queensland, Australia. *Environmental Science and Technology*, **46**:543-550.

Ahmed, W., Stewart, J., Gardner, T., Powell, D., Brooks, P., Sullivana, D., and Tindale, N. 2007, Sourcing faecal pollution: A combination of library-dependent and library-independent methods to identify human faecal pollution in non-sewered catchments. *Water Research*, **41**:3771-3779.

Ahmed, W., Stewart, J., Gsrdner, T., and Powell, D. 2008, A real-time polymerase chain reaction assay for quantitative detection of the human-specific enterococci surface protein marker in sewage and environmental waters. *Environmental Microbiology*, **10**:3255-3264.

Ahmed, W., Stewart, J., Powell, D., and Gardner, T. 2008, Evaluation of the host-specificity and prevalence of enterococci surface protein. *Journal of Environmental Quality*, **37:**1583-1588.

Ahmed, W., Tucker, J., Bettelheim, K.A., Neller, R., and Katouli, M. 2007, Detection of virulence genes in Escherichia coli of an existing metabolic fingerprint database to predict the sources of pathogenic E. coli in surface waters. *Water Research*, **41**:3785-3791.

Ahmed, W., Wan, C., Goonetilleke, A., Gardner, T. 2010, Evaluating Sewage-Associated JCV and BKV Polyomaviruses for Sourcing Human Faecal Pollution in a Coastal River in Southeast Queensland, Australia. *Journal of Environmental Quality*, **39**:1743-1750.

Ahmed, W., Yusuf, R., Hasan, I., Goonetilleke, A., and Gardner, T. 2010, Quantitative PCR Assay of Sewage-associated Bacteroides Markers to Assess Sewage Pollution in an Urban Lake in Dhaka, Bangladesh. *Canadian Journal of Microbiology*, **56**:838-845.

Ahmed, W., Powell, D,, and Gardner, T. 2008, Evaluation of Bacteroides Markers for the Detection of Human Faecal Pollution. *Letters in Applied Microbiology*, **46**:237-242.

Anastasi, E.M., Matthews, B., Gundogdu, A., Vollmerhausen, T.L., Ramos, N.L., Stratton, H., Ahmed, W., and Katouli, M. 2010, Prevalence and Persistence of Escherichia coli Strains with Uropathogenic Virulence Characteristics in Sewage Treatment Plants. *Applied and Environmental Microbiology*, **76**:5882-5886.

Aslan, A., Xagoraraki, I., Simmons, F.J., Rose, J.B., and Dorevitch, S. 2011, Occurrence of adenovirus and other enteric viruses in limited-contact freshwater recreational areas and bathing waters. *Journal of Applied Microbiology*, **111**:1250-1261.

Badgley, B.D., Nayak, B.S., Harwood, V.J. 2010, The importance of sediment and submerged aquatic vegetation as potential habitats for persistent strains of enterococci in a subtropical watershed. *Water Research*, **44**:5857-5866.

Bae, S., and Wuertz S. 2009, Rapid decay of host-specific faecal Bacteroidales cells in seawater as measured by quantitative PCR with propidium monoazide. *Water Research*, **43**:4850-4859.

Balleste, E., Bonjoch, X., Belanche, L.A., Blanch, A.R. 2010, Molecular Indicators Used in the Development of Predictive Models for Microbial Source Tracking. *Applied and Environmental Microbiology*, **76**:1789-1795.

Bernhard, A.E., and Field, K.G. 2000, Identification of nonpoint sources of faecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from faecal anaerobes. *Applied and Environmental Microbiology*, **66:**1587-1594.

Bernhard, A.E., and Field, K.G. 2000, PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. *Applied and Environmental Microbiology*, **66:**4571-4574.

bioMérieux. (2012). "Clinical Diagnostics." Retrieved 22 June 2012, 2012, from http://www.biomerieux-diagnostics.com/servlet/srt/bio/clinical-diagnostics/dynPage?doc=CNL_PRD_CPL_G_PRD_CLN_2.

Blattner, F.R. 1999, *Hot papers - Genetics - The complete genome sequence of Escherichia coli K-12 by F.R. Blattner, G. Plunkett, C.A. Bloch, N.T. Perna, V. Burland, M. Riley, J. Collado-Vides, J.D. Glasner, C.K. Rode, G.F. Mayhew, J. Gregor, N.W. Davis, H.A. Kirkpatrick, M.A. Goeden, D.J. Rose, B. Mau, Y. Shao - Comments*. Scientist, **13**:17-17.

Bonjoch, X., Ballesé, E., and Blanch, A.R. 2004, Multiplex PCR with 16S rRNA gene-targeted primers of Bifidobacterium spp to identify sources of faecal pollution. *Applied and Environmental Microbiology*, **70**:3171-3175.

Bonjoch, X., Garcia-Aljaro, C., and Blanch, A.R. 2011, Persistence and diversity of faecal coliform and enterococci populations in faecally polluted waters. *Journal of Applied Microbiology,* **111**:209-215.

Brownell, M.J., Harwood, V.J., Kurz, RC, McQuaig, S.M., Lukasik, J, and Scott, T.M. 2007, Confirmation of putative stormwater impact on water quality at a Florida beach by microbial source tracking methods and structure of indicator organism populations. *Water Research*, **41:**3747-3757.

Cao, Y., Griffith, J.F., Dorevitch, S., and Weisberg, S.B. 2012, Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring Enterococcus in environmental waters. *Journal of Applied Microbiology,* **113**: 66-75.

Cao, Y.P., Van De Werfhorst, L.C., Sercu, B., Murray, J.L.S., and Holden, P.A. 2011, Application of an Integrated Community Analysis Approach for Microbial Source Tracking in a Coastal Creek. *Environmental Science and Technology,* **45**: 7195-7201.

Carlos, C., Alexandrino, F., Stoppe, N.C., Sato, M.I.Z., and Ottoboni, L.M.M. 2012, Use of *Escherichia coli* BOX-PCR fingerprints to identify sources of faecal contamination of water bodies in the State of São Paulo, Brazil. *Journal of Environmental Management,* **93**: 38-43.

Carson, C.A., Christiansen, J.M., Yampara-Iquise, H., Benson, V.W., Baffaut, C., Davis, J.V., Broz, R.R., Kurtz, W.B., Rogers, W.M., and Fales, W.H. 2005, Specificity of a *Bacteroides thetaiotaomicron* marker for human feces. *Applied and Environmental Microbiology*, **71:** 4945-4949.

Casarez, E.A., Pillai, S.D., Di Giouanni, G.D. 2007, Genotype diversity of *Escherichia coli* isolates in natural waters determined by PFGE and ERIC-PCR. *Water Research,* **41**(16): 3643-3648.

Converse, R. R., A. D. Blackwood, Kirs, M., Griffith, J.F., and Noble, R.T. 2009, Rapid QPCR-based assay for faecal *Bacteroides* spp. as a tool for assessing faecal contamination in recreational waters. *Water Research,* **43**: 4828-4837.

Chang, T.C., and Huang S.H. 1997, A modified immuno-polymerase chain reaction for the detection of β-glucuronidase from *Escherichia coli*. *Journal of Immunological Methods,* **208**: 35-42.

Dick, L.K., Stelzer, E.A., Bertke. E.E., Fong, D.L., and Stoeckel, D.M. 2010, Relative Decay of Bacteroidales Microbial Source Tracking Markers and Cultivated *Escherichia coli* in Freshwater Microcosms. *Applied and Environmental Microbiology*, **76**: 3255-3262.

Dickerson, J.W., Hagedorn, C., aqnd Hassall, A. 2007, Detection and remediation of human-origin pollution at two public beaches in Virginia using multiple source tracking methods. *Water Research,* **41**: 3758-3770.

Dong, Y., Kim, J., and Lewis, G.D. 2010, Evaluation of methodology for detection of human adenoviruses in wastewater, drinking water, stream water and recreational waters. *Journal of Applied Microbiology,* **108**: 800-809.

Dorai-Raj, S., O'Grady, J.O., Colleran, E. 2009, Specificity and sensitivity evaluation of novel and existing *Bacteroidales* and *Bifidobacteria*-specific PCR assays on feces and sewage samples and their application for microbial source tracking in Ireland. *Water Research,* **43**: 4980-4988.

Dubinsky, E.A., Esmaili, L., Hulls. J.R., Cao, Y.P., Griffith, J.F., Andersen, G.L. 2012, Application of phylogenetic microarray analysis to discriminate sources of faecal pollution. *Environmental Science and Technology,* **46**:4340-4347.

Ebdon, J.E., Sellwood, J., Shore, J., and Taylor, H.D. 2012, Phages of *Bacteroides* (GB-124): A Novel Tool for Viral Waterborne Disease Control? *Environmental Science and Technology*, **46**: 1163-1169.

Edge, T.A., and Hill, S. 2007, Multiple lines of evidence to identify the sources of faecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario. *Water Research,* **41**: 3585-3594.

Field, K.G., and Samadpour, M. 2007, Faecal source tracking, the indicator paradigm, and managing water quality. *Water Research,* **41**: 3517-3538.

Fong, T.-T., Phanikumar, M.S., Xagorararaki, I., and Rose, J.B. 2010, Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan River. *Applied and Environmental Microbiology,* **76:**715-723.

Fremaux, B., Gritzfeld, J., Boa, T., and Yost C.K. 2009, Evaluation of host-specific Bacteroidales 16S rRNA gene markers as a complementary tool for detecting faecal pollution in a prairie watershed. *Water Research,* **43**:4838-4849.

Furukawa, T., Takahashi, H., Yoshida, T., and Suzuki, Y. 2011, Genotypic Analysis of Enterococci Isolated from Faecal-Polluted Water from Different Sources by Pulsed-Field Gel Electrophoresis (PFGE) for Application to Microbial Source Tracking. *Microbes and Environments* **26**:181-183.

Gawler, A.H., Beecher, J.E., Brandao, J., Carroll, N.M., Falcao, L., Gournelon, M., Masterson, B., Nunes, B., Porter, J., Rince, A., Rodregues, R., Thorp, M., Walters, J.M., and Meijer, W.G. 2007, Validation of host-specific Bacteriodales 16S rRNA genes as markers to determine the origin of faecal pollution in Atlantic Rim countries of the European Union. *Water Research,* **41**:3780-3784.

García-Sánchez, A., Sánchez, S., Rubio, R., Pereira, G., Alonso, J.M., Hermoso de Mendoza J., and Rey J. 2007, Presence of Shiga toxin-producing *E. coli O157:H7* in a survey of wild artiodactyls. *Veterinary Microbiology,* **121**: 373-377.

Girones, R., Ferrus, M.A., Alonso, J.L., Rodriguez-Manzano, J., Calgua, B., Correa, A.D., Hundesa, A., Carratala, A., and Bofill-Mas, S. 2010, Molecular detection of pathogens in water - The pros and cons of molecular techniques. *Water Research,* **44**:4325-4339.

Gomez-Donate, M., Payan, A., Payan, A., Cortes, I., Blanch, A.R., Lucena, F., Jofre, J., and Muniesa, M. 2011, Isolation of bacteriophage host strains of Bacteroides species suitable for tracking sources of animal faecal pollution in water. *Environmental Microbiology*, **13**:1622-1631.

Gourmelon, M., Caprais, M.P., Le Mennec, C., Mieszkin, S., Ponthoreau, C., and Gendronneau, M. 2010, Application of library-independent microbial source tracking methods for identifying the sources of faecal contamination in coastal areas. *Water Science and Technology*, **61**:1401-1409.

Gourmelon, M., Caprais, M.P., Mieszkin, S., Marti, R., Wery, N., Jarde, E., Derrien, M., Jadas-Hecart, A., Communai, P.Y., Jaffrezic, A., and Pourcher, A.M. 2010, Development of microbial and chemical MST tools to identify the origin of the faecal pollution in bathing and shellfish harvesting waters in France. *Water Research*, **44**:4812-4824.

Gourmelon, M., Caprais, M.P., Segura, R., Le Mennec, C., Lozach, S., Piriou, J.Y., and Rince, A. 2007, Evaluation of two library-independent microbial source tracking methods to identify sources of faecal contamination in french estuaries. *Applied and Environmental Microbiology,* **73:**4857-4866.

Graves, A.K., Haydorn, C., Brooks, A., Hagedorn, C., and Martin, E. 2007, Microbial source tracking in a rural watershed dominated by cattle. *Water Research,* **41**:3729-3739.

Green, H.C., Dick, L.K., Gilpin, B., Samadpour, M., and Field, K.G. 2012, Genetic markers for rapid PCR-Based identification of Gull, Canada Goose, Duck, and Chicken faecal contamination in water. *Applied and Environmental Microbiology,* **78**:503-510.

Green, H.C., Shanks, O.C., Sivaganesan, M., Haugland, R.A., and Field, K.G. 2011, Differential decay of human faecal Bacteroides in marine and freshwater. *Environmental Microbiology*, **13**:3235-3249.

Green, D.H., Lewis, G.D., Rodtong, S., and Loutit, M.W. (1991). Detection of faecal pollution in water by an *Escherichia coli* uidA gene probe. *Journal of Microbiological Methods* **13**:207-214.

Griffith University (2010). *Source Tracking and Isolate Characterisation in Lakes Somerset and Baroon Pocket Catchments*

Guan, S., Xu, R., Chen, S.C., Odumeru, J., and Gyles, C. 2002, Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. *Applied and Environmental Microbiology,* **68**:2690-2698.

Harmsen, H.J.M., Raangs, G.C., He, T., Degener, J.E., Welling, G.W. 2002, Extensive set of 16S rRNA-based probes for detection of bacteria in human feces. *Applied and Environmental Microbiology,* **68:** 2982-2990.

Harwood, V.J., Brownell, M., Wang, S., Lepo, J., Ellender, R.D., Ajidahun, A., Hellein, K.N., Kennedy, E., Ye, X.Y., and Flood, C. 2009, Validation and field testing of library-independent microbial source tracking methods in the Gulf of Mexico. *Water Research*, **43**:4812-4819.

Hassan, W.M., Ellender, R.D., and Wang, S.Y. 2007, Fidelity of bacterial source tracking: *Escherichia coli* vs *Enterococcus* spp and minimizing assignment of isolates from nonlibrary sources. *Journal of Applied Microbiology*, **102:**591-598.

Hassan, W.M., Wang, S.Y., and Ellender, R.D. 2005, Methods to increase fidelity of repetitive extragenic palindromic pcr fingerprint-based bacterial source tracking efforts. *Applied and Environmental Microbiology,* **71**:512-518.

Hundesa, A., Bofill-Mas, S., de Motes, C.M., Rodriguez-Manzano, J., Bach, A., Casas, M., and Girones, R. 2010, Development of a quantitative PCR assay for the quantitation of bovine polyomavirus as a microbial source-tracking tool. *Journal of Virological Methods,* **163**:385-389.

Ishii, S., Hansen, D.L., Hicks, R.E., and Sadowsky, M.J. 2007, Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in lake superior. *Environmental Science and Technology*, **41:**2203-2209.

Ivanetich, K.M., Hsu, P.H., Messenger, E., Walkup, W.G., Scott, T.M., Lukasik, J., and Davis, J. 2006, Microbial source tracking by DNA sequence analysis of the *Escherichia coli* malate dehydrogenase gene. *Journal of Microbiological Methods*, **67:**507-526.

Jeanneau, L., Solecki, O., Wery, N., Jarde, E., Gournelon, M., Communal, P.Y., Jadas-Hecart, A., Caprais, M.P., Gruau, G., and Pourcher, A.M. 2012, Relative decay of faecal indicator bacteria and human-associated markers: a microcosm study simulating wastewater input into seawater and freshwater. *Environmental Science and Technology,* **46**:2375-2382.

Jeong, J.Y., Park, H.D., Lee, K.H., Weon, H.Y., and Ka, J.O. 2011, Microbial community analysis and identification of alternative host-specific faecal indicators in faecal and river water samples using pyrosequencing. *Journal of Microbiology*, **49**:585-594.

Jeter, S.N., McDermott, C.M., Bower, P.A., Kinzelman, J.L., Bootsma, M.J., Goetz, G.W., and McLellan, S.L. 2009, Bacteroidales diversity in Ring-Billed Gulls (*Laurus delawarensis*) residing at Lake Michigan beaches. *Applied and Environmental Microbiology*, **75**:1525-1533.

Johnston, C., Ufnar, J.A., Griffith, J.F., Gooch, J.A., and Stewart, J.R. 2010, A real-time qPCR assay for the detection of the nifH gene of *Methanobrevibacter smithii*, a potential indicator of sewage pollution. *Journal of Applied Microbiology,* **109**:1946-1956.

Kildare, B. J., C. M. Leutenegger, McSwain, B.S., Bambic, D.G., Rajal, V.B., and Wuetz, S. 2007, 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific faecal Bacteroidales: a Bayesian approach. *Water Research*, **41:**3701-3715.

Kim, S.-Y., Lee, J.E., Lee, S., Lee, H.T., Hur, H.-G., and Ko, G. 2010, Characterization of *Enterococcus* spp. from human and animal feces using 16S rRNA Sequences, the esp Gene, and PFGE for microbial source tracking in Korea. *Environmental Science and Technology,* **44**:3423-3428.

King, E.L., Bachoon, D.S., and Gates, K.W. 2007, Rapid detection of human faecal contamination in estuarine environments by PCR targeting of *Bifidobacterium adolescentis. Journal of Microbiological Methods*, **68:** 76-81.

Kirs, M., Harwood, V.J., Fidler, A.E., Gillespie, P.A., Fyfe, W.R., Blackwood, A.D., and Cornelisen, C.D. 2011, Source tracking faecal contamination in an urbanised and a rural waterway in the Nelson-Tasman region, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **45**:43-58.

Kon, T., Weir, S.C., Howell, E.T., Lee, H., and Trevors, J.T. 2009, Repetitive element (REP)-polymerase chain reaction (PCR) analysis of *Escherichia coli* isolates from recreational waters of southeastern Lake Huron. *Canadian Journal of Microbiology*, **55**:269-276.

Korajkic, A., Brownell, M.J., and Harwood, V.J. 2011, Investigation of human sewage pollution and pathogen analysis at Florida Gulf coast Beaches. *Journal of Applied Microbiology,* **110**:174-183.

Lamendella, R., Santo Domingo, J.W., Kelty, C., and Oerther, D.B. 2008, Bifidobacteria in Feces and Environmental Waters. *Applied and Environmental Microbiology*, **74**:575-584.

Lamendella, R., Santo Domingo, J.W., Oerther, D.B., Vogel, J.R., and Stoeckel, D.M. 2007, Assessment of faecal pollution sources in a small northern-plains watershed using PCR and phylogenetic analyses of Bacteroidetes 16S rRNA gene. *Fems Microbiology Ecology*, **59:**651-660.

Lanthier, M., Scott, A., Zhang, Y., Cloutier, M., Durie, D., Henderson, V.C., Wilkes, G., Lapen, D.R., and Topp, E. 2011, Distribution of selected virulence genes and antibiotic resistance in Enterococcus species isolated from the South Nation River drainage basin, Ontario, Canada. *Journal of Applied Microbiology,* **110**:407-421.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., and Higgins, D.G. 2007, Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948.

Lasalde, C., Rodriguez, R., Toranzos, G.A., and Smith, H.H. 2005, Heterogeneity of uidA gene in environmental Escherichia coli populations. *Journal of Water and Health* **3**:297-304.

Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R., and Sayler, G. 2006, Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine faecal pollution in water. *Applied and Environmental Microbiology,* **72:**4214–4224.

Layton, B.A., Walters, S.P., Lam, L.H., and Boehm, A.B. 2010, Enterococcus species distribution among human and animal hosts using multiplex PCR. *Journal of Applied Microbiology,* **109**:539-547.

Lee, C.S., and Lee, J. 2010, Evaluation of new gyrB-based real-time PCR system for the detection of B. fragilis as an indicator of human-specific faecal contamination. *Journal of Microbiological Methods*, **82**:311-318.

Lee, D.Y., Weir, S.C., Lee, H., and Trevors, J.T. 2010, Quantitative identification of faecal water pollution sources by TaqMan real-time PCR assays using Bacteroidales 16S rRNA genetic markers. *Applied Microbiology and Biotechnology*, **88**:1373-1383.

Lee, J.E., Lee, S., Sung, J., and Ko, G. 2011, Analysis of human and animal faecal microbiota for microbial source tracking. *Isme Journal*, **5**:362-365.

Liu, R., Chan, C.F., Lun, C.H.I., and Lau, S.C.K. 2012, Improving the performance of an end-point PCR assay commonly used for the detection of Bacteroidales pertaining to cow feces. *Applied Microbiology and Biotechnology*, **93**:1703-1713.

Liu, R.L., Chiang, M.H.Y., Lun, C.H.I., Qian, P.Y., and Lau, S.C.K. 2010, Host-specific 16S rRNA gene markers of Bacteroidales for source tracking of faecal pollution in the subtropical coastal seawater of Hong Kong. *Water Research*, **44**:6164-6174.

Liwimbi, L., Graves, A.K., Israel, D.W., van Heugten, E., Robinson, B., Cahoon, C.W., and Lubbers, J.F. 2010, Microbial Source Tracking in a Watershed Dominated by Swine. *Water*, **2**:587-604.

Lyautey, E., Lu, Z., Lapen, D.R., Berkers, T.E., Edge, T.A., Topp, E. 2010, Optimization and validation of rep-PCR genotypic libraries for microbial source tracking of environmental *Escherichia coli* isolates. *Canadian Journal of Microbiology*, **56**:8-17.

Martins, M.T., Rivera, I.G., Clark, D., Stewart, M.H., Wolfe, R.L., and Olson, B.H. 1993, Distribution of *uidA* gene sequences in *Escherichia coli* isolates in water sources and comparison with the expression of beta-glucuronidase activity in 4-methylbellileryl-beta-glucuronide media. *Applied and Environmental Microbiology,* **59**: 2271-2276.

Masago, Y., Pope, J.M., Kumar, L.S., Masago, A., Omura, T. and Rose, J.B. 2011, Prevalence and Survival of *Enterococcus faecium* Populations Carrying the esp Gene as a Source-Tracking Marker. *Journal of Environmental Engineering-ASCE*, **137**:315-321.

Matthews, B, Stratton, H.M., and Higgins, J. 2008, Determining the source of *E. coli* contamination in effluent ponds. *CRC Water Quality and Treatment Report*.

McLain, J.E.T., Ryu, H., Kabiri-Badr, L., and Abbaszadegan, M. 2009, Lack of specificity for PCR assays targeting human Bacteroides 16S rRNAgene: cross-amplification with fish feces. *Fems Microbiology Letters*, **299:**38-43.

Meays, C.L., Broersma, K., Nordin, R., and Mazumder, A. 2004, Source tracking faecal bacteria in water: a critical review of current methods. *Journal of Environmental Management* **73**:71-79.

Mieszkin, S., Furet, J.P., Corthier, G., and Gournelon, M. 2009, Estimation of Pig Faecal Contamination in a River Catchment by Real-Time PCR Using Two Pig-Specific *Bacteroidales* 16S rRNA Genetic Markers. *Applied and Environmental Microbiology,* **75:**3045-3054.

Mohapatra, B.R., Broersma, K., and Mazumder, A. 2007, Comparison of five rep-PCR genomic fingerprinting methods for differentiation of faecal *Escherichia coli* from humans, poultry and wild birds. *Fems Microbiology Letters*, **277:**98-106.

Mohapatra, B.R., Broersma, K., and Mazumder, A. 2008, Differentiation of faecal *Escherichia coli* from poultry and free-living birds by GTG. *International Journal of Medical Microbiology,* **298:**245-252.

Mohapatra, B.R., Broersma, K., Nordin, R., and Mazumder, A. 2007, Evaluation of repetitive extragenic Palindromic-PCR for discrimination of faecal *Escherichia coli* from humans, and different domestic- and wild-animals. *Microbiology and Immunology*, **51:**733-740.

Mohapatra, B.R., and Mazumder, A. 2008, Comparative efficacy of five different rep-PCR methods to discriminate *Escherichia coli* populations in aquatic environments. *Water Science and Technology*, **58:**537-547.

Monday, S.R., Whittam, T.S., and Feng, P.C.H. 2001, Genetic and evolutionary analysis of mutations in the gusA gene that cause the absence of beta-glucuronidase activity in *Escherichia coli* O157:H7. *Journal of Infectious Diseases,* **184**:918-921.

Morrison, C.R., Bachoon, D.S., and Gates, K.W. 2008, Quantification of enterococci and bifidobacteria in Georgia estuaries using conventional and molecular methods. *Water Research*. **42:**4001-4009.

Nayak, B.S., Badgley B., and Harwood, V.J. 2011, Comparison of Genotypic and Phylogenetic Relationships of Environmental *Enterococcus* Isolates by BOX-PCR Typing and 16S rRNA Gene Sequencing. *Applied and Environmental Microbiology*, **77**(14): 5050-5055.

NHMCR-ARMCANZ (1996). *Australian Drinking Water Guidelines*, Commonwealth of Australia.

Okabe, S., Okayama, N., Savichtcheva, O., and Ito, T.2007, Quantification of host-specific *Bacteroides–Prevotella* 16S rRNA genetic markers for assessment of faecal pollution in freshwater. *Applied Microbiology and Biotechnology,* **74**:890-901.

Olivas, Y., and Faulkner, B.R. 2008, Faecal source tracking by antibiotic resistance analysis on a watershed exhibiting low resistance. *Environmental Monitoring and Assessment*, **139:**131-133.

Orsi, R.H., Stoppe, N.C., Sato, M.I.Z, Gomes, T.A.T, Prodo, P.I., Manfio, G.P., and Ottoboni, L.M.M. 2007, Genetic variability and pathogenicity potential of Escherichia coli isolated from recreational water reservoirs. *Research in Microbiology*, **158**:420-427.

Ogorzaly, L., Tissier, A., Bertrand, I., Maul, A. and Gantzer, C. 2009, Relationship between F-specific RNA phage genogroups, faecal pollution indicators and human adenoviruses in river water. *Water Research*. **43:**1257-1264.

Price, B., Venso, E., Frana, M., Greenberg, J., and Ware, A. 2007, A comparison of ARA and DNA data for microbial source tracking based on source-classification models developed using classification trees. *Water Research*, **41**:3575-3584.

Purnell, S.E., Ebdon, J.E., and Taylor, H.D. 2011, Bacteriophage Lysis of Enterococcus Host Strains: A Tool for Microbial Source Tracking? *Environmental Science and Technology,* **45**(24):10699-10705.

Ram, J.L., Ritchie, R.P., Fang, J., Gonzales, F., and Selegean, J.P. 2004, Sequence-based source tracking of *Escherichia coli* based on genetic diversity of beta-glucuronidase. *Journal of Environmental Quality* **33**:1024-1033.

Ram, J.L., Thompson, B., Turner, C., Nechvatal, J.M., Sheehan, H., and Bobrin, J. 2007, Identification of pets and raccoons as sources of bacterial contamination of urban storm sewers using a sequence-based bacterial source tracking method. *Water Research* **41**:3605-3614.

Reischer, G.H., Kasper, D.C. Steinborn, R., Farnleitner, A.H., and Mach, R.L. 2007, A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal influence in spring water from a large alpine catchment area. *Letters in Applied Microbiology*, **44:**351-356.

Ritchey, S.A., and Coyne, M.S. 2009, Applying MAR Analysis to Identify Human and Non-Human Faecal Sources in Small Kentucky Watersheds. *Water Air and Soil Pollution*, **196:**191-194.

Rodríguez-Lázaro, D., Cook, N., Ruggeri, F.M., Sellwood, J., Nasser, A. Nascimento, M.S., Saiz, J.C., Rzezutka, A., Bosch, A., Girones, R., Carducci, A., Muscillo, M., Kovac, K., Diez-Valcarce, M., Vantarakis, A., von Bonsdorff, C.H., Husman, A.M.D., Hernandez, M., and van der Poel, W.H.M. 2012, Virus hazards from food, water and other contaminated environments. *FEMS Microbiology Reviews*, **36**:786-814.

Roslev, P., and Bukh A.S. 2011, State of the art molecular markers for faecal pollution source tracking in water. *Applied Microbiology and Biotechnology*, **89**:1341-1355.

Roslev, P., Iversen, L., Sonderbo, H.L., Iversent, N., and Bastholm, S. 2009, Uptake and persistence of human associated *Enterococcus* in the mussel *Mytilus edulis*: relevance for faecal pollution source tracking. *Journal of Applied Microbiology,* **107:**944-953.

Saitou, N., and Nei, M. 1987, The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**:406-425.

Sandhya, S., Chen, W., and Mulchandani, A. 2008, Molecular beacons: A real-time polymerase chain reaction assay for detecting Escherichia coli from fresh produce and water. *Analytica Chimica Acta* **614**:208-212.

Sauer, E.P., VandeWalle, J.L., Bootsma, M.J., McLellan, S.L. 2011, Detection of the human specific Bacteroides genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water Research*, **45**:4081-4091.

Savichtcheva, O., Okayama, N., and Okabe, S. 2007, Relationships between Bacteroides 16S rRNA genetic markers and presence of bacterial enteric pathogens and conventional faecal indicators. *Water Research* **41**:3615-28.

Schloss, P.D. 2009, A High-Throughput DNA Sequence Aligner for Microbial Ecology Studies. *PLoS ONE,* **4**:e8230.

Sercu, B., Van De Werfhorst, L.C., Murray, J., Holden, P.A. 2009, Storm Drains are Sources of Human Faecal Pollution during Dry Weather in Three Urban Southern California Watersheds. *Environmental Science and Technology*, **43:**293-298.

Sercu, B., Van De Werfhorst, L.C., Murray, J.L.S., and Holden, P.A. 2011, Sewage Exfiltration As a Source of Storm Drain Contamination during Dry Weather in Urban Watersheds. *Environmental Science and Technology,* **45**:7151-7157.

Seurinck, S., Defoirdt, T., Verstraete, W., and Siciliano, S.D. 2005, Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environmental Microbiology*, **7**:249-259.

Shanks, O.C., Kelty, C.A., Sivaganesan, M., Varma, M., and Haugland, R.A. 2009, Quantitative PCR for Genetic Markers of Human Faecal Pollution. *Applied Environmental Microbiology*, **75**:5507-5513.

Sheludchenko, M.S. 2011, Development and validation of novel methods for microbial source tracking based on *Escherichia coli* as an indicator of water quality. Brisbane, Queensland University of Technology. **PhD**.

Sheludchenko, M.S., Huygens, F., and Hargreaves, M.H. 2010, Highly Discriminatory Single-Nucleotide Polymorphism Interrogation of *Escherichia coli* by Use of Allele-Specific Real-Time PCR and eBURST Analysis. *Applied and Environmental Microbiology*, **76**:4337-4345.

Sheludchenko, M.S., Huygens, F., and Hargreaves, M.H. 2011, Human-Specific *E.coli* Single Nucleotide Polymorphism (SNP) Genotypes Detected in a South East Queensland Waterway, Australia." *Environmental Science and Technology,* **45**:10331-10336.

Silkie, S.S., and Nelson, K.L. 2009, Concentrations of host-specific and generic faecal markers measured by quantitative PCR in raw sewage and fresh animal feces. *Water Research*, **43**:4860-4871.

Sokolova, E., Astrom, J., Pettersson, T.J.R., Bergstedt, O., and Hermansson, M. 2012, Decay of Bacteroidales Genetic Markers in Relation to Traditional Faecal Indicators for Water Quality Modeling of Drinking Water Sources. *Environmental Science and Technology,* **46**:892-900.

Solecki, O., Jeanneau, L., Jarde, E., Gourmelon, M., Marin, C., and Pourcher, A.M. 2011, Persistence of microbial and chemical pig manure markers as compared to faecal indicator bacteria survival in freshwater and seawater microcosms. *Water Research*, **45**:4623-4633.

Soule, M., Kuhn, E., Loge, F., Gay, J., and Call, D.R. 2006, Using DNA microarrays to identify library-independent markers for bacterial source tracking. *Applied and Environmental Microbiology*, **72:**1843-1851.

Srinivasan, S., Aslan, A., Xagorarki, I., Alocilja, E., and Rose, J.B. 2011, *Escherichia coli*, enterococci, and *Bacteroides thetaiotaomicron* qPCR signals through wastewater and septage treatment. *Water Research*, **45**:2561-2572.

Stapleton, C.M., Kay, D., Wyer, M.D., Davies, C., Watkins, J., Kay C., McDonald, A.T., Porter, J., and Gawler, A. 2009, Evaluating the operational utility of a *Bacteroidales* quantitative PCR-based MST approach in determining the source of faecal indicator organisms at a UK bathing water. *Water Research*, **43:**4888-4899.

Stoeckel, D.M., Mathes, M.V., Hyer, K.E., Hagedorn, C., Kator, H., Lukasik, J., O'Brien, T.L., Fenger, T.W., Smamdpour, M., Strickler, K.M., and Wiggins, B.A. 2004, Comparison of seven protocols to identify faecal contamination sources using Escherichia coli. *Environmental Science and Technology* **38**:6109.

Stoeckel, D.M., and Harwood V.J. 2007, Performance, design, and analysis in microbial source tracking studies. *Applied and Environmental Microbiology*, **73:**2405-2415.

Stratton, H., Ahmed, W., Katouli, N. 2009, A Consensus: Microbial Source Tracking in Water. *Microbiology Australia*, **30**:30-32.

Stratton, H., and Christie, M. 2010, *Tracking the source of Escherichia coli isolated from Somerset and Baroon Pocket Using the beta-glucoronidase gene.* Report to Seqwater

Stricker, A.R., Wilhartitz, I., Farnleitner, A.H., and Mach, R.L. 2008, Development of a Scorpion probe-based real-time PCR for the sensitive quantification of *Bacteroides* sp ribosomal DNA from human and cattle origin and evaluation in spring water matrices. *Microbiological Research*. **163:**140-147.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011, MEGA5: Molecular Evolutionary Genetics Analysis usig Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods, *Molecular Biology and Evolution* **28**:2731-2739.

Tsai, Y.-L., Le, J.Y., and Olson, B.H. 2003, Magnetic bead hybridization to detect enterotoxigenic *Escherichia coli* strains associated with cattle in environmental water sources. Canadian Journal of Microbiology **49**:391–398.

Ufnar, J. A., Wang, S.Y., Christiansen, J.M., Yampara-Iquise, H., Carson, C.A., Ellender, R.D. 2006, Detection of the nifH gene of *Methanobrevibacter smithii*: a potential tool to identify sewage pollution in recreational waters. *Journal of Applied Microbiology,* **101**:44-52.

Unno, T., Di, D.Y.W., Jang, J., Suh, Y.S., Sadowsky, M.J., and Hur, H.G. 2012, Integrated Online System for a Pyrosequencing-Based Microbial Source Tracking Method that Targets *Bacteroidetes* 165 rDNA. *Environmental Science and Technology* **46:**93-98.

Unno, T., Jang, J., Han, D., Kim, J.H., Sadowsky, M.J., Kim, O.S., Chun, J., and Hur, H.G. 2010, Use of Barcoded Pyrosequencing and Shared OTUs To Determine Sources of Faecal Bacteria in Watersheds. *Environmental Science and Technology* **44**:7777-7782.

USEPA. 2005, *Microbial Source Tracking Guide Document*

Vierheilig, J., Farnleitner, A.H., Kollanur, D., Bloschl, G., and Reischer, G.H. 2012, High abundance of genetic *Bacteroidetes* markers for total faecal pollution in pristine alpine soils suggests lack in specificity for feces. *Journal of Microbiological Methods*, **88**:433-435.

Vogel, J.R., Stoeckel, D.M., Lamendella, R., Zelt, R.B., Domingo, J.W.S., Walker, S.R., and Oether, D.B. 2007, Identifying faecal sources in a selected catchment reach using multiple source-tracking tools. *Journal of Environmental Quality*, **36:**718-729.

Walters, S.P., and Field. K.G. 2009, Survival and persistence of human and ruminant-specific faecal *Bacteroidales* in freshwater microcosms. *Environmental Microbiology*, **11:**1410-1421.

Wéry, N., Monteil, C., Poucher, A.M., and Godon, J.J. 2010, Human-specific faecal bacteria in wastewater treatment plant effluents. *Water Research*, **44**:1873-1883.

Wicki, M., Karabulut, F., Auckenthaler, A., Felleisen, R., Tanner, M., and Baumgertner, A. 2011, Identification of Faecal Input Sites in Spring Water by Selection and Genotyping of Multiresistant *Escherichia coli*. *Applied and Environmental Microbiology*, **77**:8427-8433.

Wong, K., and Xagoraraki, I. 2011, Evaluating the prevalence and genetic diversity of adenovirus and polyomavirus in bovine waste for microbial source tracking. *Applied Microbiology and Biotechnology*, **90**:1521-1526.

Wong, M., Kumar, L., Jenkins, T.M., Xagoraraki, I., Phanikumar, M.S., and Rose, J.B. 2009, Evaluation of public health risks at recreational beaches in Lake Michigan via detection of enteric viruses and a human-specific bacteriological marker. *Water Research*, **43:**1137-1149.

Yampara-Iquise, H., Zheng, G., Jones, J.E., and Carson, C.A. 2008, Use of a *Bacteroides thetaiotaomicron*-specific alpha-1-6, mannanase quantitative PCR to detect human faecal pollution in water. *Journal of Applied Microbiology*, **105:**1686-1693.

Zheng, G., Yampara-Iquise, H., Jones, J.E., and Carson, C.A. 2009, Development of *Faecalibacterium* 16S rRNA gene marker for identification of human faeces. *Journal of Applied Microbiology*, **106**:634-641.