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Bovine Faecal Contamination in an Irish Agricultural Catchment: sources and pathways

[Volume 1 of 1]

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for the degree of
Masters of Research

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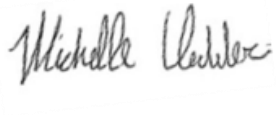
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Abstract

Agricultural impacts on surface waters have been an intensive source of contamination on freshwaters worldwide. Faecal contamination is one of the most under regulated and poorly understood pollutants within Irelands surface waters, despite is widely recorded ability to cause harm to both animals and the environment. Creating novel solutions to help rectify the negative effects of poor agricultural management is necessary for protecting the future health if Irelands waters. Although current policy under the European Union (EU) provides guidelines and regulations, Ireland has still failed to contain its faecal contaminant issue. The solution to this dilemma may lie in the origins of this pollutant; current research demonstrates Irelands agriculturally dominated catchments could be suffering from point source dissemination of contaminants via input through farmyards and direct cattle access to streams. This dissemination model differs from the current non point source model that the EU and Irish policies implicitly endorse. This study's objectives were as follows; to quantify the ability of headwater drainage channels receiving direct farmyard effluent to attenuate faecal indicator organisms (thermotolerant coliforms) within the water column and within benthic sediments, over their length; and to determine the distribution, concentration, and origin of thermotolerant coliforms (TTC's) at intensive spatial scales within in a small agriculturally dominated catchment in South West Ireland. Utilizing novel colonization substrata, results demonstrated no trends of attenuation within headwater drainage areas; subsequent data provided TTC concentration and distribution within the larger drainage catchment upon deposition from the previously studied channels. Results from catchment wide analysis demonstrated a definitive connection to faecal contamination of point source origin from farmyards as well as direct deposition from cattle access to streams. The future of faecal contamination management within Irelands waters lies within the exploration of novel and established treatment methodology in order to create effective overarching policy changes.

2.0 Introduction

2.1 Overview of Agricultural Pollution

Agricultural impacts on surface waters have been an intensive source of contamination on freshwaters worldwide (Carpenter et al., 1998; Tilman et al., 2002; Foley, 2005; Ockenden et al., 2019). Agriculturally-derived contaminants have been found to consist of organic matter, (including faeces, waste milk, silage liquor etc), mineral nutrients (largely phosphorus and nitrogen), sediment, and agrochemicals (Jarvis et al., 1996; Carpenter et al., 1998; Novotny et al., 1999; Tilman et al., 2002; Foley, 2005; Thomassen et al., 2008; Baskaran et al., 2009; Guerci et al., 2013; Ockenden et al,. 2019). All of these substances can have very serious deleterious impacts on receiving waters (Daniel et al., 1998; Henley et al., 2000; Davies-Colley & Smith, 2001; Horrigan et al., 2002; Schaller et al., 2005; Bilotta & Brazier, 2008; Monteagudo et al., 2012). Agricultural contamination of waterways also can have significant economic, as well as environmental costs (Pretty et al., 2003). With the expected global rise in agriculture projected due to population increase, it is imperative that actions should be taken to mitigate or eliminate the severe impacts of this industry on the environment (Tilman et al., 2002; Schröder et al., 2004).

2.2 Ecosystem and Human Health Risk

With agricultural pollution being such a significant issue worldwide, it is important to highlight the health risks it poses to ecosystems, as well as to human and animal health. Nutrient loading of surface waters from agriculturally-derived nitrogen and phosphorus and other contaminants such as fine sediment and agrochemicals have the potential to cause eutrophication of surface waters and harmful algal blooms, unsafe drinking and bathing water conditions as well as oxygen depletion and fish kills (Daniel et al., 1998; Horrigan et al., 2002; Schaller et al., 2004; Monteagudo et al., 2012).

Highly turbid waters (also referred to as fine particulates) have also been reported to create negative secondary effects due to their ability to decrease interactions of the periphyton layer within the hyporheic zone with the rest of the aquatic community. When these levels are elevated for prolonged periods of time, it can cause a decline in aquatic macro invertebrate populations (Henley et al., 2000; Davies-Colley & Smith, 2001; Bilotta & Brazier, 2008). This has been reported to create reductions in resource availability for other organisms that utilize them as a food source, including fish (Henley et al., 2000). Fine particulate contamination also negatively impacts periphyton and macrophytic growth within the hyporheic zone due to decreased light penetration, further destabilizing this vital niche of aquatic ecosystem (Henley et al., 2000; Davies-Colley & Smith, 2001; Bilotta & Brazier, 2008). This type of agricultural input has also been shown to create agitation within the gills of numerous fish species, predisposing them to infection from stress and decreased oxygen availability. Along with all of these negative effects, increased turbidity has also been shown to increase the mortality rates of certain species of fish eggs, including trout and salmon species, due to the settlement of fine particulates upon the eggs, causing the permeable membrane (Henley et al., 2000; Davies-Colley & Smith, 2001; Bilotta & Brazier, 2008).

Microbial loadings of faecal origin in surface waters draining agricultural catchments are also a significant risk to both aquatic ecosystems and human/animal health. These include many bacterial and protozoan organisms that have the potential to cause harm or death to humans and animals, such as *Campylobacter* bacteria, *Cryptosporidium*, *Escherichia coli* (or *E. coli*), *Giardia*, *Streptococci*, other faecal coliforms, and salmonella (Wiggins, 1996; Davies-Colley, 2004; Olsen et al., 2004; Wesley et al., 2004; Fayer, 2004; Ishii & Sadowski, 2008; Soller et al., 2010). Worldwide, illnesses of diarrheal origin cause over one million deaths annually, with 5,000 deaths recorded in the United States alone (Ishii &Sadowski, 2008). It has also been reported that while developing nations exhibit a much higher proportion of disease outbreak, developed countries still experience microbial contamination in drinking water originating from agriculturally based land use

(Smolders et al., 2015).This leaves countries in the developed world at risk of increased agricultural contamination in drinking water as the demand for food and natural resources is projected to rise due global population increase (Tilman et al., 2002; Schröder et al., 2004).

Of all of these pollutant types, faecal coliforms have been recognized as a major source of contamination and human/animal health risk (Collines et al., 2007; Ishii & Sadowski, 2008; Soller et al., 2010; Coffey et al., 2012; Bragina, 2016; Óhaiseadha et al., 2016, Bussi, 2017). Within the faecal coliform group, *E. coli* contamination of surface waters has been of particular focus due to its persistence in the environment (Ishii & Sadowski, 2008; Coffey et al., 2012.). Of the various *E. coli* strain found to occur within ecosystems, *E. coli* strain O157 has shown to be harmful to human health (Mead & Griffin, 1998, Ishii & Sadowski, 2008, Óhaiseadha et al., 2016, Health, Protection, and Surveillance Center, 2018). When combined with the knowledge that this strain shows significant persistence within the environment (more so than non-pathogenic strains), the concern for human healthy becomes apparent (O'Callaghan et al., 2014). This is also further supported by the fact that cattle shed this intestinal virus asymptomatically, leaving much of the burden of managing this virus with agricultural operations (Ishii & Sadowski, 2008). *E. coli* O157 poses risk to human health as a highly pathogenic intestinal virus can cause severe complications such as hemorrhagic colitis, hematolytic uremic syndrome, and death in the young and elderly (Mead & Griffin, 1998, O'Callaghan et al., 2014, Óhaiseadha et al., 2016; Health, Protection, and Surveillance Center, 2018).

E. coli is a widely acknowledged as a faecal indicator organism (FIO) and is a widely utilized indicator organism within water monitoring programs via collection of water and sediment samples and enumeration of *E. coli* colonies (Edberg, 2000; Coffey et al., 2007; Coffey et al., 2012). Not all *E. coli* strains are harmful to humans or animals, but their presence often is coincident with more harmful pathogens of faecal origin (Mishra et al., 2008; Coffey et al., 2012; Bradshaw et al., 2016). Although it was thought, historically,

that *E.coli* was unable to survive outside its intestinal environment, recent research has shown that the bacterium can survive in the external environment for considerable periods of time (Whitman et al., 2003; Ishii & Sadowski, 2008; Ishii, 2006, Van Elsas, 2011). Persistence of *E. coli* within surface waters upon deposition from agricultural sources poses serious risk to human and animal health.

Although faecal contamination of agricultural origin is a significant issue for human health, it is also a risk for animal health and production. It has been previously thought that the presence of *E. coli* bacteria in cattle was asymptomatic (Stott et al., 2011), however studies have shown that not only can *E. coli* cause pelvic inflammatory disease in cattle (Sheldon et al., 2010), its presence in drinking water can cause significant weight loss in cattle due to avoidance of contaminated water (O'Callaghan, 2014) and is has been suggested that FIO presence in cattle drinking water may decrease dairy yields (Socha et al., 2003). Although Ireland and the UK do not currently provide cattle drinking water regulations and guidelines for FIO levels, policies and best management practices are found in other countries. In Australia and New Zealand, it is recommended that bacterial contamination of cattle drinking water does not exceed 100 colony-forming units (CFU's) /100ml (Australian and New Zealand Environment and Conservation Council, 2000). In the United States, The US Department of Agriculture recommends less than 1000CFU/100ml for adult cattle, and less than 200CFU/100ml for calves (Pick. 2011).

Many recent studies have sought to determine the species sources of faecal coliform bacteria (i.e. whether from humans, domestic animals or wild animals) (SEPA, 2004; Bradshaw et al., 2016). Although it has been shown to be difficult to definitively track species sources of faecal contamination in aquatic catchments (Field & Samapour, 2007) determining origin is achievable, however, the methodology is logistically complex, often times laboratories do not have the necessary resources available to accomplish (SEPA, 2004; Meays et al., 2004; Bradshaw et al., 2016.

2.3 Catchment Sources and Policy Management

Although faecal contamination in agricultural catchments has generally been considered to be from diffuse sources (Novoteny et al., 1999; Collins et al., 2010; Ockendon et al., 2012), recent research has shown that small point sources may in fact contribute significantly to faecal contamination of surface waters (Shore et al., 2017; Harrison et al., 2019; Moloney et al., 2019). Farmyards, farm hard standing areas, and cattle holding areas have been shown to contribute significant amounts of FIO's to surface waters in agricultural catchments, both through the high density of faecal matter and the compacted, impermeable nature of the areas (Kay et al., 2003; Kay et al., 2007; Vinten et al., 2008; Tetzlaff et al., 2012; Monaghan et al., 2012; Kleinmen et al., 2015).

Sediment plays a key role, both as a sink and a source of FIO contamination of waterways (Sayler et al., 1975, Bohn & Buckhuse, 1985; Sherer et al., 1992 ; Davies et al., 2000 ; Haller et al., 2009). Following deposition into the environment, FIOs attach to small particles suspended within the water column, and settle into the sediment of the stream bed (Bohn & Buckhuse., 1985; Sherer et al., 1992; Howell et al., 1996; Bai & Lung, 2005; Kern et al., 2008). Studies report that waters with high sedimentation allow for greater FIO persistence (Sherer et al., 1992; Doyle et al., 1992; Buckley et al., 1998; Crabill et al., 1999; Davies and Bavor., 2000). This is further supported by studies reporting concentrations of bacteria in benthic sediment reservoirs being up to 10,000 times higher than those in the water column (Doyle et al., 1992; Sherer et al., 1992; Buckley et al., 1998; Crabill et al., 1999; Davies and Bavor, 2000). The sedimentation of FIOs is also an unstable containment method when not controlled, and the ease of re-suspension from stream beds from storm events and other animal disturbances is well documented (Sayler et al., 1975; Bohn & Buckhuse, 1985; Sherer et al., 1992; Bai & Lung., 2005; Bradshaw et al., 2016). This can create the potential for downstream contamination long after deposition.

Furthermore, the study of direct cattle deposition of FIO's in waterways has revealed this pathway to be of significant influence on faecal contamination within waterways (Hagedorn et al., 1999; Nagels et al., 2002; Collins et al., 2010; Stott et al., 2011; Smolders et al., 2015). This area of research has been helpful in elucidating the role of sediments as bacterial reservoirs; more specifically the fact that sediments release FIO's during both storm and base flow conditions, showing that all flow conditions contribute to contamination of downstream waters (Hagedorn et al., 1999; Nagels et al., 2002; Collins et al., 2010; Stott et al., 2011; Smolders et al., 2015).

During storm flow conditions, levels are found to be at their highest measurable amounts following the initial rise in stream discharge, but decline rapidly following repeat storm events due to the depletion of in stream sediment storage (Nagels et al., 2002; Stott et al., 2011). Further, FIO concentrations in the water column have been found to persist at similar concentrations over time during base flow conditions, suggesting that bacteria are being released and re-suspended at a constant rate from streambeds (Stott et al., 2011). These low but persistent concentrations of FIO's still pose risk to human and cattle health (Nagel et al., 2002). Multiple studies have shown that limiting cattle access to streams greatly reduced FIO levels in contaminated streams and rivers (Hagedorn et al., 1999; Nagels et al., 2002; Collins et al., 2010; Stott et al., 2011; Smolders et al., 2015)

Although the risk to human and animal health, as well as to the wider aquatic environment, is considerable, the issue of bacterial contamination of waters from agricultural sources is only weakly addressed within the European Union legislative framework. The European Union addresses other agricultural risks to the environment in both their Nitrate and Water Framework Directives (EU Nitrate Directive, 1991; EU Water Framework Directive, 2006). The Water Framework Directive requires member states to adhere to regular monitoring of bathing water quality, but do not require analysis of contamination in non-bathing surface waters (EPA, 2016).

The most recent Irish EPA report on bathing water quality shows a clear lack of inland bathing water sampling sites (Todd and Boyle, 2018). Although 88.9% of inland waters were reported as of sufficient quality, only nine inland freshwater sites were sampled within the country. Thus, the current nation-wide prevalence of faecal contamination of Irish freshwaters is largely unknown. This creates a disturbing precedent for Irish Policy, where the current state of faecal contamination in fresh surface waters is unknown due to a majority of Irelands waters not being categorized as "bathing waters".

In Ireland, novel approaches are needed to address this issue, as it has been shown that point source run off from agricultural sites are a larger issue than many national policies address (Wiggins, 1996; Olsen et al., 2004 ; Wesley et al., 2004; Fayer, 2004 ; Davies-Colley, 2004 ; Ishii & Sadowski, 2008 ; Soller et al., 2010 ; O'Callaghan, 2014 ; Harrison et al., 2019; Moloney et al., 2019). The Irish Good Agricultural Practice (GAP) regulations (DAFM, 2006) sets regulations for limiting agricultural waste released into the environment, particularly bovine faecal matter, although does not refer to faecal bacteria from farm animals specifically. These regulations include instructions on good record keeping, manure and slurry application limitations, fertilizer moratoriums, and storage limitations for fertilizers and manure/slurry (DAFM, 2006). The GAP regulations have also listed definitive guidelines for buffer zones near water bodies (DAFM, 2006).

Although framework exists within the EU Water Quality directives and GAP regulations, the only policy that directly addresses faecal contamination lies within the EU bathing water directive (EU Water Framework Directive, 2006). It is not so much the addressment as the overarching lack of policy framework that sets the scene for public policies view on the threat that faecal contamination poses to Irelands ecosystems, livestock, and the general public. That is not to say that the risk has not been addressed or studied in Irish literature. Overall, Ireland reports the highest recorded occurrence of pathogenic verotoxigenic *E. coli* cases in Europe (Óhaiseadha et al., 2016). A study

completed within Northern Ireland (similar ecological habitat to Ireland) enumerated high levels of faecal contaminants within coastal and inland bathing waters, with 84% of collected samples collected from inland waters testing positive for enteroviruses (Hughes et al., 1992). Along with this finding, another study confirmed faecal contamination in 58% of collected samples (N~125) from private groundwater wells (O'Dwyer et al., 2014). These findings highlight the very clear risk that these organisms pose to the public stakeholders that utilize Irelands aquatic resources.

In other countries around the world, there is a more positive approach to the problem of faecal coliforms in surface waters. In New Zealand, the government has enacted public policies to address the bacterial contamination of surface waters from agricultural sources as well as incorporate the reduction of faecal coliforms into the overall freshwater management plan of New Zealand. The action plan for good farming practice not only lists *E. coli* as a pollution threat, it also includes explicit language directing farmers to take 3-5 corrective actions within their management plans in order to have agricultural operations meet the standards put forth by the action plan (Good Farming Practice Governance Group, 2018). The New Zealand Government also includes the objective of overall reduction of *E. coli* in surface waters in the Freshwater Management Plan of 2014, listing the goal of having 90% of surface waters safe for human interaction by 2040 (New Zealand Government, 2014).

Australia also addresses faecal contamination as a pollutant in surface waters. The Australian Environment Protection: Water Quality Policy 2015 defines animal faecal waste as a class 2 pollutant and prohibits the release of this pollutant to surface waters (South Australian Environmental Protection Authority, 2015). The same guidelines also address confined animal feeding as a potential source of faecal contamination to surface waters and instructs agricultural operations to include the needed infrastructure and management into their feedlot management plans (South Australian Environmental Protection Authority, 2006).

Elsewhere, the US EPA addresses faecal contamination as a pollution type within surface waters, and prescribes its management in coastal waters through its Clean Water Act (CWA)(Copeland, 2012). The CWA also creates the framework for states to report and limit their "total mass daily loads", or daily pollutant load into state waterbodies. Within the US, individual states have enacted policy to manage faecal contamination. The state of Vermont, for example, addresses faecal contamination of surface waters as a pollutant type and explicitly states that agricultural areas are not to drain to surface waters in any capacity. It also states that any drains and ditches containing agricultural waste are to be reported and approved by the appropriate governing body (Vermont Agency of Agriculture, Food, and Markets; Water Quality Division, 2018).

2.4 Thermotolerant Coliforms and Their Characteristics in Surface Freshwaters

TTC's are faecal indicator organisms that have passed through the intestinal track of a warm blooded animal (Leclerc et al., 2001, WHO & OECD, 2003). TTC's have been shown to occur naturally within freshwater ecosystems (Laclerc et al., 2001; Hachich et al., 2012), however studies have shown that TTC's collected from the freshwater environment can consist of up to 90% *E. coli* bacteria (Robertson et al., 1998; Tallon et al., 2005; Hachich et al., 2012). Thermotolerant coliforms (TTC's) have become a widely used method of indicating the presence of faecal indicator organisms within freshwaters due to their cost-effective and rapidly replicable laboratory protocol (Ross, & Thorrold, 2004; Donnison, et al., 2005, Yeung-Chuen A.K., 2009). Although not all *E. coli* is harmful to humans, wildlife, or cattle, *E. coli O157* has the potential to be present when waters test positive, making faecal contamination a clear risk to humans, cattle, and wildlife. The presence of TTC's also indicates the potential presence of other harmful pathogens, viruses, protozoa, and algae (Donnison, Ross & Thorrold, 2004; Tallon et al., 2005).

TTC's have been shown to enter waterways in agricultural catchments from direct cattle input as well as overland flow from rainfall, especially from catchments dominated by pasture (Donnison, Ross, & Thorrold, 2004, Collins et al. 2010). Although TTCs are adapted to live in the intestines of warm-blooded animals and are thought to reproduce only above a critical temperature of 37°C (WHO &OECD 2003), there is increasing evidence that they can persist and even reproduce in the external environment, given suitable conditions (Nieme &Nieme, 1991; Davies et al., 1995; Leclerc et al., 2001; WHO & OECD, 2003; Tallon et al,. 2005; Ishii et al., 2006; Ishii & Sadowski, 2008). These conditions include thresholds of nutrient availability, temperature, sediment particle size and seasonal fluctuations (Tate, 1978; Sherer et al., 1992; Ishii et al., 2006; Haller et al., 2009).

In order to assess the risk presented by TTCs in waterways, it is important to understand their behavior in the aquatic environment.. Specifically, knowledge of how bacteria are attenuated within waterways – either by death or incorporation into sediments – will allow managers to predict the risk of faecal contamination downstream from known sources. Once released into the aquatic environment, the residence time of faecal bacteria within waterways is a critical factor determining the risk of downstream contamination, as the degree of biological, mechanical and chemical attenuation of faecal bacteria is a function of increasing residence time (Perkins & Hunter, 1999; Diaz et al., 2010; Vymazal et al., 2008). Biological attenuation occurs through antibiosis, predation from organisms like nematodes and protists, viral and lytic bacteria attack, and naturally occurring die off. Mechanical attenuation occurs though exposure to the environment, specifically UV radiation and sedimentation. Chemical attenuation occurs through the oxidation process, chemicals excreted from aquatic vegetation, and absorption by organic matter (Vymazal, 2008).

TTC persistence within aquatic environments at a particular location is strongly influenced by the particle size and organic matter content of benthic sediments at the location (Sayler et al., 1975; Tate, 1978 ; Burton et al., 1987, Sherer et al., 1992; Vymazal, 2008 ; Haller et al., 2009; Bradshaw et al., 2016). Once TTC's enter the water column, either from re-suspension or initial deposition, silt-sized particles (Wentworth scale <62.5 µm and smaller) allow for a high levels of absorption and settlement (Tate, 1978; Burton et al., 1978; Bohn and Buckhouse, 1985; Haller et al., 2009). Upon settlement within the sediment layer, high nutrient availability may allow TTCs to live for extended periods of time outside their natural intra-intestinal habitat (Tate, 1978; Haller et al., 2009; Sherer et al., 1992). Die-off of TTCs can also be affected by seasonal temperature fluxes, with die off rates within sediments being greater during winter low temperature months (Rodgers et al., 2003; Bohn & Buckhouse; 1985, Flynn et al., 2016).

The spatially and temporally-dynamic distribution of TTC's within lotic ecosystems can be visualized in Figure 1. Initial deposition of TTC's within a waterbody from point sources suspend the bacteria within the water column where they can adhere to suspended sediment, and settle out on the bed of the channel (Sayler et al., 1975; Bohn & Buckhuse, 1985; Sherer et al., 1992; Bai & Lung, 2005; Bradshaw et al., 2016). Upon deposition, TTC's can then persist for long periods of time, especially in small particle size, nutrient-rich sediments (Sayler et al., 1975; Tate, 1978; Burton et al., 1987; Sherer et al., 1992; Vymazal, 2008; Haller et al., 2009; Bradshaw et al., 2016). The TTC's can then be easily re-suspended and transported downstream, should conditions arise promoting the efflux of sediment and bound bacteria, for example, during spate flows. The process of bacteria transport can be described in terms of sink-source dynamics, where the downstream transport of FIO's in a stream can be visualized as a continual spiral. The stream sediment allows for settlement and persistence of TTC's, but its unstable nature easily allows re suspension. The bacteria then travel downstream until re-sedimented on the bed. Eventual die-off of the bacteria may occur when conditions

become unfavourable, such as when there is low availability of organic matter or where high shear stresses prevent the settlement of fine sediment. (Fig 1).

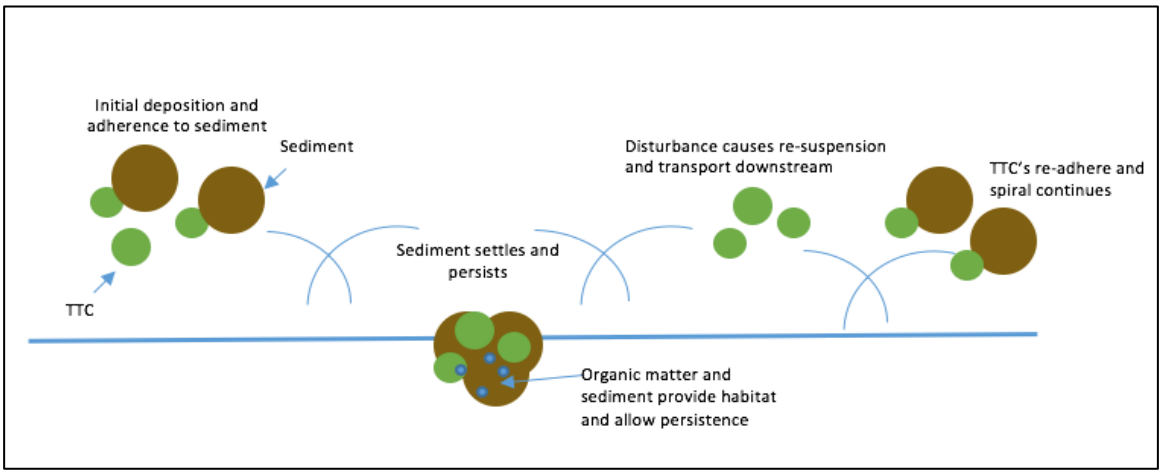


Figure 1. Visual representation of faecal contamination in fresh waters

2.5 Constructed Wetlands and Agricultural Field Drains: Their Use in Reducing Faecal Pollution

The use of constructed wetlands in attenuating agricultural pollution has been well studied. A constructed wetland (CW) is a piece of land created or adapted for the sole purpose of treating agricultural runoff, or other organic pollution. These CW's have been used successfully to reduce nutrient pollution as well as bacterial pollution (Babatunde et al., 2008; Berry et al., 200; Kavaisi, 2000; Davies & Bavor, 2000; Karathanasis et al., 2003). CW's have been shown to reduce faecal coliform levels up to 99% when inflow and outflow measurements were compared (Davies & Bavor, 2000; Kivasie, 2000; Karanthanasis et al., 2003). The most efficient constructed wetlands are of shallow nature, allowing for macrophytic vegetation growth throughout the entire treatment area and not just along the CW boundary (Wong et al., 1999; Berry et al., 2007; Davies & Bavor, 2007). Residence time has also been reported as a significant factor in bacterial attenuation within CW's (Karathanasis et al., 2003). CW's have been increasingly used

in recent years within agricultural settings, including within Ireland, and are a potential option for agricultural pollution containment and treatment (Berry et al., 2007, Babatunde et al., 2008).

Although this treatment methodology has the potential for future utilization in the reduction of agricultural contamination in Irish surface waters, much of the focus on the treatment of microbial levels within CW's has focused on the quantifying the reduction of TTC's from inflow to outflow. The change in these levels is referred to as "attenuation", however, these studies often fail to report the mortality of faecal bacteria colonies within the wetland itself (Davies & Bavor, 2000; Kavaisi et al., 2001; Karathanasis et al., 2003; Berry et al., 2007; Babatunde et al., 2008). This means that the current design models for CW's could be contributing to TTC contamination downstream due to the fact that attenuation does not necessarily indicate bacterial die off, only the CW's ability to retain bacteria within the sediment (Sayler et al., 1975; Bohn & Buckhouse, 1985; Sherer et al., 1992; Nagels et al.. 2002; Bai & Lung, 2005, Jamieson et al., 2005; Stott et al., 2011; Bradshaw et al., 2016; Pachepsky et al., 2017). Since ease of bacterial release from sediment during both base and storm flow is well documented, this further supports the notion that sediment within CW's could be creating hazardous hydrologic conditions downstream (Sayler et al., 1975; Bohn & Buckhouse, 1985; Sherer et al., 1992; Nagels et al., 2002; Bai & Lung, 2005; Jamieson et al., 2005; Stott et al., 2011; Bradshaw et al., 2016; Pachepsky et al., 2017).

Although constructed wetlands have been shown to be an effective means to attenuate agricultural pollution, they are expensive to construct, manage and maintain and may involve considerable land take from a farmer's holding. Modifying existing drainage systems which receive polluted water represents a potential cost-effective solution. Several authors report the potential for using modified agricultural drainage ditches in retaining and remediating harmful pathogens, nutrients, agricultural pesticides, and sedimentation (Needleman et al., 2007; Vymazal, 2015; Moore & Kroger et al., 2010;

Littejohn et al., 2013., Kroger et al., 2008). There has been recent interest in the role of these small headwaters in agricultural drainage management, more specifically the roles drainage ditches (either manufactured or artificially altered) and small streams play in the health of receiving waters (Pierce et al., 2012). These waters are the first to be impacted by of runoff from these sites, so their ability to lessen the effect of agricultural waste has been of special focus (Needleman et al., 2007). Artificially created or altered agricultural drainages have special potential for remediating impact, as they have been shown to be providers of dominant flow within first order headwaters (McGarrigle, 2014; Dupas et al., 2017). Agricultural drainage ditches have historically been constructed so as to drain water as quickly as possible, rather than as water treatment measures (Avery, 2012). But recent studies show that adapting these ditches into controlled wetlands may have a low cost high value association that could help to address the headwater agricultural pollution issue (Avery, 2012). The use of adapted agricultural drainage ditches has been shown to be particularly successful with the addition of engineered structures such as low weirs, in order to increase residence time(Littlejohn et al., 2013, Kroger et al., 2008). Although their effectiveness at removing FIO's from agricultural waters is not well documented, their success at attenuating other agricultural contaminants suggests that they may place a significant role in this important function.

Although literature has addressed many characteristics of TTC's within the environment, including biology, pathology, public health risk, treatment methodology, policy, and source, there remains much to be done in order to fully ameliorate the risk these organisms pose. The exact nature of TTC origins within agriculturally dominated catchments is poorly understood. Although the species source has been studied in depth, the method and location of introduction of TTCs into the environment is in need of further investigation; baseline measurement from farmyard inputs is still needed in order to determine the level of risk these inputs may pose. Furthermore, there has been little research done on the utilization of drainage ditches and small headwaters ability

to attenuate faecal input from agricultural sources. There is also no current data available in the tracking of faecal input from point source locations on a catchment wide scale, which would provide more information on the distribution and survival of TTC's within agriculturally dominated catchments. This study will aim to lessen these knowledge gaps and attempt to gain further understanding of agricultures role in the faecal contamination of fresh waters.

2.6 Study Objectives

The objectives of this study are as follows

1. To quantify the ability of headwater drainage channels receiving direct farmyard effluent to attenuate faecal indicator organisms, in (a) the water column and (b) within benthic sediments, over their length.
2. To determine the distribution, concentration, and origin of faecal indicator organisms at intensive spatial scales within in a small agriculturally dominated catchment in South West Ireland.

3.0 Methods

3.1 Physical Description of Landscape

The river Lee catchment used in this study is located within the southwest of Ireland (Figure 3). The Lee catchment has a temperate maritime climate, the west of the catchment receiving a higher level of rainfall than the eastern portions. Rainfall is heaviest in the winter months, with precipitation dropping off throughout the duration of the growing season (Gillet, 2006). Having a mix of carboniferous limestone and Devonian old red sandstone bedrock, the landscape is dominated by brown podzolic and peaty podzolic soils as classified by the USDA (USDA, 1938, Gillet, 2006). The lowland brown podzolic soil is well drained and lends itself well to agricultural use, making the primary human utilization of this landscape dairy and meat agricultural operations (Gillet, 2006). The study sub catchment is indicative of this description, with brown podzilic soils dominating the upper reaches of the catchment, and a mix of brown podzilic and acid brown earths in the lower reaches (Gillet, 2006).

3.2 Investigation 1: Quantification of Winter Water Column TTCs Within Farmyard Drainage channels

3.3 Sample Site Selection for Investigation 1

Study sites (agricultural drainage channels) were selected from sub-catchments within the Lee catchment, using prior knowledge of drainage patterns of catchments and through online map searches. Drainage channels received water from agricultural land holdings in the vicinity of farmyards and fed into small headwater tributaries of the larger Lee catchment. Four drainage channel sites were chosen on the basis of physical similarity, accessibility, prior knowledge of water chemistry parameters including phosphate, nitrate, and dissolved oxygen (Harrison et al., 2019), and observed farmyard

contamination (Figures 2, 3, and 4). Cattle density within the farmyards was notably higher than cattle density within cattle pastures throughout the duration of this investigation, as is common within Ireland during the winter months. This allowed for definitive identification of farmyard effluent.

3.4 Farmyard Drainage Channel Site 2

Farmyard drainage channel site 2, within the River Shornaugh catchment, runs approximately 400 meters in a southeast direction from its farmyard source along a physically-homogenous roadside drainage ditch. The drainage channel was characterized by abundant within-channel hydrophyte vegetation (dominated by semi-aquatic grasses). Water velocity within the channel was very uniform along its length, approximately 10cms /sec (Figures 2, 3, and 4).

3.5 Farmyard Drainage Channel Site 3

Farmyard drainage channel site 3, also within the River Shornaugh catchment, runs approximately 200 meters in a southeast direction from its farmyard source along a roadside drainage ditch. The drainage channel had abundant organic matter build up along its length, and a distinct lack of hydrophilic plant growth. Water velocity within the channel was very uniform along its length, approximately 10cms /sec (Figures 2c, 3, and 4c).

3.6 Farmyard Drainage Channel Site 4

Farmyard drainage channel site 4 within the River Dripsey catchment, runs approximately 290 meters in a southeast direction from its farmyard source along a physically homogenous semi-natural stream bed. The drainage channel was characterized by abundant amorphous benthic organic matter at the source. Growth of sewage fungus was evident for the first 50m, and was largely absent after 100m in both winter and summer months. Growth of macrophyte vegetation within the drainage channel was apparent from approximately 250m downstream in winter and summer, increasing in abundance downstream from this point. Velocity within the channel was very uniform along its length, approximately 10 cms/sec (Figures 2d, 3, and 4d).

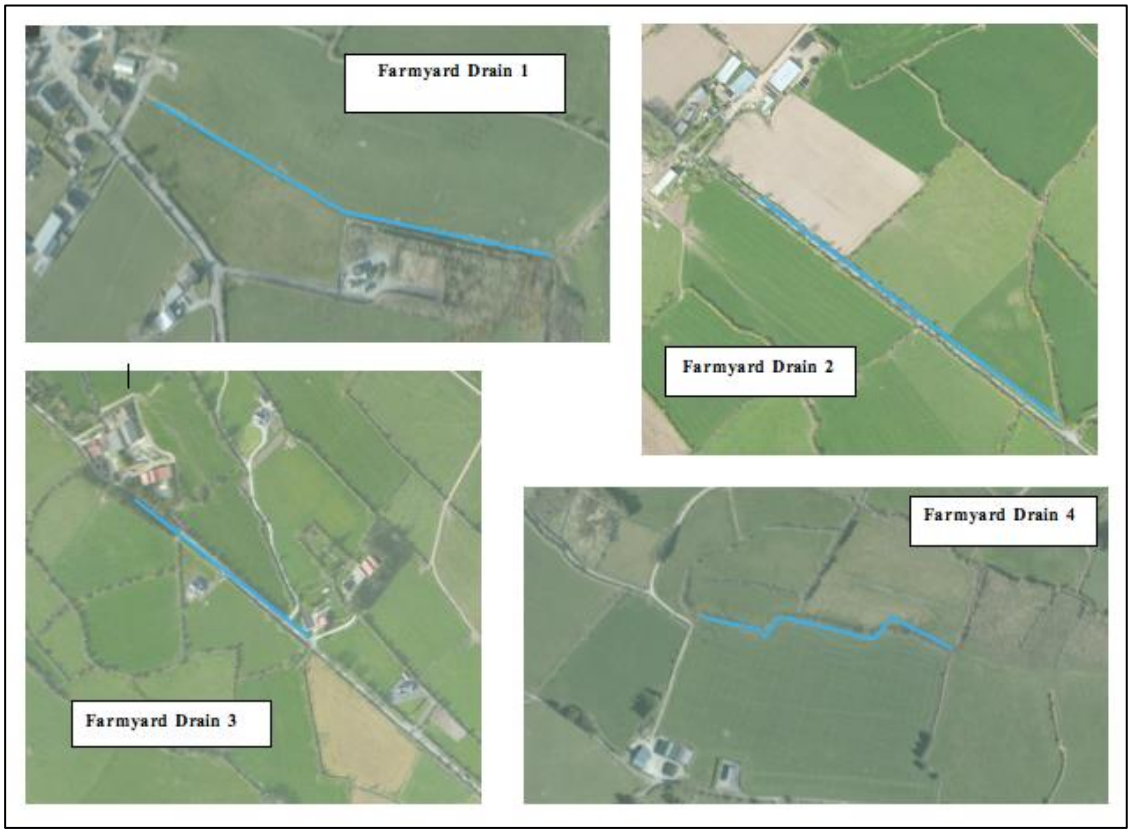


Figure 2. Composite figure of farm drainage sites 1-4. Drainage channels highlighted in blue

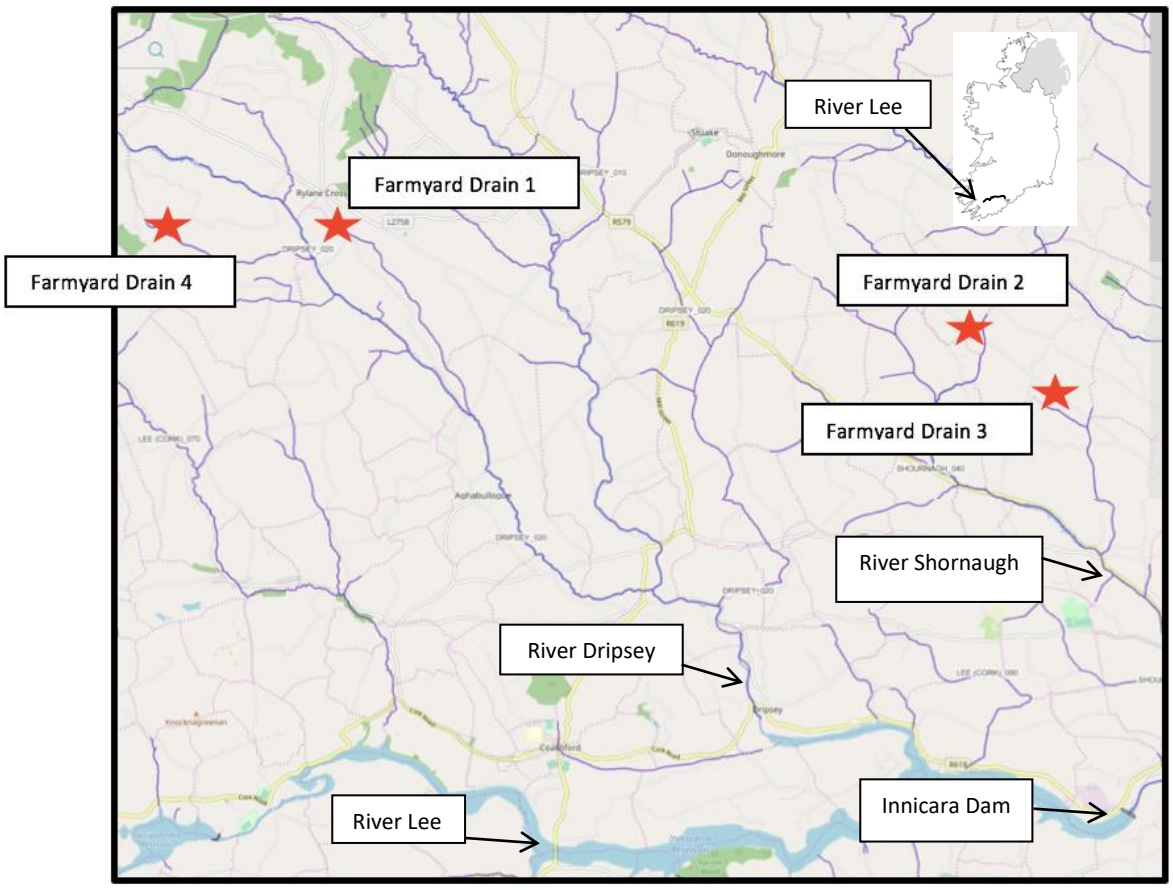


Figure 3. Locations of farmyard drainage sites 1-4. Drainage sites were within the Dripsy and Shornaugh river catchments in County Cork. Small insert map shows location of River Lee within Ireland.



Figure 4. Composite figure of photographs for farmyard drainage sites 1-4

3.7 Investigation 1 Field and laboratory methods

Thermo-tolerant coliforms (TTCs) were sampled from the water column along the lengths of the four farmyard drainage channels in February 2019. Drainage channels were sampled on days following no to light rainfall in order to collect samples that accurately depicted average hydric condition. Triplicate 200 ml water samples were taken in autoclaved sterile 200 ml bottles at the origin of input into each drainage ditch and then every 100 meters downstream until a major change of ditch/stream morphology or the termination of ditch/stream occurred. Samples were taken by inverting and submerging the sterile bottles beneath the water surface, to avoid sampling any surface biofilm. Care was taken to avoid disturbance of benthic sediment at each site. Samples were taken from downstream to upstream to avoid contamination of samples by previously disturbed sediment. Inflow samples were taken within 1 meter of the origin of farmyard input into the drainage ditches. A field 'blank' sample was also collected to ensure that there was no potential contamination of samples from aerial or other sources. Field blanks were collected by opening 200ml of autoclaved bottles containing 200 ml of sterile water onsite and exposing them to ambient air conditions for 30 seconds. Water samples were then placed in a cooler box filled with ice, transported to the laboratory, and processed within 4 hours of collection.

In the laboratory, a direct membrane filtration method was used to isolate TTC's from drainage channel water samples (Diaz et al., 2010; Kay, D et el., 2005; Smolders et al., 2015). Filtration was completed by vacuuming the final volume of 50ml through a 0.45 µm cellulose acetate membrane filter paper. This was done by first diluting samples 0-100x with sterile water depending upon observed contamination levels from initial sample collection within sample sites or from previous preliminary pilot investigation (Harrison et al., 2019). Dilutions were performed by extracting original water sample by micropipette, and adding into sterile water contained within a sterile (previously

autoclaved) 50 ml centrifuge tube. Final solutions were 50 mls. Tubes were then inverted to homogenize the sample.

Petri dishes were prepared with 2 ml of growth/cultivation lauryl sulfate broth. Water samples (final solution of 50 ml) were filtered by manual suction through 0.45 µm filter paper placed onto a Nalgene brand Polysulfone 100 ml filter apparatus. Filters with filtrate were then placed onto pre-prepared cultivation broth pads within petri dishes and incubated for 16-18 hours at 44.5°C. Following incubation, visible tan colored bacterial colonies measuring between >1mm and <10mm diameter on the filter papers were counted. Total TTC's within each sample were calculated by multiplying by necessary dilution factors and original sample size to provide a standard TTC cfu/ 100ml of sample metric (Diaz et al., 2010; Kay et al., 2005; Smolders et al., 2015).

3.8 Investigation 2: Quantification of Winter sediment TTCs Within Farmyard Drainage channels

3.9 Investigation 2 Field and laboratory methods

TTCs were sampled from the benthic sediment along the lengths of the same four farmyard drainage channels as for investigation 1, in February 2019. As for water column sampling, drainage channel sediments were sampled on days following no to light rainfall in order to collect samples that accurately depicted average hydric conditions. Benthic sediment samples were collected at the top of each farm drainage channel (nearest to the point of farmyard input) and at the defined termination point (i.e. two sites per drainage channel). At each site, the surface benthic sediment from an area approximately 100cm^2 was collected by hand in an inverted sterile plastic bag (food-grade bags purchased from local grocers and previously autoclaved), labeled and securely tied. Samples were then placed into a cooler box, transported to the laboratory

and processed within 4 hours of collection (Yeung-Chuen, A.K., 2009; Hussein et al., 2012).

In the laboratory, the methods described by Yeung-Chuen A.K. (2009) were followed. Each collected sediment sample had 10g of sediment randomly removed and placed into sterile plastic 200ml bottles. 100 ml of sterile water was placed into each 200ml bottle and firmly agitated by hand for one minute in order to thoroughly release bacteria from sediment. Samples were set to settle for 15 minutes. Following this procedure of releasing bacteria from sediment into the water, the same laboratory procedure for incubating and enumerating TTC colonies was followed as for investigation 1 above.

3.10 Investigation 3: Longitudinal distribution of TTCs within stream sediments downstream from a farmyard input

The stream system selected for the investigation of longitudinal patterns of sediment TTC's was a small tributary (Tributary 1) of a sub-catchment of the River Dripsey. The headwater of the tributary was a highly polluted farmyard drainage channel (Site 1 in investigations 1and 2 above). The tributary flowed downstream for approximately 3.5 kilometers before joining a larger channel (Figure 5 below). Benthic sediment samples of TTC's were taken from 4 sample sites along the tributary, at the head of the tributary (0m) and at 600 m, 2,000 m, and 3,500 m intervals downstream. Samples were taken on five separate occasions, from April through to June 2019.

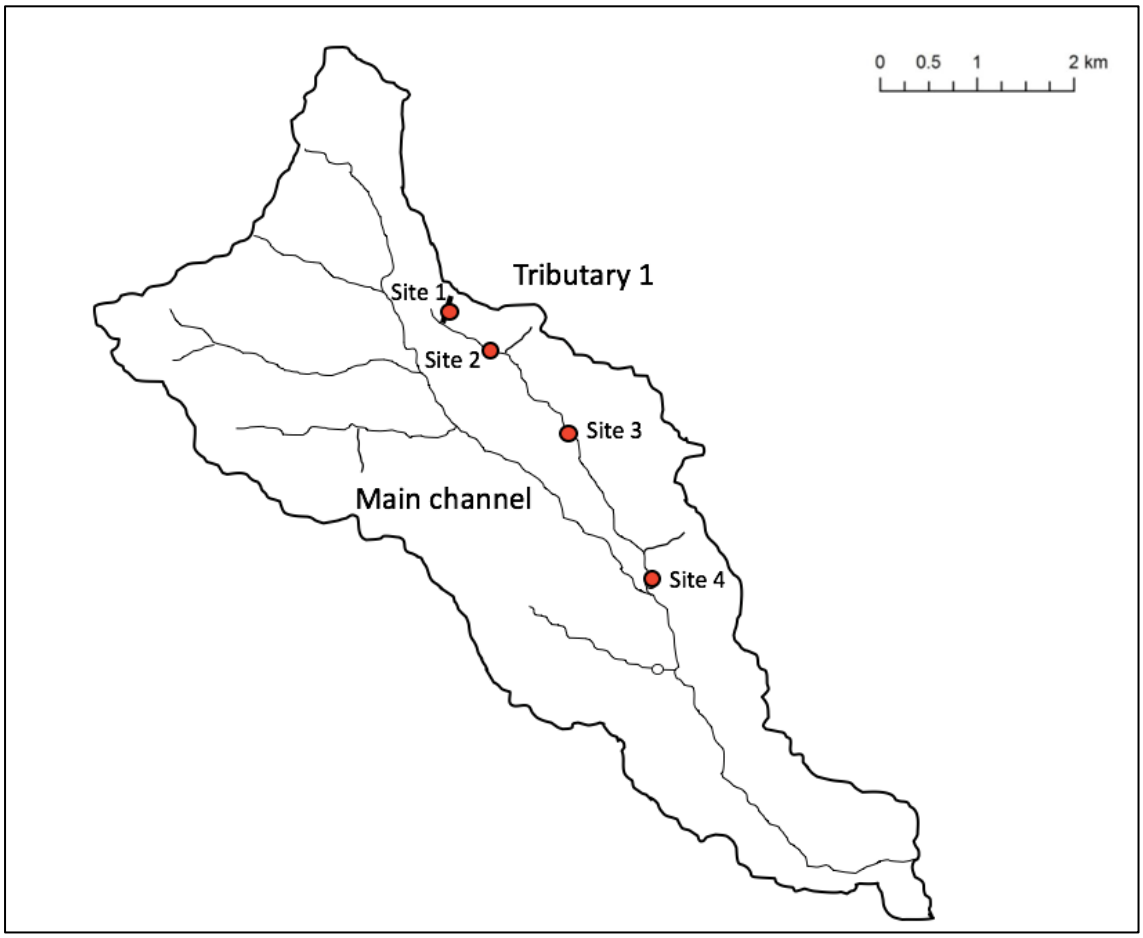


Figure 5. Location of the four sample sites along tributary 1. The location of the tributary is downstream from the farmyard drainage channel, site 1. Map retrieved from UCC School of Biological, Earth, and Environmental Sciences Faculty, Harrison et al., 2019.

3.11 Investigation 3 Field and laboratory methods

During investigations 1 and 2 above, TTC concentrations within water column and benthic sediment samples were found to be highly variable, likely due to high variability of bacteria density within the stream water itself and high heterogeneity within benthic sediments over small spatial scales. To reduce variability due to natural local within-site heterogeneity within benthic sediments, a novel bacterial colonization substratum was developed and introduced into each site.

For this investigation, the characteristics of stream benthic sediment colonized by TTCs was standardized by the use of artificial bacterial colonization substrata. The use of

artificial colonization substrata to control for substratum heterogeneity and so reduce sampling variability has been widely used in freshwaters to sample benthic algae and macroinvertebrates (Opshal et al., 2003; Sturt et al., 2011; McCall et al., 2017; Vadeboncoeur & Power, 2017). Although less commonly applied in microbial studies, artificial substrata have also been used to sample benthic bacteria in stream, including unglazed ceramic tiles (Olapade and Leff, 2006) and nylon mesh bags containing artificial mineral sediment (Santmire and Leff, 2007). We adopted the methodology of Santmire and Leff (2007) to study TTC colonization of benthic substrata in streams. Square water-permeable mesh bags (16cm x 16cm) were constructed from 25 µm nylon mesh and filled with approx. 200g of clean, TTC free coarse sand. Samples of sand were taken and tested via the previously described laboratory method, samples were found to be free of TTC contamination. The edges of the mesh bags were sealed using both wire staples and cotton thread (Figure 6 below). Mesh bags allowed the free movement of water and bacteria across the surface of the bag, but retained sand within them. Coarse sand was preferred in this experiment, as it was very close in texture and composition to the natural sand –fraction sediment within streams. The sand was obtained from a local quarry and was thus of the same geological type and origin as the natural stream sand-fraction sediment.

Three colonization bags were introduced at each of the four sites along the tributary, each sediment bag approximately 50-75 cm from its neighbouring bag. All bags were attached individually to a secure location on the stream bank by orange plastic twine, to facilitate re-location. Sediment bags were placed in the stream such that they rested securely on the bed of the stream, in a hydraulically stable location (Fig. 7). Sediment bags were left in the stream to colonise with TTC's for seven days (TTC enumeration within the lab entailed a 24-hour incubation period with optimal conditions. Stream conditions were considered varied and not laboratory grade conditions, therefore seven days would allow for bacterial colonization). After the seven day colonization period, the

three sediment bags from each sample site were retrieved from the tributary, placed into separate labelled sterile bags and transported to the laboratory.

In the laboratory, the sediment from the three colonization bags from each site were pooled together via emptying of the sediment of each bag into a single plastic bag (previously autoclaved). The sediment within this bag was then homogenized by firmly agitating by hand for one minute (Falbo et al., 2013) so as to distribute bacteria evenly throughout the sediment. 10g of this homogenized sediment was then removed from the plastic bag and added to a 200ml plastic bottle. 100ml of sterile water was then added to the bottle and firmly agitated for one minute to release bacteria from the sediment. The supernatant was then transferred to 50ml centrifuge tubes and centrifuged from 6 minutes at 1000 rpm to remove suspended fine sediment from the sample and so to prevent this sediment from clogging the filters used to collect bacteria for cultivation and quantification. All bottles/tubes used were sterilized by autoclaving. The methods used to quantify the TTCs within these centrifuged samples was analogous for investigations 1 and 2 above.

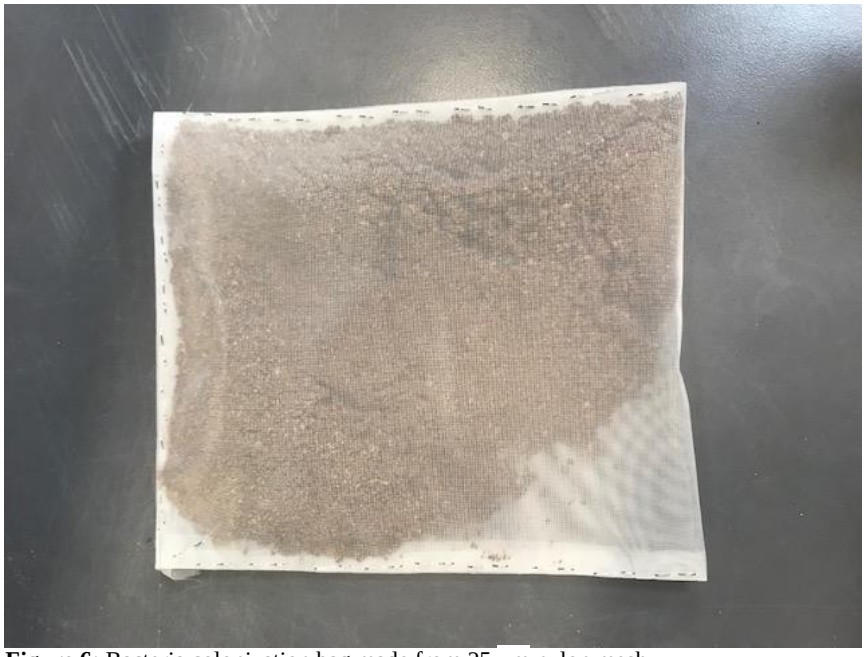


Figure 6: Bacteria colonization bag made from 25 µm nylon mesh



Figure 7. Photograph of field placement for mesh bags. Bags were secured with plastic twine to a metal bar driven into the stream bank.

3.12 Investigation 4: Distribution of benthic sediment-bound TTCs at multiple locations within a single dairy-dominated agricultural catchment.

3.13 Sample sites

The sample sites (33 in total) for investigation 4 were all located within the drainage network of a single sub-catchment of the River Dripsey, a tributary of the River Lee, County Cork, SW Ireland. The sub-catchment contained the single tributary (tributary 1) of investigation 3 (Figure 5). Sites were selected along four distinct habitat types: (a) farmyard drainage channels, (b) farmyard-polluted headwater tributaries, (c) farmyard-unpolluted headwater tributaries (henceforth first-order headwaters) and (d) the main stream channel. Seven sites were located within farmyard drainage channels, 7 sites located within headwater tributaries upstream of any known farmyard input, 9 sites were located within tributaries downstream of farmyard drainage channels, and 10 sites

were located within the main channel itself (Fig 7). TTC colonization substrata (nylon mesh bags containing coarse sand) were deployed to quantify sediment TTCs at each site (as for investigation 3 above). As for investigation 3, three mesh bags were introduced into the channel at each sample site, located 50-75cms apart, and secured to a location along the bank with orange plastic twine. Mesh bags were left to colonise with TTCs for a period of one week before removal and laboratory processing (as for investigation 3 above). Colonization substrata were introduced at each site on two occasions –the first on June 5 2019, and the second on June 25 2019. A total of 30 sites were included in the first run and 33 sites were included in the second run. The addition of sites within the second round of sampling resulted from additional observations within the catchment that had the potential to further enumerate TTC source and distribution. Each of the three colonization bags were combined into a single bag and agitated thoroughly for one minute for even distribution of bacteria. Samples were processed in the laboratory, as for investigation 3 above.

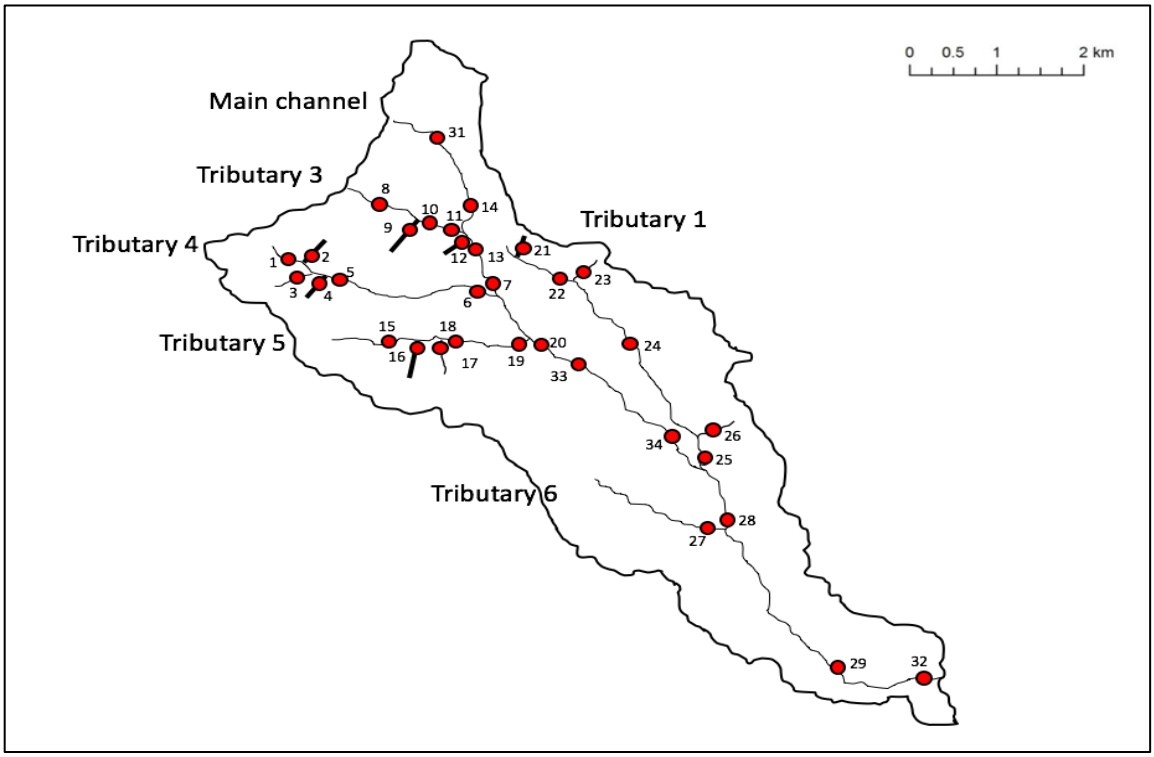


Figure 7. Locations of sample sites 1-33 for benthic sediment TTFCs within study catchment. Farmyard drainage channels are shown as thick black lines s while main channel, tributary, and upstream sites are denoted by thin black lines. Map retrieved from UCC School of Biological, Earth, and Environmental Sciences Faculty, Harrison et al., 2019.

3.14 Data Analysis Methodology

Data analysis for these investigations was performed using non-parametric Kruskall Wallis tests via IBM SPSS Statistical Analysis Program. Outliers were excluded within the statistical analysis but denoted within the provided tables and charts below. p-value was provided at <.05.

Investigations 1 and 2 analyzed any significant difference between sites along drain lengths for each sample site. Investigation 3 analyzed any significance between sample sites along the system.

Investigation 4 analyzed statistical differences between the four habitat types within the catchment- (a) farmyard drainage channels, (b) farmyard-polluted headwater tributaries, (c) farmyard-unpolluted headwater tributaries and (d) the main stream channel, with individual sites used as replicates in the analysis.

4.0 Results

4.1 Investigation 1: Quantification of Water Column TTCs Within Farmyard Drainage channels

There was little consistent pattern in the density of water-column TTFCs along the four farmyard drainage channels (Figure 8; Table 1). Although numbers showed an overall longitudinal decline from the head of the channel to the downstream site in sites 2 and 3, (Site 2; decline in averages 60 TTC, 46 TTC, 20 TTC, 15.3 TTC) there was no statistical significant difference in densities of TTFCS between sites in any of the channels (Figure 8, Table 1), and no evidence of any attenuation of water-column bacteria down the length of the farmyard drainage channels. Densities of TTCs within channels differed greatly between sites, with site 3 having by far the highest TTC density (Table 1). Densities of TTCs exceeded the suggested threshold value of 100 cfu/100ml as suggested for cattle drinking water recommendations (Australian and New Zealand Environment and Conservation Council, 2000; Pick, 2011) for many of the sampling sites in site 1 and all of the sampling sites in site 3 (Table 1).

Table 1.
Thermotolerant Coliform results reported in cfu/100ml from winter water column farm drainage sites 1-4.

Farmyard drain 1 02/2/19			Farmyard drain 2 04/2/19			Farmyard drain 3 14/2/19			Farmyard drain 4 11/2/19		
Distance down drain	Sample	Bacterial counts CFU/100ml	Distance down drain	Sample	Bacterial counts CFU/100ml	Distance down drain	Sample	Bacterial counts CFU/100ml	Distance down drain	Sample	Bacterial counts CFU/100ml
0	1	180	0	1	40	0	1	540	0	1	20
	2	60		2	60		2	1140		2	0
	3	120		3	0		3	660		3	40
100	1	120	100	1	20	100	1	720	100	1	80
	2	100		2	20		2	540		2	20
	3	120		3	20		3	620		3	20
200	1	200	200	1	0	200	1	280	200	1	20
	2	120		2	20		2	300		2	20
	3	140		3	40		3	120		3	40
300	1	40	300	1	0				300	1	20
	2	80		2	20					2	100
	3	120		3	20					3	20
			400	1	6						
				2	20						
				3	20						

Table 2.
Kruskall-Wallis results from winter water column farm drainage sites 1-4.

Site	Distance downstream (m)	Mean Rank	Kruskal H	P-value	Significance
Site 1	0	6.33	4.5	0.21	n.s.
	100	7			
	200	3.33			
	300	9.33			
Site 2	0	5.83	1.56	0.82	n.s.
	100	7.5			
	200	8			
	300	9.67			
	400	9			
Site 3	0	3.17	5.5	0.63	n.s.
	100	3.83			
	200	8			
Site 4	0	7.83	0.79	0.85	n.s.
	100	6			
	200	6.5			
	300	5.67			

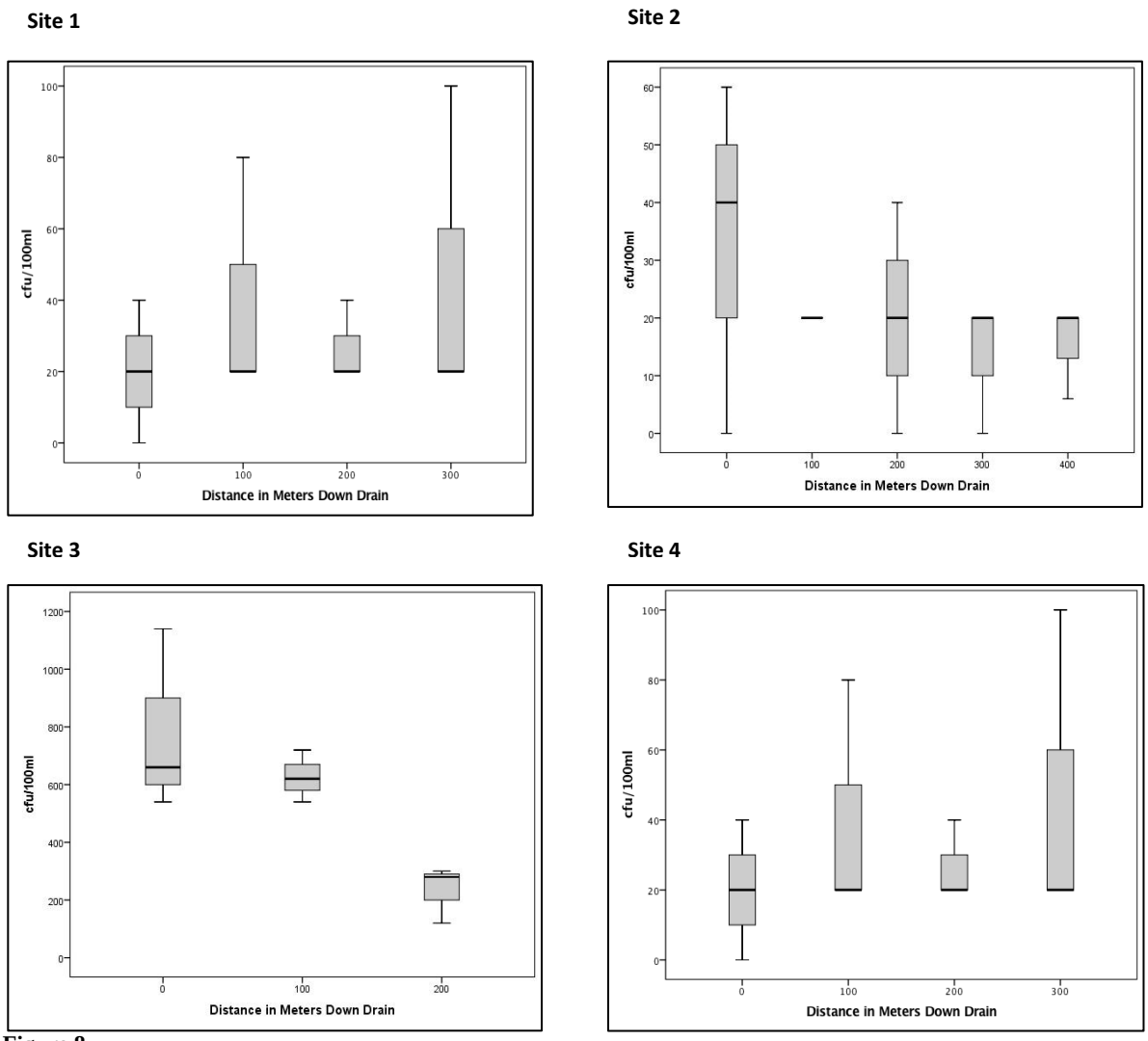


Figure 8.
Box and whisker plots for winter water column results from farmyard drainage sites 1-4. Results reported in cfu/100ml. Medians are represented by thick black lines, interquartile ranges are shown between limits of boxes. The furthest data point from median, but within 1.5x inter-quartile range are shown as whiskers.

4.2 Investigation 2: Quantification of sediment TTCs Within Farmyard Drainage channels

As for water column TTC's, there was no consistent spatial pattern in the densities of sediment-bound TTC's within drainage channels (Figure 9, Tables 3 &4). Although there were somewhat lower densities of sediment TTC's in the downstream sites in farmyard drain site 3 (as for water column TTCs at this site), there was no significant difference between upstream and downstream sites for benthic TTCS at any of the sites, with all sites providing p-values of >.05 (Table 4). There was very high variability between samples within a single sampling site across all farmyard drainage channels, demonstrating very high local (0-1m) variation in benthic TTFC densities (Table 3, Figure 9). Final results from this investigation point towards the poor attenuation capabilities of farmyard drainage channels to attenuate TTC's within their natural states.

Table 3.
Thermotolerant coliform sediment results from farm drainage channels 1-4, U/S describes upstream sires and D/S describes downstream sites

Farmyard drain 1 21/2/19		Farmyard drain 2 18/2/19		Farmyard drain 3 25/2/19		Farmyard drain 4 25/2/19	
U/S	D/S	U/S	D/S	U/S	D/S	U/S	D/S
0	36000	6000	46000	80000	2000	4000	2000
4000	0	38000	4000	28000	12000	0	2000
4000	600	4000	54000	16000	16000	0	2000

Table 4.
Thermotolerant coliform sediment Kruskall Wallis results from farm drainage channels 1-4. No significance found.

Site	Drain Location	Mean Rank	Kruskal H	P-value	Significance
Site 1	Drain Top	4.17	0.81	0.37	n.s.
	Drain Bottom	2.83			
Site 2	Drain Top	4.00	0.78	0.38	n.s.
	Drain Bottom	3.00			
Site 3	Drain Top	2.17	3.14	0.08	n.s.
	Drain Bottom	4.83			
Site 4	Drain Top	4.00	0.5	0.48	n.s.
	Drain Bottom	3.00			

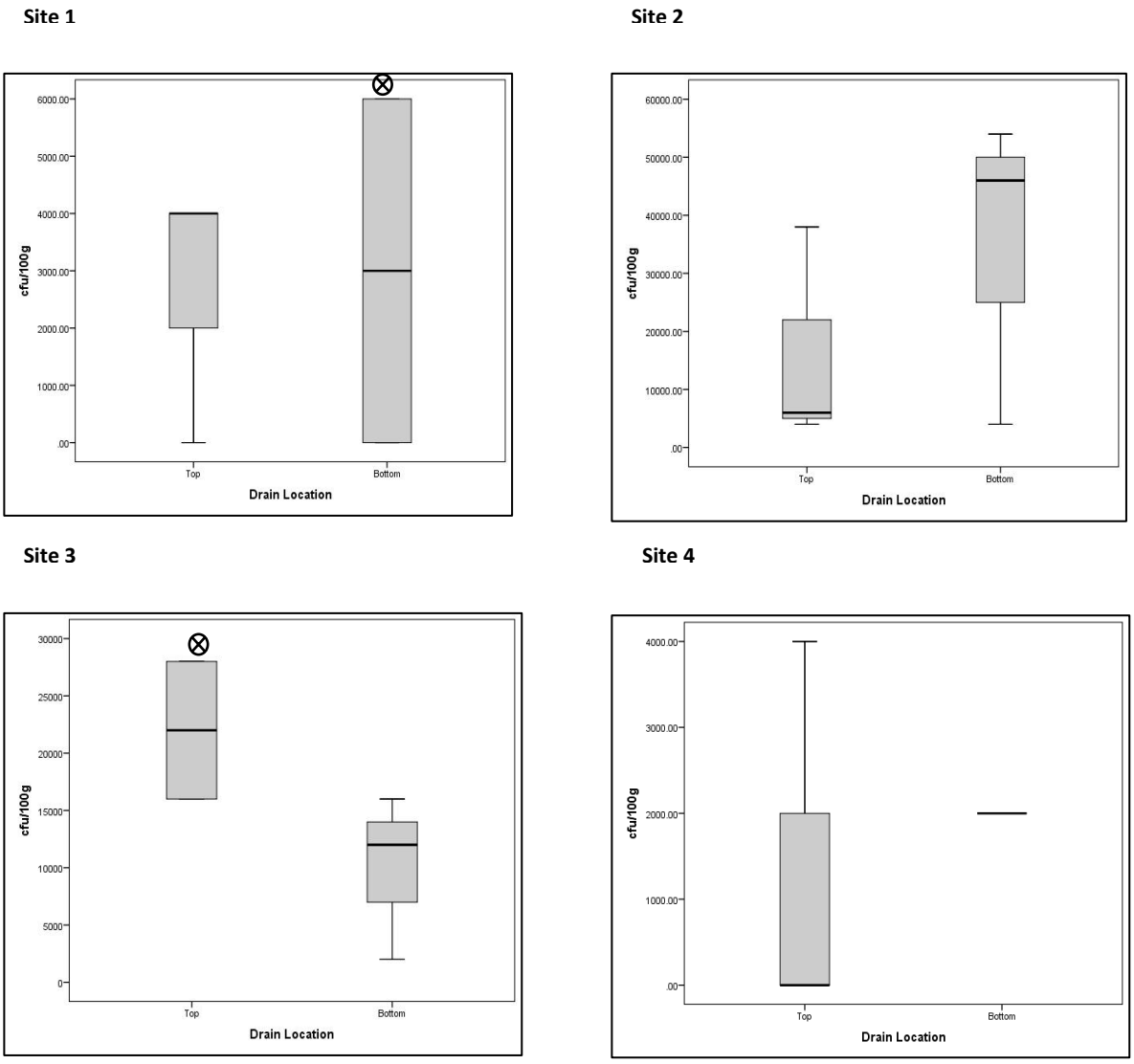
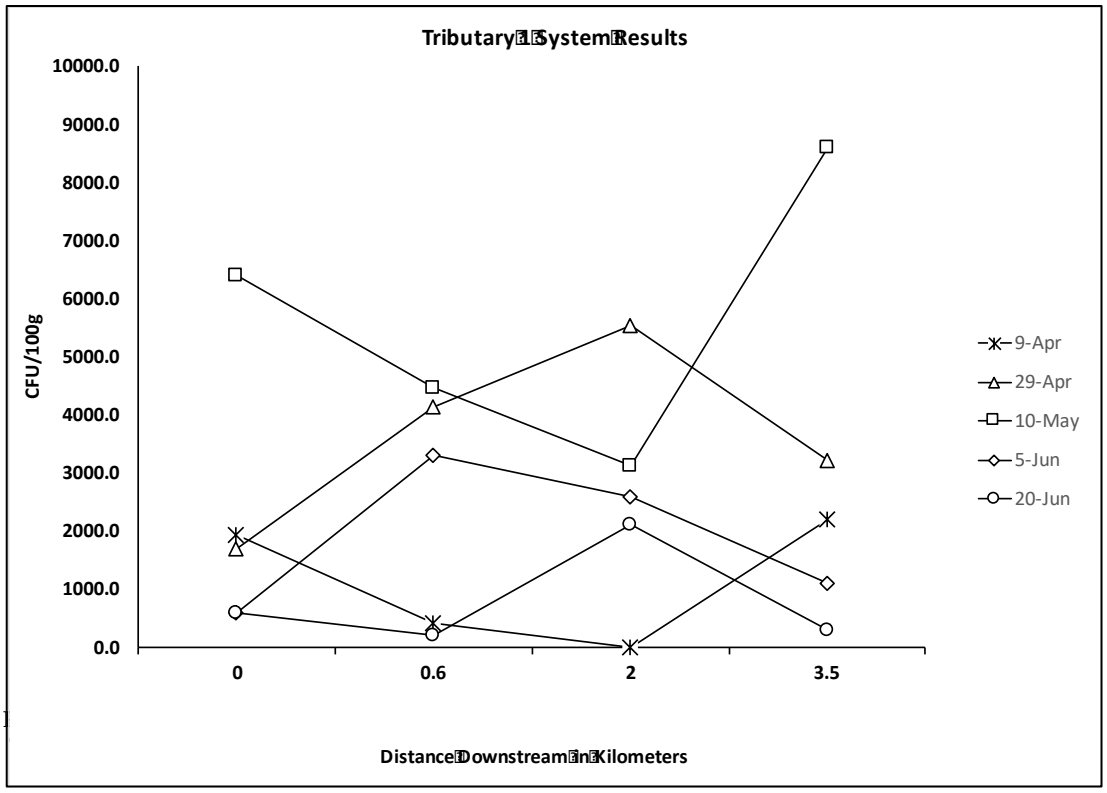


Figure 9.
Box and whisker plots for sediment TTC concentrations from farmyard drainage sites 1-4. Results reported in cfu/100ml. Medians are represented by thick black lines, interquartile ranges are shown between limits of boxes. The furthest data point from median, but within 1.5x inter-quartile range are shown as whiskers. Outliers denoted with X marking.

4.3 Investigation 3: Longitudinal distribution of TTCs within stream sediments downstream from a farmyard input

There was no consistent pattern of attenuation of sediment-bound TTC's along the length of the tributary across the five sample dates (Figure 10; Tables 5 & 6). Densities of TTC's saw a marked decrease over the course of the investigation (Figure 10; Table 5), yet no significance was displayed relative to spatial distribution within sample dates. Densities of TTCs exceeded the suggested threshold value of 100 cfu/100ml in all sample sites within all sampling runs (Table 5). No single sampling date presented results similar to any other sample date, with level rising and falling unpredictably (Figure 10, Figure 11, Table 5). Statistical analysis also shows lack of significance (P<.05) between any sampled sites (Table 6). Lack of attenuation is further supported by the grouping of sediment results within the four sites along tributary 1, with each sampling occasion grouped by site number (Figure 11).



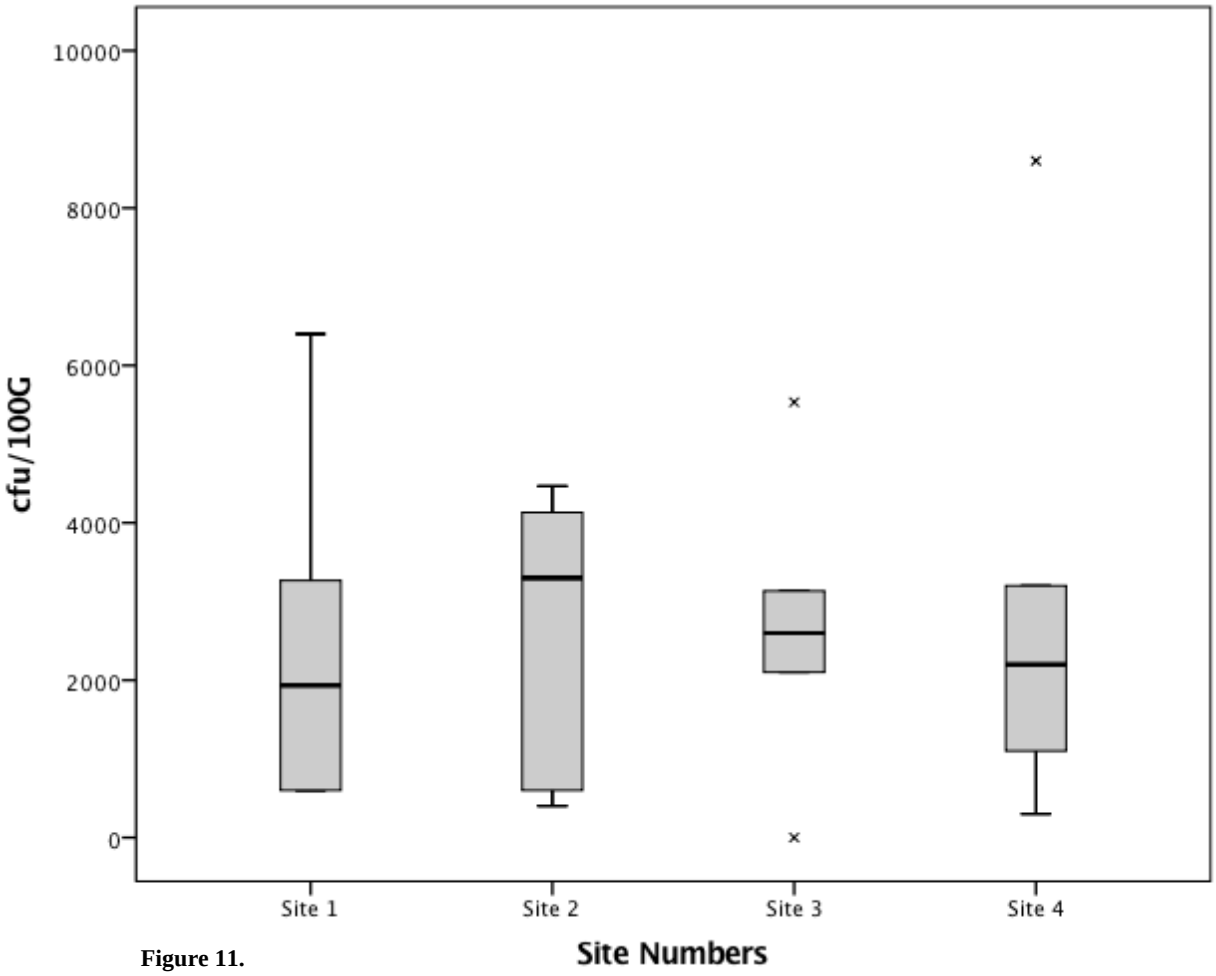


Figure 11.
Thermotolerant coliform sediment results from colonization substrata in the four sites along tributary 1, with each sampling occasion grouped by site number. Medians are represented by thick black lines, interquartile ranges are shown between limits of boxes. The furthest data point from median, but within 1.5x inter-quartile range are shown as whiskers. Note that 5 June and 20 June data are from pooled samples of 3 bags placed at sample sites. Outliers denoted with X marking.

Table 5.
TTC densities sediment at different locations downstream within tributary 1 with different dates as replicates. 6/5/19 and 6/20/19 reflect pooled samples, where all bags were homogenized as a group before reporting pooled sample results.

Date	Site	Sample 1	Sample 2	Sample 3	Mean (M) or pooled samples (P)
4/9/19	Site 1	200	5400	200	1933.3
	Site 2	600	400	200	400.0
	Site 3	0	0	0	0.0
	Site 4	1400	2200	3000	2200.0
4/24/19	Site 1	2200	5200	2400	3266.7
	Site 2	3200	5800	3400	4133.3
	Site 3	8000	1800	6800	5533.3
	Site 4	5800	3800	0	3200.0
5/10/19	Site 1	10600	4800	3800	6400.0
	Site 2	4000	3600	5800	4466.7
	Site 3	1800	4800	2800	3133.3
	Site 4	6400	15000	4400	8600.0
6/5/19	Site 1				600
	Site 2				3300
	Site 3				2600
	Site 4				1100
6/20/19	Site 1				600
	Site 2				200
	Site 3				2100
	Site 4				300

Table 6.
Summary of Kruskall Wallis test of concentrations of sediment TTCs between the four sample sites for tributary 1, for each sampling occasion.

Site	Mean Rank	Kruskal H	P-value	Significance
Site 1	19.71	1.29	0.73	n.s.
Site 2	18.11			
Site 3	15.44			
Site 4	20.72			

4.4 Investigation 4: Distribution of benthic sediment-bound TTCs at multiple locations within a single dairy-dominated agricultural catchment.

TTC contamination was clearly present as indicated by suggested cattle drinking water thresholds of 100C cfu/100ml (Australian and New Zealand Environment and Conservation Council, 2000; Pick, 2011) within many of the sites across the study catchment with tributaries and farm drains reporting the highest concentrations (Figure 12; Table 7). Although TTC counts were greater overall on the first sampling occasion, there was a significant difference between habitats with $P<.05$ on the second sampling occasion only (Table 8). Significant differences ($P<.05$) were reported between farmyard drains and main channel sites as well as farm drains and first order headwaters.

For both sampling occasions, median TTC values were highest in farmyard drains and lowest in first order headwaters (Figure 12; Tables 7 & 8) Farmyard drains exhibited the highest amount of contamination as well as the greatest variability. Tributary and main channel sites exhibited similar trends on both sampling occasions, with first order headwaters showing the least amount of contamination as well as lowest variability (Figure 12. Table 7). Areas marked as having high and intermediate cattle access showed overall increased contamination on both sampling occasions (Figures 13 & 14). Cattle access to streams appeared to be the main cause of high TTFC density in sediments within the main channel (Figure 15).

Table 7.
Catchment runs 1 and 2 (June 5th and June 20th) Results are listed by site type, number, and cfu/100g as well as median value for each site type in each catchment run.

Site Type	Site No	Bacterial counts (CFU/100g)	
		Time 1 (June 5th)	Time 2 (June 21st)
Main Channel	31	-	600
Main Channel	14	2600	200
Main Channel	13	5600	0
Main Channel	7	58400	0
Main Channel	20	200	0
Main Channel	33	-	1800
Main Channel	34	-	0
Main Channel	28	1000	300
Main Channel	29	100	300
Main Channel	32	-	300
Median Value		1800	250
Tributary 1	22	3300	200
Tributary 1	24	2900	2100
Tributary 1	25	1100	300
Tributary 2	18	400	2600
Tributary 2	19	3000	400
Tributary 3	10	500	300
Tributary 3	11	17000	800
Tributary 4	5	400	1500
Tributary 4	6	400	1000
Median value		1100	800
First order headwater	1	600	0
First order headwater	8	1000	400
First order headwater	15	400	100
First order headwater	3	0	0
First order headwater	17	0	100
First order headwater	23	2200	300
First order headwater	26	120	400
Median value		400	100
Farm Drain	4	5200	3400
Farm Drain	2	12,000	44,000
Farm Drain	12	21200	4800
Farm Drain	9	0	800
Farm Drain	27	5000	2,000
Farm Drain	21	600	600
Farm Drain	16	8400	1000
Median value		5200	2000

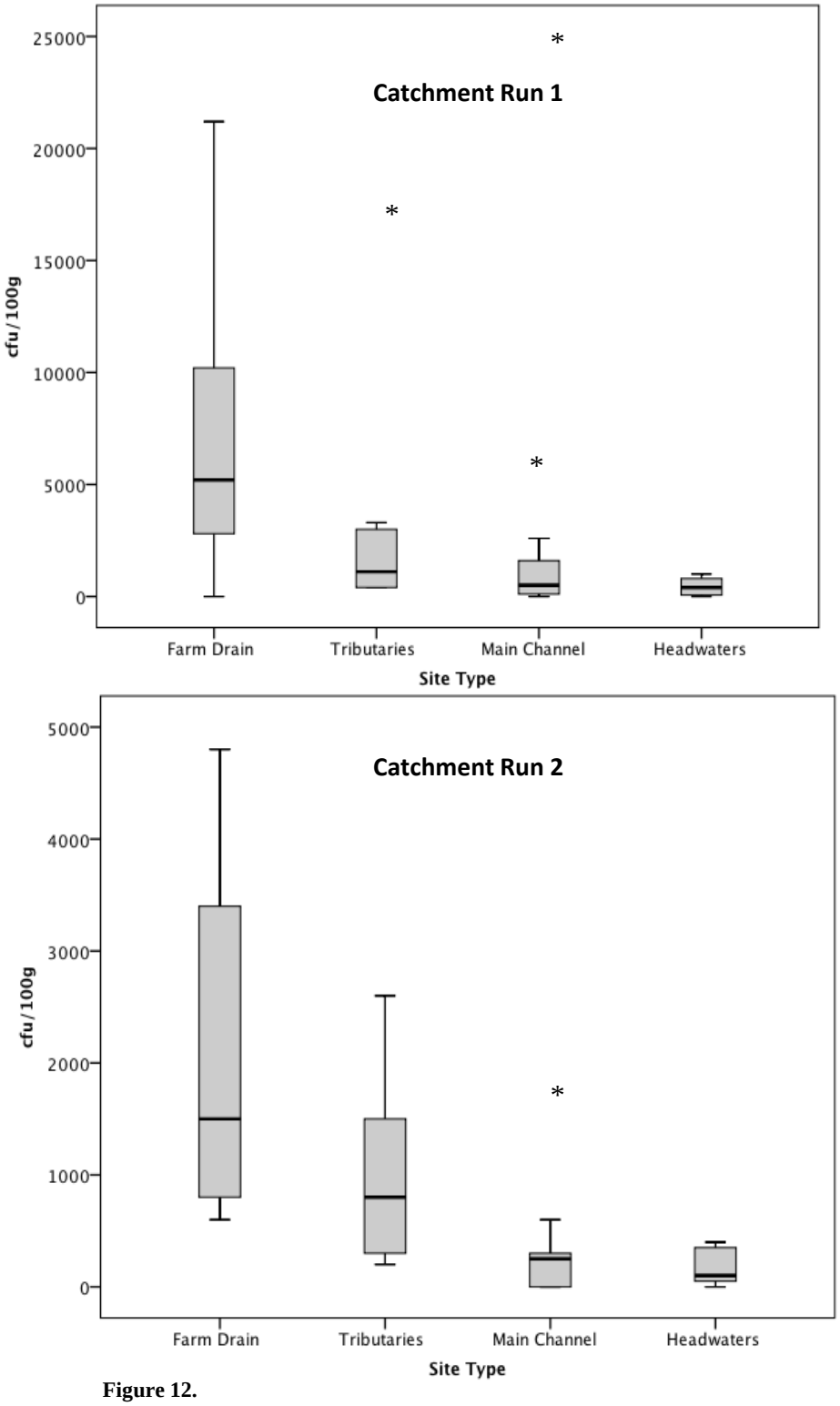


Figure 12.
Box and whisker plots of the four different site types for catchment runs 1 and 2. Site types are as follows, farm drain, tributaries, main channel, and headwaters. Medians are represented by thick black lines, interquartile ranges are shown between limits of boxes. The furthest data point from median, but within 1.5x inter-quartile range are shown as whiskers. Outliers denoted with * marking.

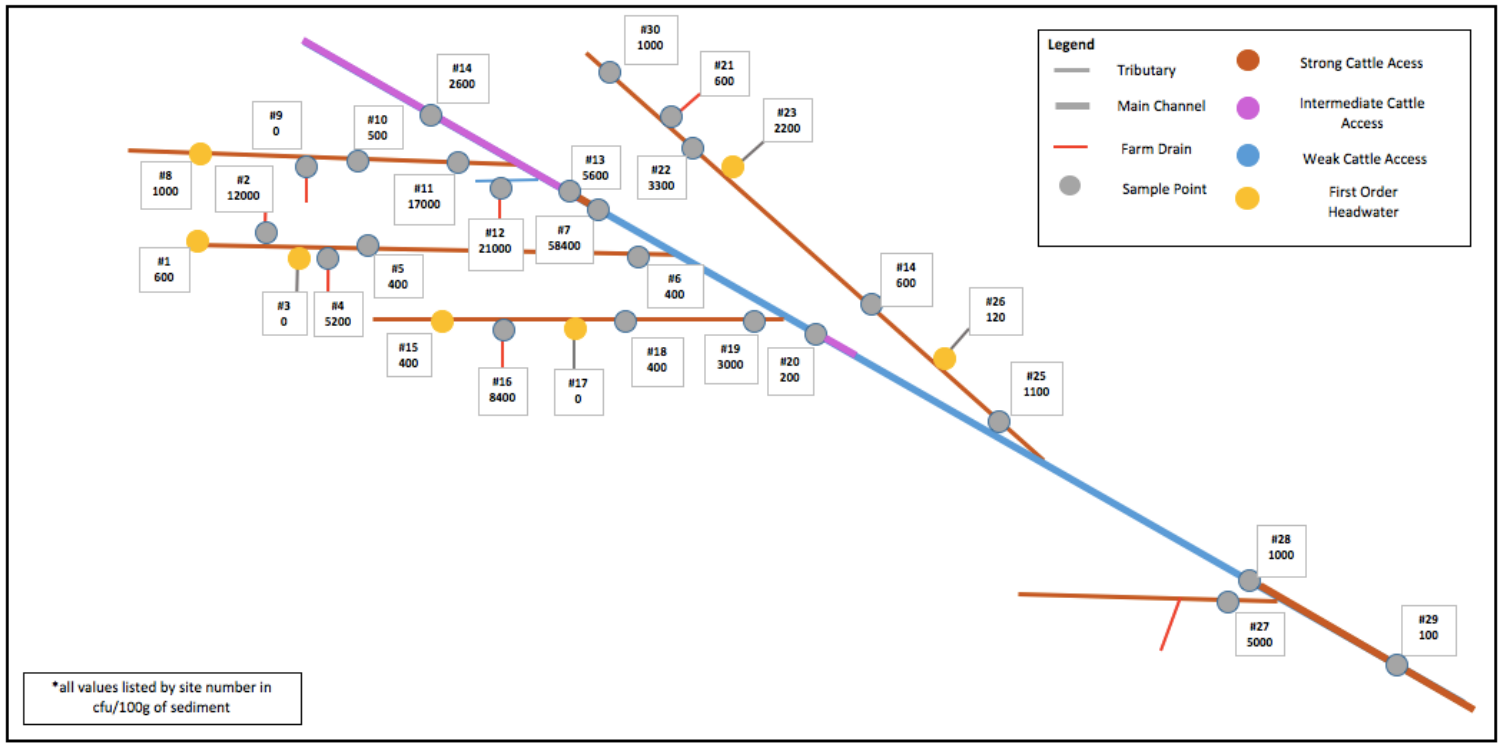


Figure 13.
First catchment run completed in early June. All values reported are in cfu/100g (see bottom of figure). Figure denotes TTC levels by sample site and includes markers for main channel, tributaries, field drains, and farm drains. Water channels are color coded to show levels of cattle access.

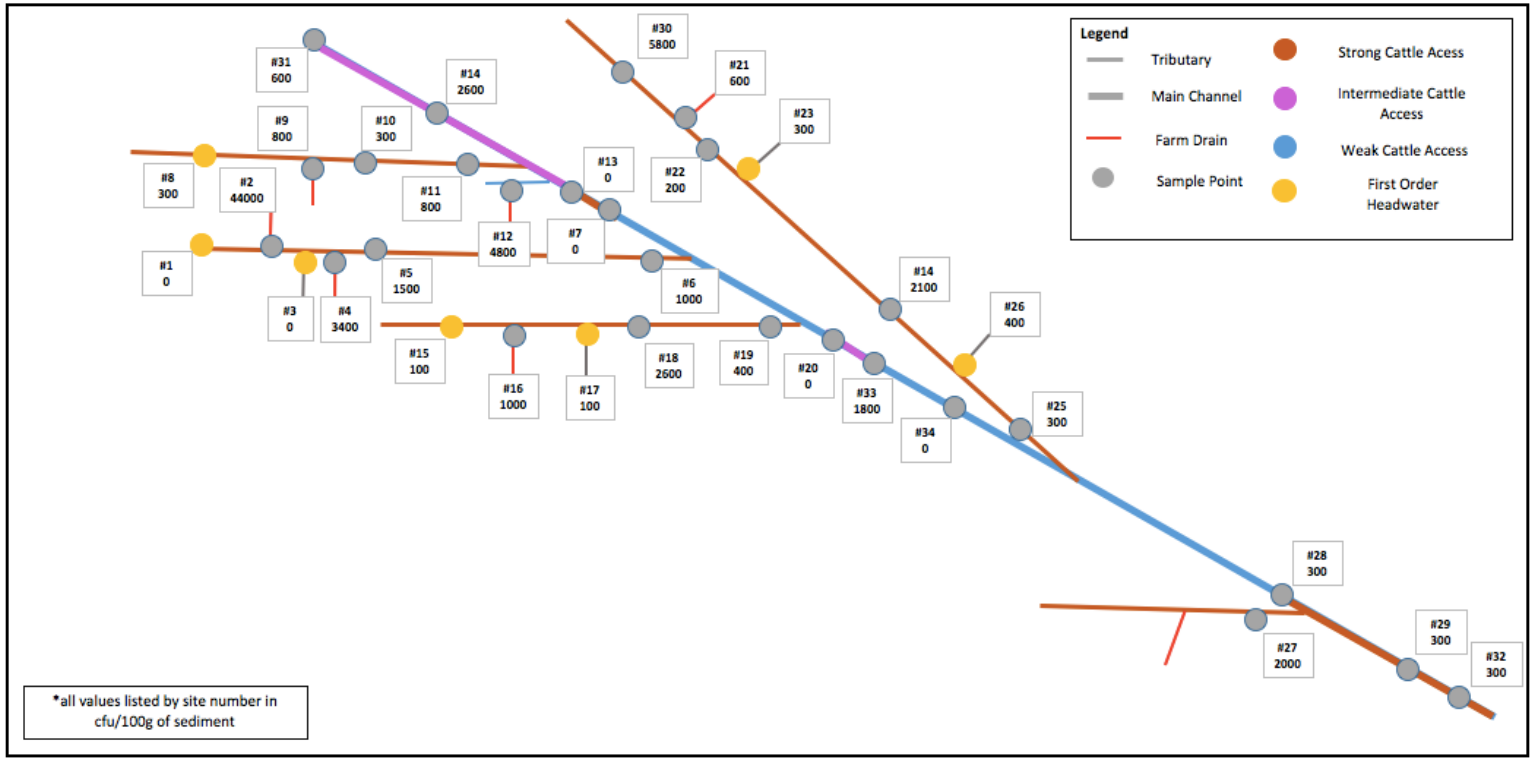


Figure 14.
Second catchment run completed in late June. All values reported are in cfu/100g (see bottom of figure). Figure denotes TTC levels by sample site and includes markers for main channel, tributaries, first order headwaters and farm drains. Water channels are color coded to show levels of cattle access.

Table 8. Summary of Kruskall Wallis test of concentrations of sediment TTCs between the four habitat types (main channel, farmyard-polluted tributaries, farmyard –unpolluted headwaters and farmyard drains) on the two sampling occasions.

	Catchment Run 1			
	Mean Rank	Kruskal H	P-value	Significance
Main Channel	**14.08**			
Tributaries	**14.50**	6.04	0.11	n.s
Headwaters	**21.21**			
Farmyard drains	**10.21**			
	Catchment Run 2			
Main Channel	**22.85**			
Tributaries	**13.28**	16.47	0.01	Sig
Headwaters	**23.79**			
Farmyard drains	**6.64**			

5.0 Discussion

5.1 Bacterial Pollution by Agriculture

Bacterial input to freshwater from agricultural sources is an under-regulated contaminant within the European Union and Ireland. This position is supported by lack of direct policies managing faecal contamination, as well as lack of effective regulation within the water framework directive in the EU as well as Ireland in regards to both farmyard effluent and direct cattle deposition (Wiggins, 1996; Olsen et al., 2004; Wesley et al., 2004; Fayer, 2004; Davies-Colley, 2004; Ishii & Sadowski, 2008; Soller et al., 2010; O'Callaghan, 2014; Harrison et al., 2019; Moloney et al., 2019). The Irish Department of Agriculture, Fisheries and the Marine provides some regulation in manure management and farmyard cleanliness, but fails to expand upon these policies (DAF, 2006). More specifically, the language utilized within the Good Agricultural Practices policy lists the needs to minimize the release and runoff of "soiled water" from farmyards (DAF, 2006). This policy does not build upon this language and fails to provide specific guidelines or and supplementary material regarding to bacterial pollutant release from yards. This leaves control of faecal contamination open to individual interpretation by agricultural sites as well as county inspectors. A portion of the bacterial contaminant from agricultural sources is indirectly addressed by the EU Nitrates Directive (Nitrates Directive, 1991).

Expected action regarding the reduction of direct cattle deposition of faecal contaminants will potentially alleviate some of this issue. The treatment of pasture drinking water locations and sources is anticipated within the Nitrates Directive update projected for 2020. The most effective change in this policy will be the moving of drinking water sources at least 20 meters from surrounding waters, within pastures with cattle stocking rates of greater than 170 kg of N/ha (Advisory Committee to the Department of Housing, Planning and Local Government and the Department of

Agriculture, Food and the Marine; 2019). The results of this study provide cursory evidence that direct cattle access (refer to Figures 13 and 14) contribute faecal contamination to surface waters in levels that would not otherwise naturally occur. Although a change in policy is a positive step forward for the management of faecal contaminants within Ireland, addressing the issue of cattle access will require proper enforcement of the new regulation.

5.2 Attenuation and Persistence of Bacteria Within the Environment

Much is understood as to the multiple interacting factors that bring about attenuation and death of these potentially harmful organisms, once depositied into the external environment. One of the most important elements in TTC attenuation is residence time within benthic sediments (Perkins & Hunter, 1999; Vymazal et al., 2008; Diaz et al., 2010) and by extension, burying by sedimentation (Tate, 1978; Burton et al., 1978; Bohn and Buckhouse, 1985; Bai & Lung, 2005; Haller et al., 2009; Bradshaw et al., 2016). A high sediment residence time gives other factors, such as UV light penetration, substratum particle size, competition and predation by naturally-occurring sediment microbial organisms, and nutrient limitation, time to influence the survivability of the bacteria (Sayler et al., 1975; Tate., 1978; Burton et al., 1987; Sherer et al., 1992; Vymazal, 2008; Haller et al., 2009; Bradshaw et al., 2016). UV light exposure is a well established factor of TTC death (Sinton et al., 2002; Vymazal, 2008). UV penetration works to kill bacteria primarily by photo oxidation and is dependent upon the amount of time they are exposed to this remediation factor (Sinton et al., 2002).

Particle size is often referred to as an important indirect survival factor. This is due to the provision of increased surface area offering shelter from predation and UV radiation. There is also a demonstrated link between the size of sediment and nutrient availability,

with smaller particles often being linked to increased nutrient availability (Sayler et al., 1975; Tate, 1978; Burton et al., 1978; Bohn and Buckhouse, 1985; Sherer et al., 1992; Ishii et al., 2006; Vymazal, 2008; Haller et al., 2009). Particle size is not only shown to be connected to persistence, but potential reproduction within the environment (Nieme &Nieme, 1991; Davies et al., 1995; Leclerc et al., 2001; WHO & OECD, 2003; Tallon et al., 2005; Ishii et al., 2006; Ishii & Sadowski; 2008).

Other factors influencing external survival include the excretion of biocides from aquatic vegetation as well as predation from protists , nematodes, and lytic bacteria, viral influence, natural uptake by organic matter (i.e. absorption of nutrients through plant uptake and binding through sediment), and naturally occurring death (Gersberg et al., 1989; Vymazal, 2008). Current research acknowledges that all of these factors effect the survivability and replication of faecal contaminants. However, the interactions between these interconnected factors and how they effect the replication and survivability is poorly understood.

Although the results of this study did not directly measure the survivability of TTC's within the study areas, the results have the potential to indicate the distance these bacterial inputs are able to travel once deposited (reference Figures 13 and 14). Results of this study also provide preliminary information on the lack of attenuation initial deposition areas (i.e. small headwater streams, roadside drainage, pasture drainage) provide (Refer to Tables 3 and 4, Figure 9). Further investigation into direct in-situ survivability and attenuation would provide a more clear picture of the exact conditions that would improve attenuation and decrease the lifespan of agriculturally deposited TTCs.

5.3 Origin of Thermotolerant Coliforms Within the Catchment and Their Contributions to Faecal Contamination

The findings of this study are a clear indication that bovine faecal contamination is a cause for concern within the study catchment (Figures 13 and 14). Elevated bacterial levels within the study catchment were likely caused by two input types, direct farmyard input and direct deposition into streams by cattle (Figures 13 and 14, Tables 1-7). Although both of these sources – farmyards and direct deposition - have similar characteristics, i.e. minimal exposure to the biological processes that may occur with non-point source origins like soil leachate and overland flow (Stevik et al., 2014; Hall, 1990; Karanthesis, 2006; Coyne et al., 1996), the key difference of these inputs lies within the persistence of location. Farmyard bacterial inputs from drainage channels or pipes represent a continuous, persistent input (albeit with seasonal variation related to temporal changes in farmyard activities) while bacterial input from direct cattle access is essentially episodic and confined to those periods in summer when cattle are grazed within a particular field (Hann et al., 2010; Smolders et al., 2015; Hagedorn et al., 1999; Stott et al., 2011; Nagels et al., 2002; Collins et al., 2010).

Within the catchment, heavy bacterial contamination of surface waters was apparent within some of the smallest, first order headwaters contaminated by farmyard inputs and continued into the main channel of the receiving river as indicated by Investigation 3(Figures 10, 13 and 14, Table 5). In contrast, small headwaters and tributaries upstream of any farmyard input and spring-fed field drains, had very low faecal loading despite draining water from intensive pastures with regular slurry application and cattle grazing as indicated by located of upstream sample points when cattle access was taken into account (Refer to Figures 13, 14, Table 7). These findings on the microbial contamination of catchment surface waters are consistent with recent research indicating that drainage ditches connecting farmyards to streams present a much greater threat to water quality than surface runoff from fields (Harrison et al., 2019; Moloney et al., 2019). Current Irish

agricultural legislation - the Good Agricultural Practice (GAP) regulations (GAP, 2016) – designed to reduce agricultural inputs into streams, focuses on management at the farmgate and field scale by regulating the application of mineral and organic fertilisers (S.I. 605, 2017). Our research indicates that an emphasis on field-scale and riparian management to reduce nutrient and faecal inputs to streams may be ineffective, given the microbial input from farmyard drainage channels(Refer to tables 1-7).

5.4 Drainage Ditches and Their Role in TTC Attenuation

Agricultural drainage ditches have the potential to attenuate agricultural pollution, notably nitrogen and phosphorus (Avery, 2012; Blackwell et al., 2002; Littlejohn et al., 2013; Kroger et al., 2008), but little information exists on their ability to attenuate faecal bacteria . The drainage ditches and streams that were the first to come into contact with the contaminated effluent from farmyard sources from investigations 1 and 2 exhibited the highest overall TTC readings within this study; they showed little evidence of bacterial attenuation within the water column or sediment at their termination(Refer to Tables 1-4, Figures 8-11). These results are further supported by the lack of significant attenuation findings within the Kruskall Wallis tests completed for investigations 1, 2, and 3 (Refer to tables 2, 4, and 6). The lack of attenuation from these sites brings into question their ability to act as treatment 'buffers' for farmyard pollution, as suggested by other published literature (Shore et al., 2015; Moloney et al., 2019).

The persistence of TTCs within the external environment can be enhanced by high organic matter within benthic sediments, and fine benthic particle substratum size (Sayler et al., 1975; Tate, 1978; Burton et al., 1978; Bohn and Buckhouse, 1985; Sherer et al., 1992; Ishii et al., 2006; Vymazal, 2008; Haller et al., 2009). Farmyard drainage ditches would typically be well supplied with organic matter and fine sediment from

farmyard soiled water and their generally low gradient would facilitate the accumulation of both within the drainage channel. Rather than acting as a bacterial attenuation zone, therefore, the drainage ditches may represent a zone of high potential re-suspension and contamination to downstream waters. In the same manner, Moloney et al., (2019) have suggested that phosphorus-rich farmyard drainage channels represent a risk of P supply to downstream waters, during episodes facilitating the release of P from sediments, such as anoxia or high turbulent flows. Ease of re suspension of TTC's is well documented (Sherer et al., 1992; Sayler et al., 1975; Bradshaw et al., 2016; Bai & Lung, 2005; Bohn & Buckhuse, 1985), and sediment reserves of bacteria posing risk to water quality is further supported by reports that TTC levels have been shown to exhibit levels over 10,000 times higher than that of the water column (Doyle et al., 1992; Sherer et al., 1992; Buckley et al., 1998; Crabill et al., 1999; Davies and Bavor, 2000). Furthermore, bacteria can be re-mobilised from benthic sediments at both baseline and high flow hydraulic conditions (Nagels et al,, 2002; Jamieson et al., 2005; Davies-Colley, 2008; Stott et al., 2011; Pachepsky et al., 2017). The farmyard drainage channels within the study catchment may therefore likely harbour high concentrations of faecal bacteria entrained within sediments which represent a potential chronic risk to downstream waters, irrespective of any mitigation measures to reduce bacterial input downstream, such as bankside fencing to exclude cattle from watercourses. Investigation 3, which quantified the longitudinal distribution of TTC's within the length of the stream, from a concentrated farmyard source, also found little evidence of any attenuation of TTCs within the channel, despite the semi-natural conditions of the stream over nearly 2.5km, downstream from the contamination source(Refer to Figures 10 and 11, Tables 5 and 6). Further, these results suggest that smaller tributaries receiving effluent from farmyard drainage ditches may themselves become heavily contaminated by TTCs residing within benthic sediments.

5.5 Thermotolerant Coliform Distribution Within the Catchment

The tributaries within the catchment as reported by Investigation 4 received high levels of bacterial contamination from both farmyard drains and cattle access to surface waters (Refer to Figures 14 and 15, Table 7). Sediment TTC levels in these water bodies however showed little consistent pattern of downstream attenuation or die off over their lengths (Refer to Figures 13 and 14). Effective attenuation would be more likely in faster moving waters, in sediments with decreased organic matter availability, or larger sediment size within the larger main channel. Reduction of TTC's in downstream waters (2nd order streams and rivers) can also be attributed to reported accounts showing that higher water velocity can strip nutrient-rich organic matter from the top sediment layer, the area of the sediment horizon with the highest TTC density and also prevent the deposition of new nutrients (Jamieson et al., 2005; Pachepsky et al., 2007). The higher energy conditions of the larger river provided faster moving waters which could contribute to the lowering of TTC levels further down the catchment.

5.6 Artificial Substrata for Microbial Benthic Colonisation

Although this research shows that colonization substrata can be a valuable tool by which to investigate benthic faecal bacteria, further research is needed to determine the most suitable methodology. The survivability of TTC's within benthic sediments is a key area of uncertainty, as the many interactions within the environment that directly effect persistence and replication are poorly understood.

Colonisation substrata for in-stream bacteria has been studied and presented definitive connections between bacterial levels and nutrient availability; as well particle size in the utilisation of natural and artificial substrata (Claret & Fontvieille, 1997; Sliva & Williams, 2005; Olapade & Leff, 2006; Santmire & Leff, 2006). Reports show that particle size

alone does not effect bacterial levels; however, it has been shown to be a primary factor. Other influencing environmental conditions that have been suggested are permeability (packing of substrata), porosity, chemistry of the space between substrata, and other unknown biological factors (Santmir &Leff, 2006; Navel et al., 2010, Mueller et al., 2013).

Also to be noted is the permeability of substrate within the surface of the hyporheic zone has been shown to effect the movement of water, nutrients, and oxygen to stream substrate as well as reduce leaf litter bio mass (Navel et al., 2010). This is a factor that needs to be taken into account when utilizing colonisation substrata for faecal bacteria. The use of fine particulates could positively effect growth and persistence by providing nutrients to colonies, as well as have the potential to create an effective impermeable layer between substrata and the surrounding aquatic ecosystem and cause colony collapse (Meuller et al., 2013, Navel et al., 2010). Larger particle size has been shown to allow the proper cycling of nitrogen, allowing for lower levels of ammonium as well as higher oxygen levels. However, reports also show that well sorted substrata showed the most balanced interaction with the water column (Navel et al., 2010). This may further lend the utilisation of similar geomorphic substrata more merit, however properly sorting substrata may be key in providing optimal habitat (Navel et al., 2010).

5.7 Project Limitations

The results that this study has provided are promising and have provided cursory evaluation of the distribution and source of TTC's within Irelands agriculturally dominated water catchments. In order to explore this topic further, further enumeration of effective lab techniques and novel colonisation bags would be useful.

The sand utilized within this study was chosen due to its locality (sourced within the study catchment) but contained higher levels of fine particulates that made centrifuging the supernatant necessary. In the future further preparing the colonisation substrata

before placement could help to provide less turbulent samples. Although the condition existed for TTC's to be entrained within the bottom sediment of the centrifuged supernatant, all samples within this study were centrifuged to provide clearer samples, so results were comparable even if the conditions for their bio load to not be fully represented existed. Also to be noted are the extraordinarily high TTC results provided within this study. Should the colonization substrate be further prepped in the future, reported results have the potential to be further elevated due to the need for centrifuging to be eliminated.

6.0 Conclusion

Faecal contamination of surface waters within catchments will only worsen with the expected global increase in agriculture (Tilman et al., 2002; Schröder et al., 2004), especially if policies and solutions are not enacted to minimize their impacts. Current legislation and guidelines for faecal input are rooted in literature that support a non-point source contaminant model. This is further supported by the implicit language utilised by the Irish DAF Agricultural Best Practices Guidebook as well as the EU Nitrates Directive for the control of run-off from yards and other farm areas. This language indicates that point source input has not historically been viewed as a valid threat to Irelands waters (The Department of Agriculture and Food, 2006; Nitrates Directive, 1991). Although the shift in the Nitrates directive will limit the point source issue created by the episodic direct deposition of faeces to streams by cattle, not addressing the consistent input issue of farmyards will mean the future of Irelands agriculturally dominated catchments will remain at risk for increased contamination of this potentially dangerous pollutant.

The data this study has elucidated provides more information on the previously misunderstood origin and distribution of FIO's within agriculturally dominated catchments in Ireland, and helps to provide a foundation for future research within this field. Building upon these findings is crucial to creating effective and long-lasting change rooted in solid policy modification and treatment methodology.

This research has also provided a novel field technique and potential treatment methods to minimize and better understand faecal contaminants in Irelands waters. Utilizing experimental substrata to measure TCC presence and density has been studied and considered effective in the past, however, the specifically developed method for this study has not been previously executed and is therefore in need of further investigation. Development of these substrata bags would enable those interested in exploring the

role that direct deposition of TTC's play in faecal presence of Irelands waters and their ability to potentially monitor not only density and distribution, but also die off.

Survivability of TTC's is poorly understood within lotic ecosystems and wetlands, although laboratory research has shown singular survivability factor influence on persistence. Other in field studies using experimental substrata have also enumerated information as to which factors influence the reduction of TTC's within the sediment which are believed to be the secondary source of TTC's after primary deposition (Claret & Fontvieille, 1997; Sliva & Williams, 2005; Olapade & Leff, 2006; Santmire & Leff, 2006). These same studies suggest the potential connections that these factors may demonstrate, however, they do not currently provide in depth evaluation of the interconnected survivability factors that need to be understood in order to manage faecal contaminants within Irelands agriculturally dominated waters.

Although this study has provided several treatment methodology suggestions, it would be unwise to assume that a singular solution to this previously misunderstood issue will ameliorate this complicated problem. Along with these physical treatment options, the overall reduction of meat and dairy consumption within the country could have a role to play in this multifaceted environmental challenge. Exploring the feasibility of diversifying Irelands agricultural industry by supporting plant and vegetable growth has the potential to provide some much-needed support in the reduction of faecal contamination within Irelands waters.

With current policies in place, the future of Irelands agriculturally dominated waters remains murky at best. In order to revitalize the health of its aquatic ecosystems, further development of TTC management and analysis of density, distribution, and die off is needed in order to create a healthier future for Irelands environments and people.

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