Assessing how rainfall and other environmental factors affect the level of *E. coli* contamination in two species of bivalve.

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I certify that material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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1. Introduction

Growing and harvesting of bivalves is a worldwide trade, with an estimated 13.1 million tonnes produced in 2008 alone and past trends have identified dramatic increases in demand with the majority of production owing to oysters (31.8%) carpet shells and clams (24.6%) mussels (12.4%) and scallop species (10.7%)(FAO, 2010). In the UK aquaculture production is dominated by *Crassostrea gigas* (Pacific Oyster) and *Mytilus edulis* (Common Mussel). However, clam, cockle and scallop species are also harvested (Laing and Smith, 2011) and in terms of landings, scallops are the most important species in the UK as It's one of the top three landed along with crabs and nephrops (MMO, 2011). Due to the increasing demand for bivalve shellfish a greater number of coastal locations are being utilized and are often situated near to human populated or agricultural areas. The location of shellfisheries thus renders them increasingly vulnerable to contamination by pathogenic micro-organisms from an increase of diffuse and point pollution sources (Kelsey *et al*. 2003).

Bivalves are defined as filter feeding lamelibranch molluscs (Brusca and Brusca, 2003) which can filter large quantities of water depending on species. Through these filtration processes bivalves may bio accumulate particles making them prone to the uptake of pathogenic micro-organisms which can be held as particulates within the water (Bitton, 1999). This uptake is a major cause for concern for consumers and shellfish harvesters alike as contaminated bivalves that are eaten raw such as oysters or those that are undercooked can result in illness. The severity of the illness will depend on the pathogen and can be categorised under those of protozoa (*Cryptosporidium* and *Giarda*) viruses (*Enterovirus* and *Norovirus*) or bacteria (*Campylobacter* and *Escherichia coli* 0157:H7) which are thought to be those of highest risk to human health (Dechesne and Soyeux, 2007). The extent of faecal contamination in shellfish is usually estimated by determining the concentrations of faecal coliforms and/or *Escherichia coli* in a water body or shellfish sample. *E. coli* is a bacterium which is found present in both humans and animal faeces (McAllister and Topp, 2012) and therefore can contribute significantly to water and shellfish contamination and disease risk. For that reason, *E. coli* are often used as an indicator or Faecal Indicator Organism (FIO) of faecal contamination.

Pollutant material, which may be rich in protozoa, viruses or bacteria, will originate from a variety of point sources such as sewage discharges. Discharges from sewage treatment works are a significant point source, in which the risk of contamination will depend on the type of treatment the effluent is subjected to i.e. primary, secondary and tertiary treatments. Primary treatments are the first stage of the process and involve the removal of organic or inorganic material. Further secondary treatments continue to remove organics and suspended solid by the use of sludge activators and biological filters. Tertiary treatments are advanced treatments and used to remove remaining elements such as pathogenic bacteria that cannot then be released into the environment. These processes can involve chlorination or UV disinfection (FAO, 1992). Factors such as extreme rainfall or high levels of turbidity within the treated water can alter the effectiveness of the treatment, generally decreasing the efficiency of the treatments. Combined sewer overflows that catch sewage water and storm water run-off as intermittent discharges represent the biggest risk of contamination to a water body as they discharge crude untreated sewage into the environment (Kay et al. 2008c). Septic tanks are another source of sewage-related contamination: these essentially deliver primary treatment and the level of risk will depend on the level of use and maintenance.

A number of environmental pathways as outlined by Quilliam et al. (2011) can transfer faecal bacteria such as *E. coli* from diffuse pollution sources into the wider environment through freshwater systems. Agricultural run-off, from processes such as land spreading of farm wastes (dirty water/ sewage sludge and other organic wastes) and other land uses such as grazing for livestock (Bilotta *et al*. 2007; Oliver *et al*. 2007b) are two major factors in this pathway system. Wildlife such as deer can act as vectors (Fischer *et al*. 2009; Renter *et al*. 2001) and bird species (Alderisio and DeLuca, 1999) can also contribute significantly to faecal loading. This transfer of pollutants from land to surface waters is dramatically increased by rainfall, particularly extreme rainfall events which, due to global warming, are increasing in their magnitude and intensity (Osborn *et al*. 2000; Maraun *et al*. 2008). As global temperatures increase through the emission of greenhouse gases its effects are causing increased atmospheric moisture and evapotranspiration which are in turn affecting the hydrological cycle (Chahine, 1992). After a rainfall event transport pathways then carry the pollutants by means of storm water, surface run off, lateral near surface flow and often sub surface drainage into the nearest water body at a much faster rate. The response to the increased level of rainfall is determined by catchment and watershed specific characteristics (Crowther *et al*. 2001). Rainfall is the major contributing factor, however, other environmental parameters can also play significant roles; for example the watershed in which the water drains in to can differ in its topography/elevation, soil type and geology (Kay *et al*, 2005). The hydrodynamics of the near-shore basin which determines the movement of pollutants, the depth of water and currents in the surrounding area of surface waters will determine the dilution (Seiler, 1986) and level of mixing (Alkan *et al*. 1995) as well as the extent to which the pollutants will impact at the point(s) of interest, such as a shellfishery. The hydrodynamics will also affect water temperature and salinity which are known to effect survival rates of *E. coli* in seawater (Troussellier et al. 1998; Rozen and Belkini, 2001) and the physiological behaviour of bivalves.

Understanding the association between rainfall and environmental factors to faecal pollution and shellfish hygiene status is important to safeguard public health and industry. In order to assess this, aims and objectives were formulated (see Section 2) and the review attempts to answer these by describing the environmental factors in greater detail and how they have been reported to influence microbial contamination of water and shellfish. The review also looks specifically at water quality in terms of the survival and retention times of *E. coli* in both fresh and sea water, but also the relationship between environmental factors. Similarly, shellfish quality is also an area of focus due to the differences in physiological/ biological processes and behaviour of individual species, but also the differing impact of harvesting methods for these different species.

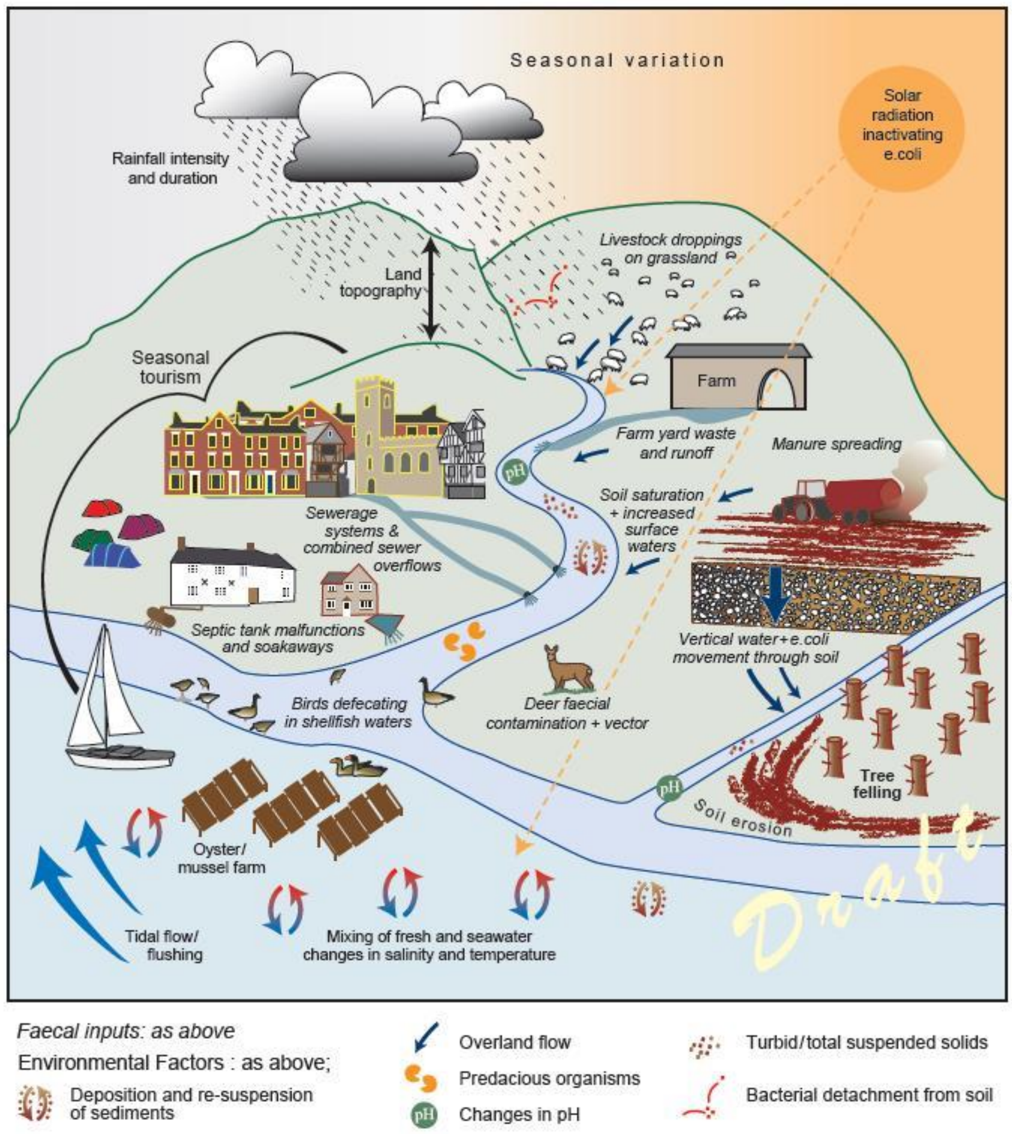

Figure 1. A conceptual diagram that shows the transfer process and fate of *E. coli* as it moves from its source into coastal waters. Both point and diffuse sources of *E. coli* are influenced by rainfall, which acts as a trigger and increases the rate at which *E. coli* moves from land to water. Other environmental factors affect this transfer process and are differentiated between land based (i.e. soil characteristics) and water based factors (i.e. turbidity). The amount of *E. coli* available for uptake by shellfish is affected by local hydrodynamics of the coastal area and associated changes in physiochemical properties of the water.

2. Aims and Objectives

The aim of this review is:

(1) To determine which environmental factors may influence the spread of *E. coli* contamination after a rainfall event

Objectives are therefore to establish:

- Whether there is an association between environmental factors, such as, rainfall intensity, duration and frequency and *E. coli* contamination in surface waters.
- Where the pathogens originate from; i.e. whether they are products of point source or diffuse pollution.
- How existing literature suggests the entrainment, transport and delivery of *E. coli* from land to water is best monitored and understood.

(2) To determine how environmental factors may differentiate in their impact on water and shellfish microbial quality.

Objectives are to understand:

- The impact of both freshwater and seawater on the survival of *E. coli*.
- The difference in accumulation and elimination of *E. coli* between different shellfish species (mussels and oysters).

3. Factors influencing transport of *E. coli* from land to water

The transport of *E. coli* form land to water involves a number of processes that have been identified and introduced in the conceptual model (Figure 1). Rainfall is most often the trigger of this transport process in which its effects are seen from both point source and diffuse pollution. These contamination sources, especially those of diffuse pollution which can be from wildlife, grazing livestock and farmyard manures/slurry applications are influenced by other environmental factors such as soil characteristics, erosion and topography of the land, all of which are discussed in detail below.

3.1 Rainfall characteristics

Rainfall around the UK is highly variable and regional differences are attributed to a number of factors. Northern Scotland is exposed to westerly winds which bring heavy rain to the area especially in autumn where frontal rainfall rolls in from the frequent Atlantic depressions. Data from the meteorological office show that over a 30 year period (averaging 1971 - 2000) annual rainfall for west facing areas averages1700mm. Western Scotland has shown great variability in rainfall with some parts of the highlands reaching an annual figure of 4000mm. Most of east coast of Scotland is sheltered from the rain and average annual rain has been shown at 700mm, although areas of mountainous region in north eastern Scotland have considerably higher rainfall. Wales is also subjected to Atlantic depressions and the mountainous parts have shown an annual average of 3000mm, where lower lying land and coastal areas are of around 1000mm. A similar trend was shown for North West England with an annual average rainfall of 3200mm was recorded, whereas north east England was drier with only 600mm of rain. Southern parts of UK are influenced by the warmer climate of the continent, where eastern England has an annual rainfall average of less than 700mm in areas that are to some extent protected from the Atlantic depressions. The south west is wetter which an annual average rainfall has been recorded at 950mm (Met Office, n.d).

Further studies on UK rainfall have shown that several factors interact to determine the distribution and magnitude of rainfall over time. Seasonal variation, regional location and other physical processes can control the frequency and most importantly the intensity of rainfall for a given area, which can then affect processes on a local scale e.g. surface runoff/flooding, soil erosion and diffuse pollution.

The analysis of rainfall data in the UK undertaken by (Osborn *et al*. 2000; Osborn and Hulme, 2002; Maraun *et al*. 2008) have all shown that in recent years the number of wet days has increased across the UK for both autumn and winter months. Summer for the most part showed a decreasing trend of total rainfall, whereas for spring months a general trend saw rainfall increase to both the north and west coast of the UK but a decrease in rainfall to the south. The most significant trend observed was rainfall increasing in its intensity through the winter months and to a lesser extent in the spring and summer months. The above studies show that large proportions of the monthly or annual rainfall occurred as high intensity daily precipitation. Osborn and Maraun (2008) noted that this intensity accounted for the increases in annual rainfall even when a decrease in annual number of wet days occurred.

Extreme daily precipitation events were investigated further by (Maraun *et al*. 2009) who modelled spatial rainfall intensity throughout the UK. Extreme rainfall events occurred at their heaviest in the late autumn and winter months on the west coast, whereas on the east coast rainfall extremes were less frequent, but occurred throughout the summer months. Rust *et al.* (2009) modelled seasonal extreme rainfall and their results support the ideas that higher rainfall extremes are observed in the north western regions. Daily precipitation was seen of up to 100mm per day in the winter of the Scottish highlands compared to winter in eastern England at 15mm per day. The reasoning behind regional and seasonal variation may be down to the type of rainfall; eastern and southern parts of the UK are prone to convective rainfall due to warmer climate off the continent and would explain a higher level of extreme precipitation within the summer months. However most of the UK is subjected to frontal precipitation where the temperature of the air determines the warm/cold front. The western part of the UK typically receives the cold front from the Atlantic and an increase in heavy precipitation especially in the winter (Rust *et al*.2009). Northern and western areas are typically mountainous regions and therefore also subjected to orographic rainfall (Malby *et al*. 2007) .

High intensity rainfall events occur when the atmosphere becomes saturated. In order for saturation to occur there must be an increase in atmospheric moisture content which is caused from increased evaporation and evapotranspiration. These processes are affected by increases in surface temperatures which have been exacerbated by the release of greenhouse gas emissions and have therefore contributed to global warming (Trenberth, 1999; Chahine, 1992). Predictions made by Hulme *et al.* (2002) suggest that annual temperature may rise between 2 and 3.5 ˚C, and winter daily precipitation intensities may become between 5 and 20 percent higher by 2080 depending on the level of emissions (Low, medium, high scenarios). Therefore, significant increases in surface temperatures in the future are likely to enhance precipitation extremes. A review by Callaway et al. (2012) noted that these precipitation extremes were likely to cause high levels of surface runoff and flooding, a major cause of increased transport of pollutants and nutrients into coastal waters from both point and diffuse pollution sources causing a multitude of water and shellfish quality issues.

3.2 Point Sources

The discharge of faecal bacteria from point sources of pollution is well documented in causing severe water and shellfish quality issues (Kay *et al*. 2008a; Kay *et al*. 2008b). Sewage treatment works and consented discharges, combined sewer overflows and septic tank systems all contribute towards faecal contamination. Their severity is dependent on the level of discharge being put into the system i.e. population density within the watershed catchment and ultimately the level of treatment they are subjected to before being released into the environment. The risk of contamination is then based on the proximity of the fishery to sewage outlet points and the environmental factors that continue to modify this.

3.2.1 Sewage treatment plants, combined sewer overflows and other sewage sources.

Before wastewater is released into the environment, it undergoes a series of treatment processes that are designed depending on the system, to remove contaminants and produce environmentally safe effluent and solid waste.

Sewage is subjected to different treatment processes before it is released through consented discharges into nearby waterways or directly into ocean outfalls. The level of treatment is dependent on the type of system, which is dictated by the space available and volume of treatment required for a given area (amongst other socioeconomic factors). The treatments which can then be available are based on three levels of primary, secondary and tertiary processes. The majority of wastewater treatments involve primary and secondary processes, however where high quality effluents are required tertiary treatments are also used.

In some cases untreated sewage is released into the environment through combined sewer overflows where it has only been subjected to simple preliminary treatments such as screening (Nitio and Clarke, 2006). Crude discharges are released into the environment from the duration of high impact rainfall and extreme precipitation events. Treatment efficiency varies with flow as well as other factors. Kay *et al*. (2008b) examined the levels of total and faecal coliforms discharged from the different treatment processes under base flow and high flow conditions, from several catchment areas around the UK and Jersey. A summary of the findings are shown in Table 1.

Table 1. Summary of faecal coliforms (CFU $100ml^{-1}$) discharging from different sewage treatment processes under base-flow and high-flow conditions. Those values that are marked with (+) indicate that statistically they were found to be higher than values marked with (-) p value = <0.05).

Treatment Level	**No of valid samples**	**Geometric mean Base flow**	**Geometric mean High flow**
Untreated	252	1.7×10^7 (+)	2.8×10^6 (-)
Primary	127	1.0×10^7 (+)	4.6×10^6 (-)
Secondary	864	3.3×10^5 (-)	5.0×10^5 (+)
Tertiary	179	1.3×10^3	9.1×10^2

The units presented in Table 1 assist in the assessment of the potential effect that environmental factors such as rainfall will have on the amount of contamination entering the water body. Primary and untreated sewage contained significantly higher levels of faecal coliforms in base flow conditions than high flow conditions. Secondary treatment contained significantly higher levels in the samples taken at high flow compared to those in the base flow. Values were seen to be higher under base flow for tertiary treatments but there was no significant differences found. The amount of rainfall under high flow conditions is likely to decrease concentrations of faecal coliforms in untreated and primary effluent due to the dilution effect of increased freshwater input into the system. The velocity of water, especially on days of extreme precipitation may often cause higher levels of faecal coliforms to be released because of the reduced retention times in the sewerage system.

Many sewage systems in the UK and Europe are able to accommodate land drainage after a rainfall event, where excess water is stored within the system or in separate storage tanks. They are treated to capacity, after which point any excess water is discharged through combined sewer overflows (CSO) or the storage overflows (STO). These intermittent discharges are of particular importance to shellfish growing areas as when treatment plants fail to cope with the volume of water entering the system (often after extreme rainfall events) it results in untreated sewage entering the environment and therefore a dramatic increase in potential contamination to a fishery. Another study by Kay *et al.* (2008a) specifically looking at storm overflows and microbial quality of wild mussels, showed that concentrations of faecal coliforms and *E. coli* in the shellfish and surrounding water increased very quickly after a combined sewer overflow (CSO) discharge.

Storm driven flows are also associated with the 'first flush' phenomenon. The first flush is generally described as the discharge of higher concentrations of contaminants at the start of a rainfall event compared to the end (Stenstrom and Kayhanian, 2005). Several different definitions have been used to quantify the first flush phenomenon and the term is used for most water quality constituents such as turbidity, total suspended solids and pH as well as for faecal contaminants (Deltic, 1998). Bertrand-Krajewski *et al.* (1998) derived a definition from their own analysis of mass (volume) curves, as a significant first flush to be 80% of the total pollutants transported in the first 30% of the storm run -off.

The first flush of faecal indicator organisms from urban storm waters was investigated by McCarthy (2009), the results suggested that the first flush was only present in one out of the four sites studied due to factors such as magnitude of rainfall and the presence of 'end flushes' that resulted from slower moving wastewater entering the system from highly contaminated sources. Hathaway and Hunt (2011) showed similar results with the first flush effect being relatively weak for *E. coli*, which is thought to be attributed towards factors affecting transport and survival that were otherwise not accounted for in the study. There is much debate on the effects of first flushes as they can be dependent on catchment specific parameters such as how it responds to rainfall, the level of wastewater treatment and the level of *E. coli* already present in the system.

Wastewater treatments efficiency can be altered by seasonal differences in tourism and in environmental temperature (Leitao *et al*. 2006). The efficiency of biological filtration systems can be distorted by the seasonal changes in tourism. In the summer months, populations in coastal areas tend to double in size, which requires a suitable sized infrastructure to cope with the increase in demand. In order to manage, the system must build on the natural organisms already present, and therefore problems tend to occur at the start of the tourist season when there is inadequate biological filtration. Conversely in the winter months, when the demand has decreased, the natural stock of bacteria in the system depletes, as the amount of sewage required to feed the natural micro-organisms is not fulfilled by the amount of sewage available, thus also reducing efficiency (Castillo *et al.* 1997; Orhon *et al.* 1999).

In more rural areas connection to main sewerage treatment networks is not always feasible. In such locations, septic tanks are often used as the method of waste water treatment; whilst some discharge to soakaways (as explained in section 3.3.1) others discharge treated effluent into nearby watercourses or directly into coastal waters.

3.3 Diffuse pollution, land use and land management

3.3.1 Diffuse septic systems

Septic tanks that discharge to land through subsurface irrigation pipes or soakaways are sources of diffuse pollution and both rely on soil properties for absorption and filtration to decrease contaminant levels before it reaches groundwater. Risk levels of contamination from septic discharges can depend on a variety of factors. These include the size and type of tank and drainage system, the level of use and maintenance, the topography of the land, and also the underlying soil quality and geology of the area used for drainage (Lindbo *et al*. 2005; Butler and Payne, 1995).

Solid particles from overflowing septic tanks (caused by poor maintenance) will block the pore space within the soil matrix of the land drainage system. Blocked drainage causes the system to become increasingly inefficient over time and especially during heavy or prolonged rainfall. Due to this, the soil becomes saturated at a much faster rate, which results in untreated effluent being transported as contaminated as groundwater or as surface water into nearby waterways (Harris, 1995). The size of the tank and soil type of the drainage field will therefore determine the saturation limits and holding capacity of a septic tank system. Cahoon *et al.* (2006) found that malfunctioning septic tanks were the main cause of shellfish contamination by faecal pollution compared to pollution transported from storm water runoff. The high density of septic tanks in areas with unsuitable soil and high slopes were the primary cause of concern. Lipp *et al.* (2001) concluded that areas with several septic tank systems were also the reason for elevated bacterial levels of coastal waters. Their study confirmed that the release of faecal coliforms into the environment was through subsurface transport in ground water but also due to the age of the septic tank systems in place. Ahmed *et al.* (2005) used a unique biochemical fingerprinting technique (BPT) to identify specific faecal indicator bacteria in septic tanks to compare with samples taken from nearby waterways. Their results showed that identical *E. coli* (BPTs) were found from septic tanks that were classified as defective and from water samples taken downstream. Unique BPTs found in well maintained septic tanks were not present in nearby waterways.

Other studies of onsite sewage treatment systems have shown that appropriate, well maintained septic systems do not cause significant water quality problems, for example, Weiskel et al. (1996) found that despite there being a large number of septic tanks in one area their faecal load from discharge run off was minimal and did not contribute largely to contamination of the near shore waters due to the gradual loss of contamination prior to reaching the water. Reneau and Pettry (1975) also concluded that faecal pollution from drainage fields with suitable soil types was unlikely to cause permanent contamination to groundwater, even with fluctuations in the water table.

Like municipal sewage works, seasonal use of onsite sewage treatment e.g. holiday homes in coastal areas should also be recognised for contributing to water quality problems, usually occurring in the summer months. A study conducted by Postma *et* *a.l* (1992) noted that seasonal elevation of faecal coliforms in groundwater that exceeded water quality regulations were attributed towards heavy effluent loading of the drainage field resulting in inefficient treatment. The risk potential of faecal pollution from diffuse septic systems is site specific, in which several of the key factors mentioned play a significant role in the treatment and transport of faecal matter to nearby waterways. Rainfall is a major contributor because of its effects on the soil that are important to the treatment process. The other major factor is the density of septic systems in a given area and their distance to nearby watercourses or coastal waters which will ultimately determine their microbiological impact (Yates, 1985).

3.3.2 Wildlife

There is some evidence that the spread of contamination may also occur through the faecal shedding of wild deer. As deer are warm blooded animals they would be expected to shed *E. coli*, however research on the level of faecal coliforms produced by deer has not been widely explored in the UK. In a study by Fischer *et al.* (2001) several White-Tailed Deer were inoculated with 10^8 CFU of *E. coli* O157: H7to try and determine this faecal output. The results showed that the deer started to shed 3-5 $\log_{10}$ of *E. coli* per day for up to 26 days of the study, similar to that seen in inoculated cattle. These authors also looked at the *E. coli* content in faeces of free ranging deer across different locations and concluded that overall prevalence of *E. coli* O157: H7 was low in the faecal samples. Renter *et al.* (2001) conducted a similar study on free ranging deer and found that even though prevalence of this particular strain was low, contamination to watercourses from deer faeces was still considered to be significant in terms of protection to public health regulations.

Coastal locations support a number of shellfish farms, which coexist alongside natural habitats and feeding grounds for a variety of seabird species. The direct defecation into surface waters from these birds (depending on number of birds and species) can result in major contamination to a shellfishery, especially when they perch and feed directly on the farmed shellfish ropes or trestles. Several studies have reported birds to have a significant influence on water quality. Alderisio and DeLuca (1999) conducted a study on Ring-Billed Gulls (*Larus delawarensis*) and Canada Geese (*Branta canadensis*) to determine the faecal coliforms output of these species. Their results showed that on average gull samples (249) contained 3.68×10^8 FC/gram and geese samples (236) contained 1.53 x 10^4 FC/ gram of droppings. Gould and Fletcher (1978) noted the number of faecal coliforms in four species of Gull (Table 2).

Table 2. Faecal coliforms output (faecal coliforms per gram and daily loadings) of faeces from four species of Gull. Adapted from Gould and Fletcher (1978)

Bird Species	**Total weight of droppings wet weight (g/day)**	**Average number of faecal coliforms per gram of faeces (millions)**	**Daily loadings (24h) Faecal coliforms (10^8)**
Herring gull	24.9	71.1	18
Lesser black-backed gull	13.4	374	50
Common gull	11.8	52.6	6.2
Black headed gull	11.2	27.1	3.0

Although literature on faecal contamination from bird species is limited, the above studies have identified that a number of gull species that frequent coastal waters in large numbers are likely to cause faecal contamination in areas where shellfish beds are present.

3.3.2 Livestock

Faecal pollution from livestock is often a primary source of contamination for bivalve fisheries especially in remote areas where agricultural farming activities are prevalent and in close proximity to coastal areas. Contamination occurs through the transport of bacteria into nearby watercourses from faecal matter deposited to land. The methods of application, management practises and the effects of environmental factors all facilitate movement and survival of faecal bacteria in the transport process from land to water (Oliver *et al*. 2007a).

These application methods occur through three main pathways; direct deposition from livestock onto pasture (Avery *et al*. 2004), via transport of farm yard manures and dirty run off from housed livestock, or through the application of slurries and manures to the land (Nicholson *et al*. 2005).

The most commonly farmed livestock in the UK are cattle for beef and dairy, sheep, pigs and poultry. In most farming systems, cattle and sheep are put out to pasture, where pigs and poultry often occupy indoor farming systems (Hooda *et al*. 2000). These indoor systems affect the environment through farm yard run off and the application of manure to the land (see Section 3.3.3) however in some situations, livestock that is put out to pasture can also occupy indoor farming systems, usually in the winter months (Hutchinson, et al. 2000) and so sources of contamination can change seasonally. The type and size of farming system will often dictate the level of contamination into the environment where the different species harbour different levels of bacteria in their faeces. Table 3 shows the concentrations of faecal coliforms found in the faeces of the four most prevalent livestock animals, as presented by Cox et al. (2005), Moyer and Hyer (2003), Metcalf and Eddy (1991) as cited in Moench *et al.* (2009). The quantity of faecal coliforms deposited on the land is directly proportional to amount of excreta being discharged from the type of animal present. Daily faecal production values per animal unit (AU) are also presented in Table 3; these values were sourced from Moench *et al.* (2009) which contains further detail.

Table 3. Faecal coliforms and total coliforms of different animal species discharged per day, presented as colony forming units (CFU) per gram of faeces. Daily faecal production values are also presented in grams per day per animal unit ([AU] is a standardized measure of an animal).

Livestock	**Median Faecal Coliform Concentrations (CFU g^{-1} [wet weight]) Cox *et al* (2005)**	**Faecal coliform densities (CFU/g) Moyer and Hyer (2003)**	**Faecal coliform densities (CFU/g) Metcalf & Eddy (1991)**	**Daily faecal production (g/day/AU) Moench *et al* (2003)**
Poultry	1.1×10^8	1.8×10^9	1.3×10^6	28, 916*
Cattle - Beef	1.8×10^5	1.8×10^6	2.3×10^5	37,195
Pig	7.1×10^6	-	3.3×10^6	29,484
Sheep	6.6×10^5	1.8×10^7	1.6×10^7	18,144

* is an average value taken from the daily values given for boilers, layers, pullets and turkey to be representative of poultry.
- Value not available.

The values in Table 3 show that sheep faeces contain more faecal coliforms per gram then cattle and therefore could cause a higher risk to water quality. Even though daily faecal production is higher in cattle which may result in a higher daily loading, it will ultimately depend on stocking densities of a given area. Looking specifically at *E. coli* O157, Hutchinson *et al.* (2004) noted that 20.8% of fresh sheep faecal samples (n = 24) contained the pathogen compared to only 13.2% of the cattle samples (n = 810). The size of animal and quantity of faeces produced is highly variable. For example, the difference in the amount of excretion produced between the dairy and beef cow per day is approximately 21 litres (dairy = 53 and beef = 32 litres per day) (MAFF, 1998). It is also important to take into consideration the differences in faecal shedding of *E. coli* between juvenile and mature animals as noted by Mechie *et al.* (1997) and Shere *et al.* (1998).

On pasture, livestock often have direct access to waterways, which results in direct defecation into the water causing extreme spikes in contamination. Davies-Coley *et al.* (2004) noted extreme increases of *E. coli* in water samples taken from a stream that was used by cattle as a crossing section. Samples were found to be in excessive of billions (CFU) at this section, whilst samples up stream only contained background levels of *E. coli*. The threat to water quality from the defecation onto land is determined by factors such as the length of time the animals are put out to pasture and the type of grazing system in place. Hutchison *et al.* (2000) described six methods of grazing which includes the two-sward system, set stocking, continuous grazing, the three field system, block grazing and paddock grazing. The risk level varies because of the impact on the land, some of the systems such as the block grazing and paddock grazing rotate the livestock from field to field on a regular basis. These methods typically result in less faecal build up or extensive trampling from the high densities of livestock, unlike continuous grazing systems that graze the same (usually larger) area for two to three months. A study by Thorn et al. (2011) also showed that high intensity grazing of livestock increased the risk of *E. coli* O157 being found present in surrounding freshwaters compared to low intensity grazing. However, their results showed that *E. coli* O157 were able to survive for longer in waters where livestock were less intensively grazed. It was concluded that this may be attributed to competition and microbial grazing (as discussed in section 4.6).

Other considerably important factors are the land type (and presence of land drainage systems) soil characteristics and land topography, these apply to both the application of faeces from grazing livestock as well as that from farmyard manures and slurries. The impacts are specific to the site and farming system and are often exacerbated by changes in season, either through housing livestock or changes in weather conditions.

3.3.3 Farm Yard Manures (FYM) Slurries and Dirty Water Runoff

The application of faecal matter to agricultural lands occurs mainly from the spreading of faeces collected from indoor farming systems, but in some areas the spread of sewage sludge from sewage treatment plants also occurs (MAFF, 1998). Waste products from farming are usually (but not always) stored prior to spreading, in aid of reducing the amount of pathogenic bacteria being released onto land and so that timing of the application can be managed. Depending on the type of waste (farm yard manures, slurry or sludge) will determine how it is applied to the land and how it is likely to affect water quality. Other seasonal influences such as rainfall and temperature also affect both storage and the application of farm and sewage wastes.

Farm yard manures (FYM), a term collectively used for both solid faeces and other materials used for livestock bedding or feed, are gathered and stored in field heaps or onsite solid manure stores. Slurries are a mixture of urine and manure that form a liquid (Hutchinson *et al*. 2005; 2000) and most farming systems have direct pathways from animal housings into slurry tanks. Similar storage tanks are used for other effluents produced as farm waste such as dirty water from washing down animal housing and farm vehicles (Hutchinson *et al*. 2000). The storage of these different types of manures affects the survival rate of pathogenic bacteria because of the differences in conditions. In the case of solid manures, extended stockpiling and composting (involving aeration through turning) is effective in assisting the decline of bacteria as they are able to reach extremely high temperatures, McAllister and Topp (2012). McAllister and Topp (2012) noted temperatures reached between 55°C and 70°C in the central areas of manure piles. Turner (2002) using both laboratory and field studies found that pig FYM kept at 55°C for two hours is sufficient in reducing bacteria to a safe level. However, both studies noted that temperatures of the surrounding manures did not reach as high a temperatures and so complete elimination was not possible unless stockpiles were turned frequently (composted) and left for longer periods. Such a finding was supported by Shepherd et al. (2007) who conducted similar studies on two field based cattle FYM piles. Temperatures reached above 50°C in all central samples over a seven day period, but reduced to varying degrees around the outside. *E. coli* were detected for up to 14 days in the first pile and with up to five days within the second pile. They noted that without frequent turning of the stockpile *E. coli* could survive for up to four months on the top of the heap. Kudva *et al.* (1998) supported the need for aeration to successfully reduce bacterial numbers as they detected *E. coli* O157: H7 for up to 12 months in a non-aerated sheep manure pile compared to up five months in an aerated pile. Another study by Hutchison, *et al.* (2005a) recorded a decimal reduction time (1-log reduction in *E. coli*) of no more than 2.3 (approx) days calculated for each of the different (cattle, pig, sheep, poultry) livestock FYM. In the study most bacterial die-off was seen within two weeks.

Due to the increase in demand for farming produce, farming systems have become bigger and increasing numbers of livestock have led to manure management systems converting to slurry-based systems. Slurries have different compositions (wet: dry ratios) depending on the livestock and type of feed, this can results in different treatment methods. Some slurry undergoes mechanical separation in which the liquid is siphoned off and used for irrigation and the remaining solid stored as FYM. Alternatively and the most common method is treatment by batch storage as other methods involving anaerobic digestion are expensive and not used widely (MAFF, 1998). A study by Nicholson et al. (2004) showed that the survival time of *E. coli* in batch storage of slurries are generally longer than composted manure as shown in Table 4. This is because slurries are unable to reach as high a temperature as farmyard manures. The values presented in Table 4 show the maximum survival of *E. coli* O157:H7 for both solid manures and slurry for different livestock species.

Table 4 Shows the maximum number of days that E. coli O157: H7 can survive in different types of manure storage methods. Manures taken from both dairy and pigs, were stored both as FYM; turned and unturned, as well as two types of slurry. Dirty water from farm yard runoff was also used in the study (Nicholson et al. 2004).

Manure Type	**Days of maximum *E. coli* survival**
Dairy FYM turned	8
Dairy FYM unturned	4
Pig FYM turned	4
Pig FYM unturned	32
Dairy slurry (7% dry matter)	32
Dairy slurry (2% dry matter)	93
Dirty Water	16

Slurry storage comprises of either above ground circular stores, weeping walls, and earth banked stores or below ground tanks and pits (MAFF, 1998). There is not much research available on the temperatures reached inside these slurry pits, but Hutchison *et al*. (2000) suggests that they would be close to the external environmental temperatures. The rate of bacterial reduction could therefore be influenced by seasonal changes in temperature. The rise in temperatures in the summer months will contribute to a greater bacterial die off, but conversely in the winter time when temperatures are much lower the time needed for bacterial reduction will be much longer. The decimal reduction times (1-log reduction in *E. coli*) were again calculated in another study by Hutchison *et al.* (2005b) which resulted in D values of between six and 44 days in pig and cattle slurries as well as dirty water within the range of survival times noted in Table 4. The results concluded that a minimum of 6 months batch storage was required for slurry manures. A significant consequence of this is the lack of space availability and cost of storage, especially in the winter months when more livestock are put into housing. One storage tank would result in a continued supply of fresh manure re-seeding the manure already in the tanks that would have naturally declined over time.

A further environmental pressure is the infiltration of rainfall into some slurry systems. This causes a dramatic increase in the volume of waste needing storage and may result in it being spread to land straight from collection or before it has had adequate time to reduce bacterial content (Aitkin, 2003). Rainfall can also causes the spread of contamination from field manure piles into both ground and surface waters when there is no barrier between the manure and soil interface. In some cases, solid manures are stored on concrete bases that have a runoff control system that is collected, stored and used for irrigation. These have the least impact on the environment, but are seldom used (Nicholson *et al*. 2002).

3.3.4 Application of faecal bacteria to land.

The application of slurries and farm yard manures are spread onto agricultural lands as a method of waste disposal, but are also used as fertilizers when applied to crops at specific stages in their growth. Late winter/ spring is when this most often occurs as the crops are more likely to uptake nutrients at this time; however it is very dependent on the type of crop (Tried & Tested, 2001). Most farmers will apply manures as a method of waste disposal in the spring and summer months when conditions are more suitable as the ground is drier and heavy or prolonged rainfall events are less frequent than in the winter months. This is particularly important in nitrate vulnerable zones where application of manure is only permitted within specific times and is under strict guidelines (Defra, 2009).

Summer storms (depending on their magnitude) will promote the movement of bacteria either horizontally or vertically through the soil. This is largely dependent on the method of manure application, as manure that is incorporated into the soil is less likely to be entrained in overland flow after a rainfall event unlike just surface applied manure (Quinton *et al*. 2003). If no rain occurs than survival of bacteria is much shorter than if it were to be injected into the soil, this would be attributed to increased UV radiation on the surface, desiccation and lack of nutrients (described further in the next section).

3.3.5 Soil Characteristics

Soil plays a significant role in the transport of bacteria to neighbouring waterways and the conditions in which it provides dictates the survival rate of the bacteria that has been deposited onto land. Survival of bacteria is determined by temperature and pH of the soil, nutrient availability, competition and moisture content (Jamieson *et al*. 2002). The movement of bacteria is determined mainly by soil type, particle size and saturation thresholds which influence both surface and subsurface runoff and entrapment of faecal particles (Mawdsley *et al.* 1995).

The transfer of bacteria through agricultural soils occurs through both horizontal and vertical movement and is directly related to soil type, soil moisture content and saturation thresholds that determine the lands ability to withstand high intensity rainfall (Mawdsley et al. 1995). Different soil types have also shown to have differing effects on bacterial survival, mostly due to particle size and nutrient availability. Tate (1978) noted that survival of *E. coli* was greater a week after manure application in organic soils than in sandy soil, due to the presence of organic matter which promoted the retention of nutrients which aided not only survival but growth of *E. coli* within the soil.

Particle size of the differing soil types also play a major role in bacterial attachment and therefore transport. Oliver et al. (2007a) conducted a study on preferential attachment of *E. coli* to different particle sizes using clay loam soil. They concluded that *E. coli* preferred attachment to sizes 30 - 16µm, but overall found *E. coli* did associate with sizes of varying proportion. *E. coli* were therefore able to move through different soil types either attached or as free organisms. The state in which bacteria exist may also include attachment to manure particles or as free microorganisms (Tyrell and Quinton, 2003).

Particle size distribution of soil will also help to determine the pore size and moisture content which promotes survival of bacteria and ascertains the saturation potential and subsequent transport of pathogens. Mubiru *et al*. (2000) found that soils exhibiting a higher matric potential had a higher bacterial survival rate as bacteria could move through the soil more freely. Larger pore sizes are associated with coarse soils and so it is likely that these soil types will have scope for greater movement and less water retention than finer or heavier soils. Hagedorn *et al.* (1978) showed that faecal bacteria moved faster in coarse soils and Patni *et al.* (1984) showed that coarse soils had a greater drainage capacity than finer textured soils, both of which promote the accelerated movement of bacteria from the soil to receiving waters.

Overland runoff is directly associated with moisture content which is affected by the amount of rainfall. When rainfall exceeds the water retention capacity of the soil it becomes saturated, typical soil field capacities were taken from Boorman et al. (1995) and can be seen in Table 5. Field capacities are defined as the amount of water that remains in the soil after drainage whereas the wilting point is the minimal point of soil moisture that a plant requires not to wilt.

Table 5 Field capacity, wilting points and available water for different soil types.

Soil Texture	Field Capacity	Wilting Point	Available Water
Coarse sand	0.06	0.02	0.04
Fine sand	0.10	0.04	0.06
Loamy sand	0.14	0.06	0.08
Sandy loam	0.20	0.08	0.12
Light sandy clay loam	0.23	0.10	0.13
Loam	0.27	0.12	0.15
Sandy clay loam	0.28	0.13	0.15
Clay loam	0.32	0.14	0.18
Clay	0.40	0.25	0.15
Self-mulching clay	0.45	0.25	0.20

Once the soil is saturated the filtering effect of the soil is removed and the build-up of surface waters leads to transportation of soils and movement of bacteria (attached to particles or free moving) into nearby streams by overland flow and erosion.

3.3.6 Soil erosion and topography

Soil loss occurs through erosion by overland flow, throughflow (combined as interflow) and drainage systems (Billotta and Brazier, 2008) which moves as sediment and become deposited into nearby stream beds. They either remain in the stream bed or become re-suspended and transported downstream consequently increasing levels of turbidity and total suspended solids in the process. Rainfall and soil saturation is a significant contributor to erosion, however Billotta *et al.* (2007) has also contributed ideas that livestock and farm vehicles add to this erosion process by the physical detachment of particles through trampling and compaction that may accelerate the 'natural' erosion process from rainfall. The topography of the land significantly affects the rate at which overland flow or surface runoff can push *E. coli* and soil particles into nearby streams or coastal waters. Alongside rainfall Collins *et al*. (2005) concluded that the steepness of the slope significantly contributed to the amount of *E. coli* found in a pastoral stream. Another study by Abu-Ashour and Lee (2000) also found that steeper slopes provided higher velocity and pushed higher amount of *E. coli* further downhill after intense rain compared to the smaller slopes.

4. Factors affecting the survival of *E. coli* in water

4.1 Sediment properties/ characteristics

Studies have shown that the presence of faecal bacteria in sediments can influence the nearby or overlying water quality. Sediment contamination occurs principally through the same methods of diffuse and point source pollution as previously described and many of the factors influencing bacterial survival are identical to those of soils. The concern is that sediments may act as bacterial reservoirs and some studies have shown that they can contain more than one hundred to one thousand times as many bacteria compared to the surrounding water column, as shown by

Obiri-Danso and Jones (2000) and therefore poses significant risks in becoming bio – available to shellfish when frequent storms, strong winds and turbulence will help to re-suspend the sediment and release the bacteria back into the water column (Nagels *et al*. 2002). The risk of re-suspension will therefore be catchment specific and will depend on how heavily the water body is affected by these environmental factors. Shellfish sites that are largely unaffected by these conditions will alternatively act as a sink and essentially remove the bacteria from the water. Faecal bacteria attach themselves to the particles in sewage and in turbid areas to the suspended sediments, this continued attachment and their deposition leads to a concentration of bacteria in the sediment and it is there that other sediment characteristics may permit the survival even further. The first characteristic would be that of protection from the environmental factors, especially UV radiation that would otherwise prevent their survival in the water column as mentioned above through being buried in the sediment, but also turbid waters will also lessen the amount of UV penetrating (Davies *et al*. 1995). Another such characteristic would be that of the anaerobic conditions present in sediments, some studies have suggested that these conditions would restrict the activity of predators that would otherwise consume the bacteria and maintain equilibrium between them. Howell *et al.* (1996) looked at the influence of sediment size and noted that *E. coli* was mainly associated with particle size of 0.45 – 10Fm and that decreasing particle size indicated increased survival of faecal indicator bacteria.

The following studies have concluded that both fresh and marine water sediments harbour more bacteria then in the overlying water (Table 6). McDonald *et al.* (1982) found that bacteria concentrations increased 10 fold in response to increases in resuspension through artificial storm hydrographs. Pettibone and Irvine (1996) also found that sediments played a role in microbial transport where faecal coliforms in river sediments were one to five logs higher than the overlying water.

Table 6. The difference in number of faecal coliforms found between sediment and the overlying water. Sediment types used in this study were both intertidal marine sediments.

Author	**Faecal Coliforms Water**	**Faecal Coliforms Sediment**
Gerba and Mcleod, (1976)	Range; 70-170 (MPN) per 100 ml	Range; 70-2,600 per 100 ml volumes of wet sediment
Obiri-Danso and Jones, (2000)	Geometric mean (100 ml^{-1})	Geometric mean (100g dry weight cm^{-3})
Site		
1	2951	75624
2	3981	57196
3	758	8132

4.2 Salinity

The salinity of seawater is dependent on the degree of dilution and mixing in a given area, however in general, it is approximately between 30 to 35 parts per thousand. The ability of bacteria, specifically *E. coli*, to survive changes in the environment from low salinities to high salinities is attributed to their ability to osmoregulate. *E. coli* cells by nature are freely permeable to water and when under hyper or hypo-osmotic stress they react by the intake or removal of water into or out of the cell, which automatically changes the internal solute concentrations and turgor pressure. Bacterial cell membranes are able to cope with the pressure of water created from inside the cell and under hypo-osmotic conditions this pressure is low and cell volume only increases a small amount (Csonka, 1989). In higher saline (hyper-osmotic) conditions the cell is at risk of dehydration, where the cell undertakes plasmolysis (cell shrinking) in order for the water activity to be at equilibrium with the external water activity (Csonka, 1989).

Changes in water content and solute concentration are important for growth and the survival of bacteria, as it will affect other cellular processes such as the effective uptake of nutrients necessary for life. (Csonka, 1989) (Record Jr *et al*. 1998) and (Pommepuy *et al*. 1992) have well documented the active and passive responses of bacteria to changes in external osmolarity. An important active response is the accumulation of osmoprotectors which are crucial in terms of survival when under stress from salinity as they actively helped to restore osmotic equilibrium. However, they also help to stabilize proteins and membranes in the cell when under both osmotic and temperature shock. Detailed information on these processes is outside the scope of this review and further information should be sought from the authors Csonka (1989); Record *et al* (1989) and Pommepuy *et al* (1992).

Enteric bacteria have the adaptations to tolerate extreme changes in conditions and several studies have looked at the response of bacteria to differing concentrations of salinity in order to determine the T90 and survival times. (Anderson et al. 1979) looked at the survival of *E. coli* when subjected to waters of differing salinities, they noted that overall survival decreased as salinity increased. They experimented with salinities of 10, 15, 25, and 30 $^{0}/_{00}$ over an exposure period of 2, 5 and 8 days and as salinities increased the percentage of survival decreased more dramatically but the percentage of survival between exposure times decreased. Interestingly at 30 $^{0}/_{00}$ although survival was considerably lower than the other concentrations, after 8 days of exposure the results showed to be higher than that of 2 and 5 days. Carlucci et al. (1961) also showed similar results, where *E. coli* was subjected to four different strengths of seawater and the percentage of survival was assessed after 48 hours. The optimum concentration of seawater was 25% where 74.5% of *E. coli* survived, compared to 50 and 75% concentrations where survival rates were 34.6 and 22.5% respectively. As could be expected, the survival rate at 100% seawater decreased dramatically to 8.2% where 0% seawater had the second highest survival rate at 59.9%., indicating that freshwater does also cause some degree of osmotic stress. Solic and Krstulovic (1992) supported these conclusions using faecal coliforms, but emphasized that increases in salinity is more destructive to bacteria that is subject to lower ranges of salinity I.e. 7-15 $^{0}/_{00}$ than those that are subjected to salinities of 15-40 $^{0}/_{00.}$ The experiment took the two ranges and noted the T90 (time it takes to kill 90% of the bacteria) values as the salinity was increased by a series of 5%. These values showed that at the lower salinities, the T90 decreased by approximately 55%, whereas at the higher salinity range the T90 only decreased by 15%.

The similarities between the results are evidence that seawater is more detrimental to bacterial survival than freshwater when isolating salinity as factor. The method of entry into these saline environments may influence the survival rate i.e. directly from freshwater into seawater from sewage pipes, or gradually from streams and tributaries where gradual changes in salinity can occur. Intertidal areas often provide this gradual change, especially with larger influxes of freshwater from these streams and tributaries. It is therefore important to determine how other environmental factors work alongside or against each other in affecting the salinity concentration in a given area.

4.3 Potential Hydrogen (pH)

The pH is a measure of the acidity or alkalinity of a solution. With the pH of 7 being neutral, any lower value denotes acidic conditions and is created by the addition of hydrogen ions. Values above 7 and up to 14 is described as alkaline and created by the addition of hydroxyl ions. The pH of seawater is normally in the range of 7.5 – 8.5 as the interaction between CO_2 and water in the sea acts as a buffering system so that the seawater can resist extreme changes in pH when there is an addition of acids or bases to the water (Rozen and Belkin, 2001). The pH of river and stream waters is also around pH 7 and the surrounding soil will have a significant role in maintaining these neutral waters but only if the soil is rich in minerals. As the water flows through, the minerals combine with the hydrogen or hydroxyl atoms and the pH can then be regulated (Mesner and Geiger, 2010). Under conditions of heavy rainfall though, this process may be bypassed as the soils become saturated. Naturally rainwater has a pH of approximately 5.6, the slight acidity is caused by the interaction of rainfall and CO_2 creating carbonic acid and therefore if not naturally buffered by soils, the pH of stream/ river waters may be lowered (Neal et al. 1992). Under normal condition, changes in pH may still occur through the addition of pollution from groundwater, sewage systems or surface run off.

The changing pH of seawater is also caused by the release of anthropogenic carbon dioxide (CO_2) into the atmosphere. The estimated net emission of CO_2 in the UK for 2011 was 456.3 million tonnes from the burning of fossil fuels (DECC, 2012).The released Carbon dioxide dissolves in the ocean and forms carbonic acid (H_2CO3)

which increases the acidity and lowers the pH levels. Caldeira and Wickett (2003) indicate that continued release of the CO_2 into the atmosphere will result in a pH decrease of 0.7 units over the next two or three centuries.

Increasingly acidic waters would encourage more suitable conditions for the survival of bacteria as suggested by Carlucci and Pramer (1960). Their results showed that the death of *E. coli is* more rapid in alkaline solutions than acid solutions. They subjected *E. coli* to pH concentrations of 5.0 – 9 in which the percentage of survival decreased as the pH level increased. The pH 5.0 concentration showed a survival rate of 58.3%, whereas pH 9.0 showed a survival percentage of <0.01. Rozen and Belkin (2001) supported these results where they found a pH of 5 to be optimum for survival. Solic and Krstulovic (1992) had conflicting results in that preferred pH levels for faecal coliforms was between 6 and 7 as rapid die off occurred when subjected to levels either side of these figures, their study also demonstrated that high acidity had a more detrimental effect then high alkalinity. Acidity causes damage to membranes and more importantly cell DNA and so *E. coli* have developed an acid stress response. Swenson et al. (2012) reviewed acid tolerance in environmental strains of *E. coli* and discovered that the two mechanisms were involved in the response to low pH in which both involved amino acid antiporters; glutamate which help to maintain internal pH by alteration in membrane phospholipid composition and also by synthesis of acid shock proteins. The second mechanism involved amino acid antiporters; arginine-agmatine which contribute to the extreme acid response.

4.4 Sunlight and Temperature

Many studies have shown that the presence of sunlight is a major contributing factor for the survival of *E. coli* in both fresh and seawater. Whilst there are differences in the physiochemical characteristics of both marine and fresh water which assist in decreasing bacterial numbers, (Solic & Krstulovic 1992) has shown solar radiation to be more effective than temperature, salinity and pH at bacterial removal. Sunlight is described in three categories: infrared, visible light and ultraviolet (UV) and two (UV and visible light) have both been described as having bactericidal effects (Hollaender, 1943).

The wavelengths of ultraviolet are split into UV-A, UV-B and UV-C and are classed by their intensity in nanometres (nm). UVA has the strongest wavelength at 320 – 400nm compared to UVB (290 – 320nm) (Muela et al. 2000). UV-A and UV-B are likely to be responsible for a number of damaging processes to *E. coli* affecting both survival rate and viability. Due in part to the intensity of radiation, a receiving bacterial cell will absorb photons causing ionisation and disruption to cell membranes and transport processes (Koch et al. 1976) by damage to nucleic acids and toxicity within the cell causing damage within. Muela et al. (2000) determined that UV-B radiation was the most detrimental to survival as some of the above effects were seen within a short time span, whereas prolonged exposure to UV-A bacterial cells were still able to divide and multiply. It was concluded that the different wavelengths affected different parts of the cell and therefore its ability to cause increased damage and death of the bacteria.

Fujioka et al. (1981) suggests that visible light is more detrimental to bacteria in the aquatic environment as UV light is readily absorbed and does not penetrate into water, where visible light does. Results here showed that light could penetrate to a depth of 3.3m of clear seawater and that 90% of the bacteria were inactivated within 30 to 90 minutes, whereas under dark conditions they survived for several days. Another important conclusion from this study was the difference in survivability between freshwater and seawater. Freshwater bacteria had increased resistance to the effects of solar radiation compared to those in seawater, highlighting that seawater is more detrimental to bacteria than freshwater. Other environmental factors such as cloud cover, depth of water, level of mixing, turbidity and temperature were also considered to impact on the amount of radiation penetrating the water and the position of the bacteria in the water column (Alkan et al. 1995).

Solar radiation has a strong association with surface water temperatures, (Solic & Krstulovic 1992) found that the two factors combined could explain 96.6% of the variability of the T_{90} value that were used to convey survival of faecal coliforms. However when solar radiation was removed temperature did not contribute largely to bacterial die-off. Nevertheless, temperature especially at depth where solar radiation cannot infiltrate may contribute extensively to survivability.

Upon entering the environment from the host organisms of a temperature of 37°C, *E. coli* undergo a temperature shock and quickly adapt to the different temperatures found in both fresh and seawater. *E. coli* have the ability to survive a range of temperatures however slower rates of inactivation are demonstrated at lower temperatures. This is because cold shock in *E. coli* causes changes to cell membrane fluid and causes translational block (Yamanaka, 1999). In order to prevent this becoming critical to the cell, the cellular response of *E. coli* is the release of cold shock proteins and to increase the production of fatty acids. The major cold shock protein in *E. coli* is cspA and has been attributed to the regulation of transcription, translation and mRNA stability within the cell. See, Phadtare et al. (1999); Jones and Inouye (1994); Jones et al. (1987) for further detail. These studies have suggested that these proteins allow growth of *E. coli* to occur as low as 10°C which may have implications for bacteria to multiply within temperate waters of UK given the right conditions. Further work would be required in order to determine whether this is viable due to the nature of bacteria and their response to multiple stressors. Optimum temperature for survival of *E. coli* is different to that required for growth, survival studies were conducted by Vasconcelos and Swartz (1976) where *E. coli* were subjected to differing temperatures (8 .9, 10.7, 12.6 and 14.5). Their results showed that higher die off rates were of those exposed to temperatures of 14.5°C compared to temperatures of 8.9°C. Overall as temperature increased, survival of *E. coli* decreased. Fraser and Argall (1954) using similar techniques also noted that 50% of *E. coli* survived temperatures of 6°C over a period of 24 hours, whereas those subjected to a 2°C increase did not survive for more than 8 hours. Factors that enhance die off at higher temperatures, especially in nutrient limited environments will be attributed to increases in kinetic energy of the bacterial cells, without adequate nutrition die off will occur rapidly. Competition and the predatory actions of other bacteria and protozoa will also accelerate under higher temperatures and ultimately decrease survival rates of *E. coli*.

4.5 Turbidity and Total Suspended Solids

Turbidity has been described by Austin (1973) as the scattering of suspended particles in the water column. Whereas total suspended solids (TSS) is the measured weight of these suspended solids that include both mineral and organic particles (Davies-Coley and Smith, 2001).The level of turbidity and total suspended solids in a water body often occurs through increased surface run-off from urban and agricultural areas after rainfall. Sewage from both consented and storm driven discharges that also cause algal blooms from the increase in nutrients provided by sewage and the re-suspension of bottom sediments are also factors causing increased turbidity, processes of which are described in the above sections.

An important aspect of turbidity for the survival of *E. coli* is the amount of solar radiation that is able to penetrate the water column. In waters of high turbidity, the bactericidal properties of sunlight are not as effective and therefore die-off rates would be lower than under normal conditions (Alkan et al. 1995). This coupled with suspended particles that assist in the transport of bacteria can determine the level of *E. coli* reaching coastal waters and subsequent shellfish production areas.

Huey and Meyer (2010) looked at both turbidity and total suspended solids and their relationship with water quality in several watersheds. Their results demonstrated that water flow after a rainfall event caused an increase in turbidity, TSS and *E. coli* concentrations. Significant positive correlations were seen between all three factors for all of the catchment areas, indicating that as one of the variables increased so did the other. Irvine et al. (2002) also documented strong relationships between TSS and turbidity and TSS and faecal coliforms in a freshwater system which was attributed towards some of the samples taken during storm conditions.

4.6 Predation

Predacious bacteria and Protozoa are two types of organisms that are known to reduce numbers of *E. coli* present in both sediments, and within the water column. Natural bacteria and protozoa are thought to affect *E. coli* through competitions for nutrients but more importantly the role of these organisms as predators. Predacious bacteria that have been identified in several predation studies include myxobacteria, bdellovibrios and other types of lytic bacteria (Enzinger and Cooper, 1976; Roper and Marshall, 1977).

Numerous studies have suggested that the role of protozoa is more significant than the role of bacteria in the removal of *E. coli.* McCambridge and McMeekin (1979) looked at survival of *E. coli* in difference concentrations of protozoans and found a negative relationship between the two. As the level of protozoa increased the number of *E. coli* decreased from 7 x 10^6 to 10 within 10-12 days. Roper and Marshall (1977) conducted a study on the bacterial predation against *E. coli* from sewage outfalls and found that numbers of *E. coli* declined rapidly after incubation for 24 hours whilst the number of predacious bacteria in this case a myxobacteria; *Polyangium* increased.

Ezinger and Cooper (1976) used an antibiotic resistant strain of *E. coli* to determine survival rates when placed in natural estuarine water with the known presence of both natural bacteria and protozoa. The addition of antibiotics removed the (known) predacious bacteria from the sample but levels of *E. coli* still decreased in their absence, which was linked to the increase in numbers of protozoa. Furthermore, a filtration experiment concluded these results and noted a logarithmic death for *E. coli* after a 2 day lag time in the presence of protozoa; here bacterial presence did not significantly affect *E. coli*. McCambridge and McMeekin (1980; 1981) supported these conclusions but deduced that bacterial predation does occur, just at a lower level than protozoa due to also be under threat from predacious protozoa.

4.7 Hydrographic Factors

Physical processes of a water body have considerable influence on the contamination entering coastal areas and therefore the relative uptake by shellfish. Inputs from both point source and diffuse pollution are dependent on site specific characteristics and are subjected to the influence of the tidal cycle (spring/ neap, high/low) (Mallin et al. 2000) and water circulation patterns which will contribute to the degree of mixing (Alkan et al. 1995) physical dilution and dispersion (Maalouf and Pommepuy, 2010) of bacteria which is exacerbated by other environmental factors such as the amount of rainfall and the level of freshwater input into the system. Depth, which is associated with changes in temperature and salinity, are also key factors in bacterial survival within coastal environments and are also influenced by tidal/water movements as explained further on in this section.

Mallin et al. (2000) conducted a study on the functions of tide on faecal coliforms concentration and salinity in three different coastal areas. The authors concluded that for all three sites higher abundance of faecal coliforms were found at low tide compared to high tide and that faecal coliforms were inversely correlated with both tide and salinity. This is likely to be due to the effects of dilution from the incoming tide, but also influences from other physiochemical properties influenced by tide. Mill et al. (2006) showed that levels of bacteria were lower in water samples taken at high tide, which was attributed to the higher recordings of pH, temperature and salinity taken at the flood tide compared to the ebb tide. Water circulation and the degree of mixing is a process that also combines with the movement of tides. Alkan et al. (1995) in an experiment of *E. coli* survival rates showed that high levels of vertical mixing within an environment, increased die off rates due to the transportation of bacteria through different depths of the water where other factors such as solar radiation and temperature caused increased die off. Therefore, environments with large levels of vertical mixing will contribute considerably to decrease bacterial survival by working synergistically with other environmental factors such as salinity and temperature.

5. Factors affecting the uptake and elimination of *E. coli* in bivalves.

The uptake and elimination of faecal bacteria as part of their feeding process in bivalves are two features that determine the level of contamination and the associated transfer of food borne disease (Wood, 1976). Controlling these factors is a series of biological and physiological processes that are themselves influenced by factors in the surrounding environment. The most influential environmental factors controlling the feeding process in bivalves are temperature and salinity (Wood, 1959). These are discussed below along with other environmental components that alter feeding behaviour, with focus on mussels and oysters.

5.1 Temperature and filtration rate

Water temperature can alter filtration and clearance rates of both mussels and oysters and therefore will control the uptake and elimination of bacteria. Pacific oysters are thought to be able to survive and grow in temperatures between 4 and 24°C (Pauly et al. 1998) but filtration rate is limiting at temperatures below 10° C. Loosanoff (1958) conducted a study that measured the filtering capacity of 24 oysters in the temperature ranges between 0 and 30°C. Optimum temperature for maximum filtration was at 28.1 – 30.0°C, all 24 oysters remained open and pumped on average

12.983 (units not specified). At temperatures as low as 2.0°C, 22 of the 24 oysters opened their shells but only ascertained an average pumping rate of 863. The author conducted a further study to determine whether oysters at these low temperatures (between 3 and 4°C) were in the active process of feeding, which resulted in only one oyster eliminating true faeces (sample of 90 oysters).

Mussels are less susceptible to temperature changes than oysters and have a wider tolerance especially to lower temperatures. Kittner and Rusguard (2005) showed that mussels that were cold adapted through a period of acclimation of 11°C were able to filter feed at temperatures as low as 4.1°C. Those that were acclimated to a higher temperature of 18°C could not filter below 6°C suggesting that the range of temperature tolerance of mussels to filter is a function of what temperature they are adjusted to in their natural surroundings. Loo (1992) also conducted a study to determine whether the mussel was capable of filtering and absorbing particles at these low temperatures and the results showed that they were able to filter as low as -1°C. Upper limiting temperatures for mussels were considered to be in the range of 27 - 29° C (Read and Cumming, 1967).

Filtration rates of algae by both oysters and mussels of various body weights, with two different temperatures were set out by Gerde (1983) and can be seen in Table 7.

Table 7 Filtration rates of two species of bivalve molluscs; *Mytilus edulis* and *Crassostrea gigas*. Algal concentrations were used to measure the rate of filtration of the different sized species under two different temperatures.

Species	Dry tissue weight(ng)	Concentration of Algae (10^6 cells 1^{-1})	Temperature (°C)	Filtration Rate (h^{-1}) ml mg^{-1} dtw^a	Author
Mytilus edulis	160.0	110	18	22.5	Bayne, 1965
	380.0	60	18	26.3	
	380.0	25	18	23.2	
Crassostrea gigas	50.5	100	25	55.7	Gerde, 1983
	92.5	100	25	20.7	
	285.0	100	25	24.6	

Differences in temperature can be down to seasonal affects Wood (1957) established that the higher temperatures of the summer months (10-16.5° C) caused increases in filtration rate in both oysters and mussels as both species were able to purify themselves from extreme pollution with incoming seawater within six and one hours respectively. Compared to winter temperatures of 1.5 – 2.1°C oysters did not become polluted as the temperature was too low to facilitate feeding whereas mussels were less affected and continued to filter at the same rate and therefore became more polluted. The range of thermal tolerance for mussels dictates that temperature will not significantly alter the mussel's ability to uptake faecal bacteria whereas in oysters low temperatures significantly decrease the rate of bacterial uptake. Mussels are therefore at a much higher risk of becoming contaminated, especially during the winter period.

5.2 Salinity

The response of oysters and mussels to changes in salinity is species specific and determined by the external environment to which they exist. Mussels and oysters are euryhaline species and so are considered to be able to tolerate a wide range of salinities due to their ability to adapt to fluctuations in the natural environment (Gosling, 2003).

Bohle (1972) subjected the mussel *Mytilus edulis* to differing seawater strengths to determine its rate of filtration under differing salinities, measured by the addition of algae to the experimental tanks. The salinity concentrations were at 100% seawater (34 ppt), 75% seawater (26 ppt) and 50% seawater (18 ppt). At the beginning of the experiment, mussels subjected to both 26ppt and 18ppt filtration rates were low as the mussels did not fully open their shells, unlike those at 34ppt where algal uptake was rapid. Upon the fourth week of study, mussels in 75% seawater acclimatised to feed at the same rate as those at a 100% whereas even by week 7 of the study those as 50% seawater still did not acclimatise to the same filtration rate as the other concentrations, but algal uptake did increase suggesting that over a longer time period, these mussels may still manage to increase their filtration rates as they acclimatise. This study as well as one by Schlieper (1955) also noted that acclimation is also dependent on water temperature and the controlling factor of the physiological response to changes in salinity is dependent on enzymatic adaptation. Kinne (1970) noted that mussel acclimation to salinity would happen at a faster rate under higher temperatures, but it dependent on the degree of salinity change.

These study suggests that if inputs of freshwater from nearby water ways into coastal areas are constant than mussels can adapt to lowering salinities and therefore filtration and the potential uptake of bacteria is as feasible as mussels at higher salinities. Theede (1963) confirmed that mussels taken from two different populations based in differing salinities, one population at 15ppt and the other at 30ppt had the same filtering rate and so confirms that the optimum salinity is of the surrounding water to which they are accustomed, therefore either decreasing as well as increasing salinities may alter the uptake of bacteria. Oysters also respond to changes in salinity stress by changing the degree in which they open their shells and their filtering capacity. Quayle, 1969 conducted a study subjecting the pacific oyster to different salinities and from this he noted that maximum filtration rate occurs at 25-35ppt. When the oysters were subjected to lower salinities of 13 – 20ppt oysters became sensitive and only small amounts of water were able to be filtered over the gills.

7. Conclusions

Studies have shown that increases in global warming will lead to more extreme precipitation events in the future. In regions of high rainfall, such as North Western parts of the UK and where considerable numbers of shellfish are harvested, these precipitation extremes could become even more detrimental to both water and shellfish quality. Rainfall has shown to trigger the release of untreated sewage and the extremely high concentrations of faecal bacteria/ *E. coli* into the environment as wastewater treatment plants fail to cope with the volumes of water passing through the system. It is understood that the number of diffuse sources of *E. coli* has increased due to the demand for the intensive production of livestock. Most farming methods result in the application of faecal matter to the lands where rainfall plays a highly significant role in transferring it into nearby waterways. The level of contamination is dependent on several factors which are both social and environmental.
Environmental variables such as soil characteristics and topography are the land based factors which determine the rate of transfer of *E. coli* to water and will dictate the speed in which a catchment responds to rainfall. Both these factors are catchment

specific and also determine the amount of soil and soil associated bacteria that end up depositing as stream bed sediments. It is clear that sediments act as a source of *E. coli* and that re-suspension of sediments can significantly alter water quality.

Salinity, solar radiation and temperature are three factors that are well known to combat bacterial survival and have been widely studied in both lab and field conditions. The bacterial die off caused by high salinities in marine waters is probably the most important factor in terms of levels of *E. coli* available for uptake by shellfish. This review has shown conflicting evidence as to whether bacteria survive better in more acid or alkaline conditions. Either way changes in pH in stream and seawater could be of increasing concern if global warming continues to cause extreme rainfall events and ocean acidification. The influence of turbidity and total suspended solids has not been extensively researched like some of the other environment factors. They each play a significant role in both survival, and with regards to turbidity the transport of bacteria in natural waters. Suggestions have been made that the relationship between turbidity and faecal bacteria may be significant enough to use turbidity as a water quality indicator. However, further research between the two variables is required to validate this.

Local hydrodynamics have been shown to affect shellfish contamination by altering the amount of bacteria available for uptake and the external environmental e.g differing salinities. Few studies have documented these effects on shellfish quality as the factors are very specific to individual shellfish sites. But it is important that they are considered because of the potential effects on the filtering capacity and therefore bioaccumulation of bacteria in shellfish. Changes in salinity and temperature have been shown to be the two factors that are most influential on this filtration rate of both oysters and mussels. Overall oysters are more sensitive to changes in conditions and physiologically their response time is slower, indicating that mussels are at higher risk to contamination. Up to date research on shellfish response to contamination from *E. coli* under environmental conditions and especially from rainfall requires further in-depth research . Some studies have found associations between rainfall and shellfish quality using point pollution sources, but fewer studies have looked at a direct association between rainfall and diffuse pollution and shellfish quality in the UK. Due to the increases in intensive farming and extreme precipitation events, the effects of agriculture is increasingly problematic to shellfish quality and further research is required to aid in the understanding the association between these variables.

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Section two – Research Project

Assessing how rainfall and other environmental factors affect the level of *Escherichia coli* contamination in two species of bivalve.

Abstract

The purpose of this study was to obtain an understanding of the association between environmental variables, particularly rainfall and the faecal contamination of bivalve shellfish. Diffuse pollution is an important source of this contamination, in which the transfer of faecal bacteria from land downstream to coastal waters is exacerbated by the magnitude of rainfall and other environmental factors. Oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*) were set up on a small intertidal oyster farm that received inputs from two streams draining a headwater agricultural catchment. The oysters/mussels, stream and seawater were sampled under rainfall event and baseline conditions for bacteriological quality using the faecal indicator bacteria *Escherichia coli*. Turbidity (NTU) and total suspended solids (TSS, mgl^{-1}) were also monitored. Further, in situ measurements were recorded which included; temperature (°C), salinity (ppt) flow rate (ms^{-1}) and flow depth (m).

Flow rate, flow depth, turbidity and TSS were significantly correlated with rainfall in both streams and regression analysis showed that the preceding 12 hour rainfall and turbidity could explain 68.3% of the variability of *E. coli* found in stream one (F = 21.51, p = <0.001), whereas in stream two, preceding 12 hour rainfall and total suspended solids could explain 66.5% of the *E. coli* present (F = 19.86, p = <0.001). Levels of *E. coli* in the surrounding seawater were significantly correlated with preceding 12 hour rainfall (R = 0.530, p = <0.05). No significant relationships were found between rainfall and levels of *E. coli* in mussels and seawater (F = 8.22, p = <0.05). Overall, oysters exhibited higher levels of *E. coli* than Mussels but no significant relationship could be found with environmental variables to explain these elevated *E. coli* values. The data highlights the need for future sampling strategies to be tailored to individual species (Oysters, Mussels or other bivalves) and suggests that several rainfall events are required in order to capture the variability in bivalve response to rainfall through the year.

1. Introduction

Shellfish production areas are at risk of faecal contamination due to the effects of rainfall and other environmental factors that are responsible for the movement and survival of pathogenic bacteria from its source in to coastal waters. Several studies have shown that diffuse pollution, with focus on contamination from livestock in agricultural areas is a major source of faecal contamination to streams and rivers (Avery, 2004; Collins et al. 2005; McAllister and Topp, 2012). The transfer process from land to water has been shown to be dependent on several environmental factors which include soil characteristics, topography and level of rainfall. The water holding capacity of soil is important in determining how much water after a rainfall event ends up as overland flow. Fine grain soils such as sandy or clay soils are known to be easily saturated and any faecal matter deposited here are likely to be washed away as water pools on the surface (Brouwer et al. 1985). Compared to peaty organic soils, the water holding capacity is higher which prevents large amounts of surface runoff. Freely draining soils do however allow for any faecal bacteria deposited on the surface to be transported downwards through the soil and travel through groundwater into nearby waterways (Jamieson et al. 2002, 2005; Tyrell and Quinton, 2003; Muirhead et al. 2006). When extreme precipitation events occur, all soils can become saturated and the topography of the land which dictates the speed at which transfer occurs (Abu-Ashour and Lee, 2000) modifies how a particular catchment responds to rainfall.

The combination of high rainfall and topography are known to increase rates of surface runoff from farm buildings (Edwards et al. 2008) and changes in the landscape from increases in urbanization are also providing quick route of transfer into water (Arnold and Gibbons, 1996).

The fate of faecal bacteria once in stream/river waters is dictated by a further series of environmental factors. Once in stream waters, bacteria attached to soil or manure particles either sediment out and form part of the stream bed sediments or are carried downstream (Jamieson et al. 2005; Collins, 2004). Two initial factors that affect the process are the rate of flow and depth of the water body, both of which are influenced by the level of rainfall and hydrological connectivity of the catchment (Mallin, 2001, Brock, 1985). Salinity, temperature, solar radiation, and pH are four factors that have been frequently used in researched of bacterial survival in both freshwater and seawater (Anderson et al. 1979; Record et al. 1998; Jones and Inouye, 1994; Phadtare et al. 1999; Fujioka et al. 1981; Trousellier et al. 1990; Swenson et al. 2012, Solic and Krstulovic, 1992). Turbidity and total suspended solids have not been researched extensively but are believed to play a considerable role in this survival process (Huey and Meyer, 2010; Irvine et al. 2002).

In coastal waters the uptake of faecal bacteria by shellfish is a function of the external environmental conditions and the physiological response of individual species. The process involved in bioaccumulation is mainly associated with the filtration capacity. Two environmental factors that affect filtration are the salinity and temperature of the surrounding water (Gosling, 2005). Pacific oysters (*Crassostrea gigas*) are more susceptible to changes in environmental conditions than the common mussel (*Mytilus edulis*). Mussels have been shown to filter in temperatures from -1 - 29°C (Loo, 1992; Read and Cumming, 1967) whereas oysters could not tolerate lower temperatures and could only filter from 10- 30°C (Pauly et al. 1998; Loosanoff, 1958). Both oysters and mussels showed optimum filtration rates at higher salinities and are much more sensitive to salinity changes than temperature. Oysters severely restrict filtering abilities in salinities of 13-20ppt, where 25ppt has been shown to be the optimum for filtration (Quayle, 1969). Mussels are unable to filter at salinities as low as 18ppt, however have shown the ability to acclimatise to changes in salinity after a period of time(Schliepper, 1955 as shown in Bohle,1972;) and therefore mussels are at much higher risk of contamination all year round because of this tolerance to changing conditions.

The significance of this shellfish contamination is the potential health problems presented to the public if highly contaminated shellfish are consumed raw (oysters are often eaten this way) or undercooked. In Scotland, 251 tonnes of pacific oyster, 28 tonnes of native oyster and 6,996 tonnes of mussels were produced in 2011 with shellfish aquaculture worth 9.8 million to the UK industry (Scottish Government, 2011). For this reason contaminated shellfish are detrimental to both public health and industry. Therefore the up to date research on the association between rainfall and shellfish quality would be a useful addition to what is already known to safeguard health and industry. Whilst studies have looked at the effects of rainfall on these different factors individually, and few studies have found any direct association between rain events, environmental factors and shellfish quality as a whole in the UK. The aim of this study was to incorporate as many of these environmental factors mentioned above to try and get a clearer understanding of the association between rainfall and shellfish quality. Using a small oyster farm off Loch Fyne, West coast Scotland as a study site, both Pacific oysters (*Crassostrea gigas*) and Common mussels (*Myttilus edulis*) were monitored for contamination using the faecal indicator bacteria *Escherichia coli*. Two of the main sources of faecal pollution that were likely to be affected by rainfall were identified as two streams entering onto the shoreline of the shellfishery set up. These were also monitored for *E. coli* and other environmental factors under both base line and rain event conditions to test the following hypotheses

- H1 It was hypothesised that the preceding rainfall would have a significant relationship with the levels of *E. coli* found in both streams one and two
- H2, The environmental factors; rainfall, flow rate, flow depth, turbidity and total suspended solids would significantly contribute towards increased levels of *E. coli* in both streams one and two.
- H3, Each of the environmental factors; rainfall, flow rate, flow depth, turbidity and total suspended solids will show significant positive relationships with one another.
- H4, It was hypothesised that stream one would discharge a significantly higher loading of *E. coli* than stream two.
- H5, It was hypothesised that levels of *E. coli* taken from water samples at the trestle area would be significantly lower than samples taken from the streams.
- H6, It was hypothesised that there would be a significant relationship between rainfall and levels of *E. coli* in seawater.
- H7, It was hypothesised that there would be a significant relationship between rainfall and levels of *E. coli* in mussels.
- H8, It was therefore hypothesised that a significant relationship would exist between the *E. coli* levels in seawater and the levels found present in mussels.
- H9, It was hypothesised that oysters contained significantly higher levels of *E. coli* than mussels and the surrounding seawater.

- H10, It was also hypothesised that rainfall would significantly affect the level of *E. coli* found in oysters.
- H11, It was hypothesised that *E. coli* levels in each sediment type would significantly different from one another.
- H12, It was further hypothesised that there would be a significant difference between levels of *E. coli* before and after rainfall for all three sediment types.

2. Materials and methods

2.1 Study Area

The chosen study area was positioned on the Ballimore Estate of Otter Ferry, which is situated on the eastern shore of Loch Fyne, Argyll and Bute, west coast of Scotland (Figure 1).

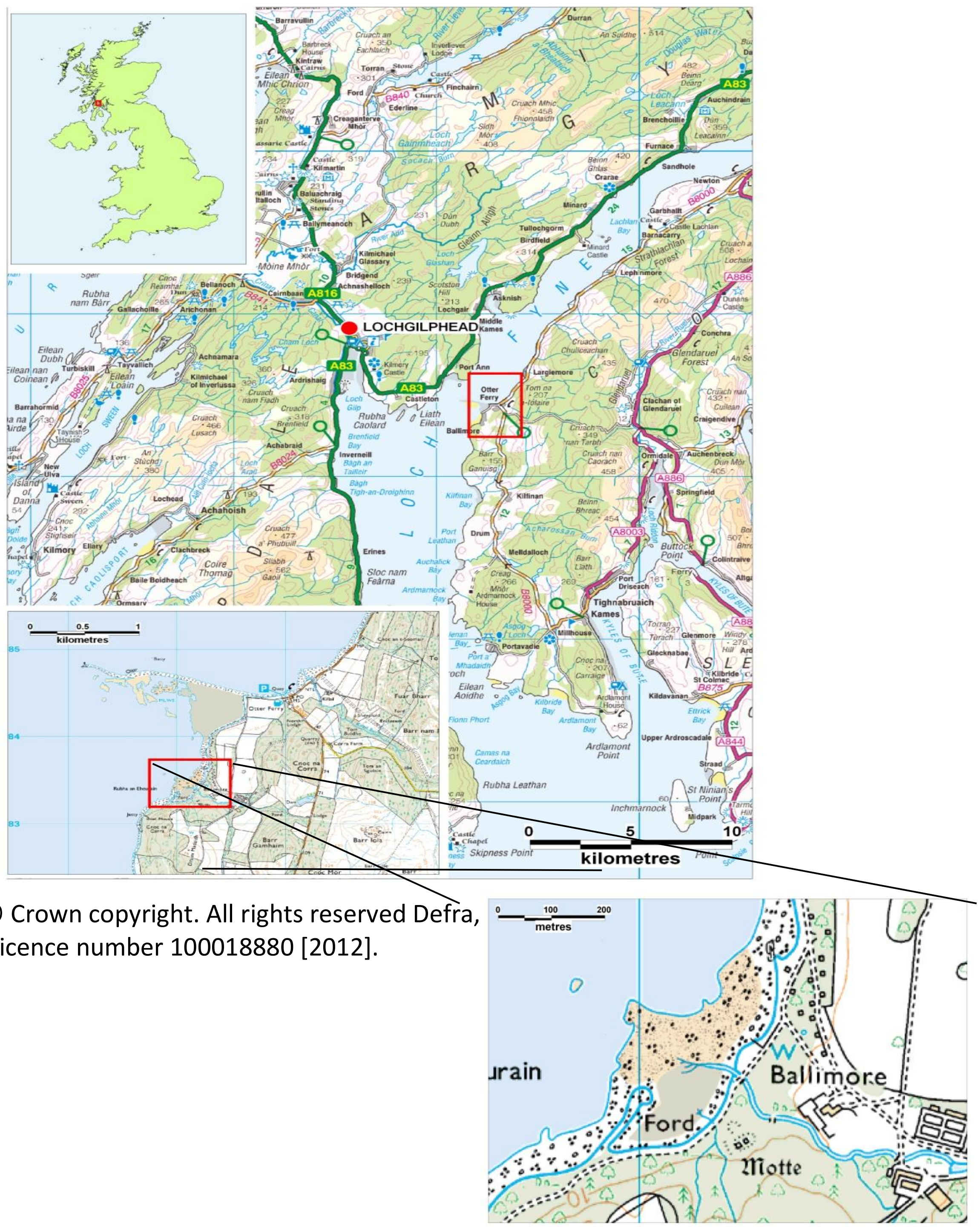


Figure 1 Map of study area, Loch Fyne Otter Ferry, Scotland. The study area is situated to the south of the sand spit as shown in the insert which delineates the area of Figure 4.

2.2 Site selection criteria

Several factors were taken into consideration when deciding on a suitable site to be able to achieve the aims of the project. The criteria that were used in site selection are presented in Table 1. Sanitary surveys undertaken by Cefas (2010) provided detailed information in relation to the criteria for the potential sites.

The presence of a predominant watercourse would be likely to bring increased levels of freshwater and associated faecal contamination into the coastal water.

The catchment needed to be big enough to ensure flow throughout the study period so that sampling and measurements could be undertaken. A lower elevation of the land and freely draining soils would provide a slower response to rainfall and therefore give a greater chance of capturing the effects of a rainfall event. Logistical considerations needed to be taken into account to ensure that both baseline and intensive sampling within a selected time scale and samples could be transported to the laboratory within the required timescale for microbiological analysis.

Table 1 Criteria used to determine a suitable site for the study including both scientific and logistical requirements.

Scientific Criteria	
High Rainfall	Sites in areas with known high rainfall e.g. North west Scotland.
Contamination sources	Diffuse/ point sources.
Catchment type and size	Presence of a predominant watercourse. Large enough to yield sizeable flow throughout the study period. Relatively low land elevation.
Coastal water dynamics	Limited complexity, so that sources of contamination are easily identified.
Cooperation with harvester	Willingness to take part in the study. Allow the setup of equipment on the land i.e. weather station, CTD meters.
Species harvested and techniques	Require the setup of both oysters and mussels, as species differ in their ability to uptake and eliminate so need to be representative of both.
Species harvested and techniques	Require the setup of both oysters and mussels, as species differ in their ability to uptake and eliminate so need to be representative of both.
Nearby UK Meteorological Office weather station	The ability to obtain comprehensive rainfall data set for the period of the study if required.

Logistical Criteria	
Site logistics and accessibility	Easy access to the shellfish and other sampling points.
Transport distance	Suitable driving distance to sample drop off points and post offices to send samples within designated times and cut-off points.

A previous survey (Cefas, 2010) noted that the Loch Fyne, Ballimore oyster farm could accommodate the majority of the specified criteria over the other sites surveyed in Scotland. The harvester was likely to be helpful (pers. comm.) and accommodate the use of scientific equipment and be able to sample both oysters and mussels, whether they were wild or provided from an alternative source.

The rainfall level of the area was assessed by looking at the rainfall history obtained for use in the previous sanitary survey of the area. The closest station with the most comprehensive rainfall data sets was Skipness House located 25km to the south of the fishery. During the period of 2003-2009 (time in which data were available) higher rainfall was evident between the months of August and January (Figure 2) which identified a period where sampling should take place in order to capture a rainfall event. Previous analysis between preceding seven day rainfall and *E. coli* samples noted a positive correlation and therefore indicated a potential for future correlations between rainfall and *E. coli* to occur at this site.

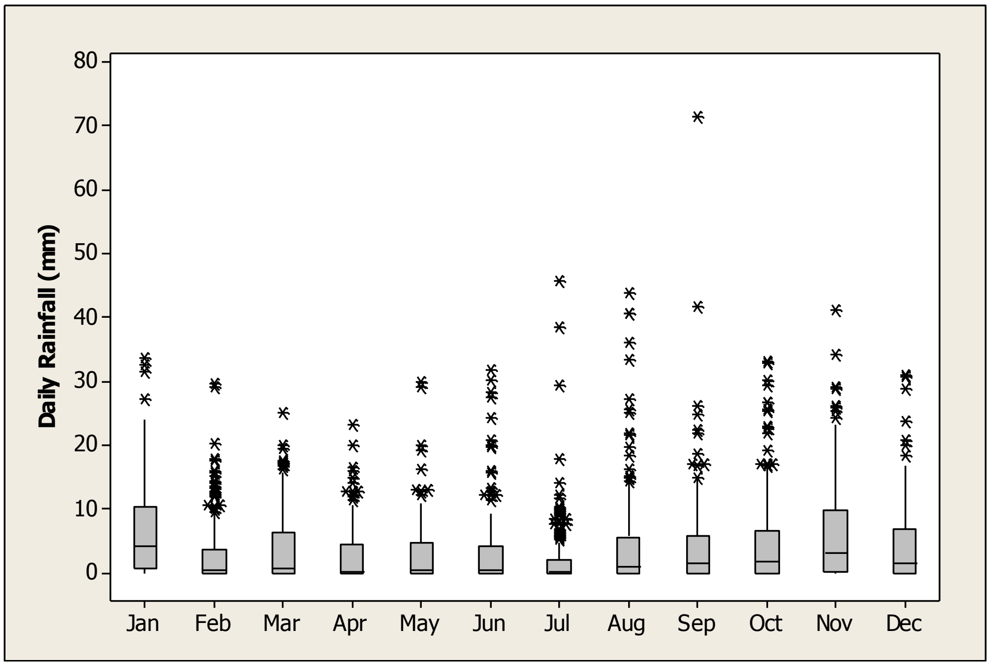


Figure 2 The distribution of daily rainfall values by month through the years of 2003-2009 as measured at Skipness House (UKMO). Data is missing for the months of January and December 2006. The median number is represented by the black line in the grey box which is the 50th percentile of rainfall values. The whisker represents the 1.5 interquartile range of the upper quartile. Any rainfall observations outside of this range are represented as the symbol * (Minitab 15, 2010).

To address the hypotheses that preceding rainfall would have a significant relationship with *E. coli* in streams one and two (H1) and seawater (H7), historical rainfall data from Skipness House were analysed. The aim was to determine a study period where baseline data (levels found under dry conditions, prior to a rainfall event) and event based sampling (after a high rainfall event) was most likely to be obtained. The three months that were shown to have the highest level of rainfall (as shown in the full sanitary report for the fishery) and most suitable for sampling were September, October and November (Figure 2). Although January showed to have high levels of rainfall, it was not considered a suitable time to sample due to other factors such as the drop in temperature and the possibility of snow. Using the most recent data from 2008 and 2009 rainfall values from these three months were placed into a time series chart (Figure 3) to look at the frequency, duration and intensity of rainfall for a given month.

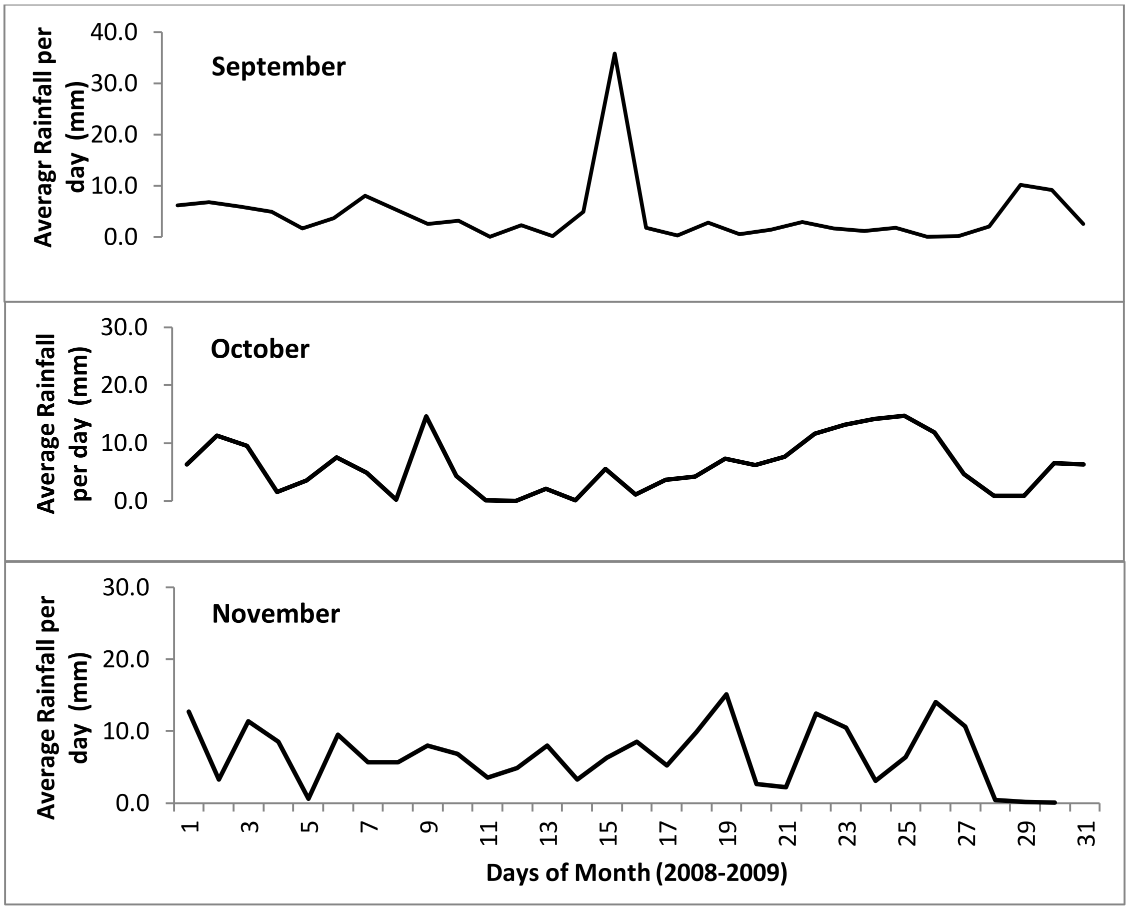


Figure 3 Daily rainfall values across the months of September October and November, taken on average from the years 2008 and 2009.

The outcome showed that over a two year period October had a higher number of wet days (total = 55) compared to September (total = 45) and November (total = 52). Rainfall intensities were lower in September, whereas October and November saw several more peaks and troughs in rainfall. Taking the above into consideration, October and November were both deemed suitable for sampling. To increase the possibility of catching a rainfall event, it was decided that a sample period of two weeks would be sufficient upon looking at the number of days between peaks in rainfall in Figure 3. In order to classify a rainfall event, a trigger level was determined by looking at the 90^{th} percentile of Octobers and Novembers rainfall data from the year 2009. The 90th percentiles were 13.1 and 22.3mm respectively and so rainfall above 13.1mm would be classified as an event.

The site was also simplistic in terms of the number of different pollution sources, the previous survey (2010) and the survey conducted for the present study (2011) (presented in Figure 4) shows that diffuse pollution was prevalent over human sewage outputs. Major point sources were not present in close proximity of the fishery, previous assessment of discharges other than those in the direct vicinity of the fishery were not thought to cause any significant contamination. Only the four septic tank (1 – 4, Figure 4) outputs as seen on the shoreline surrounding the fishery had the potential to contribute. Diffuse pollution was largely present with approximately 400 sheep (5-10, Figure 4) present within the catchment. The harvester indicated that approximately 10 pigs were present on the farm.

The catchment was relatively small with three streams discharging on the shoreline (numbers 17-19, Figure 4). Stream number 17, Figure 4, seeped across the shoreline and a water sample was taken to determine any *E. coli* loadings. Stream numbers 18 and 19 on Figure 4 were considered to be potential contributors of faecal contamination, especially as the main stream (19) flows through the estate and farm buildings and directly through the trestles. The loadings per days and constant stream flow from both stream 1 and stream 2 from the original survey indicated that a) there was a constant flow from the catchment and b) *E. coli* was present and was a representation of the diffuse pollution in the area, considering there are no sewage discharges into these waterways within the catchment area. See Figure 4 and Table 2 for further detail. The dominant soil types along the shoreline were freely draining humus-iron podzols and brown forest soils, indicating that surface run-off is less likely due to the high permeability of the soil, however high rainfall is likely to cause saturation and therefore saturation excess overland flow and surface erosion (Boorman *et al*. 1995).

Hydrometric information obtained from the previous survey (Cefas, 2010) illustrated that the bay within which the trestles are located will not be subjected to outside contamination as the currents are likely to divert any particles around the site due to the presence of the sand bar (see Figure 1).

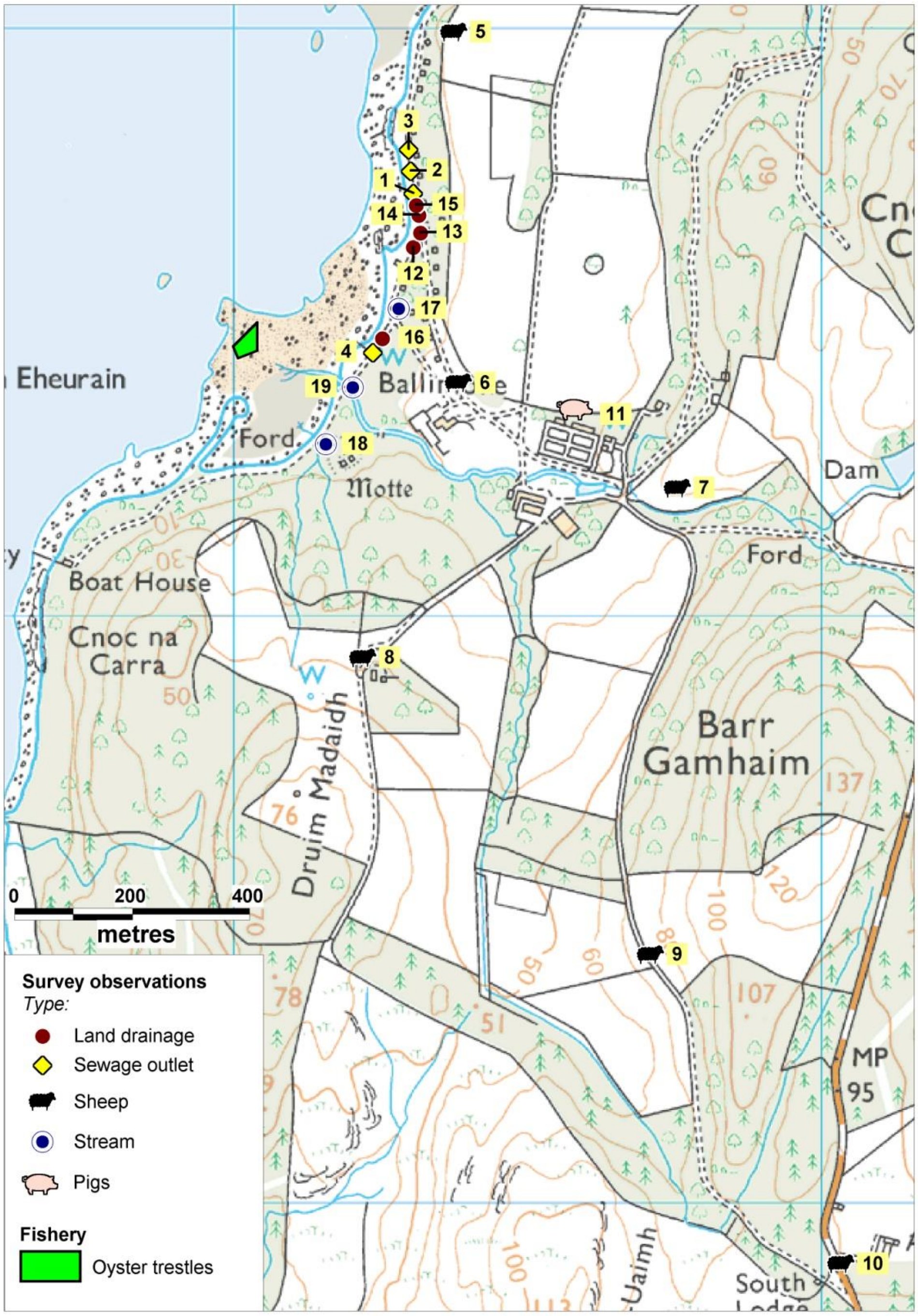


Figure 4 Survey of the catchment undertaken as part of the study which shows the relative contribution of faecal contamination to the fishery. The survey identifies the land drainage and sewage outlets in close proximity to the fishery as well the natural watercourses within the catchment. Position of livestock in the area is also shown to identify the potential sources of contamination to the watercourse or drainage systems.

Table 2 Shoreline observations taken from the present study, supplemented with data from the original shoreline survey that was used to determine the site selection.

Number	Observation	Comments
Field Observations		
1-3	Septic tank discharges	Released onto the shoreline, evident by the presence of sewage sludge, foul smelling puddles on shore and the presence of green growth on the rocks and sewage fungus in the direct line of the output. Water samples could not be taken.
4	Septic tank	Discharge from main estate house, pipe was 15cm in diameter and was flowing 100ml = 10 seconds.
5	Livestock	130 sheep were noted across the upper and lower field east of the recorded position.
6	Livestock	Approximately 35 sheep were recorded east and uphill of this position. Approximately 40 sheep were downhill west of this position.
7	Livestock	Approximately 50 sheep present
8	Livestock	Approximately 30 sheep present
9	Livestock	Approximately 70 sheep west of the recorded position
10	Livestock	Approximately 50 sheep.
11	Pigs	Ten pigs were said to be present on the farm by the owner. The position shown on the map is approximate as the pigs were not seen.
12-15	Land Drainage	Seeps across shoreline, no foul smell present, but green algal covered rocks were seen in the vicinity of the drainage pathways.
Sanitary Survey Observations		
16	Land Drainage	Freshwater drainage closest to the trestles. Insufficient flow for sample to be taken on original survey.
17	Stream	Small stream that runs between the houses and under the road and seeps across foreshore. E. coli levels in water sample from original survey sample = 160(cfu/ 100ml).
18	Stream	Stream measurements and water samples from the original survey indicated that the flow in m/sec per day = 493 and therefore E. coli loading = 1.6×10^9.
19	Stream	Stream measurements and water samples from the original survey indicated that the flow in m/sec per day = 3230 and E. coli loading = 5.2×10^9.

2.3 Site Set-up

Prior to the sampling period, a preliminary visit to the site was undertaken with the aim to discuss the project with the harvester and to determine any differences with the site in comparison to the original survey. This visit was also used to help decide on project set up, site and transport logistics for the study. It was agreed with the harvester to collect approximately 300 wild mussels from the surrounding area and placed them into shellfish bags. Along with the 200 oysters required for the project they were placed on to one upper and one lower trestle (Figure 5) in the direct vicinity of stream one to equilibrate with the environment. The trestles were positioned this way so that the upper trestle could be accessed at higher tidal states. Both trestles were dug into the ground and stabilised with rocks to try and ensure limited movement from any adverse weather conditions and one buoy was attached to each trestle for identification.

DST CTD meters are microprocessor-controlled temperature, depth and conductivity (salinity) recorders with electrodes housed in a waterproof housing. One of these meters was attached to both the upper and lower trestle, positioned halfway down the outside of the middle leg of the trestle. They were positioned this way to try and allow a free flow of the seawater to the sensors. Prior to the sampling period, both CTD meters were set up and given a trial run to decide sufficient intervals of time between recordings and to ensure they were working prior to their deployment in the study. Recordings of conductivity, temperature and depth were taken every 15 minutes during the course of the sampling period.

A weather station was erected at the site (Figure 5) in an area of grassland that was open from trees and buildings. The weather station was set up to include a tipping bucket rain gauge, which was fixed to a flat board in order to keep it stable for accurate measurements and to prevent movement from strong winds. The rain gauge was set to tip every 0.2mm and information was recorded on the Delta T data logger in which it was attached. Other measurements that were recorded in one minute intervals were solar radiation (kWm-2) air temperature (˚C) and relative humidity (%). Both sensors for solar and air were securely attached to a wooden post fixed firmly into the ground. A conventional rain gauge was also placed near the weather station as a back-up for rain measurement and to determine when a high rainfall event had occurred without having to access the weather station data in the field. Rainfall data was also obtained from a United Kingdom Meteorological Office (UKMO) located 7km to the north west of the fishery at Lochgilphead (Figure 1). The rainfall data were available as daily rainfall values for Monday to Thursday and accumulated values Friday – Sunday for the period of the 01/09/2011 – 30/11/2011.

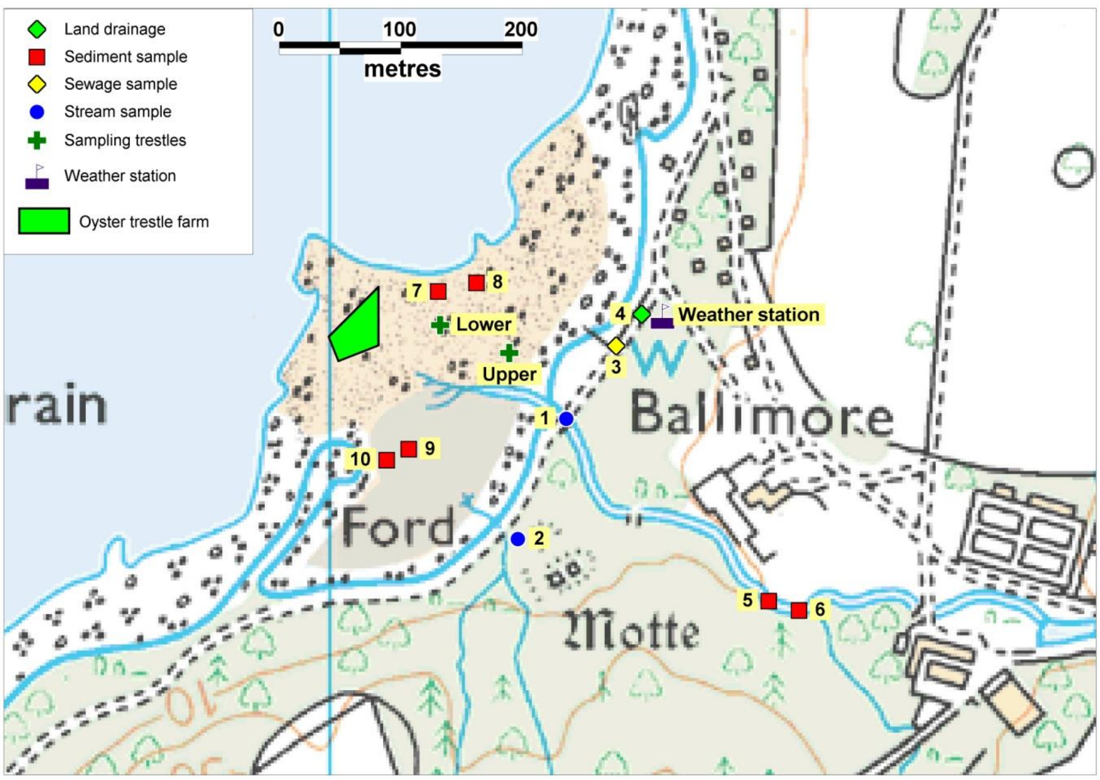


Figure 5 Map of sampling locations. The upper and lower sampling trestles at the site are positioned directly in the path of the stream, which differs to that on the map above. Number 1 = main stream one sampling point. Number 2 = stream two sampling point. Number 3 = Septic tank. Number 4 = freshwater land drainage. Number 5- 6 = fresh sandy sediment samples. Numbers 7 – 8 = marine sandy samples. Numbers 9 – 10 = fresh sandy samples. The weather station and the oyster farm are also labelled.

2.3 Sampling

The project was set up to run for two weeks in which one rainfall event was captured. The rainfall level at which the event was triggered in this study was recorded at 16.8mm, dictated from the previous 12 hours of rainfall on the 29th October 2011 prior to 08:00hours.

Tidal information was acquired from the United Kingdom Hydrographic Office (UKHO) which was consulted to establish dates in which suitable tides would allow greatest access to the shoreline. The information was also required to provide times of high and low tide during the sampling period.

2.3.1 Water quality monitoring

Baseline sampling took place twice daily for stream one (no. 1, Figure 5) stream two (no. 2, Figure 5) and the production area (closest point to the upper or lower trestle depending on where the sample was taken from) and once daily for the freshwater land drainage and septic tank outlet as recorded by GPS and outlined in the site map. In order to determine whether the selected environmental factors present would significantly affect the levels of *E. coli* in both streams one and two as well as influence each other, flow depth, flow rate, turbidity, total suspended solids, temperature, pH and salinity were measured (H1 – H4).

Sampling in both streams took place at a fixed point. For stream one, width was kept constant by choosing a location where the same width was maintained due to a concrete bridge. Pegs were inserted into the ground for the chosen point at stream two to keep consistency in sampling the same area, and to act as a guide for stream width. Depth was recorded in centimetres using the flow meter pole at the deepest part of the channel for stream one and an average depth was taken across stream two. Flow was measured in meters per second, by placing the flow paddle at half the depth of the stream and holding the flow pole steady to get an accurate reading, using the standard deviation of the flow to determine accuracy. Other environmental variables were recorded in situ such as temperature (˚C) and salinity (ppt). Prior to each am/pm sampling, the meter that recorded both variables was calibrated and measurements were taken at the same sampling points.

Two types of water sample were obtained, using sampling pots, the sampling pole and latex gloves. To avoid any contamination between the samples, the pole was rinsed clean or gloves were changed between samples. Water samples were taken prior to any other in stream measurement to also avoid any contamination from the sampler, or disturbance to stream that would otherwise not have been present. One litre water samples (chemical standard) were taken to send for testing within the laboratory, first to measure for turbidity by reporting the Nephelometric Turbidity Units (NTU), total suspended solids (mg/L) and pH. Second 500ml water samples (microbiological) were taken to test for presumptive *E. coli* and presumptive coliforms. Both microbiological and chemical water samples were also taken at the trestle area in order to determine relationships between the environmental factors (rainfall, turbidity, total suspended solids, temperature, salinity and pH) and *E. coli* in seawater (H5, H6)

Sampling took place whilst standing downwind of the tide in order to avoid any contamination from the sampler. Temperature and salinity measurements were taken by using a hand held device with cable attachment; this allowed the sensor to be placed further into the undisturbed stream channel or undisturbed seawater in order to keep measurements as accurate as possible.

The freshwater land drainage was also sampled for both microbiological and chemical standards and flow rate was measured by timing on a stopwatch the amount of time it took in seconds to fill a bucket of a capacity of 7 L, this was repeated three times to gain an average flow rate. The septic tank was only sampled for microbiological analysis and the flow rate taken by timing on a stopwatch as the time in seconds it took to fill a 500ml bottle. The septic tank was sampled last, and placed into a sampling bag to avoid cross contamination and ensuring protective gloves were worn at all times.

Once rainfall had reached its trigger level, the sampling strategy remained the same but sampling took place every hour for both stream one and stream two for seven hours. Water samples from the production area were taken every two hours and stored in cool boxes to keep them at a temperature between 2-8˚C. The freshwater outlet and the septic tank were not sampled. All water samples for both microbiological and chemical analysis were issued with unique sampling numbers from the testing laboratory; these were coupled with another project sample number and recorded with the date, time and location of sample.

2.3.2 Shellfish sampling

The bivalves used in the study were pacific oysters (*Crassostrea gigas*) and common mussels (*Mytilus edulis)*. Samples were taken in a way to ascertain the hypotheses H7 –H10. Both oyster and mussel samples were taken twice daily for all sampling days apart from 31/10/2011 – 03/11/2011 in which only morning samples were taken. Afternoon/ evening samples could not be taken because accessibility to the trestles within daylight hours/ dusk hours was not possible because of the tide. On the rainfall event, samples were taken in the morning and a further two samples from both the lower and upper trestles were taken once the trestles were accessible. Upon collection each one was lightly scrubbed to remove grit and dirt, with care not to re-submerge them into water to promote their opening. They were placed into a sampling bag labelled with the time and date. The shellfish samples were then stored in temperature controlled boxes between the temperature range of 2-8˚C before sending to the lab for testing within 24 hours. Project sample numbers were assigned to both oysters and mussels samples and recorded with the date, time and whether they were from the upper or lower trestle.

2.3.3 Sediment sampling

In order to find out H11 two sediment samples were collected in three different locations as illustrated in Figure 5. The sediment types were; marine mud (no.'s 9/10) and marine sand (no.'s 7/8) and fresh sand (no.'s5/6). The location of the freshwater samples was chosen to be the most accessible point to the bottom sediments along the stream. 20g of surface sediment (1 cm deep) were collected per sample (split into two tubes) by using the tube as a corer and two rulers to guide the depth of the sample. For both marine mud and marine sand, samples were chosen at random taking into account suitable sediment type and ease of accessibility. Gloves were worn to take the samples to avoid cross contamination and placed into separate zip-lock bags and clearly labelled. To establish H12each sampling point was recorded with GPS locations so that the same area could be sampled again. The first lot of sediment samples were taken on the 26/10/2011 and the second on the 31/10/2011. They were assigned project sample numbers and recorded with the date, time and position of sample.

2.4 Laboratory Analysis

2.4.1 Water samples

All microbiological water samples were analysed using the membrane filtration method. Filtration of the 500ml water sample occurred through a 0.45µm pore size membrane filter, in which the filter was then transferred to a selective culture medium and incubated. Presumptive coliforms and *E. coli* recognised by their yellow and green colours respectively were then subjected to further confirmatory tests which include subculturing to Trytone Nutrient Agar including an ONPG disc to test for the expression of B-galactosidae at 37˚C and at 44˚C and testing for indole production at 44˚C. After considering the volume of sample filtered and the number of colonies counted and confirmed, a final confirmed count for both coliform bacteria and *E. coli* was calculated and reported as colony forming units (CFU) per 100ml of water (CFU/100ml).

The chemical analysis of the water samples included three different tests for hydrogen ion (pH), turbidity and total suspended solids. The pH(x) content of the water was determined by measuring the electromotive force (e.m.f.) Ex, of a cell containing the sample and comparing it with the e.m.f of a similar cell, Es in which the sample is replaced by a standard buffer solution. Turbidity was measured by light from a tungsten source scattered by the suspended and/or colloidal material present in the sample and is measured at 90 degrees relevant to the incident beam. The intensity of the light scattered is compared with that measured for standard formazin suspension and expressed as nephelometric turbidity units (NTU). To measure suspended solids, the water sample was filtered through a pre-weighted glass fibre paper under vacuum. The weight of the retained material was then determined by drying at 105 ± 5˚C.

2.4.2 Shellfish samples

The enumeration of *E. coli* in shellfish was conducted by the 5-tube most probable number method. The methodology included the dilution of the shellfish sample by

2ml of peptone water per 1g of shellfish. The samples were homogenised and made into a 10^{-1} and 10^{-2} dilution. Five universals containing 10ml of MMGBx2 placed in the first row and ten universals containing MMGBx1 in the next two rows and inoculated with 10ml of the 10^{-1} dilution in the first row, 1ml of the 10^{-1} dilution in the second and then 1ml of the 10^{-2} dilution in the third. They were then incubated at 37˚C for 44h. Positive results were inoculated onto brilliant green bile broth (BBGB) and incubated at 44± 1˚C for 24 h. Results were reported at the most probable number per 100g of shellfish (MPN/100g).

2.4.3 Sediment samples

The enumeration of *E. coli* required 10g of top 1cm sediment per sample which was mixed with 40ml of 0.1% peptone water and then ultrasonicated. A further 50ml of peptone water was added and mixed before filtering using a 0.45µm pore-size filter and then placed on a tryptone-soy agar plate supplemented with 0.1% yeast extract. The samples were then incubated at 37˚C for three hours. After this period of incubation each membrane was transferred onto a membrane faecal coliform agar and incubated further at 44˚C for 18 h. After incubation any blue colonies present were sub-cultured onto tryptone bile glucuronidae agar (TBGA) plates and incubated at 44˚C for 22 h. *E. coli* results were determined per 100ml of water and reported as colony forming units per 100ml (cfu/100ml). Sediments were not analysed to type in the laboratory.

2.4.4 Transport of samples

Water samples that were collected in the evening were stored overnight in a cool box and shellfish samples were stored in biotherm boxes. They were kept at the recommended temperature of between 2 and 8°C by the use of ice packs. A tube of water was placed in each of the boxes so that the temperature could be checked easily and accurately. Each day along with the samples collected in the morning, both microbiological and chemical water samples were driven to Scottish Water and the shellfish samples to Glasgow Scientific services both situated within 15minutes of each other in Glasgow. Upon arrival at Glasgow Scientific Services the shellfish samples were temperature checked to ensure that on receipt the shellfish were at a temperature of between 2 and 8°C (at no point did they exceed these temperatures). The agreement set up between Cefas and Glasgow Scientific Services was for the samples to be tested as soon as possible after arrival. Water samples that were dropped off at Scottish water were also dealt with on the same day of arrival. The two lots of sediment samples (before and after rainfall) were collected in the morning and sent in biotherm boxes by Royal Mail using their next day special delivery service to Cefas Weymouth for analysis. Samples were tested on the day of arrival.

2.5 Data Analysis

2.5.1 Quality control and exploration of the data

The first step of data analysis was to quality control the data. After inputting all data into a spreadsheet, it was checked for obvious errors and any missing values. All data sets were complete apart from the chemical and microbiological data taken for the land drainage and septic tank on the 30/10/2011.

The Geometric mean, standard error, standard deviation, plus minimum and maximum values were used to provide an overview of the input of *E. coli* into the trestle area from the four sources identified; stream one, stream two, land drainage and the septic tank. Time series chart were then formulated between *E. coli* and each of the environmental factors measured (flow rate, flow depth, turbidity, total suspended solids, temperature and salinity) for both streams one and two. Rainfall data was manipulated into total rainfall per day (mm/d^{-1}) and intensity per hour of rain per day ($mm /hr/ d^{-1}$) and presented as a bar chart to look for relationships between *E. coli* and environmental factors, along with any influence from rainfall. The median values for all the environmental factors were calculated and compared against their minimum and maximum values to show any major differences that may have been influenced by rainfall. Those factors that did not show a relationship with *E. coli* and little difference between median, minimum and maximum values were not considered for any further analysis.

2.5.2 Hypothesis testing using stream data

To test H1, preceding rainfall values were calculated. Rainfall data were calculated into preceding 12 hour (R-12), 24 hour (R-24), 48 hour (R-48) prior to both morning and afternoon sampling. Weighted rainfall values (Rw48) were also calculated for the 48 hour period prior to sampling with the most recent rainfall receiving the highest weight as set out by Francy and Darner (2006). Weighted rainfall was included as it was shown to improve the correlations between *E. coli* and rainfall and so it was pertinent to apply a similar process in this study. Although in this study, it was adjusted to include 12 hour rainfall and the weighted calculation was as follows: Rw48 = (3*R-12 +2*R-24 + R-48).

Rainfall data obtained from Lochgilphead UKMO was used to supplement data from the onsite weather station on and prior to the 25th October 2011 where data was not available. As the data from Lochgilphead was only available in daily not hourly amounts, the previous 24 and 48 hour values for the 26th and 27th October are approximate. Preceding 12 hour rainfall values were not available to compare against *E. coli* in water samples taken on the 25th October and so was not included in the analysis. Prior to any further statistical treatment, *E. coli* data was log base 10 transformed and both sets of data were tested for normality and equal variances using the Kolmogorov-Smirnov test and Levene's test respectively to determine which test could be performed (parametric versus non parametric). As the preceding rainfall values did not conform to a normal distribution, the values were ranked in order to compute a Spearman rank correlation between rainfall values and *E. coli* in both streams one and two. The probability (alpha) level at which significance was determined was 0.05.

To test H2, each of the factors were tested for normality and equal variances with *E. coli*. Those factors that met the assumptions were ranked and computed under Spearman Rank correlation. Total suspended solids and *E. coli* were computed under Pearsons'' correlation, even though total suspended solids did not meet the assumptions of the test. However upon looking at what is known from the literature and the scatter plot of the relationship, the correlation was justified. In order to test

H3, that the environmental factors would significantly impact on each other the same process occurred with Spearman Rank correlations. The correlations between total suspended solids and the other environmental factors were computed as Pearsons' correlations. Regression models were then applied to a further series of hypothesis based on the results from the correlations, to find out which environmental factors could explain the highest amount of *E. coli* in streams one and two. Due to problems with multicollinearity between flow rate and flow depth with rainfall, the first two factors could not be added to the model. Using *E. coli* as the response variable and a series of two environmental factors as the predictor variables (as set out by the hypotheses) the models were assessed by looking at the goodness of fit and significance of the model as shown by analysis of variance. Significance level was set at 0.05. The R-sq (%) value was looked at to determine how much variation could be explained by the particular model. The model which had a significant fit and could explain the highest percentage of variation was then chosen to represent each stream (Dytham, 2003).

Daily faecal loadings from both streams were presented by firstly working out the measured flow per stream per day (m^3/day) which equalled width*depth*flow*86400. Loadings (E. coli cfu/ 100ml/d^{-1}) were then calculated as the amount of *E. coli* in a sample, divided by the value of 0.0001, and then multiplied by the measured flow of the stream per day. As two samples were taken on each day of sampling and a further five on the event day, averages were used as the amount of *E. coli*. These formulae were adapted from those used in sanitary surveys undertaken by Cefas (2010). The faecal loadings of stream one and two were then applied to a T-test to answer H4

To test H5, one way analysis of variance (ANOVA) was computed to test the mean differences in *E. coli* found present in all three sources. All three data sets were log transformed and the assumptions of the one way ANOVA were met prior to computation. A significant fit, as indicated by p = <0.05 was ensued by Tukeys post hoc analysis which confirmed where the significant differences were found between the variables.

2.5.3 Hypothesis testing using seawater and shellfish data

A time series plot was constructed to show *E. coli* levels found in oyster, mussels and seawater, against the total/rainfall intensity chart to look for any visual relationships between the variables. From this, it was hypothesised that there would be a significant relationship between rainfall and levels of *E. coli* in seawater (H6). As it is already known that the rainfall data do not meet the required assumptions, spearman rank correlations were used directly to test for a relationship. The time series plot lead to the same process of generating the hypotheses that there would be a significant relationship found between rainfall and levels of *E. coli* in mussels (H7), and that between rainfall and *E. coli* in Oysters (H9).

The data for *E. coli* in seawater and *E. coli* in mussels were log transformed and met the assumptions to undertake a Pearson correlation to look for a relationship between the two variables. Significant correlations lead to a single regression analysis to conclude H8 that a significant relationship would exist between *E. coli* in seawater and *E. coli* in mussels. The regression was evaluated as done previously, where *E. coli* in mussels is the response variable and *E. coli* in seawater the predictor variable. *E. coli* data for mussels, oysters and seawater were also put into a one way ANOVA to validate H10 . The differences between mean *E. coli* were shown statistically by a Tukeys post hoc test and visually through formulation of a bar chart.

To test H11,a one way ANOVA was performed after a log base 10 transformation of all the samples taken for each sediment type. After a significant difference was found between the mean levels of *E. coli* for each sediment type, a post hoc Tukeys test was computed to determine where the significance was found. The data was then split into values taken before and after rainfall for the three sediment types and a paired T test was computed to determine H12 t. Pre- test checks were computed prior to both the one way ANOVA and T test in order for the parametric tests to be used and significance was measured at the alpha level of 0.05. All statistical analysis was undertaken using Minitab 15 (2010) statistical software and Excel (2007).

3. Results

3.1 Water and Shellfish Quality

A summary of the concentrations of *E. coli* found in the four main sources of pollution identified as stream one, stream two, land drainage (diffuse) and the septic tank output (point) are presented in Table 3. Both streams saw elevated levels of *E. coli*, with considerable differences between minimum and maximum values. *E. coli* Levels found in the land drainage samples were minimal i.e. < 100 (cfu/100ml) and not considered to cause any significant contamination to the water body and therefore were not analysed further. The septic tank discharged high levels of *E. coli* (>10,000 cfu/100ml) as samples were taken only for reference because they are not likely to be affected by rainfall, they were not considered in any further analysis.

Table 3 Geometric mean of *E. coli* results from the four identified areas of faecal contamination (Stream one, stream two, land drainage, septic tank shown in figure. The table shows log standard error/ deviation and the minimum/maximum values of the combined baseline and event samples.

Location	N	Geometric Mean (cfu/100ml)	Log St Error	Log St Dev	Minimum (cfu/100ml)	Maximum (cfu/100ml)
Stream one	25	224.5	0.107	0.533	45	4200
Stream two	25	172.6	0.138	0.689	10	7200
Land Drainage	8	8.64	0.216	0.611	1	100
Septic Tank	7	4.8×10^4	0.191	0.506	11×10^4	2.4×10^5

Stream one and two are two of the main sources of contamination in the area of the trestles and average *E. coli* levels were greater for samples taken from the bigger stream one compared to stream two. Peaks in *E. coli* and peaks in other environmental factors such as flow rate and depth, turbidity and total suspended solids were mostly attributed to peaks in rainfall when scatter plots of these environmental variables against *E. coli* were compared with daily rainfall values. Figure 6 shows an example of the association between flow rate and *E. coli* in stream one (middle) stream two (bottom) with rainfall totals and intensities (top) also shown. The highest total rainfall occurred on the 29th October, with the majority of rainfall taking place in the morning. Stream one responded with maximum flow rate and a corresponding increase in *E. coli* concentrations. Similar results were found for stream two. Neither stream showed an increase in flow rate during the second highest peak in rainfall, which occurred on the 3rd November. Turbidity and total suspended solids for stream one reached their maximum after both peaks in rainfall along with levels of *E. coli.* In stream two, turbidity and total suspended solids only peaked during the main rainfall event but not during the second event. Both streams were at their deepest during the main rainfall event but did respond to the second peak in rainfall. Scatter plots showing these relationships can be found in appendix one.

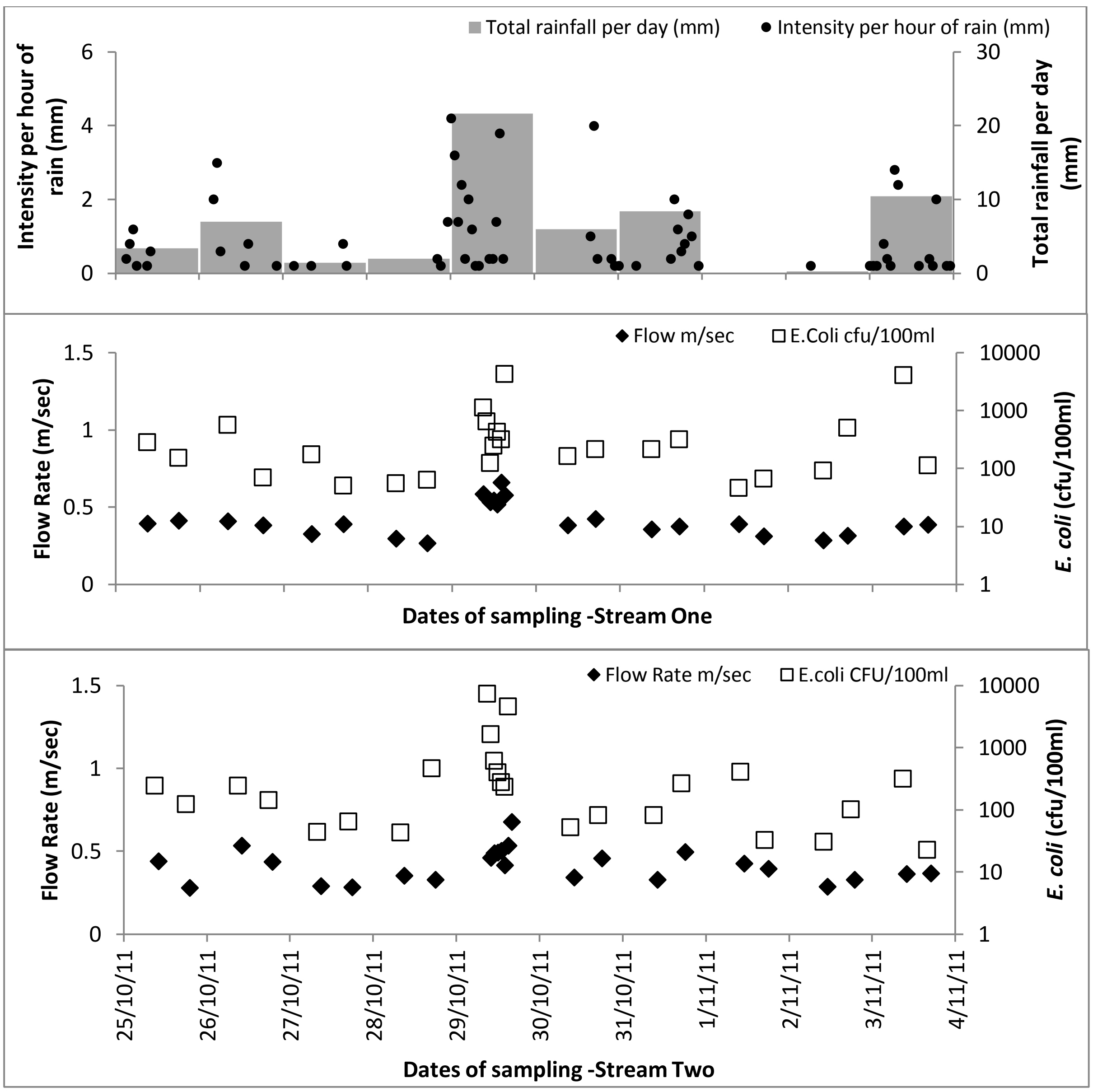


Figure 6 Scatter plot to show the relationship between flow rate and *E. coli* for both stream one (middle) and stream two (bottom). The top graph exhibits the total amounts and differing intensities of daily rainfall.

The median values as shown in Table 4 are representative of the characteristics of both streams under normal conditions as the median number is not influenced by extreme values that have occurred due to intense rainfall. For those variables that are to some extent affected by rainfall the maximum values are considerably higher than the median. The range of temperature readings was small and was not considered likely to affect levels of *E. coli* in either stream over the period covered by the fieldwork. Both salinity and pH decreased to a minimum value after peaks in rainfall. As there was no discernible relationship between temperature, salinity, pH and *E. coli (*appendix one) they were not considered for further analysis.

Table 4 Minimum, median and maximum values of each environmental variable measured in both streams one and two.

	Minimum		Median		Maximum	
	Stream 1	Stream 2	Stream 1	Stream 2	Stream 1	Stream 2
Flow Depth (cm)	11	4.5	18	6	46	13
Flow Rate (m/sec)	0.265	0.277	0.388	0.413	0.659	0.675
Turbidity (NTU)	2.1	0.9	2.7	2.1	24.4	38.4
Total suspended solids (mg/l)	3	3	8	11	56	59
Temperature (˚C)	9.2	9.2	10.5	10.7	11.6	11.6
Salinity (ppt)	0.02	0.02	0.07	0.06	0.08	0.07
pH	7.3	7.3	7.6	7.6	7.7	7.7

To test H1, spearman rank correlations were conducted. The outcome showed that preceding 12, 24 and weighted 48 hour rainfall values were found to be significantly correlated with *E. coli* in both streams, whereas previous 48 hour rainfall was not (Table 5). Stream one exhibited the strongest correlation with preceding 12 hour rainfall whereas stream two responded with a stronger correlation between preceding 24 hour rainfall and *E. coli*.

To H2, both Spearman rank and Pearson's correlations were carried out. Flow rate, flow depth, turbidity and total suspended solids all showed significant correlations with *E. coli* (Table 5). Turbidity displayed a much stronger relationship with *E. coli* in comparison to the other variables in stream one; all other variables were weak to moderately correlated, with preceding 24 hour rainfall displaying the weakest . Interestingly in stream two, turbidity had the weakest and lowest significance compared to the other variables, where preceding 24 hour rainfall exhibited the strongest relationship with *E. coli.* For those relationships that

showed to play a significant role in the levels of *E. coli* found present in both streams, it seemed reasonable to hypothesise that these environmental factors have a marked influence on each other. To test H3Spearman rank correlations were calculated. Significant correlations were seen between the variables as shown in the correlation matrix in Table 6.

A significant relationship between total suspended solids was only permitted between preceding 24 hour rainfall, flow rate and depth in stream two; however a high significant correlation was seen between turbidity and total suspended solids in stream one. Turbidity values were correlated with all other environmental factors.

Table 5. Correlation coefficients between log *E.coli* concentrations and other environmental variables for both stream inputs.

Variables	Stream One		Stream Two	
	Coefficient	**P - Value**	**Coefficient**	**P - Value**
R-12(mm)	0.687	<0.001*	0.644	0.001*
R-24(mm)	0.484	0.019*	0.680	<0.001*
R-48(mm)	0.360	0.091	0.450	0.055
$Rw48^2$(mm)	0.658	0.001*	0.669	<0.001*
Flow Rate (m/sec)	0.542	0.005*	0.598	0.002*
Flow Depth (cm)	0.513	0.004*	0.525	0.007*
Turbidity (NTU)	0.719	<0.001*	0.435	0.030*
TSS (mg/L)	0.558	0.005*	0.534	0.006*

** Significantly correlated with E. coli using Spearman rank correlations, except total suspended solids in which a Pearson's correlation was used.*

Table 6 Spearman rank correlation matrix between the environmental factors.

	R-12		**R-24**		**Rw48**		**Flow Rate (m/sec)**		**Flow Depth (cm)**		**TSS (mg/L)**	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
R-24	**0.79**	**0.79**	-	-	-	-	-	-	-	-	-	-
Rw48	**0.89**	**0.89**	**0.89**	**0.89**	-	-	-	-	-	-	-	-
Flow Rate (m/sec)	**0.81**	**0.68**	**0.78**	**0.77**	**0.86**	**0.75**	-	-	-	-	-	-
Flow Depth (cm)	**0.73**	**0.67**	**0.71**	**0.78**	**0.81**	**0.77**	**0.57**	**0.74**	-	-	-	-
TSS (mg/L)	0.16	0.27	0.13	**0.42**	0.58	0.38	0.05	**0.52**	0.11	**0.70**	-	-
Turbidity (NTU)	**0.72**	**0.52**	**0.55**	**0.54**	**0.65**	**0.70**	**0.56**	**0.60**	**0.49**	**0.46**	**0.78**	**0.81**

Bold *values denote those correlation coefficients that were significant at the 5% level.*

In order to determine which of the variables had the most influence on *E. coli* for both stream one and stream two several hypotheses were generated and statistically analysed using regression analysis. The best fitting model for stream one was identified from the hypothesis that the amount of *E. coli* present in stream one is a function of the two variables; turbidity and preceding 12 hour rainfall. The outcome is presented in Table 7 and shows that, in combination, both these variables explained 68.3% of the *E. coli* in stream one compared to the outcome of other models in (appendix two). For stream two the best fitting model was identified from the hypothesis that the amount of *E. coli* present in stream two is a function of the two variables; preceding 12 hour rainfall and total suspended solids. The outcome (Table 7) shows that both these variables were able to explain the highest amount of variability at 66.5% compared to the other models generated. Due to colinearity between rainfall and flow rate and flow depth, the latter two variables could not be added to the model, however it is likely that both variables would play a significant part in the transport of *E. coli* downstream.

Table 7 Regression analysis showing the variables that could explain the highest amount of variability in *E. coli* for both stream one and stream two.

Predictor	T-Value	P - Value	R- Sq (%)	Anova, F	Anova, P
Stream one					
Constant	19	<0.001			
Turbidity	4.89	<0.001			
Preceding 12 hour rainfall	2.49	0.022	68.3	21.51	<0.001
Stream two					
Constant	10.22	<0.001			
Preceding 12 hour rainfall	4.67	<0.001			
Total suspended solids	2.78	0.012	66.5	19.86	<0.001

The amount of *E. coli* being discharged into the trestle area from both stream one and two was determined by calculating the faecal loading per day for each stream over the sampling period (Table 8). From these values it was hypothesised that stream one discharges a significantly higher loading of *E. coli* than stream two (H4). The resultant t test (t = 7.49, df = 18, p = <0.001) demonstrated that the hypothesis could be accepted, concluding stream one to be a greater source of *E. coli* to the trestle area.

Table 8 Faecal loadings per day for both stream one and stream two.

Date	**Stream one** Loading (*E. coli* cfu/100ml) per day.	**Stream two** Loading (*E. coli* cfu/100ml) per day.
25/10/2011	3.3×10^{12}	1.7×10^{11}
26/10/2011	3×10^{12}	4.9×10^{11}
27/10/2011	1.7×10^{12}	3.9×10^{9}
28/10/2011	3.3×10^{11}	2.3×10^{11}
29/10/2011	3.5×10^{13}	4.2×10^{12}
30/10/2011	2.3×10^{12}	7.4×10^{9}
31/10/2011	2.3×10^{12}	1.9×10^{2}
01/11/2011	4.2×10^{11}	2.5×10^{12}
02/11/2011	1.6×10^{12}	1.3×10^{11}
03/11/2011	2.4×10^{13}	1.75×10^{12}

The level of *E. coli* being discharged from the streams and the actual levels found present in the trestle water samples differed dramatically when *E. coli* numbers were evaluated. Analysis of variance was used to test H5 and the outcome identified significantly lower levels (ANOVA, $F_{2,70} = 8.68$, $p = <0.001$). The geometric mean of *E. coli* for seawater was as low at 53 CFU/100ml compared to stream one (224.5 CFU/100ml) and stream two (172.6 CFU/100ml). The decreased numbers of *E. coli* present in the trestle area indicate that other environmental factors are playing a significant role; however scatter plots did not demonstrate any visual correlations between the environmental factors measured and *E. coli* in seawater apart from preceding rainfall and turbidity (appendix 3). The general trend showed an increase in *E. coli* where there were peaks in rainfall (Figure 7).

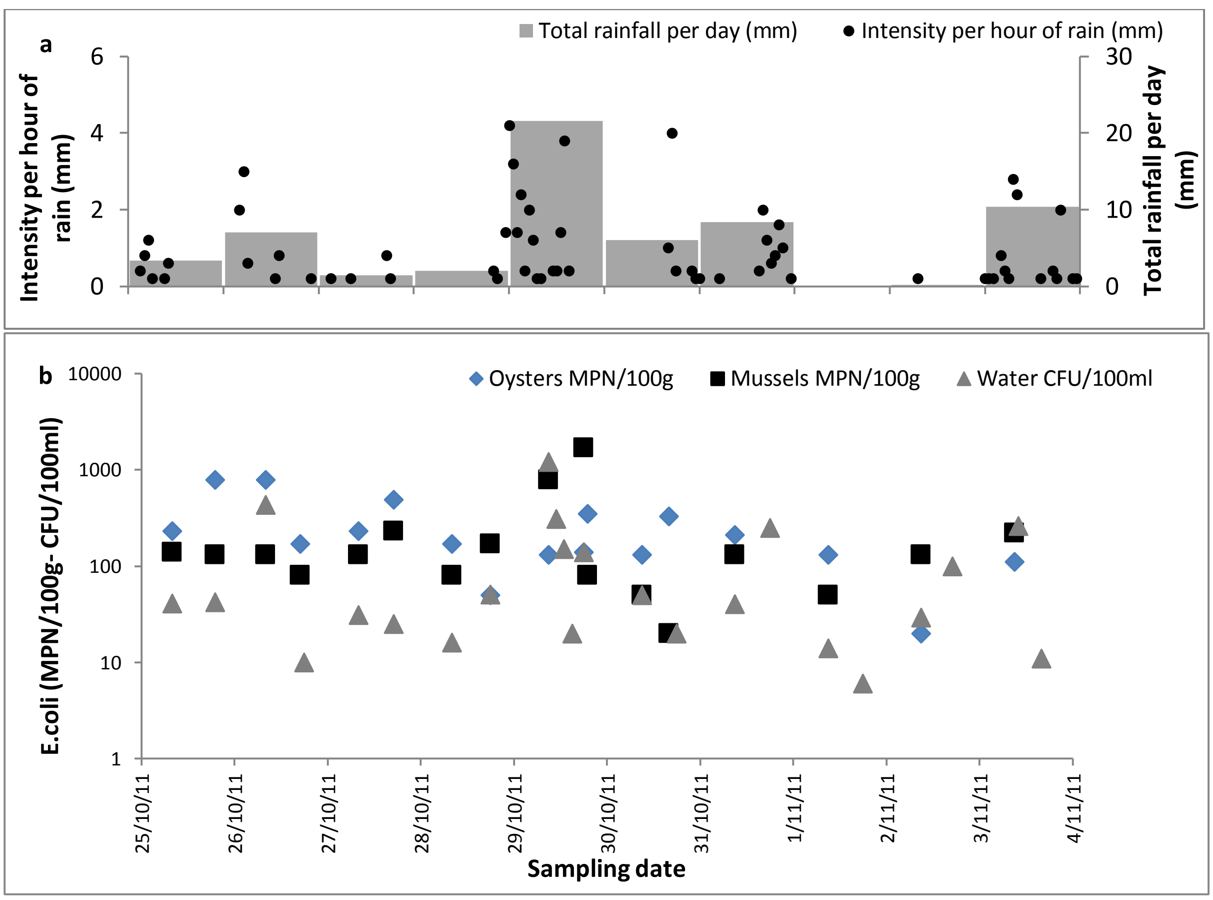


Figure 7 a. Time series plot of total rainfall and rainfall intensity per day for the sampling period of 25/10/2011 to 03/11/2011. **b.** Time series plot of E. coli levels found present in oysters, mussels and seawater for the duration of the sampling period, as above.

The peak in *E. coli* from the seawater samples (1200 CFU/100ml) taken on the rain event of the 29th occurred at the first morning sample in which the associated mussel samples also showed a considerable rise in *E. coli* (790 MPN/100g) (Figure 7). *E. coli* levels in seawater decreased as the sampling continued, it was hard to find a direct association between levels of *E. coli* in seawater and that of Mussels; however during the hours of which the mussels were submerged they accumulated to a peak level of 1700 MPN/100g (samples taken at the upper trestle). *E. coli* concentrations in samples taken from the lower trestles which were submerged for longer had dropped markedly to only 80MPN/100g, exhibiting similar levels to that of the surrounding seawater. From this H6 and H7 were tested in which a Spearman rank correlation showed that seawater was positively correlated with previous 12 hour and weighted 48 hour rainfall, whereas mussels showed no correlation. It was further hypothesised that there would be a significant relationship between *E. coli* levels in mussels and seawater (H8). Spearman rank correlation analysis returned a positive moderate correlation (Table 9).

Table 9. Correlation coefficients between preceding rainfall and levels of *E. coli* in seawater, oysters and mussels.

Variables	Mussels	Oysters	Seawater
R-12[1]	0.426	0.204	**0.530***
R-24[1]	0.118	0.315	0.332
R-48[1]	0.307	0.309	0.079
Rw48[2]	0.168	0.484	**0.458***
Mussels	-	-0.148	**0.595***
Oysters	-	**-**	0.132

***Bold** values denote the correlation coefficients that show the significant relationships.*

Regression analysis was used to further investigate the hypothesis that a relationship would be found between levels of *E. coli* found in seawater and mussels. A significant regression was found (Regression ANOVA, $F_{1,16} = 8.22$, $p = <0.05$; the equation for the fitted line was Mussels = 1.31 + 0.467 seawater). The fitted line only accounted for 35.4% of the variability between the two variables.

Overall oysters exhibited higher levels of *E. coli* than seawater and mussel samples when values were observed on the time series plot (Figure 7). Analysis of variance was used to test H9The results indicate that oysters did contain significantly higher levels than the surrounding seawater but were not significantly different from mussels (ANOVA, $F_{2,48} = 5.61$, $p = <0.05$). Geometric means and standard deviations of the three are shown in Figure 8.

It was originally hypothesised that rainfall would also significantly affect the level of *E. coli* found in oysters (H10). No visual relationship was evident on the time series plot, and spearman rank showed no significant correlation so the hypothesis could be rejected. However, oysters did showed a peak in E. *coli* of 790 MPN/100g in the samples taken on the evening of the 25th and morning samples of the 26th October.

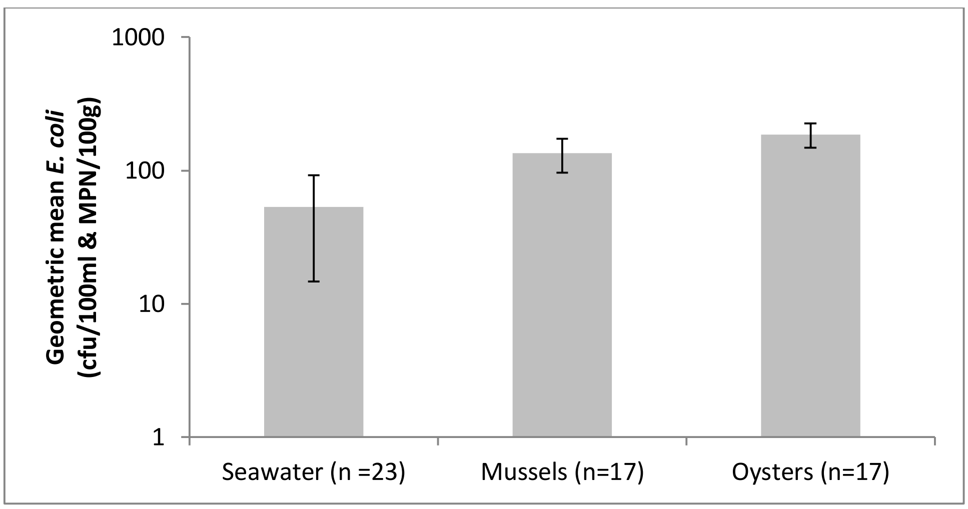
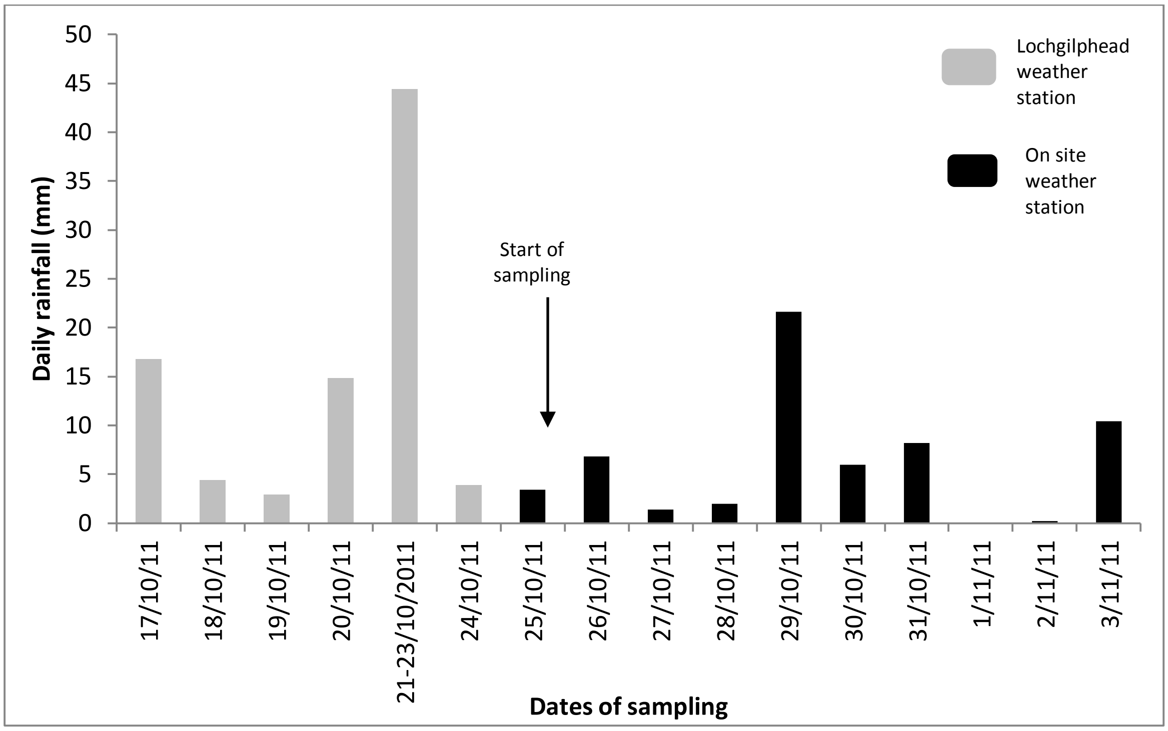


Figure 8 Geometric mean of *E. coli* levels found in the seawater samples and mussel and oyster samples.

Rainfall values for the whole of the sampling period were taken from both the onsite and Lochgilphead weather station (Figure 9) and compared against one another in order to determine reliability of the data used from the onsite station. It was hypothesised that there would be no significant differences between levels of rainfall at the two sites in which a t-test confirmed that rainfall levels recorded at the site were similar to that of the nearby met office station (T test = 0.29, df = 13, p = 0.777).



Lochgilphead data is presented for the eight days (17^{th}-24^{th}) prior to the start of the sampling period which accumulated values were given for the days of the 21^{st} – 23^{rd}.

Figure 9 Daily rainfall values obtained from the Lochgilphead weather station and the onsite weather station.

3.2 Sediments

All three sediment types were found to harbour *E. coli* in which the results are presented in Table 10. To answer H11, Analysis of variance confirmed that the fresh sandy sediments contained significantly higher levels of *E. coli* than both marine sand and marine mud (ANOVA, $F_{2, 21} = 70.21$, $P = <0.001$). The geometric mean and standard deviation for all samples (including before and after) of each sediment type are shown in Table 10.

Table 10 Levels of *E.coli* found present in all three sediment types (marine sand, marine mud and fresh sand).

	Marine sand E. coli (cfu/100ml)	Marine mud E. coli (cfu/100ml)	Fresh sand E. coli (cfu/100ml)
Before rainfall (26/10/2011)			
1	220	1250	6900
2	160	1155	6450
3	160	845	86000
4	250	1004	85000
After rainfall (31/10/2011)			
1	64	250	6600
2	99	329	6250
3	140	460	9550
4	125	575	9500
Geometric Mean	**141**	**635.6**	**1.3×10^3**
Standard Deviation	**60.7**	**383.1**	**3.6×10^3**

Four sets of results were produced for each sediment type for both the before and after rainfall samples. The geometric mean and standard deviation are presented for each of the sediment types results combined.

As *E. coli* levels were found to be present, a further hypothesis H12 was identified.The first set of samples was taken two days prior to the rainfall event on the 26/10/2011 29th and the second set two days after on the 31/10/2011. A t-test showed that differences were found for both the marine sand and marine mud sediments where on average levels of *E. coli* were lower after rainfall. Fresh sandy sediments showed no significant difference in levels before and after rainfall in which results to the t-tests can be seen in Table 11.

Table 11 Mean and standard deviation of *E. coli* found in sediment samples taken before and after a rainfall event.

	SEDIMENT TYPE		
	Fresh Sand	**Marine Mud**	**Marine Sand**

	(cfu/100ml)		(cfu/100ml)		(cfu/100ml)	
	Before	**After**	**Before**	**After**	**Before**	**After**
Mean	46087.5	7975	1063.5	403.5	197.5	107
St Dev	45511.8	1795.6	177.4	143.4	45	33.3
T Value	1.50		3.07		4.97	
DF	6		6		6	
P Value	0.185		0.022		0.003	

3.3 Summary of results

- H1,It was hypothesised that the preceding rainfall would have a significant relationship with the levels of *E. coli* found in both streams one and two in which preceding 12, 24 and weighted 48 hour rainfall values were found to be significantly correlated.
- H2, It was hypothesised that the other environmental factors measured would significantly contribute towards increased levels of *E. coli* in both streams. Results showed that flow rate, flow depth, turbidity and total suspended solids all showed significant correlations with *E. coli*.
- H3, It was further hypothesised that each of the environmental factors measured will show significant positive relationships with one another. All of the factors showed to be significantly correlated, apart from total suspended solids.
- For stream one regression modelling indicated that turbidity and preceding 12 hour rainfall could explain 68.3% of the *E. coli* present.
- For stream two regression modelling indicated that total suspended solids and preceding 12 hour rainfall could explain 66.5% of the *E. coli* present.
- H4, It was hypothesised that stream one discharged a significantly higher loading of *E. coli* than stream two. A t-test confirmed stream one to be higher.
- H5, It was hypothesised that levels of *E. coli* taken from water samples at the trestle area would be significantly lower than samples taken from the streams. The results confirmed that trestle samples were significantly lower.
- H6, It was hypothesised that there would be a significant relationship between rainfall and levels of *E. coli* in seawater. Results showed that preceding 12 and 48 hour weighted rainfall values were correlated with *E. coli* levels in seawater samples.

- H7, It was hypothesised that there would be a significant relationship between rainfall and levels of *E. coli* in mussels. No significant correlation was found.
- H8, It was therefore hypothesised that a significant relationship would exist between the *E. coli* levels in seawater and the levels found present in mussels. A significant correlation was found between the two, regression analysis specified that seawater could only explain 35.4% of the *E. coli* in mussels.
- H9, It was hypothesised that oysters contained significantly higher levels of *E. coli* than mussels and the surrounding seawater.
- H10, It was also hypothesised that rainfall would significantly affect the level of *E. coli* found in oysters. No significant correlation was found.
- H11, It was hypothesised that *E. coli* levels in each sediment type were significantly different from one another. The results showed that fresh sandy sediments contained significantly higher levels than marine sand and marine mud sediments.
- H12, It was further hypothesised that there would be a significant difference between levels of *E. coli* before and after rainfall for all three sediment types. Marine sand and marine muddy sediments were significantly lower after rainfall whereas no difference was seen between fresh sediments.

4. Discussion

The purpose of this study was to achieve an understanding of the association between rainfall and selected environmental factors such as turbidity, total suspended solids, temperature, salinity and pH that could contribute to the faecal contamination of both water and shellfish. Determining this association is important because of the consequences to human health and industry if contaminated shellfish are consumed as detailed in (Wittman and Flick, 1995). In the UK many shellfish farms are in close proximity to livestock areas especially in Scotland and due to increasing farming intensity livestock waste enters the environment through a number of channels which include, grazing livestock, slurry application and farm yard runoff (Oliver et al. 2007). The transfer of this faecal matter into nearby waterways is triggered by rainfall and is modified by a range of environmental factors that are specific to the catchment and occur on land such as soil type and land topography as well as in stream waters. The factors that play a role in this transfer and survival of bacteria, have been identified in several different studies and include but are not limited to temperature, solar radiation and turbidity (Pommepuy et al. 1992; Solic and Krstulovic, 1992; Huey and Myer, 2010). In coastal waters, the survival of bacteria is then determined by factors such as salinity and temperature as governed by the local hydrodynamics and inputs of freshwater (Trousselier et al. 1998; Mallin et al. 2000). These factors also influence the rate of uptake and elimination of *E. coli* in shellfish (Wood, 1957).

In order to capture the effects of rainfall on faecal contamination of shellfish, this research focussed on two stream inputs of diffuse pollution from a small catchment into a coastal area set up with both mussels and oysters. Along with rainfall the selected set of environmental factors (flow rate, flow depth, turbidity, total suspended solids, temperature, salinity and pH) were also sampled to test a series of hypotheses of which the outcomes are discussed below.

4.1 Effects if the selected environmental factors on the levels of E. coli found in streams one and two (addressing hypotheses H1-H4)

The response to high intensity rain that fell on the morning of the 29th October 2012 compared to the lower intensities throughout the sampling period as shown in Figure 7 and Table 5 gave a good indication of how the catchment responds to rainfall. The lower amounts of *E. coli* transferred from land to both streams under low rainfall/ base flow conditions is likely to be a function of subsurface drainage in groundwater (Stevik et al. 2004). Although soil moisture levels were not measured, it is known that the soils were freely draining and highly permeable, meaning that rainwater can infiltrate and transport bacteria through the soil column at a steady pace. The speed at which it is transferred depends on the distance and rate of movement from the source as described by Jamieson *et al* (2002). The first small peak in rainfall (figure 1) did not elicit a corresponding peak in *E. coli* in either stream. The preceding rainfall values were not large enough to cause any significant movement which was confirmed by the lack of increased flow rate in both streams. Under high rainfall/ event based conditions the dramatic increases in *E. coli* in both streams could therefore be attributed to overland flow, controlled by saturation of the soil and topography of the land. Even in freely draining soils, intense rainfall can cause either infiltration excess overland flow or saturated overland flow and initiate the rapid transfer of *E. coli* and rainwater. Tyrell and Quinton (2003) described these transport mechanism involved in overland flow and concluded that it is the major hydrological pathway of faecal bacteria from agricultural lands following rainfall. A study by Vinten *et al*. (2004) looked at soil transport models that assumed all *E. coli* movement from land to river was through soils. They concluded that after rainfall the dominant transport route was as overland flow rather than through the soils as when the soils become saturated they significantly reduce their infiltration capacity and bacteria can no longer move downwards. Any bacteria deposited on the surface are then easily entrained into overland flow. The work of Muirhead *et al* (2006) showed that rapid transfer of *E. coli* occurred when the soil became saturated because the bacteria have less chance to interact and attach to soil particles.

The lands surrounding stream one and two exhibited differing topography, which is shown in Figure 3 and is thought to contribute to the rate at which bacteria was transported after rainfall as overland flow. Collins *et al* (2005) noted that heavy rainfall was a significant contributor to *E. coli* found in a hillside pastoral stream due to the increase in overland flow and steepness of the land. Another study by Abu-Ashour and Lee (2000) compared the level of *E. coli* in the runoff from two different sized slopes after a rainfall event. The runoff from the steeper slope contained more *E. coli* than the shallower slope and *E. coli* was found for much longer distances downhill. In addition, rainwater washed from impervious areas such as farm buildings and track areas would reach the stream at a much faster rate because of the gravel paths and paved areas. These areas would promote surface runoff due to less soil being available for infiltration into the ground. Several studies (Mallin *et al*. 2001) have indicated that impervious cover is a major contributor to increases in the rate of overland flow after rainfall and the subsequent contamination to nearby streams.

The combination of surface runoff and sloping of the land could explain why stream one had a stronger correlation between *E. coli* and preceding 12 hour rainfall. In stream two it is likely that preceding 24 hour rainfall had a stronger relationship with *E. coli* due to the soil characteristics and the ability to withstand low intensity rainfall. Towards the end of the sampling period a further smaller peak in rainfall confirmed these relationships where a peak of *E. coli* was seen in stream one but not in stream two. The intensity of rainfall was lower and so *E. coli* found in stream one could be attributed to the quick response of surface runoff to the preceding 12 hour rainfall rather than overland flow. Minimal rainfall fell in the previous two days to this peak in rainfall, which would have contributed to drying up of the soils and therefore they would be less susceptible to saturation and saturation excess overland flow. This could indicate why stream two did not show this second peak in *E. coli*. However, the preceding 12 hour rainfall was the factor that in regression modelling (Table 7) could explain most of the variance in *E. coli* values in both streams.

Of all the environmental factors measured, flow rate, flow depth, turbidity and total suspended solids were the factors which displayed significant relationships with *E. coli* as shown in (Table 5). The rate of flow and flow depth are a function of the influx of water coming off the land after rainfall. Crowther *et al* (2003) noted that faecal indicator concentrations were higher at high flow compared to base flow because a higher proportion of land generated surface runoff. Depending on the state in which the *E. coli* exists (attached to soil particles or free in suspension) high flow and depth can reduce the amount of sedimentation that occurs as it is transferred from land into stream waters. Along with freely suspended bacteria already in the water column, high flow rates also transfer the bacteria downstream at a much faster rate, thus reducing the amount of time available for bacterial cell die off (Jamieson et al. 2005; Pachepsky et al. 2006). Considering high levels of *E. coli* were found in stream one bed sediments it is possible that re-suspension under event conditions is in part responsible for the rise in *E. coli*. The work of Wilkinson et al. (1995; 2006) and Barillier et al. (1993) showed that increasing flow rate in an agricultural stream by releasing water from a reservoir after a series of dry days still lead to peak concentrations in faecal coliforms. The rise in faecal concentrations coincided with the rising flow rate as more coliforms became entrained in the turbulent waters. They found rapid decline in concentrations after peak flow as coliforms were washed or redistributed downstream. The authors concluded that high flow caused by rainfall events is likely to cause these episodic loadings from stream bed sediments.

Turbidity was significantly correlated with *E. coli* in both streams and in stream one showed to be one of the factors that could explain most of the variability of the *E. coli* found as shown in the regression analysis in Table 7. Collins (2003) found turbidity to be a strong predictor of *E. coli* across all 73 streams sampled within one region of New Zealand. Strong correlations were also seen between *E. coli* and turbidity for all sites sampled in a study by Huey and Myer (2010) and Reeves et al. 2004). In this study the high levels of turbidity is likely to be a result of both erosion and re-suspension of sediments that occur after intense rainfall which is indicated by the significant correlations seen between rainfall and turbidity in both streams. As described previously the increase in flow rate and depth in both streams from antecedent rainfall is a considerable contributor to erosion from both surface and subsurface pathways as illustrated in a study by Deasy et al. (2009). Sediment loading and re-suspension of stream channel sediment creating fluxes in stream turbidity has been identified by several authors to severely impact water quality (McKergrow and Davies-Coley).

The correlation between TSS and *E. coli* was shown to be significant in both streams which are in accordance with research by Murray et al. (2001) and Irvine et al. (2003). There are several possibilities as to why turbidity and TSS influence *E. coli* and these are the association with sediment particles that provide a place of attachment to free living bacteria (Jamieson et al, 2005), protection from solar radiation (Alkan et al. 1995). Protection from other predacious organisms also occurs as attachment to sediment particles increases their size and improves their chance of survival (Roper and Marshall, 1979). Total suspended solids are also made up of nutrients and therefore available food to bacteria (Davies et al. 1995). Rapid increases of TSS also cause eutrophication which will also increase turbidity levels within stream waters (Rast and Thornton, 1996).

Stream one contained higher levels of *E. coli* and a higher daily faecal loading as shown in Table 8 because it was at higher risk from contamination due to the close proximity of the farm buildings and increased numbers of grazing livestock. Run off from farm buildings have been shown to cause significant impairment to stream water quality. In a study by Edward et al. (2008) storm water runoff from farm roofs, tracks and animal collection areas was considered to be a long term source of faecal contamination to stream headwaters as response to rainfall of both high and low intensities. Extreme rainfall events caused gross contamination to stream waters, but lower intensity rainfall also caused a gradual input of bacteria and other water quality problems. Stream one is bigger and has a tributary that runs through a valley into it as shown in Figure 4. Approximately 70 sheep were recorded to the top of this tributary (no. 9. Figure 4) and therefore, it is likely to be a big contributor to the faecal loadings in stream one especially due to the lie of the land and movement after rainfall. The livestock at no. 7 (Figure 4) also had direct access to the stream, although it is not likely for livestock to enter the stream due to its position. The lack of boundary has been shown by Collins (2005) and Collins and Rutherford (2004) to be significant to water quality because there is no filter strip for run off from the land into the stream. Compared to stream two, there were lower numbers of livestock in the surrounding area of the stream and no other sources found during the survey to increase faecal loadings in stream two.

4.2 Effects of selected environmental factors on the levels of *E. coli* in seawater and the uptake in oysters and mussels (addressing hypotheses H5-H10).

Although seawater did not return any significant relationships between *E. coli* and the other environmental factors apart from rainfall, it is thought that significantly lower levels of *E. coli* found in seawater samples compared to the stream is attributed to a number of characteristics. The work of Wcislo and Chrost (2000) and Rozen and Belkin (2001) illustrate a few ideas that could account for this drop in *E. coli*. Firstly, the changes in salinity from stream waters of around 0.06 – 0.07 ppt to seawater at around 26ppt which could account for a certain amount of osmotic shock and therefore reduce the number of *E. coli* to considerably lower levels compared to the streams. However, *E. coli* do have the ability to osmoregulate which help them to cope with osmotic shock and aid survival which would indicate why some *E. coli* are still found present as shown by Pommepuy et al. (1992). The effects of salinity on bacterial survival are explained in further detail in section 4.4 of the literature review, but in brief, high salinities cause lethal stress in bacterial cells through rapid changes in internal solute concentrations and turgor pressure resulting in cell die off.

The significant relationship between rainfall and *E. coli* in seawater is due to the inputs from both streams after the rainfall event, both in terms of faecal loadings and the increasing amount of freshwater entering the trestle area. Carlucci et al (1961) noted that 25% seawater was the optimum level in which maximum number of *E. coli* survived, so the mixing of freshwater to seawater is likely to provide better conditions for survival. Other factors which include a certain amount of dilution and dispersion upon water entering into the trestle area could account for the overall reduction in *E. coli*. Tidal influences were not analysed in this study but it is likely that they would have contributed to the dilution and mixing of *E. coli* as Riou et al. (2007) found that the tidal flushing and the dilution were the main reasons behind the drop in contamination of *E. coli* when a rainfall event caused increased flow into a shellfish production area from a series of adjoining tributaries.

The significant relationship seen between *E. coli* in mussels and seawater (H8) is in agreement with studies by Brock et al (1985) Sasikumar and Krishnamoorthy (2010) who both found linear relationships between *E. coli* in seawater samples and mussel samples taken on the same day. Overall their findings showed that mussels maintained higher concentrations of *E. coli* than the surrounding seawater as did the results in this study. Oysters were also found to have significantly higher concentrations of *E. coli* than the surrounding seawater; however the relationship was not linear (H9). The similarities and differences between species are attributed to their feeding processes and their response and tolerance to changes in the external environment.

Oysters and mussels filter particles, including bacteria that are carried in suspension in the water column; the level of accumulated bacteria in the gut is a function of the filtration efficiency, capacity of the pump which is affected by several factors which include the concentration of food in the water column (Jorgenson, 1990; 1996) and environmental factors which alter their behavioural responses such as temperature and salinity (Wood, 1957). Both salinity and temperature remained fairly constant throughout the baseline sampling in which salinity recorded a median value of 26ppt and temperature remained at between 10 and 12°C. Mussels are able to filter at a wide range of salinities if they are acclimatised to their surroundings. Bohle (1972) showed that after a period of acclimatisation mussels were able to fully open their valves and filter to a lower salinity of 75% seawater (26ppt) to the same capacity and constant rate as full strength seawater. Laing and Spencer (2006) also noted that for mussel's filtration occurred at salinities between 20 and 35ppt. The same occurs with acclimatisation to temperature, after a period of acclimatisation the temperature range at which mussels are able to filter feed is between 4.1 -18°C with their temperature tolerance being dictated by the temperature of their natural surroundings (Kittner and Riisguard, 2005). This suggests that in terms of salinity and temperature the two major factors controlling filtration rates, mussels in this study were under conditions which would not be inhibitive and could filter at full capacity. The rate at which mussels uptake and eliminate bacteria has been shown by Wood (1957) at temperatures above 10°C mussels were able to purify themselves within 1 hour of contamination. This is evident from the difference between the samples taken at the upper and lower trestles on the second sample collection of the event day. After being submerged for 6 hours due to the tide E. coli concentrations at each trestle were markedly different (Figure 3). The point at which the mussels had stopped feeding from the upper trestle as the tide receded to the point in which the mussels stopped feeding at the lower trestle a distance of approximately five metres meant that they were able to filter out higher quantities of bacteria. Alternatively the differences seen between the upper and lower trestle could be due to the fact the upper trestle was closer to the contamination source of stream one and so the mussels were subjected to higher amounts of contamination and therefore were likely to accumulate more *E. coli*. Mussels did not show a significant relationship with rainfall (H7) which could be due to the limited number of samples taken on the event day and the missing samples that occurred towards the end of the sampling period. However, the spike in contamination on the event day and the significant relation between *E. coli* in mussels and *E. coli* in seawater suggests that there is an indirect relationship present.

A study by Younger and Reese (2011) showed that average *E. coli* accumulation in mussels is one to two times greater than in oysters and is consistent with higher filtration activities (Campos and Kershaw, 2012) (Gerdes, 1983) linked to their higher tolerance to changes in external conditions, where oysters are more susceptible (Gosling, 2005). This is evident for oyster samples taken under event conditions where they failed to respond to any increases in *E. coli* from the influence of rainfall (H10) whereas mussels did. Salinity was unlikely to inhibit the filtration process as Laing and Spencer (1996) noted that pacific oysters preferred salinity levels of 25ppt, however as the median salinity value was 26ppt any lower salinity values could mean a decrease in the rate of filtration. At one sampling stage during the event day, salinity dropped to 15ppt but increased at the next stage to 25ppt these fluctuations in salinity may cause oyster pump valves to close temporarily in order to mitigate the effects of low salinity, thus filtration rates are not constant. The range in water temperature was also not considered to significantly affect the oyster's behaviour even though it was in the lower limiting end of their filtration capacity as in a study by Pauly et al. (1998) filtration rates were limiting at 10°C and under. However, in contrast Wood (1957) noted that filtration rates increased dramatically in the range of 10-18°C. Under optimum conditions the time taken for oysters to accumulate and eliminate bacteria is approximately 6 hours. It is likely that this slower rate is the reason why oysters did not show any peaks in *E. coli* during the rain event as the influence of the tide and the number of samples taken on this day was limiting. A second rain event with measures to take shellfish samples throughout the tidal cycle may have shown a better response between oysters and rainfall as oysters did show a peak in *E. coli* at the beginning of the sampling (25^{th} /26^{th} October, Figure 7a) which could have been influenced by rainfall. As only accumulated values were available for the previous 3 days of the weekend (Figure 9), preceding 12 hour rainfall analysis was not included for the 25^{th} and values for the 26^{th} were approximate it was hard to find a statistical relationship as rainfall intensities were not clear. However, this spike in *E. coli* for oysters could be further evidence that they take longer to respond to a rainfall event compared with mussels who could have already filtered through any bacteria present in the water.

Another factor that may contribute to the concentration levels found in oysters is turbidity and total suspended solids. Even though no relationship was found between *E. coli* in oysters and turbidity and TSS, the fact that both levels were found to be higher during the event day could indicate that oysters were feeding on other suspended matter in the water column which make up natural food sources. Filter feeding bivalves are not selective in terms of bacteria, they feed by actively sorting particles of different sizes (Cefas, 2008) and therefore the ratio of particle density to bacterial density in the water column could ultimately influence what is available for the oysters (as well as mussels) to uptake (Plusquelle et al. 1990) (Barille et al. 1997). As oysters filter more slowly than mussels, the amount of bacteria passing through alongside other suspended particles may be less than what mussels are able to process within a six hour tidal cycle.

4.3 Sediments and E. coli levels, before and after rainfall (addressing hypotheses H11 – H12)

All sediments were shown to harbour *E. coli* which is in accordance with several other studies (Gerba and McLeod, 1976; Haller et al. 2009; Craig et al. 2001). The levels of *E. coli* found in stream one were significantly higher than those found in marine mud and marine sand (Table 10) which is attributed to the higher number of sources but also the processes involved in the transport and deposition of the bacteria into the stream but also the sediment characteristics that promote survival. The transport process of *E. coli* into stream one may be as free bacteria or attached to soil or manure particles through overland and subsurface runoff. *E. coli* already attached to particles in run off from the farm yard could also contribute to the *E. coli* concentrations in stream sediments (Steets and Holden, 2003). The survival of *E. coli* in sediments is attributed to the protection from both solar radiation (Davies et al. 1995) and predation (Roper and Marshall, 1979) and the organic and nutrient content (Brown et al. 1977; Ghoul et al. 1990). Marine mud and marine sand contained significantly lower levels of *E. coli* because survival rates in seawater are much lower as described in Pommepuy et al. (1992) and due to tidal movements natural settling velocities would be higher. It is also possible that the number of samples were too few to be entirely representative of all three areas and the amount of *E. coli* being discharged into stream one were a lot higher than receiving waters for both the marine mud and marine sediments due to cell die off and the other environmental factors mentioned.

Analysis of before and after samples showed that both the marine sediments were significantly lower after rainfall, however, due to the small amount of samples and the time between the first sediment sample and the second after rainfall the results may just be a response of natural variation in the sediment levels. No significant difference was seen in the fresh sediments before and after rainfall (Table 11), the area which is most likely to be affected by changes in volume and velocity of water entering the stream channel and therefore re-suspension of sediment associated bacteria. Sediment samples were taken as an indicator as to whether sediments in this area could be contributing to concentration of *E. coli* in both stream water and sea water in this particular catchment. As several authors have shown that sediments have contributed significantly to water quality problems (Bai and Lung, 2005; Gazio-Hadzick et al. 2010). It is thought that rainfall does not show a significant influence on stream bed sediments in this particular study, but may contribute to concentration in the trestle area. A previous study by Martinez-Manzanares et al. (1992) illustrated an association between faecal pollution in marine sediments and seawater but no association between faecal pollution and shellfish could be determined. Therefore, especially with regards to this study further in depth analysis is required.

5. Conclusions

The findings of this research aid in the understanding of how rainfall and other environmental factors are able to influence bacterial contamination of water and the subsequent uptake by shellfish. By focusing on diffuse pollution as a source of contamination to stream waters that enter into coastal shellfish areas (a common situation for many rural locations), this research is able to show original contributions of the environmental factors involved as the faecal indicator bacteria is transferred from its source to the accumulated levels found in both oysters and mussels.
Regression modelling showed that preceding rainfall, turbidity and total suspended solids were the most influential factors on *E. coli* found in stream waters, which has provided further evidence towards previously suggested ideas that turbidity could be a useful indicator of bacterial water quality and of total suspended solids because of the strong association seen between them. Intensity of rainfall has clearly shown to be the trigger of the environmental factors that significantly affect *E. coli* in stream waters and is responsible for peaks in concentrations. Intensity of rainfall and turbidity are therefore two factors which could allow for potential small scale assessment on water quality monitoring.
The microbiological monitoring of mussels showed that they exhibit similar levels of *E. coli* to the surrounding seawater, which confirms that the filtration rate of mussels is fairly rapid under suitable conditions. Because of this relationship and the significance found between rainfall and *E. coli* in seawater, with more advanced monitoring a direct association may be found between *E. coli* contaminations in mussels after rainfall. The results from this study could not find a significant association between *E. coli* in oysters and *E. coli* in the surrounding seawater or between preceding rainfall values. However, it was shown that microbiological monitoring of oysters requires a longer period of study of more than one rain event and round the clock sampling to try and capture the oyster's response to rainfall. The main findings from the sediment analysis revealed that freshwater sediments contained a high level of *E. coli* which confirms the role of sediments as sources of bacteria. Although lower levels were found in both marine mud and marine sand sediments, the results lead the way for

further research and more comprehensive sampling between *E. coli* and sediment type in the future.

6. Limitations to study and further work

The study incurred a few limitations which could have potentially enhanced the outcome of the research. On the day of the rain event the rising limb of the hydrograph for both streams was not captured, due to reasons that could not be controlled by the sampler. This meant that the response time between rainfall and the steady increase in *E. coli* concentrations in both streams was not determined. Similarly the falling limb back to baseline concentrations was also not captured due to the restriction on number of samples. In order for this to be accomplished in the future, a telemetry system would enable efficient stream water sampling.

A similar occurrence was seen with the rise in *E. coli* concentrations in seawater and shellfish. Limitations here were not being able to access the trestles due to the tide. This meant that in the hours the oysters and mussels were feeding the rise and fall in *E. coli* concentrations could not be systematically monitored. Future monitoring of mussels and especially oysters would require an arrangement where they could be accessed at all times. One factor that was not measured directly in this study was the influence of the tide on *E. coli* concentrations in shellfish which may have helped link the association between rainfall and *E. coli* uptake. As different shellfish farming areas vary in their environmental conditions and harvesting set up, future work would require the use of several sites with varying characteristics in order to compare those factors that are thought to be of most significance.

Limitations to the sediment study were found due to cost, which made it difficult to show variation across the sediment types. It was also not possible to gain comparisons between *E. coli* content in sediment and the overlying water. Both these factors could have potentially contributed to the understanding between rainfall and water quality with sediment as the source of contamination and is an important factor that could be considered further in water and shellfish quality monitoring.

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Appendix one

Scatter plots to show relationship between *E. coli* and the remaining environmental factors in streams one and two.

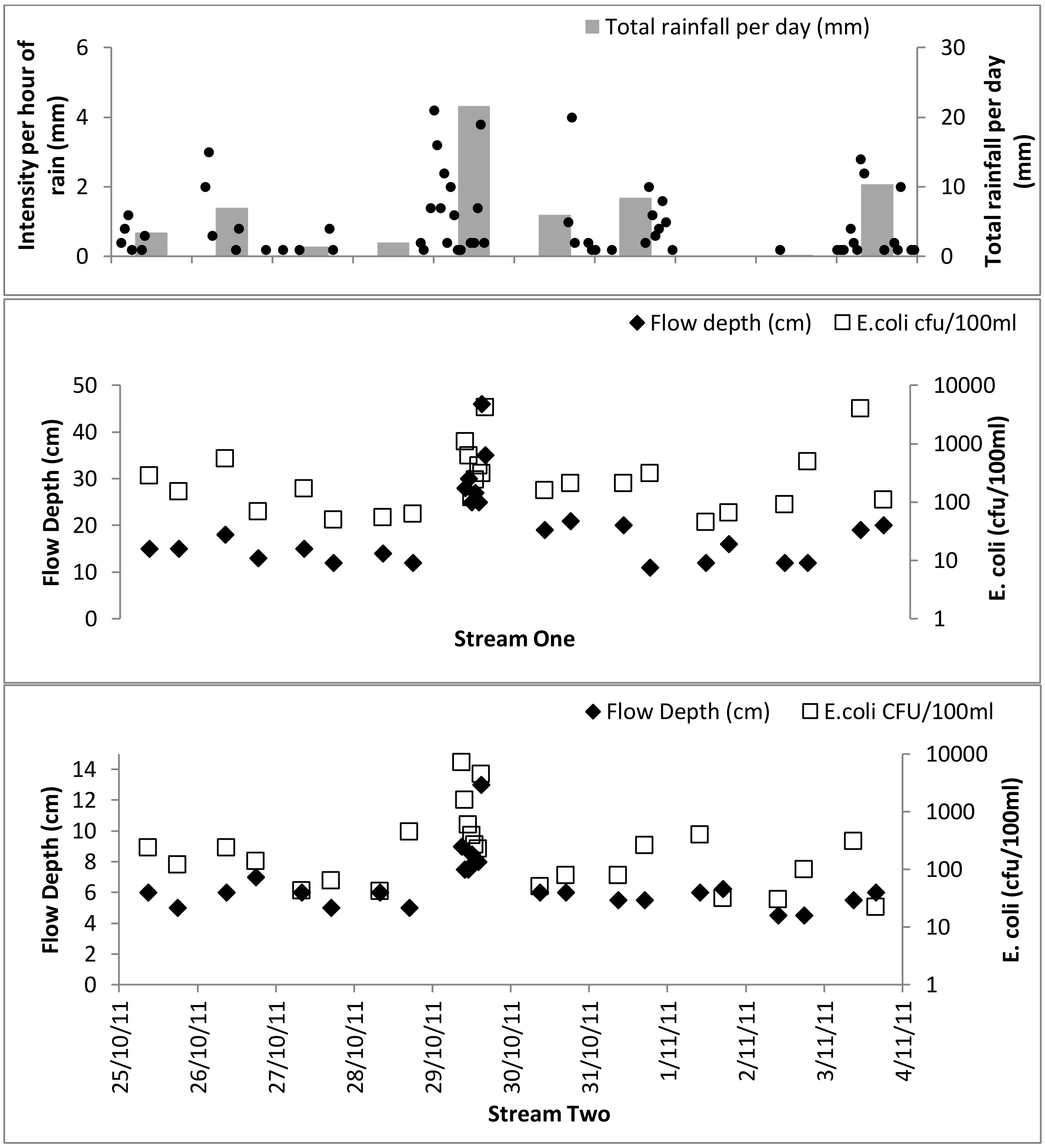

Figure 1 Scatter plot to show the relationship between flow depth (cm) and *E. coli* for both streams one (middle) and stream two (bottom). The top graph exhibits the total and differing intensities of daily rainfall.

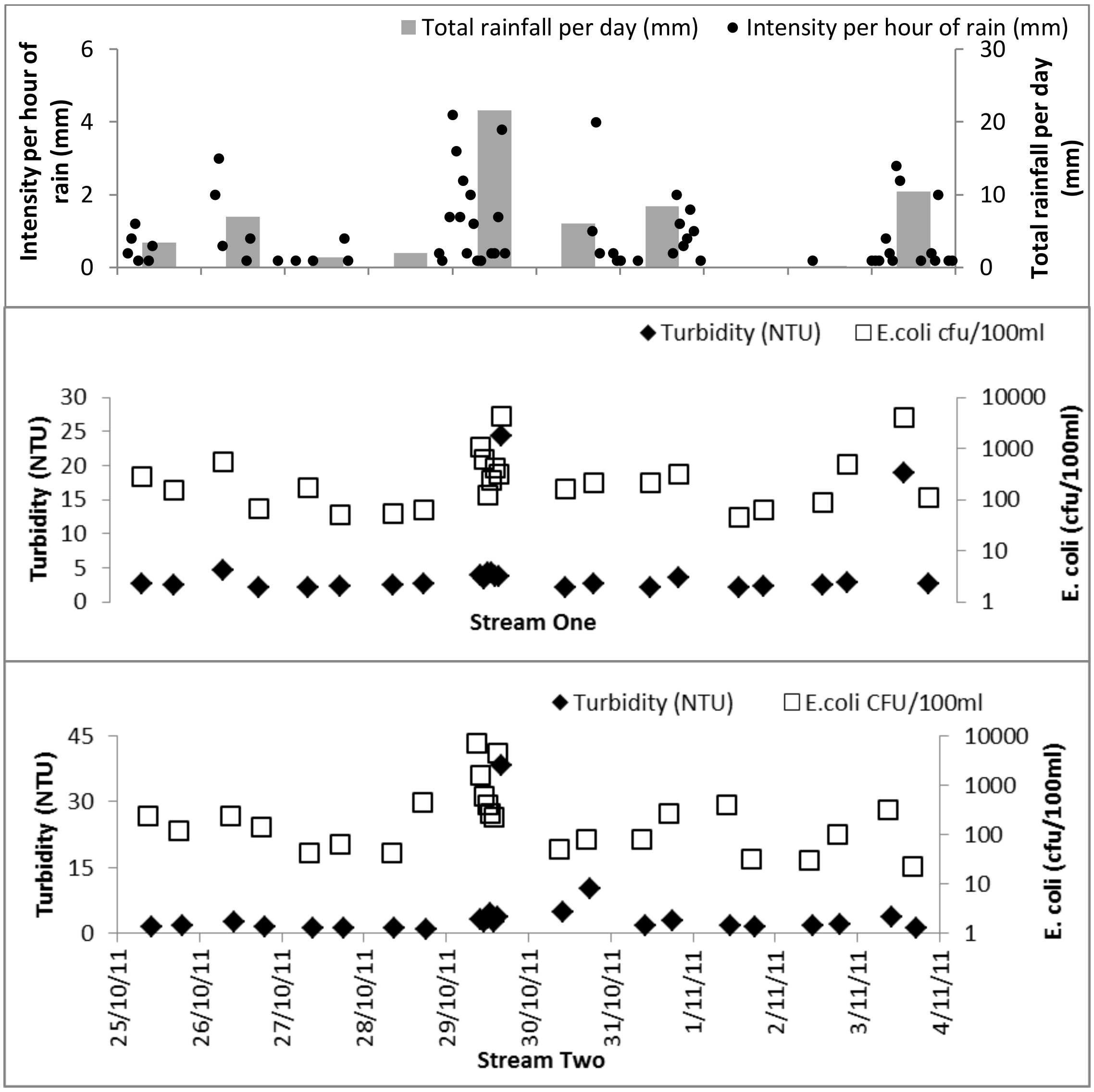

Figure 2 Time series plot to show the relationship between turbidity and *E. coli* for both stream one (middle) and stream two (bottom). The top graph shows the total and intensity of rainfall per day.

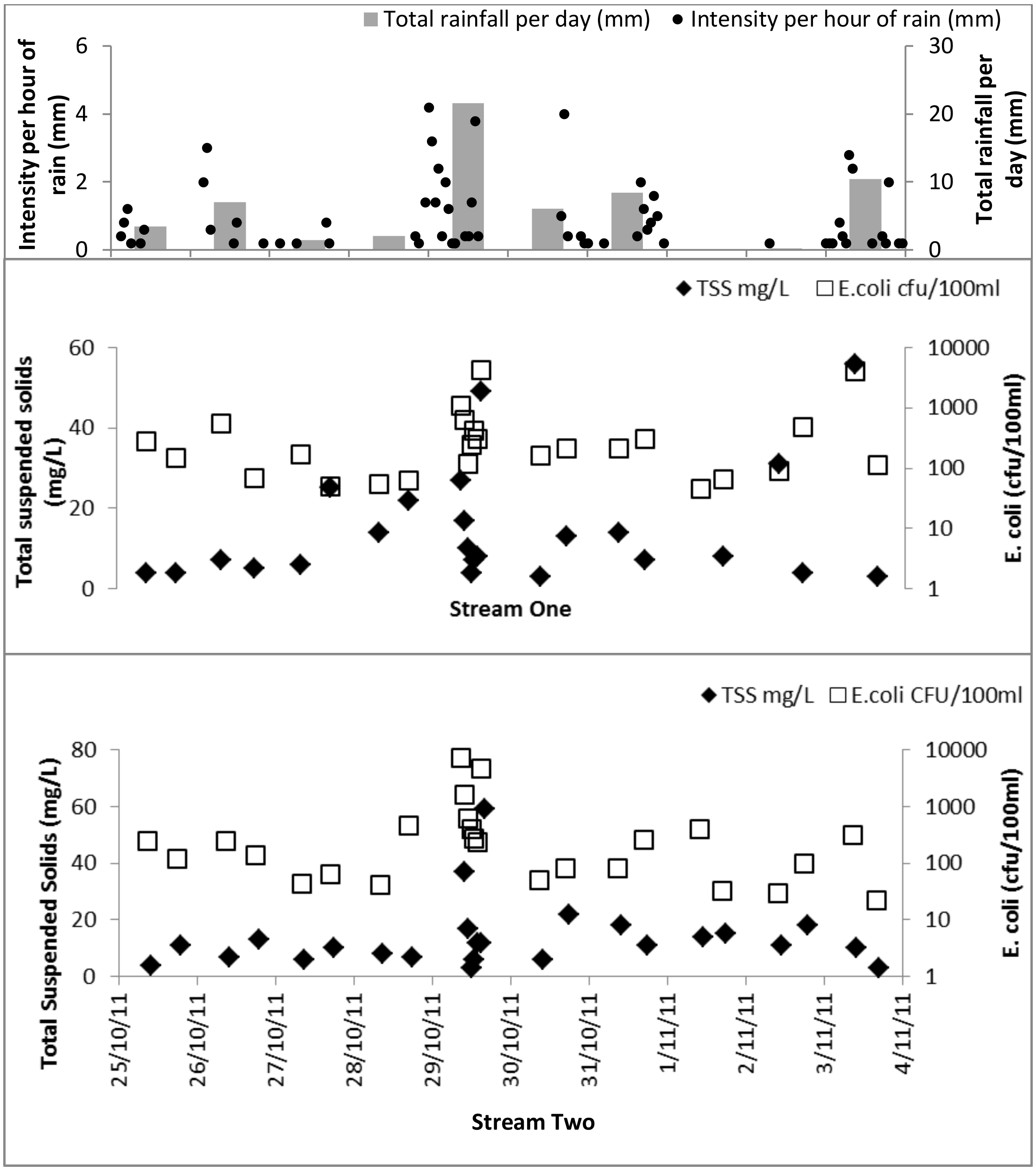

Figure 3 Time series plot to show the relationship between total suspended solids and *E. coli* for both stream one (middle) and stream two (bottom). The top graph shows the total and intensity of rainfall per day.

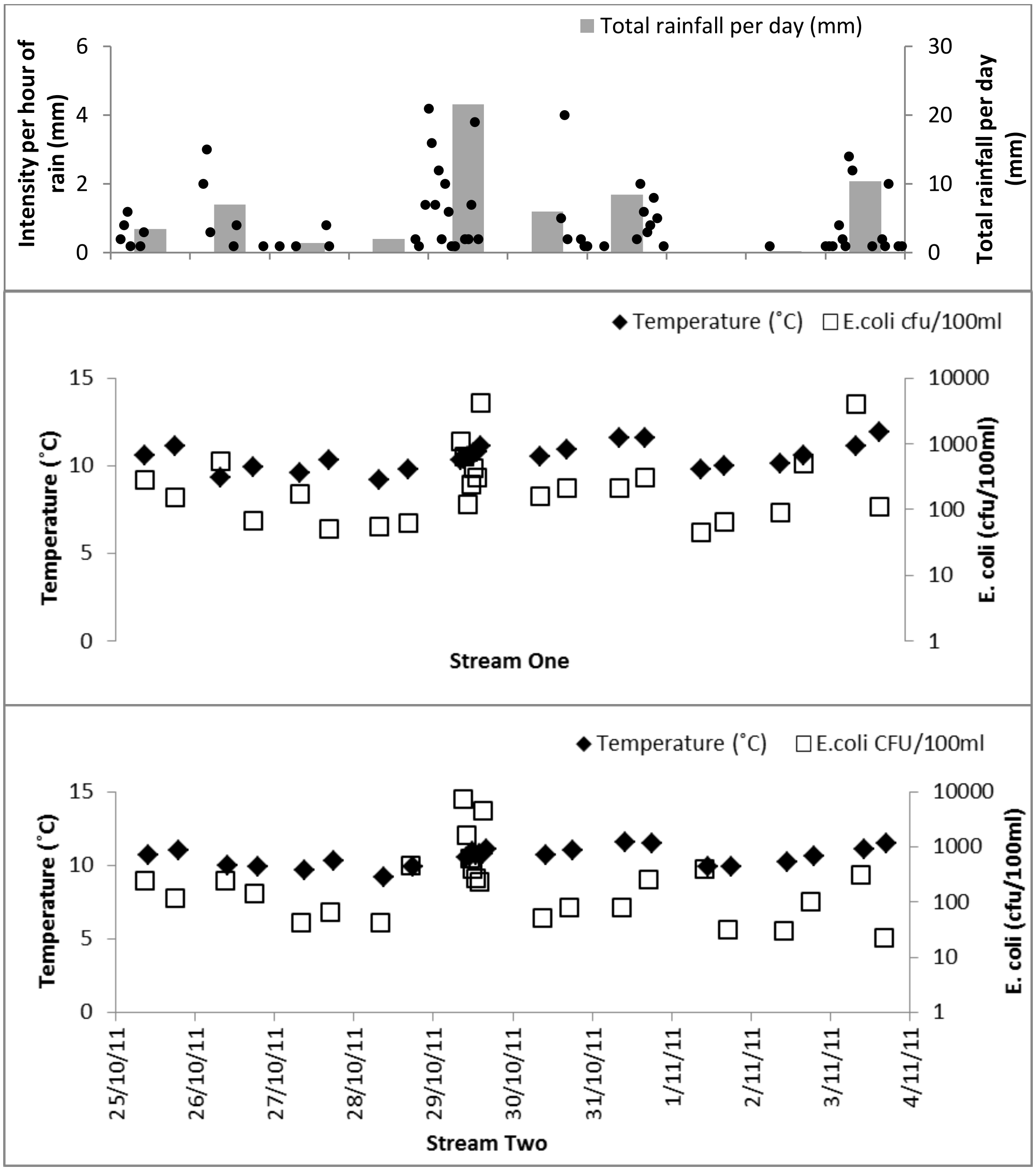

Figure 4 Time series plot to show the relationship between temperature and *E. coli* for both stream one (middle) and stream two (bottom). The top graph shows the total and intensity of rainfall per day.

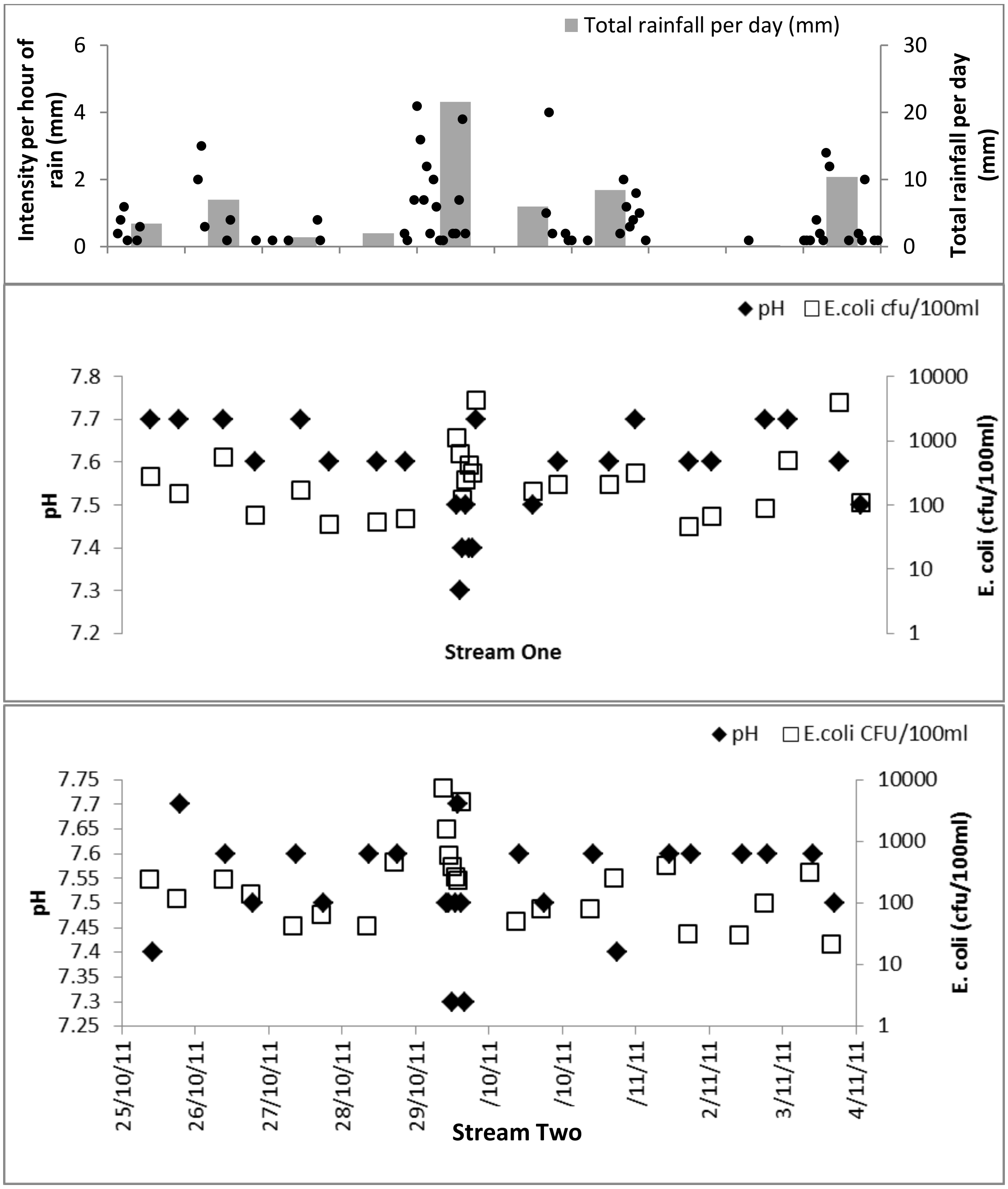

Figure 5 Time series plot to show the relationship between pH and *E. coli* for both stream one (middle) and stream two (bottom). The top graph shows the total and intensity of rainfall per day.

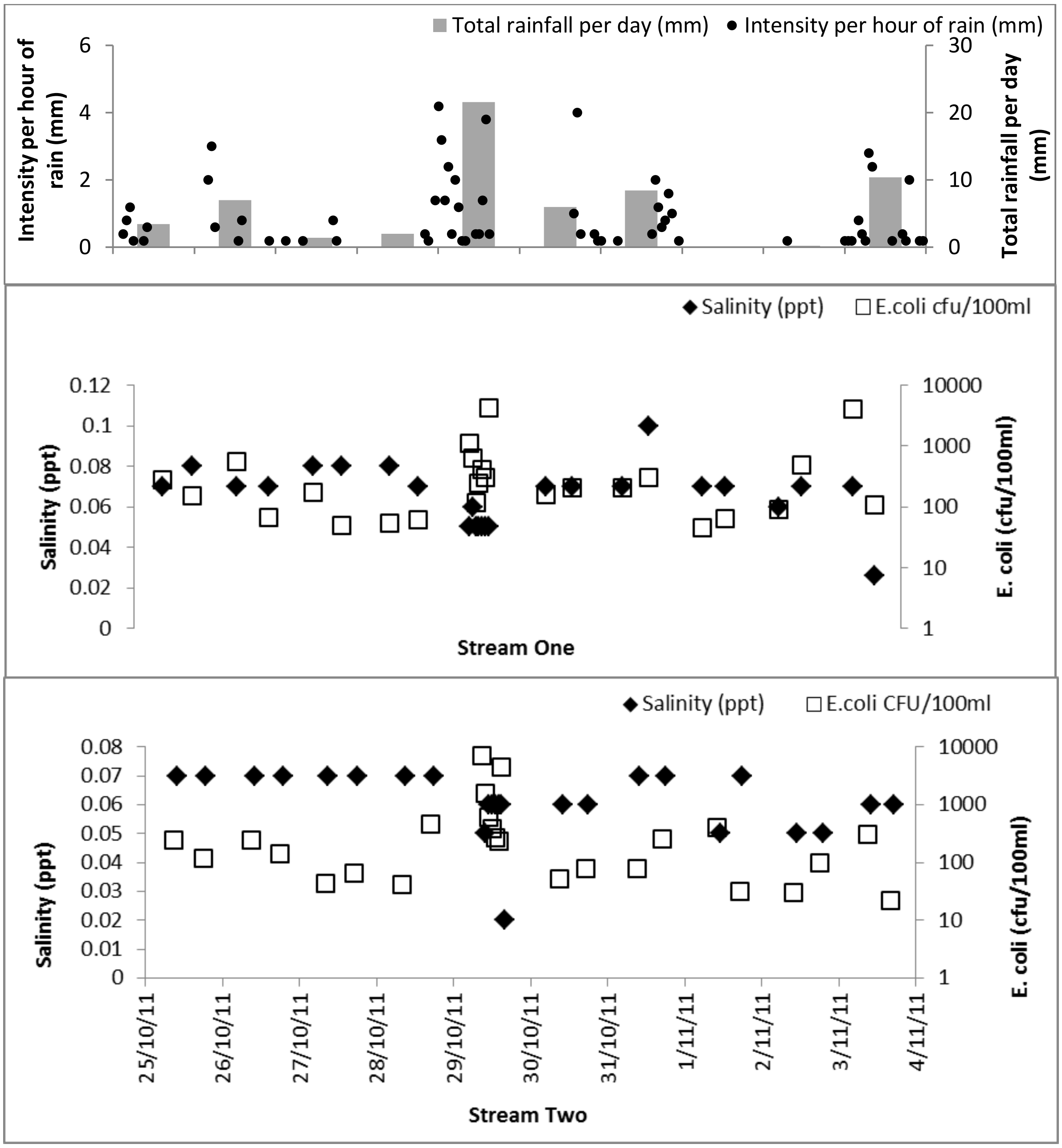

Figure 6 Time series plot to show the relationship between salinity and *E. coli* for both stream one (middle) and stream two (bottom). The top graph shows the total and intensity of rainfall per day.

Appendix two

Regression models used in determining those factors that showed the biggest influence on *E. coli* in both stream one and two.

Stream One					
Predictor	**T- value**	**P- value**	**R-Sq (%)**	**Anova, F**	**Anova, P**
Model 1					
Constant	-260	0.017			
Preceding 12 hour rainfall	-4.53	<0.001	50.9	10.25	0.001
pH	2.87	0.009			
Model 2					
Constant	11.59	<0.001			
Preceding 12 hour rainfall	2.17	0.013			
Total suspended solids	2.81	0.012	51.4	8.98	0.002
Model 3					
Constant	-2.65	0.015			
Weighted 48 hour rainfall	4.59	<0.001			
pH	2.91	0.009	51.3	10.54	0.001
Model 4					
Constant	10.19	<0.001			
Weighted 48 hour rainfall	2.99	0.008			
Total suspended solids	2.93	0.009	53.8	9.89	0.001
Model 5					
Constant	15.74	<0.001			
Weighted 48 hour rainfall	2.25	0.036			
Turbidity	4.66	<0.001	66.8	20.13	<0.001
Model 6					
Constant	3.07	0.007			
Flow rate	2.77	0.013			
Total suspended solids	3.08	0.007	51.4	9.00	0.002
Model 7					
Constant	9.30	<0.001			
Flow depth	2.01	0.058			
Turbidity	4.72	<0.001	65.4	18.89	<0.001
Model 8					
Constant	6.06	<0.001			
Flow depth	2.48	0.024			
Total suspended solids	2.80	0.012	48.2	7.92	0.004

Stream two					
Predictor	**T- value**	**P- value**	**R-Sq (%)**	**Anova, F**	**Anova, P**
Model 1					
Constant	8.46	<0.001			
Weighted 48 hour rainfall	4.39	<0.001			
Total suspended solids	2.08	0.05	64.3	14.83	<0.001

Appendix three

Scatter plots to show relationship between *E. coli* and the environmental factors measured in seawater.

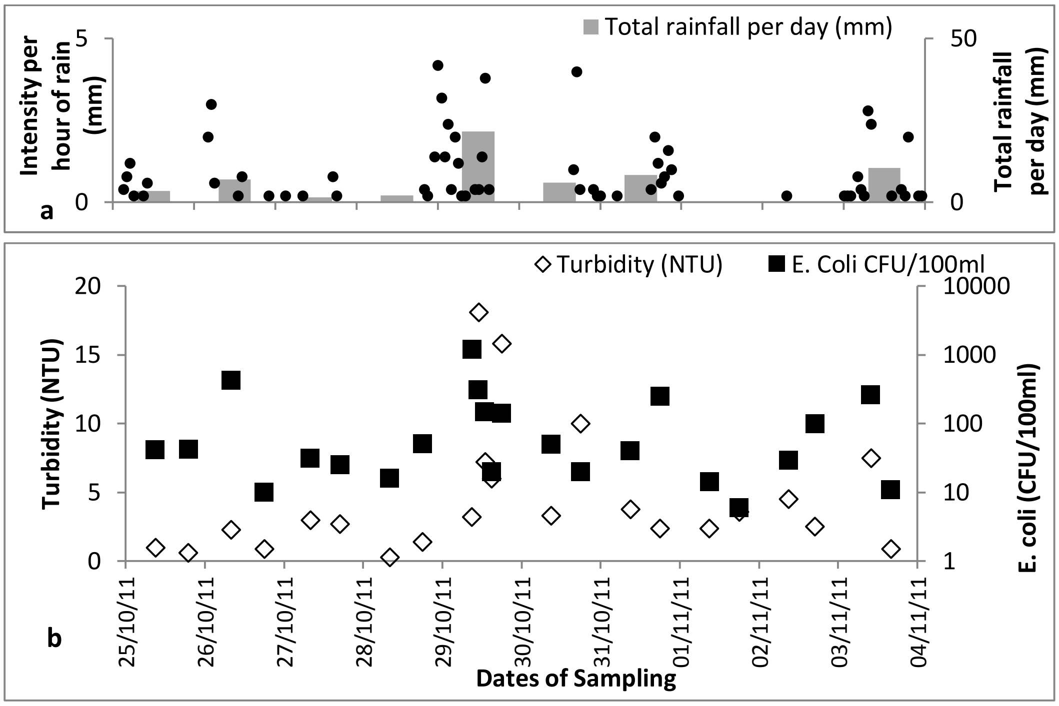

Figure 1 a Time series plot of total rainfall and rainfall intensity per day. **b** Time series plot of the relationship between *E. coli* and levels of turbidity found in seawater.

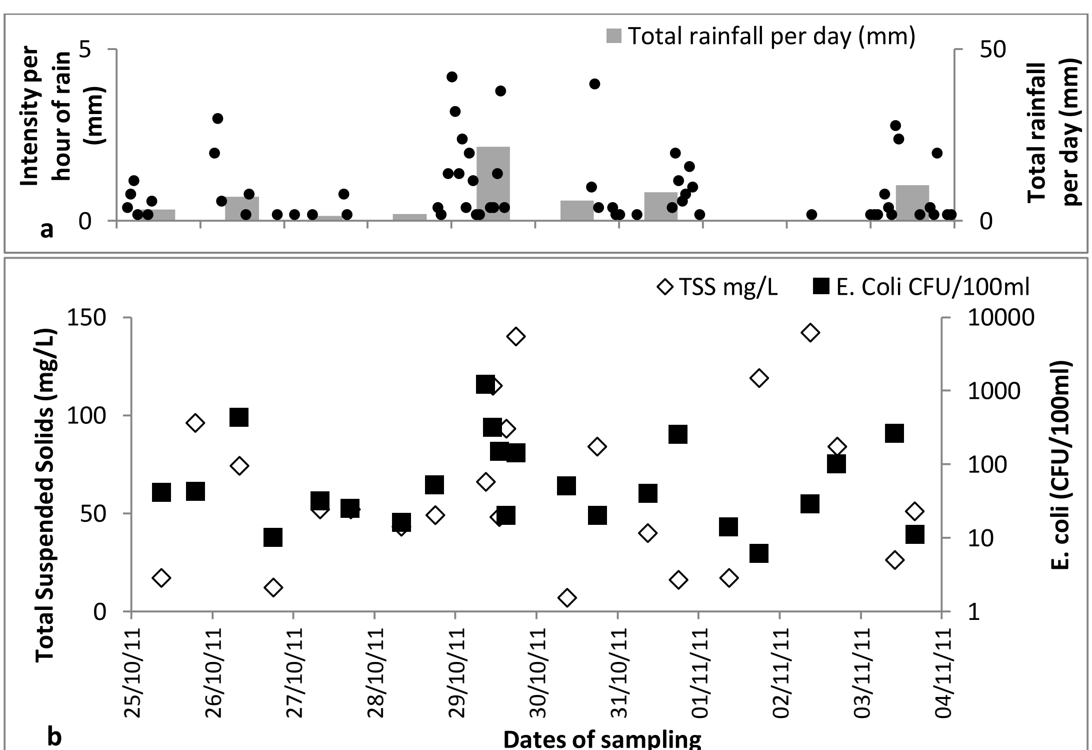

Figure 2 **a** Time series plot of total rainfall and rainfall intensity per day. **b** Time series plot to show the association between E. coli and levels of total suspended solids in seawater

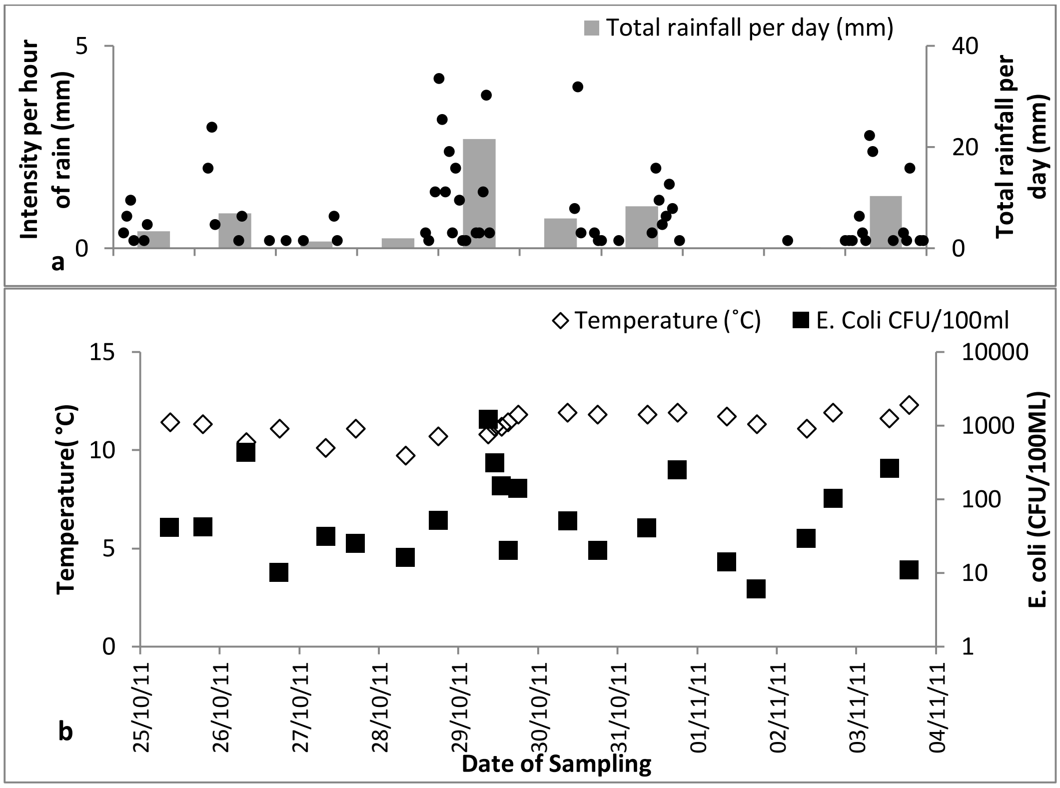

Figure 3 a Time series plot of total rainfall and rainfall intensity per day. **b** Time series plot to show the relationship between temperature and *E. coli* levels found in seawater.

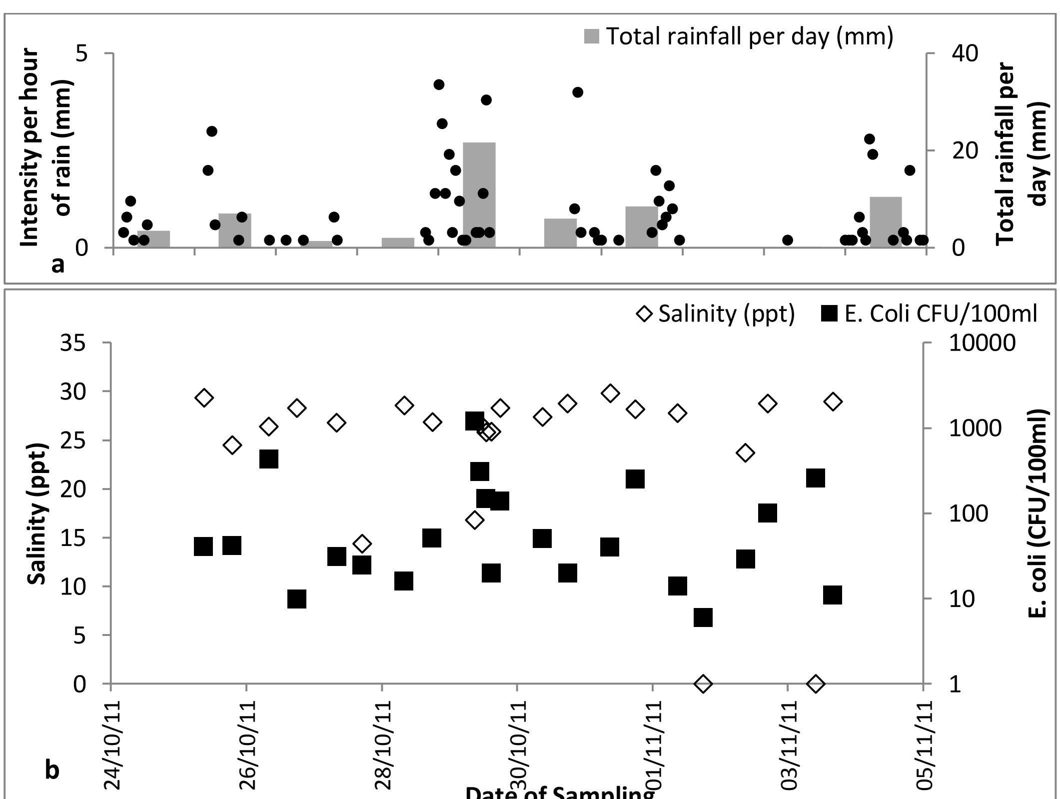

Figure 4 a Time series plot of total rainfall and intensity per day. **b** Time series plot to show the relationship between salinity and *E. coli* levels found in seawater.

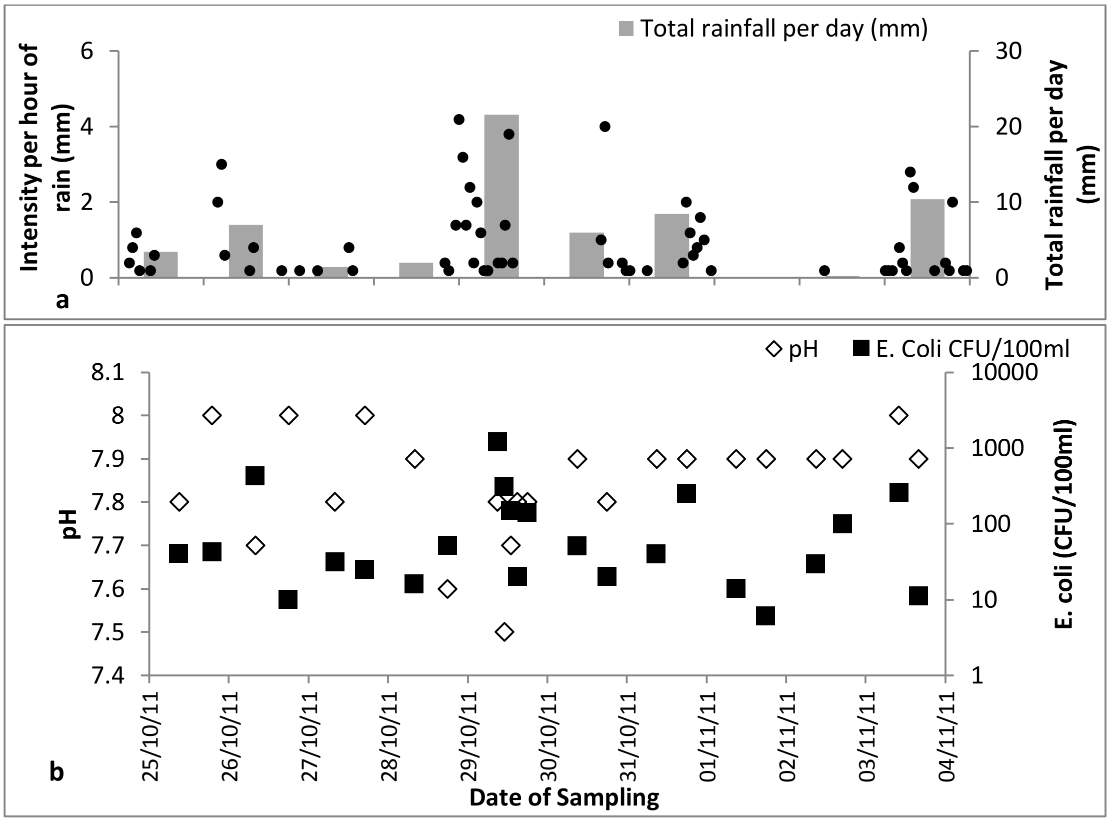

Figure 5 a Time series plot of total rainfall and intensity per day. **b** Time series plot of relationship between pH and *E. coli* levels found in seawater.