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Decay rates of faecal indicator bacteria from sewage and ovine faeces in brackish and freshwater microcosms with contrasting suspended particulate matter concentrations

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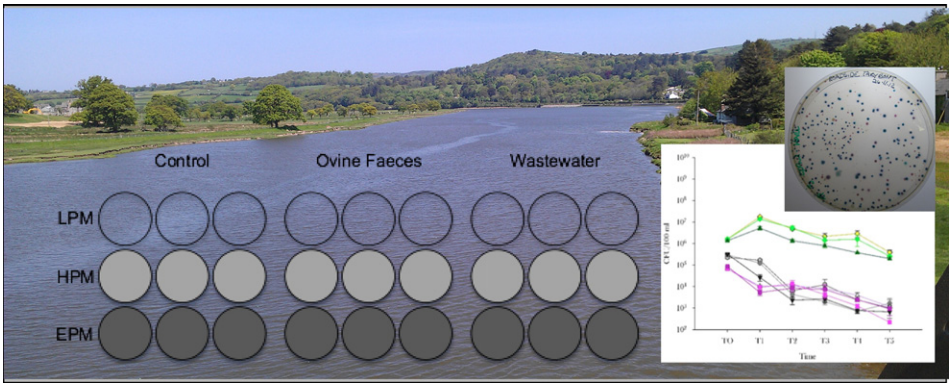
HIGHLIGHTS

- Suspended particulate material (SPM) affects the survival of faecal bacteria (FB).
- FB decay was measured in fresh and brackish water with three SPM concentrations.
- Ovine- and sewage-derived FB had contrasting decay/proliferation rates.
- SPM concentration had a significant effect on FB decay rate in brackish water.
- System, species, source and SPM concentration influence estuarine FIB survival.

GRAPHICAL ABSTRACT



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ABSTRACT

To safeguard human health, legislative measures require the monitoring of faecal indicator bacteria (FIB) concentrations in recreational and shellfish waters. Consequently, numerous studies have focussed on FIB survival in the water column and more recently in estuarine sediments. However, there is a paucity of information regarding the influence of contrasting suspended particulate matter (SPM) concentrations on the survival of FIB in the water column of estuaries. Here, microcosms containing freshwater or brackish water with low, high and extreme SPM concentrations were inoculated with sewage and ovine faeces and the decay rate of *Escherichia coli*, coliforms and enterococci were determined by enumeration over five consecutive days. *E. coli* derived from ovine faeces proliferated and persisted at high levels in both freshwater and brackish microcosms (no decay), whereas ovine enterococci demonstrated a net decay over the duration of the experiment. Furthermore, SPM concentration had a significant effect on the decay rates of both *E. coli* and enterococci from ovine faeces in brackish microcosms, but decay rate was greater at low SPM concentrations for *E. coli*, whereas the opposite was observed for enterococci, whose decay rates increased as SPM concentration increased. *E. coli*, enterococci and coliforms derived from wastewater demonstrated

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Freshwater
Coliforms
E. coli
Enterococci

a net decay in both freshwater and brackish microcosms, with contrasting effects of SPM concentration on decay rate. In addition, some FIB groups demonstrated contrasting responses (decay or proliferation) in the first 24 h following inoculation into freshwater versus brackish microcosms. Overall, SPM concentrations influenced the proliferation and decay rates of FIB in brackish waters, but had minimal influence in freshwater. These results demonstrate that the survival rates of FIB in aquatic environments are system specific, species and source dependent, and influenced by SPM concentration. This study has important implications for catchment-based risk assessments and source apportionment of FIB pollution in aquatic environments.

1. Introduction

Estuarine environments are important ecosystems that sustain numerous socioeconomic activities and provide vital resources such as food, habitat and areas for recreation (Costanza et al., 1997). However, estuarine and coastal ecosystems are increasingly exposed to environmental pressures, particularly from anthropogenic activities (GESAMP, 2001). Human populations are concentrated in coastal areas and consequently, the close proximity of industrial sites, extensive agriculture, sewage discharge and recreation can result in an increase in the flow of microbial pollutants to the sea (GESAMP, 2001).

A decline in the microbiological water quality of highly productive coastal systems can have significant social and economic consequences, and this is particularly true for catchment areas that incorporate blue flag beaches and shellfish harvesting waters (GESAMP, 2001). The current EU standard to assess microbial pollution in bathing waters involves the enumeration of *Escherichia coli* and enterococci as indicators of pathogen content (E.C., 2006), and consequently, the main focus of research has historically been on the survival and persistence of faecal indicator bacteria (FIB) in the water column (Jin et al., 2004; Kay et al., 2005). However, recent studies have demonstrated that sediments act as a significant reservoir of faecal bacteria, harbouring much greater concentrations than the overlying water column (Perkins et al., 2014). Attachment of FIB to suspended solids in the water column can provide protection, particularly from sunlight (Kay et al., 2005), and result in the downward flux of particle-bound microbial pathogens to benthic sediments, where they accumulate. Furthermore, the increase in nutrient and organic matter concentrations promotes the persistence of sediment-associated FIB (Lee et al., 2006).

Currently, there is a lack of information regarding the influence of suspended particulate matter (SPM) concentrations in the water column on FIB survival in aquatic systems, and this is particularly true for extreme levels of SPM that may arise due to future climate scenarios such as extreme precipitation events that potentiate increased turbidity in the water column by sediment resuspension, and the increased flow of particulate material and FIB from land to the river system (Rose et al., 2001).

Estuaries are the interface between rivers and the sea, resulting in a complex and dynamic ecosystem that regularly processes a large exchange of fresh and sea water, causing the resuspension of bottom sediments and the constant mixing of particulate material (Malham et al., 2014). Sediment transport and resuspension within an estuarine environment can be influenced by many environmental and human factors (Liu and Huang, 2009). For example, the water column of a tide-dominated estuary is usually high in particulate matter concentration, as fine sediments are persistently resuspended (Wells, 1995). In addition, recent climate observations have revealed that episodes of extreme weather events such as storms and hurricanes have increased in many parts of the world (Wetz and Yoskowitz, 2013), which can exacerbate the remobilisation of sediments in estuarine and other aquatic environments (Williams et al., 2008; Liu and Huang, 2009). Liu and Huang (2009) recorded levels of SPM reaching 4 g/L in Apalachicola Bay, Florida, USA, under severe storm conditions, whilst a reduction in wind-induced currents resulted in levels of SPM concentrations as low as 0.2 g/L. Furthermore, human activities such as the felling of forests and the building of roads can lead to soil erosion and the subsequent transport of particulate material into river systems where it becomes deposited in estuarine and coastal environments (GESAMP, 2001). To ensure that management strategies used to assess microbial pollution of environmental waters are adequate to safeguard human health, it is therefore important to identify how varying levels of SPM concentrations could impact upon FIB survival in the coastal zone, particularly in a changing climate. Due to the heterogeneous nature of microbial pollution sources in estuarine environments, it is important to determine if the survival and decay rates of FIB in environmental waters of varying SPM concentrations is source dependent.

The Conwy catchment, North Wales, UK, has a population of ~112,000, with over 75% living near the coast. Approximately 5.4 million tourists visit the Conwy catchment annually, with many partaking in water sports and swimming (Thorn et al., 2011). The Conwy estuary is an extremely productive system that incorporates blue flag beaches and directly impacts on a commercial shellfishery. Subsequently, a decline in microbial water quality could have significant consequences for both the tourist and shellfish industries, and for human health. The catchment covers ~300 km^2 and includes large areas of land utilised for agribusiness, private residence and commercial properties. Subsequently, the Conwy estuary is subject to inputs of waste-water effluent both directly and indirectly from its tributaries. Additional microbiological inputs can be attributed to wildfowl such as migratory birds that utilise the Conwy estuary as a feeding ground, ineffective septic tank systems, agricultural runoff and storm water runoff.

A previous study of FIB concentrations in the Conwy estuary revealed that the abundance of FIB and other potentially pathogenic bacteria in sediments had a positive correlation with finer sediment fractions such as clay (<4 μm), silt (4 μm–63 μm) and organic matter content (Perkins et al., 2014). Fine sediment particles of <63 μm are easily mobilised due to their large surface area and geochemical composition (Droppo and Stone, 1994) thus agitation of FIB-contaminated sediments by processes such as tidal fluctuations and water level flux, can release particulate matter (Wetz and Yoskowitz, 2013) and microorganisms back into the water column (Jamieson et al., 2005). Complex processes in aquatic systems make it difficult to assess the fate of FIB, however, and little is known about the survival and persistence of estuarine FIB in relation to varying SPM concentrations in the water column. The aim of this study was to investigate the decay rates of FIB (*E. coli*, enterococci and coliforms) derived from two contrasting sources; ovine faeces (representing a diffuse pollution source) and crude human sewage (representing a point pollution source) in relation to three SPM concentrations (low, high and extreme SPM) in microcosms containing freshwater and brackish water from the Conwy estuary, UK.

2. Materials and methods

2.1. Sample collection

Estuarine (brackish) sediment and water samples were collected from the Conwy estuary, North Wales, UK, during the flood tide to ensure saline conditions. Freshwater sediment and water samples were collected from the river Llugwy (Betws-y-Coed, North Wales). Crude wastewater influent (untreated, solids removed) was collected from a local wastewater treatment plant (Treborth Wastewater Treatment

Works, Caernarfon, North Wales). In addition, 10 independent samples of fresh ovine faeces were collected and pooled to ensure microbial variation for each experiment from local farms (Conwy and Anglesey).

2.2. Microcosm setup

Water and sediment samples from each environment (fresh and brackish water) were mixed and homogenised by shaking. SPM concentrations were determined by filter paper weight difference using glass microfibre filters (Whatman® grade GF/F pore size 0.8 μm, Sigma-Aldrich, Gillingham, Dorset, UK), hardened by ignition at 500 °C, soaked in distilled water for 5 min, dried at 80 °C for 1 h and weighed. 10 mL of each water sample (fresh and brackish) was filtered under vacuum through the prepared filters that were then dried for 1 h at 80 °C and reweighed. Dilutions of the fresh and brackish water stocks were performed to give a suspended particulate matter content of 16 mg/L for low particulate matter (LPM) treatment, 160 mg/L for high particulate matter (HPM) treatment, and 1600 mg/L for extreme particulate matter (EPM) treatment for freshwater microcosms and 16.5 mg/L for LPM 165 mg/L for HPM and 1654 mg/L for EPM for brackish water microcosms. The LPM and HPM concentrations were analogous to a range of concentrations found over a previous 12-month survey of the Conwy estuary showing a range of 5 to 178 mg/L SPM and of a freshwater site at Dolgarrog, North Wales, UK showing a range of 1 to 482 mg/L SPM (data not shown).

Ovine faeces was prepared for inoculation of microcosms by mixing 5 g of each faecal sample and adding fresh or brackish water, as appropriate, in a ratio of (1:2 w/v). Suspensions were thoroughly vortex mixed and pulse centrifuged to pellet solids. Crude wastewater required no initial processing.

Microcosms were prepared in triplicate in 250 mL sterile opaque bottles (Nalgene® Sigma-Aldrich Company Ltd., Gillingham, Dorset) (Fig. 1). Freshwater control microcosms contained 100 mL of each SPM concentration (16, 160 and 1600 mg/L) and brackish water control microcosms contained 100 mL of each SPM concentration (16.5, 165 and 1654 mg/L). Ovine faecal microcosms were set up in the same manner as the controls, with the exception that 5 mL of the volume was replaced with 5 mL of prepared ovine faecal suspension. For wastewater faecal microcosms, 2 mL of the final volume was replaced with 2 mL crude wastewater. Microcosms were incubated at 10 °C in the dark on a shaking platform (at 100 rpm) to ensure constant agitation of the contents.

2.3. Isolation and enumeration of FIB from microcosms

To determine the decay rates of FIB in the microcosms, 100 μL samples from each microcosm were inoculated onto agar plates containing selective culture media using the spread-plate technique. *E. coli* and non-*E. coli* coliforms were enumerated on Harlequin™ *E. coli*/coliform medium (LabM, Haywood, UK), prepared as per the manufacturers recommendations. Inoculated plates were incubated at 37 °C for 24 h ± 2 h. Enterococci were enumerated using Slanetz and Bartley medium (Oxoid, UK), prepared as per the manufacturers recommendations. Inoculated plates were incubated at 37 °C for 48 h ± 2 h. The resulting colony forming units (CFUs) were counted. Samples were taken from each of the microcosms at the beginning of the experiment (T0) and every 24 ± 2 h for 5 days (T1–T5). A dilution series was performed where needed to obtain countable CFUs throughout the experiment.

2.4. Statistical analysis

Although the data in this study did not meet the assumption for equal variances for a two-way factorial analysis of variance (ANOVA) to be truly valid, distinct differences between bacterial decay rates and between treatments were observed throughout this study. Therefore, ANOVA tests were performed at the 0.05 level of significance to determine if there was at least one mean that was significantly different from the rest. If a significant difference was reported the post hoc Tukey test was performed to report pairwise comparisons at the 0.05 level of significance. Although the statistical results cannot be reported as official values, they are reported to enhance the obvious observed differences between bacterial decay rates and varying SPM concentrations. In addition, the p-values (usually <0.001) were extremely low, thus giving extra confidence that the indication of difference can be acknowledged even if not reported as statistically different.

3. Results

3.1. Decay rates of *E. coli* and enterococci derived from ovine faeces in brackish water with LPM, HPM and EPM concentrations

Enumeration of *E. coli* derived from ovine faeces over 5 consecutive days in brackish water containing low, high and extreme concentrations of SPM revealed an overall increase in their concentrations (Fig. 2). Furthermore, *E. coli* concentrations increased with decreasing SPM concentrations, with mean percentage increases of 6170%, 4286% and 1210% for LPM, HPM and EPM, respectively between T0 and T1 (Fig. 3A). In addition, another increase in the concentration of *E. coli* for all levels of SPM was observed from T3 to T4 (Fig. 3A). A post hoc Tukey test revealed differences in *E. coli* concentrations in brackish water between LPM and HPM ($p = 0.003$), LPM and EPM ($p < 0.001$) and HPM and EPM ($p < 0.001$) over time. CFU counts taken at T5 showed that after the initial increase of *E. coli* between T0 and T1 there was an increase in decay rates with a decrease in SPM, as the percentage decrease from T1 to T5 was 44% for LPM, 39% for HPM and 37% for EPM. Despite the observed increase in the decay rates of *E. coli* in LPM concentrations CFU counts remained higher in LPM than HPM and EPM concentrations throughout the experiment (Fig. 2).

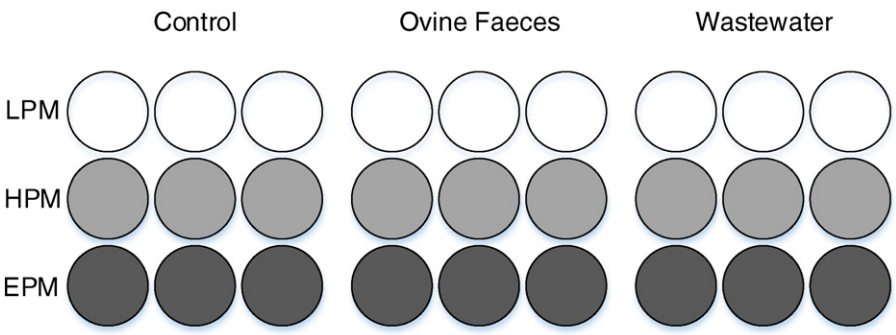

Fig. 1. Schematic diagram showing microcosm experimental overview. Circles represent individual microcosms. Shading represents level of suspended particulate matter concentration. LPM, low particulate matter concentration; HPM, high particulate matter concentration; EPM extreme particulate matter concentration. Controls, 100 mL source water; ovine faeces, 95 mL source water + 5 mL ovine faecal suspension; wastewater, 98 mL source water + 2 mL crude sewage.

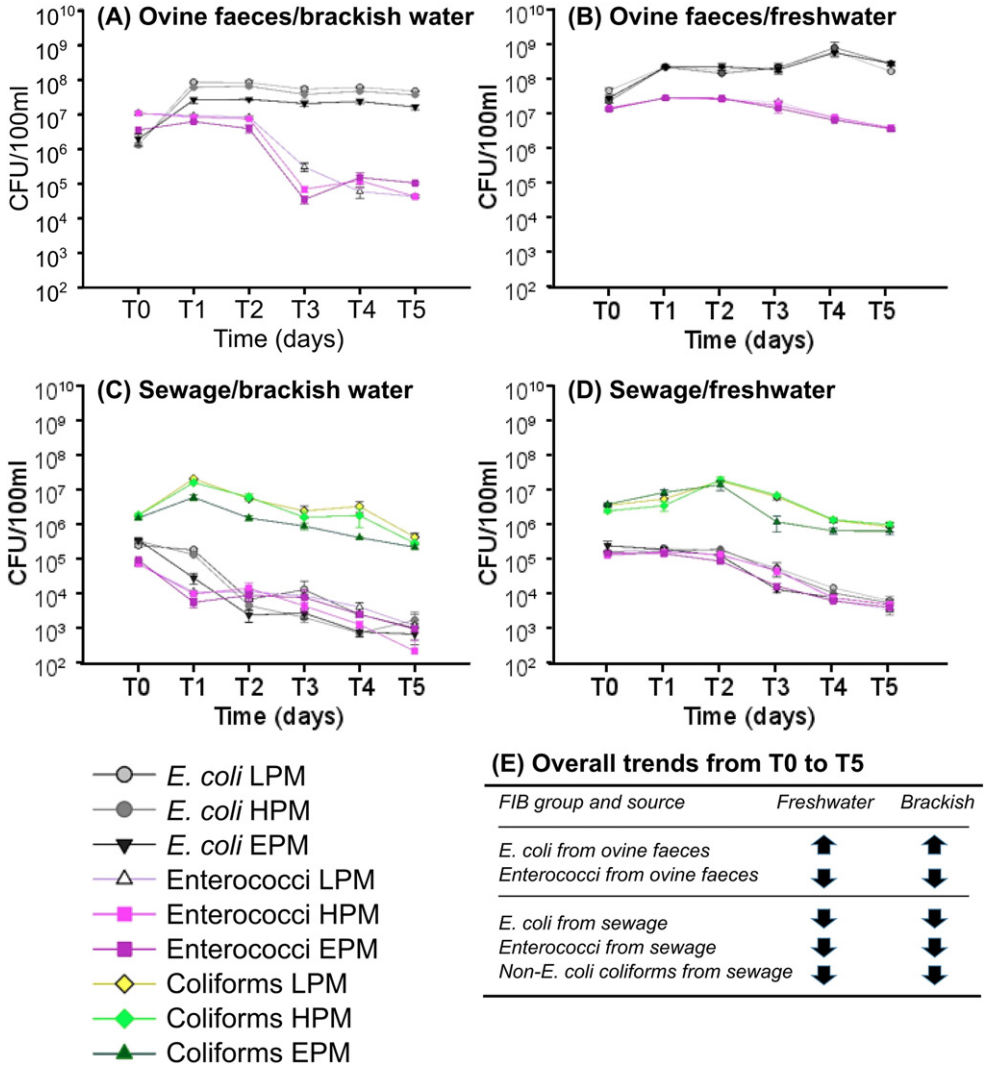

Fig. 2. (A) Decay rates of *E. coli* and enterococci from ovine faeces in brackish water. (B) Decay rates of *E. coli* and enterococci from ovine faeces in freshwater. (C) Decay rates of *E. coli*, enterococci and coliforms from sewage in brackish water. (D) Decay rates of *E. coli*, enterococci and coliforms from sewage in freshwater. (E) The overall trend in decay rates of faecal indicator bacteria from sewage and ovine faeces in fresh and brackish water. CFU, colony forming units; LPM, low particulate matter; HPM, high particulate matter; EPM, extreme particulate matter.

An overall decrease in ovine enterococci concentrations in brackish water containing LPM, HPM and EPM concentrations was observed, and differences in enterococci concentrations between all three SPM concentrations were also recorded. Enumeration by selective medium resulted in only one countable replicate for both high and extreme SPM concentrations for enterococci at T0, and consequently, the increase in enterococci concentrations observed for EPM from T0 to T1 may be an artefact. There was however, an observed decrease in enterococci of 11% for LPM, 16% for HPM and 31% for HPM at T3. Decay rates of ovine enterococci increased with an increase in SPM concentration. A post hoc Tukey test reported a difference between all three SPM concentrations ($p < 0.001$) based on enterococci concentrations over time. In addition, a post hoc Tukey test revealed a reported difference between the decay rates of *E. coli* compared with enterococci over time for all SPM concentrations ($p < 0.003$).

3.2. Decay rates of E. coli, enterococci and non-E. coli coliforms derived from human sewage in brackish water with LPM, HPM and EPM concentrations

The enumeration of *E. coli*, enterococci and non-*E. coli* coliforms derived from human sewage over 5 consecutive days in brackish water containing low, high and extreme concentrations of SPM revealed that overall there was a decrease in their concentrations (Fig. 2C, D, E). The decay rates of human sewage-derived *E. coli* displayed a different trend over time to that of ovine faeces derived *E. coli* in brackish water. In comparison, levels of sewage derived *E. coli* did not show any initial increase from T0 to T1 but exhibited a reduction of 28%, 59% and 92% for LPM, HPM and EPM concentrations, respectively (Fig. 3B). These initial reductions reveal that decay rates increased with increasing SPM concentrations.

Enterococci from human sewage revealed the same initial trend in decay rates as sewage-derived *E. coli* from T0 to T1. However, the differences between SPM concentration and the reduction in enterococci from T0 to T1 were much smaller compared with the reduction in *E. coli* concentrations over the same time period. Enterococci concentrations reduced by 84% for LPM, 86% for HPM and 94% for EPM concentrations from T0 to T1. However, between T1 and T2 an increase in concentration of 3% for LPM, 45% for HPM and 57% EPM was observed.

The percentage decrease in *E. coli* concentrations at T5 based on the initial counts at T0 revealed an overall reduction of 100% for LPM, 99% for HPM and 100% for EPM and for enterococci, an overall reduction of 98%, 100% and 99% for LPM, HPM and EPM, respectively, was observed (Fig. 2). The total decay rates based on CFUs taken at T0 and T5 have to be reported with caution, due to the proliferation of some bacterial

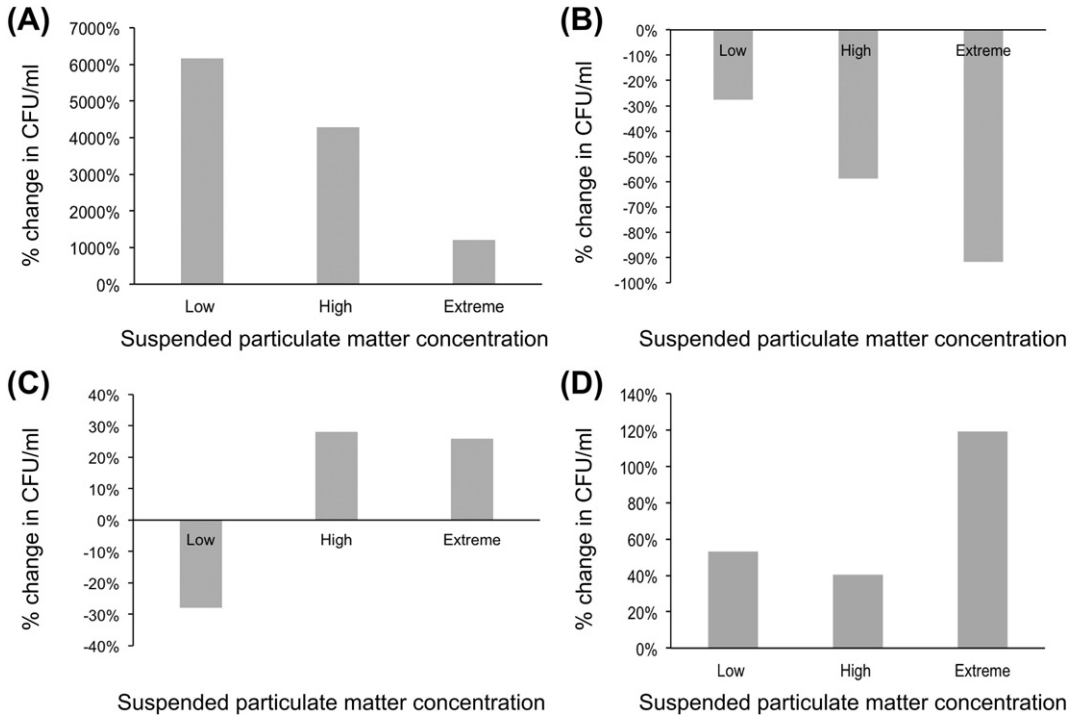

Fig. 3. Percentage change in CFUs per 100 mL at low, high and extreme suspended particulate matter concentrations: A) ovine derived *E. coli* at T0–T1 in brackish water, B) sewage derived *E. coli* at T0–T1 in brackish water, C) ovine derived *E. coli* at T1–T5 in freshwater, and D) sewage derived coliforms at T0–T2 in freshwater.

groups at certain time points during this five-day study; therefore, these results do not necessarily indicate total eradication from the water column. Despite differences in *E. coli* concentration between varying SPM concentrations, a post hoc Tukey test reported no difference between SPM concentrations over time. In addition, a post hoc Tukey test also revealed no difference between the decay rates of enterococci over time between all three SPM concentrations.

Non-*E. coli* coliforms from human sewage revealed an increase in concentration from T0 to T1 and their concentrations increased with a decrease in SPM (987% for LPM, 743% for HPM and 281% for EPM). The increase of coliforms at T1 was followed by a rapid reduction where concentrations dropped by 88% for LPM, 90% for HPM and 85% for EPM at T3. With the exception of T2, coliform concentrations were consistently higher in LPM compared with concentrations in HPM and EPM. A post hoc Tukey test revealed that there was no difference between *E. coli* and enterococci concentrations over time for all three SPM concentrations but there was a difference between *E. coli* and non-*E. coli* coliform concentrations ($p < 0.001$) and enterococci and non-*E. coli* coliform concentrations ($p < 0.001$) for all SPM concentrations.

3.3. Decay rates of E. coli and enterococci derived from ovine faeces in freshwater with LPM, HPM and EPM concentrations

E. coli derived from ovine faeces increased in concentration in freshwater containing LPM, HPM and EPM concentrations over the 5-day experiment, whereas there was an overall decrease in the concentrations of ovine enterococci (Fig. 2E).

Ovine *E. coli* CFU counts in freshwater microcosms increased from T0 to T1 by 399%, 851% and 686% for LPM, HPM and EPM concentrations, respectively, and this was followed by a second substantial increase in *E. coli* levels between T3 and T4 (184%, 286% and 211% for LPM, HPM and EPM, respectively). At T5, concentrations of *E. coli* were still higher than the initial concentrations at T0 (260%, 1118% and 888% for LPM, HPM and EPM concentrations, respectively). However, between T1 and T5 *E. coli* concentrations varied with SPM concentration, demonstrating a 28% decrease in CFU counts for LPM but an increase of 28% for HPM and 26% for EPM (Fig. 3c).

Despite these differences, the results from a post hoc Tukey test indicated no significant difference between SPM concentrations over time. However, based on the percentage increase of *E. coli* between T0 and T1, and the percentage increase between T3 and T4, it appears that *E. coli* concentrations increase at a higher rate in freshwater with HPM concentrations. Ovine enterococci also increased in concentration from T0 to T1 (104% for LPM, 95% for HPM and 112% for EPM concentration) and continued to increase between T1 and T2, but exhibited a decline in concentrations thereafter, which continued throughout the experiment. At T5, enterococci levels had decreased by 88%, 86% and 87% for LPM, HPM and EPM respectively. Enterococci concentrations displayed the same trends in decay rate for all three SPM concentrations, supported by a post hoc Tukey test that revealed no difference between the decay rates of enterococci in relation to varying SPM concentrations.

3.4. Decay rates of E. coli, enterococci and non-E. coli coliforms derived from sewage in freshwater with LPM, HPM and EPM concentrations

Overall, there was a decrease in the concentration of *E. coli*, enterococci and non-*E. coli* coliforms derived from human sewage over 5 consecutive days in freshwater microcosms containing LPM, HPM and EPM concentrations (Fig. 3A). Concentrations of sewage-derived *E. coli* in freshwater were similar between all three SPM concentrations over time (Fig. 2). However, initially *E. coli* CFU counts in LPM and HPM concentrations increased from T0 to T1 by 30% and 5% respectively, compared with a decrease of 21% for EPM concentrations and a second increase was observed between T1 and T2 for *E. coli* in HPM concentrations. Although there was a difference in decay rates from T0 to T1, the overall percentage decrease in concentrations from T1 to T5 was 97% for all three SPM concentrations, showing the same trend in decay rates over time.

A post hoc Tukey test confirmed that there were no differences between enterococci decay in all three SPM concentrations over time. The enumeration of sewage-derived enterococci in freshwater with

varying SPM concentrations revealed a similar trend to that displayed by *E. coli* from T0 to T1, with enterococci CFU counts increasing by 23% for LPM and 14% for HPM, whilst a reduction of 1% was observed for EPM. Subsequently, enterococci concentrations remained similar throughout the duration of the experiment. Results from the post hoc Tukey test indicated that there was a difference between enterococci decay rates between LPM and EPM ($p < 0.001$) and HPM and EPM ($p = 0.006$), indicating that the decay rate of sewage-derived enterococci in freshwater is similar for LPM and HPM concentrations but increases with EPM.

Enumeration of non-*E. coli* coliforms concentrations derived from human sewage revealed an increase in concentrations from T0 to T2 for all three SPM concentrations. An increase of 53%, 40% and 119% was observed for LPM, HPM and EPM concentrations between T0 and T1, followed by a further increase of 227% 448% and 64%, from T1 to T2 respectively. This corresponded to an overall increase of 401%, 670 and 260% for LPM, HPM and EPM, respectively, between T0 and T2.

A decline of 95% in non-*E. coli* coliform concentrations was observed from T2 to T5 for all SPM concentrations. These data indicate that the proliferation of sewage derived coliform bacteria in freshwater is similar for low and high concentrations of particulate matter but increases with extreme concentrations. However, despite this increase, a post hoc Tukey test reported no difference between coliform decay rates for all SPM concentrations over time. A post hoc Tukey test reported no difference in the decay rates of *E. coli* and enterococci, but reported a difference between *E. coli* and coliform concentrations ($p < 0.001$) and enterococci and coliform concentrations ($p < 0.001$) for all SPM concentrations.

3.5. Decay rates of E. coli derived from ovine faeces in fresh and brackish water

Substantial differences were observed between the decay rates of *E. coli* from ovine faeces in fresh and brackish water, although similar overall trends were observed over time. There was an initial increase from T0 to T1 of ovine derived *E. coli* for all SPM concentrations, in both fresh and brackish water. However, the percentage increase in the levels of *E. coli* across all three SPM concentrations was substantially higher in brackish water compared with freshwater. *E. coli* concentrations in brackish water increased from T0 to T1 by 6170% 4286% and 1210% for LPM, HPM and EPM respectively, compared to an increase of 399%, 851% and 686% for LPM, HPM and EPM respectively in freshwater.

3.6. Decay rates of enterococci derived from ovine faeces in fresh and brackish water

Enterococci levels in freshwater increased from T0 to T1 for all 3 SPM concentrations, then showed a gradual decline (Fig. 2). In comparison, enterococci levels in brackish water gradually reduced from T0 to T2, then rapidly declined from T2 to T3 by 96%, 99% and 99% for LPM, HPM and EPM respectively. However an increase in CFU counts was observed between T3 and T4 of 75% for HPM and 330% for EPM concentrations compared to a continued reduction of a further 80% for LPM concentrations.

3.7. Decay rates of E. coli derived from wastewater in fresh and brackish water

The decay rate of sewage-borne *E. coli* exhibited different trends in freshwater compared with brackish water (Fig. 2). In freshwater, *E. coli* concentrations increased from T0 to T1 by 53% for LPM, 40% for HPM and 119% for EPM concentrations, followed by a decline. In contrast, *E. coli* CFU counts in brackish water declined by 28% for LPM, 59% for HPM and 92% from T0 to T1. *E. coli* concentrations in freshwater microcosms with HPM continued to increase, but subsequently declined after T2 and continued to decline throughout the duration of the experiment. However, an increase in *E. coli* levels was observed from T2 to T3 in LPM and EPM concentrations in brackish water (Fig. 2).

3.8. Decay rates of enterococci derived from wastewater in fresh and brackish water

Sewage-derived enterococci in brackish water declined in concentration between T0 and T1 (84% for LPM, 86% for HPM and 94% for EPM). However, in freshwater, enterococci concentrations increased by 23% at LPM and 24% at HPM concentrations from T0–T1, but subsequently declined in all SPM concentrations (Fig. 2).

3.9. Decay rates of coliform bacteria derived from ,wastewater in fresh and brackish water

Coliform concentrations in brackish water increased significantly from T0 to T1 (987%, 743% and 281% for LPM, HPM and EPM concentrations, respectively) compared with a smaller increase of 53% 40% 119% for LPM, HPM and EPM concentrations in freshwater (Fig. 4B). However, coliform concentrations continued to increase to T2 for all three SPM concentrations in freshwater (227%, 448% and 64% for LPM, HPM and EPM concentrations, respectively), followed by a decline. In comparison, levels of coliform bacteria in brackish water decreased from T1 for all three SPM concentrations, but another increase for LPM and HPM of 34% and 11% respectively was observed between T3 and T4.

3.10. Control microcosms

All control microcosms demonstrated negligible background concentrations of the target bacteria. Average concentrations did not exceed 254 CFU/mL for coliform bacteria, 133 CFU/mL for *E. coli* and 2 CFU/mL for enterococci concentrations (data not shown).

4. Discussion

To address the paucity of information regarding the effects of SPM concentration on the survival of FIB, the decay rates of three FIB groups (*E. coli*, enterococci and coliforms) derived from ovine faeces and crude human sewage in both freshwater and brackish water microcosms were determined for three different SPM concentrations (low, high and extreme). Results from this study show that SPM concentrations influence the proliferation and decay rates of FIB in brackish waters but have minimal influence in freshwater (Fig. 2). The general lack of an effect of SPM concentration on FIB decay rate in freshwater is interesting given that inland waters are subjected to the same water quality compliance regulations as coastal zones where SPM has a much greater influence on FIB decay rate. Furthermore, contrasting trends in decay rates were both source- and species-dependent.

In general, decay rates in brackish water increased with an increase in SPM concentration. In addition, *E. coli* derived from ovine faeces exhibited an initial increase in concentration, which reduced with an increase in SPM concentration. In comparison, both enterococci and *E. coli* exhibited an initial increase in CFU counts in freshwater, but no difference in decay rates in relation to SPM concentrations were reported over time. It is important to note that although a post hoc Tukey test reported no difference in decay rates over time, for ovine-derived *E. coli* in freshwater there was a 100% increase in *E. coli* levels from LPM to HPM and a 50% increase from LPM to EPM suggesting that initially, levels of HPM provide more favourable conditions for the proliferation of ovine derived *E. coli* in freshwater.

Overall, concentrations of *E. coli* from ovine faeces increased at a much higher rate in both brackish and freshwater compared with ovine faeces derived enterococci. Although *E. coli* from ovine faeces exhibited an increase in CFU counts in both brackish and freshwater, the initial percentage increase was much greater in brackish water. High

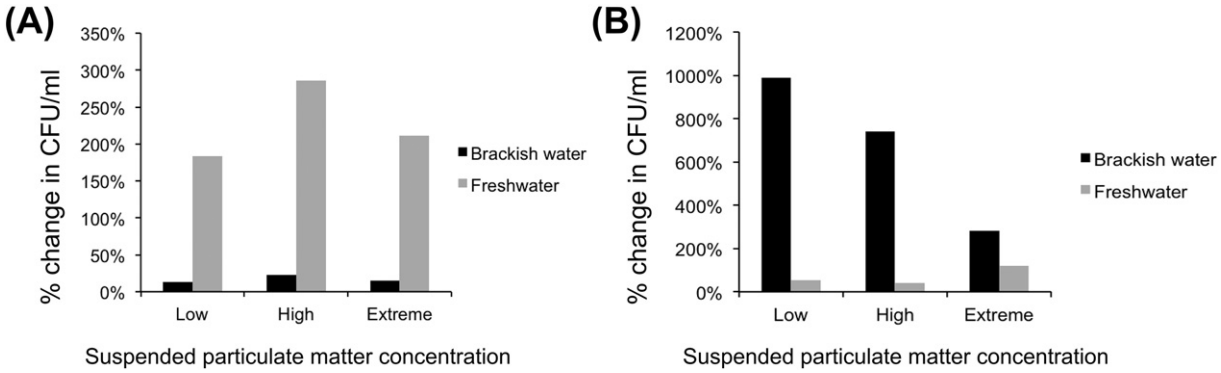

Fig. 4. Percentage increase in CFUs per 100 mL at low, high and extreme suspended particulate matter concentrations in A) ovine derived *E. coli* and B) sewage derived coliform bacteria.

concentrations of *E. coli* were then sustained in brackish water for the duration of the experiment compared to fluctuating concentrations of *E. coli* in freshwater. In comparison, enterococci from ovine faeces decayed faster in brackish water than freshwater and SPM concentrations impact the proliferation of ovine derived *E. coli* and enterococci in brackish water, but had minimal impact in freshwater.

FIB from ovine faeces proliferated in both brackish and freshwater matrices and also displayed lower decay rates than sewage-derived FIB. Furthermore, SPM concentration in freshwater had no effect on the decay rate of *E. coli* and coliform bacteria from sewage, and in brackish water SPM concentration had no effect on *E. coli* and enterococci decay rates. In contrast, sewage-derived enterococci in freshwater and coliform bacteria in brackish water generally displayed a decrease in concentration concurrent with a decrease in SPM concentration. These observations are concordant with the study of Korajkic et al. (2013) which demonstrated differential decay rates of *E. coli* and enterococci originating from cattle manure and human wastewater effluent in marine and freshwater under ambient light and dark conditions with and without exposure to the indigenous aquatic microbiota. Korajkic et al. (2013) concluded that sunlight and the indigenous aquatic microbiota contributed to the decay rates of FIB under varying conditions, but the faecal source appears to be the most important influence on FIB decay rates. In addition, manure-derived FIB persisted for longer than sewage-derived FIB, and this was particularly distinct for *E. coli* in freshwater and enterococci in marine water (Korajkic et al., 2013).

Noble et al. (2004) determined the inactivation rate of *E. coli*, enterococci and total coliform bacteria from various sewage sources (sewage influent and effluent and storm drain run-off) in both seawater and freshwater, revealing that SPM concentration (ranging from 100 mg/L to 500 mg/L) and faecal source were not significant factors in the inactivation of FIB. In contrast, we show that SPM concentrations do indeed impact on the decay or proliferation of FIB in both brackish and freshwater, and that the difference in these trends over time is source dependent. Previous studies have suggested that enterococci survive longer in brackish water compared with *E. coli* (Jin et al., 2004), whereas results from this study reveal no difference between the decay rates of *E. coli* and enterococci from sewage in brackish and freshwater. Furthermore, *E. coli* from ovine faeces persisted at much higher concentrations in both water matrices compared with enterococci from ovine faeces.

It is generally considered that the association of FIB with particles in environmental waters can enhance survival, and in particular, provide protection from the bactericidal effects of sunlight. Walters et al. (2014) performed in vitro experiments to determine the effect of total suspended solid (TSS) concentrations on the UV inactivation of *E. coli* and enterococci from river water. They observed that as TSS concentrations increased, inactivation rates of *E. coli* and enterococci decreased, but tailed off as SPM concentrations reached 100 mg/L, concluding that bactericidal effect of sunlight is severely reduced in environmental waters with concentrations of TSS exceeding 100 mg/L (Walters et al., 2014). It was also noted that under high rainfall, TSS concentrations in river water can quite often substantially exceed 100 mg/L. In addition, the release of sewage into environmental waters from combined sewage overflow systems in particular is associated with higher sediment loads. However, here we have demonstrated that in the absence of UV light, SPM concentrations impact on proliferation and decay rates of FIB from ovine faeces-derived, sewage-derived coliforms in brackish water, and sewage-derived enterococci in freshwater. UV inactivation plays a significant role in the reduction of FIB in environmental waters (Burkhardt et al., 2000; Pommepuy et al., 1992), but this effect is severely reduced when SPM concentrations exceed 100 mg/L (Walters et al., 2014). This is of particular importance under extreme weather conditions, which can lead to high SPM concentrations in environmental waters (Wong et al., 2010; Wetz and Yoskowitz, 2013).

FIB inactivation is a complex process governed by numerous environmental parameters. In general, we observed that the initial response of FIB entering the water column (T0 to T1) was either proliferation, or inactivation (but at a lower rate when compared with subsequent time periods, i.e. T1 onwards). These data suggest that viability is optimal at this early time point, and one explanation for this phenomenon could be that the decay rates of FIB are influenced by their attachment to particulate matter in the water column, which does not occur immediately on the initial addition of FIB to the microcosms. Flocs are aggregates of organic detritus and inorganic materials such as clay and silt (Malham et al., 2014) that accumulate exopolymers or extracellular polymeric substances (EPS) exuded by aquatic organisms (Wotton, 2004). Exopolymers mainly comprise carbohydrates, which aid in the flotation of the aggregates, providing attachment sites and nutrients for bacterial growth (Wotton, 2004). It is well documented that bacteria in the water column preferentially attach to suspended material (Malham et al., 2014; Kiorboe and Jackson, 2001). Kiorboe and Jackson (2001) suggested that these tiny aggregates (termed marine snow or marine aggregates) are extremely nutrient rich and can support bacterial production, in addition to attracting protozoa that predate on bacterial cells. Thus the association of FIB with flocs in the water column can result in competition for nutrients and the subsequent grazing by protozoa. Many studies have attributed the decay of enteric bacteria in environmental waters to predation from protozoa (Barcina et al., 1997; Iriberri et al., 1994; Gonzalez et al., 1990, 1992). However, the susceptibility to grazing can differ between bacteria (Iriberri et al., 1994).

Kiorboe and Jackson (2001) also showed that the constant movement of these highly productive aggregates results in a plume of dissolved organic matter (DOM) in the surrounding water column. It was suggested that the release of DOM from the aggregates could have a positive impact on the growth of bacteria in the surrounding water column (Kiorboe and Jackson, 2001). The proliferation of FIB in the early stages of this study (between T0 and T1) supports the idea that the growth of bacteria is greater in the water column and subsequent predation occurs upon attachment to SPM. This is also highlighted in concentrations of EPM where decay rates tend to be much greater. FIB

derived from ovine faeces proliferated under certain conditions and persisted at higher levels than sewage-derived FIB. One explanation for this decrease in decay rates is that FIB from ovine faeces are already present in a tight consortium bound together by faecal matter, this tight consortium may protect the contained FIB and the high nutrient levels contained in the faeces may sustain their survival. These data therefore suggest that SPM influences the survival of FIB in the water column by the possible facilitation of predation and concur with the theory of bacterial proliferation and predation in aquatic environments as proposed by Kiorboe and Jackson (2001).

This study suggests that catchment-based risk assessments of FIB pollution need to take into account the source of the FIB, the influence of SPM concentration on survival, and the hydrodynamic properties of the environment, all of which will influence the decay rate or proliferation of FIB in aquatic environments. The inactivation of FIB in aquatic systems is tremendously complex and this study demonstrates the need for further investigations to fully understand the factors influencing their survival and persistence so that human health may be safeguarded.

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