Sediment Composition Influences Spatial Variation in the Abundance of Human Pathogen Indicator Bacteria within an Estuarine Environment

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Abstract

Faecal contamination of estuarine and coastal waters can pose a risk to human health, particularly in areas used for shellfish production or recreation. Routine microbiological water quality testing highlights areas of faecal indicator bacteria (FIB) contamination within the water column, but fails to consider the abundance of FIB in sediments, which under certain hydrodynamic conditions can become resuspended. Sediments can enhance the survival of FIB in estuarine environments, but the influence of sediment composition on the ecology and abundance of FIB is poorly understood. To determine the relationship between sediment composition (grain size and organic matter) and the abundance of pathogen indicator bacteria (PIB), sediments were collected from four transverse transects of the Conwy estuary, UK. The abundance of culturable Escherichia coli, total coliforms, enterococci, Campylobacter, Salmonella and Vibrio spp. in sediments was determined in relation to sediment grain size, organic matter content, salinity, depth and temperature. Sediments that contained higher proportions of silt and/or clay and associated organic matter content showed significant positive correlations with the abundance of PIB. Furthermore, the abundance of each bacterial group was positively correlated with the presence of all other groups enumerated. Campylobacter spp. were not isolated from estuarine sediments. Comparisons of the number of culturable E. coli, total coliforms and Vibrio spp. in sediments and the water column revealed that their abundance was 281, 433 and 58-fold greater in sediments (colony forming units (CFU)/100 g) when compared with the water column (CFU/100 ml), respectively. These data provide important insights into sediment compositions that promote the abundance of PIB in estuarine environments, with important implications for the modelling and prediction of public health risk based on sediment resuspension and transport.

Introduction

Estuarine environments represent some of the most biologically productive systems in the biosphere and consequently provide a wealth of economic, social and natural ecosystem services that include food, employment, recreation and habitat [1]. However, the sustainability of such systems can be severely compromised along developed and urbanised coastlines, and this is predominantly due to anthropogenic influences [2]. Almost half of the world’s population are thought to live within a few hundred kilometers of the coast [3] and consequently, anthropogenic activities have a significant influence upon the health of estuarine and ocean ecosystems. Furthermore, as the global climate changes, meteorological events such as storms and floods present further impacts upon estuarine environments, and increased rainfall will significantly impact the flow and transportation of microbial pollution from the terrestrial environment into the coastal zone [4]. Human pathogenic microorganisms are introduced into estuarine ecosystems via the release of effluent from wastewater treatment plants, ineffective septic tank systems and storm water runoff [5]. Agricultural runoff from livestock farming can also represent a major source of microbial pollution, particularly when excreted waste from poultry and livestock is re-applied to land [6]. Wildlife, especially migratory wildfowl and other birds also represent an important source of zoonotic bacterial pathogens in natural environments [7].

Human pathogenic bacteria often occur at low levels in the environment [8] and their specific detection is a laborious and costly process [9]. Consequently, the detection and enumeration of faecal indicator bacteria that are present in the gastrointestinal
Pathogen Indicator Bacteria in Estuarine Sediments

Triggered by E. coli, Vibrio spp., Campylobacter spp., Salmonella spp., and Campylobacter spp. are ubiquitous in aquatic environments and some strains are pathogens of humans; *V. cholerae* has been responsible for several previous pandemics, resulting in high human mortality rates [20], some of which originated from the consumption of contaminated seafood [21]. Consequently, filter-feeding shellfish such as mussels, scallops and oysters are especially susceptible to contamination with bacterial pathogens if grown in contaminated waters [22].

Spatial and temporal variations in bacterial abundance in an environment are controlled by the interactions of complex physical, chemical and biological parameters, such as available nutrients [23], organic matter [24], sediment grain size [25], clay content [26], heavy metal content [27], predation by protozoa [28], competition [29], temperature [30], salinity [31], sunlight intensity [32] and seasonal variations [33]. Consequently, association with particulate material and sediments offers several advantages in terms of the survival and persistence of bacterial pathogens, such as protection from UV light [34,35], protection from phage attack in saline conditions [36], shelter from predation [37] and a greater organic matter content compared to the water column [38]. However, hydrodynamic processes (e.g. tides and storms), recreational activities and mechanical disturbances such as commercial dredging all have the potential to re-suspend sediment particles and their associated pathogens back into the water column, resulting in periodic elevated levels of pollution [39,40]. Currently, classification of both bathing and shellfish water quality relies solely upon the enumeration of FIB in water samples, despite the well-established paradigm that allochthonous bacterial pathogens in the water column become preferentially attached to particulate material, which promotes their downward flux to the bottom sediments where they are typically found in greater abundance [41,42].

The Conwy catchment, North Wales, UK, has a population of approximately 112,000, 80% of which live in coastal resorts that represent the main economic and tourist areas, with over 5.4 million visitors per annum. The Conwy estuary directly impacts flux to the bottom sediments where they are typically found in attached to particulate material, which promotes their downward movement. Samples, despite the well-established paradigm that allochthonous water quality relies solely upon the enumeration of FIB in water column, resulting in periodic elevated levels of pollution worldwide [16] but their presence in bathing waters is not routinely monitored [17]. Pathogenic strains of *Campylobacter* spp. are also thought to be responsible for a large proportion of enteric illnesses in humans living in developed and industrialized countries [18]. Contamination of surface and coastal waters by *Salmonella* and *Campylobacter* spp. is thought to occur predominantly via faecal contamination from wildfowl and other birds, although other animals including domestic livestock are also reservoirs [19]. *Vibrio* spp. are ubiquitous in aquatic environments and some strains are pathogens of humans; *V. cholerae* has been responsible for several previous pandemics, resulting in high human mortality rates [20], some of which originated from the consumption of contaminated seafood [21]. Consequently, filter-feeding shellfish such as mussels, scallops and oysters are especially susceptible to contamination with bacterial pathogens if grown in contaminated waters [22].

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triplicate from 0.2 m below the water surface using a sterile 1 L polypropylene container, then transported to the laboratory where water samples were processed within 4 h and sediment samples within 6 h.

Isolation and cultivation of target bacterial groups from sediment

One gram of each sediment sample was transferred to a 7 ml sterile bijou tube (Starlab UK Ltd., Milton Keynes, UK) and suspended in 5 ml Ringers solution (Oxoid Ltd., Basingstoke, UK) to obtain a 1:5 (w/v) dilution. Each sample was vortexed for 90 s to disassociate and resuspend bacteria from the sediment. Aliquots of the resulting supernatant for each sample were transferred aseptically onto agar plates containing a selective medium for E. coli, total coliforms, enterococci, Vibrio spp., Campylobacter spp., Salmonella spp. and total heterotrophs. The optimum volume of supernatant used to inoculate each selective medium was determined in a previous study (data not shown). All selective medium plates were inverted and incubated according to manufacturer’s recommendations. Resulting colony forming units (CFUs) provided enumeration of bacterial groups. Details describing the selective media used and incubation times are described in Table S1 in the supplemental material. The CFU data for sample point 13 represents the average of only two sediment samples due to one of the replicates being lost as a result

Figure 1. A map of the study site; the Conwy Estuary, North Wales, UK. Water and sediment samples were collected in triplicate from four transverse transects of the Conwy estuary (twenty one sampling sites). doi:10.1371/journal.pone.0112951.g001
of the presence of gravel clasts. All other CFU data represent the average of three independent replicate samples.

Isolation and cultivation of target bacterial groups from water

Water samples were processed within 4 h in accordance with the Revised Bathing Water Directive (2006/7/EC). Enumeration of bacteria in water samples was achieved by using vacuum–filtration as described in [45]. Briefly, water samples were homogenised by shaking and 50 ml of water filtered under vacuum through a 0.2 μm cellulose acetate membrane (Sartorius Stedim Biotech., Gottingen, Germany). Subsequently, the membranes were aseptically transferred onto sterile agar plates containing selective medium for the enumeration of *E. coli*, total coliforms, *Vibrio* spp. and heterotrophic bacteria. Agar plates were inverted and incubated according to manufacturer’s recommendations (Table S1). Resulting Colony Forming Units (CFUs) were enumerated 24 h post incubation.

Sediment particle size analysis

Sediment grain size was determined by laser diffraction after 1 min sonication to separate particles, using a Malvern Hydro 2000 MU particle size analyser in conjunction with the Mastersizer 2000 software. Three replicate sediment samples from each site were pooled and homogenised. Approximately 1 g of sediment was added to the particle size analyser and 3 independent size determinations were made. This was repeated 3 times using the same pooled sample to determine an overall average.

Determination of sediment organic matter content

The loss on ignition method (LOI) was used to determine organic matter content of sediment samples. Three replicate sediment samples from each site were pooled and homogenised. Approximately 20 g of fresh sediment from each sample was placed in a pre-weighed crucible and dried at 95°C for 24 h. Approximately 4 g of the resultant dried sediment was re-weighed, transferred to another crucible and placed into a muffle furnace at 550°C for 4 h. Organic matter content was calculated as the difference between the weight of the dry sediment and weight of the residue post-combustion. This was repeated 5 times using the same pooled sample to determine an overall average. Moisture content per g of fresh sediment was determined by calculating the percentage difference between wet weight and dry weight after 24 h at 95°C (Table S2).

In situ physico-chemical measurements of estuarine transect sample sites

Conductivity (calculated to practical salinity units (PSU)), temperature and depth measurements were recorded in situ using a YSI 6000S CTD scanner attached to a YSI 650 MDS data logger. This was deployed at each sample site in parallel with collection of water and sediment samples. Salinity and temperature measurements were recorded from the water immediately above the sediment bed and 0.2 m below the water surface. (Conductivity readings were calculated to determine salinity) (Table S3).

DNA extraction, 16S rRNA gene PCR and sequencing of bacterial isolates

To validate the identity of bacterial colonies on selective microbiological medium, DNA was extracted from a total of 30 isolates that matched or were similar to the expected colony morphology/phenotype for *E. coli*, enterococci, *Campylobacter* and *Vibrio* spp. for 16S rRNA gene PCR amplification and sequencing. For *E. coli* and enterococci, little variation in colony morphology and phenotype was observed across all of the colonies counted, and so only a small number of isolates were sequenced for confirmation.

DNA was extracted from 30 isolated bacterial colonies using the ISOLATE genomic DNA mini kit (Bioline Reagents Ltd., London, UK) following the manufacturer’s protocol. Agarose gel electrophoresis (1%) was used to visualise the DNA extracted from each bacterial isolate. Subsequently, the 16S rRNA gene of each isolate was amplified via PCR using the primer pair pA (5′-AGAGTTTGATCTTGCCAGCTA-3′) and pB (5′-AAGGAGTGTACGGAAGCGCAC-3′) (45). PCR reactions consisted of 10 pmol of each primer (pA and pB), 1x MyTaq red mix (Bioline), approximately 100 ng of template DNA and ddH2O to a total reaction volume of 50 μl. The PCR conditions were as follows: an initial denaturation step at 95°C for 1 min, followed by 35 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 10 s and a final hold at 4°C. PCR amplicons were visualised using 1% agarose gel. The expected 16S rRNA gene amplicon size was approximately 1500 bp and amplicons of the expected size were subsequently excised from the agarose gel and purified using the Isolate PCR and Gel Kit (Bioline Reagents Ltd., London, UK) following the manufacturer’s instructions. Purified 16S rRNA gene amplification products for each bacterial isolate were sequenced in both the forward and reverse orientation using Macrogen Europe (Netherlands). The Gениous Pro software package, Gениous 6.1 version created by Biomatters, (Available from http://www.geneious.com/) was used to quality clip each sequence and assemble the forward and reverse reads of each strain into a contiguous sequence. The sequence identity of each contig was determined using NCBI BLASTn. Identification of sequenced strains is given in Table S4 of the supplemental material.

Statistical analysis

Using the Statistical Package for Social Sciences SPSS v20, (IBM Corp., Armonk. NY), basic correlations were performed using the average data calculated for each site to determine the relationships between cultured bacterial abundance in sediments and water with different tested parameters. The non-parametric Spearman Rank Correlation Coefficient (rs) was used due to the data being not normally distributed.

Results

The relationship between PIB abundance, sediment grain size and organic content

There were marked spatial differences in the abundance of sediment-associated PIB across all of the 21 sample sites within the estuary as determined by culture counts on selective medium (Table 1). Mean densities of *E. coli* and total coliforms in sediments ranged from 0 to 2.4×10⁵ CFU/100 g and 0 to 5.4×10⁴ CFU/100 g wet weight, respectively (for all mean bacterial densities from all sampling sites see Table S5 in the supplemental material). Enterococci and *Salmonella* abundance varied from 0 to 1×10⁶ CFU/100 g and 0 to 2.5×10⁶ CFU/100 g wet weight, respectively. *Vibrio* spp. were detected at all 21 sample sites (6.7×10⁵ to 1.2×10⁶ CFU/100 g wet weight) and this reflects their status as indigenous members of marine and aquatic environments. In addition, direct colony counts on *Campylobacter* selective media indicated the presence of 0 to 3.5×10⁴ CFU/100 g wet weight. However, 16S rRNA gene sequencing of putative *Campylobacter* spp. isolated from a subsequent sampling survey on
the same selective medium revealed that none of the sequenced isolates were *Campylobacter* spp. (Table S4). Culturable heterotrophic bacteria were enumerated as a proxy for the abundance of the indigenous estuarine microbial community and their abundance ranged from $6.0 \times 10^3$ to $8.7 \times 10^5$ CFU/100 g wet weight. The enumeration of culturable heterotrophic bacteria alongside our target PIB groups enabled analysis of the relationship between total culturable heterotrophic bacteria counts and PIB counts in sediments, these data demonstrate that the abundance of heterotrophic bacteria within the sediments showed significant negative correlations with the abundance of all PIB groups measured (Table S6). Sediment grain size composition within sediment samples ranged from clay (0 to 18%), silt (0 to 65%), very fine sand (0 to 15%), fine sand (3 to 67%) medium sand (0 to 59%), coarse sand (0 to 17%) and very coarse sand (0 to 16%). The organic matter content of the sediment samples varied from 0.3 to 6% across the sample sites (Figure 2).

The abundance of each cultured bacterial group within the sediments showed significant positive correlations with the abundance of all other measured bacterial groups (Table S6). *Vibrio* spp. were also more abundant in sediments that had higher densities of FIB. In addition, the abundance of all isolated PIB groups showed a significant positive correlation with both sediment clay content (grain size $<4 \mu m$) (*E. coli*, enterococci, total coliforms, and *Vibrio* spp., $r_s = 0.543$ ($p < 0.011$), $r_s = 0.864$ ($p < 0.001$), $r_s = 0.495$ ($p < 0.023$), $r_s = 0.663$ ($p < 0.001$) respectively) and silt content (grain size 4 $\mu m$–63 $\mu m$) (*E. coli*, enterococci, total coliforms and *Vibrio* spp., $r_s = 0.570$ ($p < 0.007$), $r_s = 0.687$ ($p < 0.001$), $r_s = 0.547$ ($p < 0.010$), $r_s = 0.668$ ($p < 0.001$) respectively). Significant negative correlations were observed between the abundance of all PIB groups with fine sand (125 $\mu m$–250 $\mu m$) and medium sand (250 $\mu m$–500 $\mu m$) (Table S7). Sediments with high amounts of clay and silt along with very fine sand contained the greatest proportion of organic material (significant positive correlation; organic matter content and clay, $r_s = 0.917$ ($p < 0.001$), organic matter content and silt, $r_s = 0.926$ ($p < 0.001$), organic matter content and very fine sand, $r_s = 0.810$ ($p < 0.001$). Significant negative correlations were evident between organic matter content and fine sand and also organic matter content and medium sand (Table S8). Sediments with high organic matter therefore also showed significant positive correlations with the abundance of all isolated PIB (Table S9).

### Comparison of PIB abundance between sediment and water samples

The average abundance data for culturable PIB (*E. coli*, total coliforms and *Vibrio* spp.) in sediment and water samples across all 21 sample sites revealed that *E. coli*, coliforms and *Vibrio* spp. were $2.8 \times 10^3$, 433 and 58-fold more abundant in the sediment (CFU/100 g) than the water column (CFU/100 ml), respectively (Table 1).

| Bacterial group | Sediment | Water | Fold - difference |
|-----------------|----------|-------|-------------------|
| *E. coli*       | $5.9 \times 10^5$ | $2.1 \times 10^3$ | 281               |
| Total coliforms | $1.3 \times 10^5$ | $3.0 \times 10^2$ | 433               |
| *Vibrio* spp.   | $4.5 \times 10^5$ | $7.8 \times 10^3$ | 58                |

![Pathogen Indicator Bacteria in Estuarine Sediments](https://example.com/pathogen.png)
effects of environmental stresses, such as providing protection from UV light (34), which in turn can augment the survival and even growth of FIB. For example, DNA fingerprinting analyses performed on *E. coli* populations in beach sand and sediment revealed the possibility that some strains may have become naturalized to this environment [52] and growing evidence also...
suggests that there may be free-living strains of FIB surviving and multiplying within the water column of environmental waters, independently of a host [33].

The abundance of *E. coli* and enterococci in sediments had a significant positive correlation with the abundance of other PIB. Consequently, the enumeration of FIB as the ‘classic’ bacterial indicators currently conducted as part of routine water quality monitoring represent suitable indicators for the presence of other PIB groups. *E. coli* and enterococci are the predominant indicator species for monitoring faecal pollution of aquatic environments within the European Union (EU) despite numerous studies highlighting the differential environmental survival of different FIB groups [48,51], in addition to different strains of the same group [34]. In this study, the presence and abundance of enterococci had a stronger significant positive correlation with silt and clay when compared with other FIB, which may suggest greater survival times of enterococci under favourable conditions that certain sediment types may provide. However, it is well established that the survival times of different species, and even strains of the same species, varies considerably in aquatic environments, both between and within species, and this must be taken into consideration. For example, in comparison to other FIB, enterococci survive for longer periods in sediments [48] and within estuarine environments [31], and it has been proposed that the increase in survival under harsh conditions may be due to their membrane composition [55,56]. Such evidence suggests that enterococci may be a more robust indicator of the persistence of faecal contamination rather than more recent contamination within coastal and estuarine environments.

The enumeration of total heterotrophs revealed significant positive correlations with all other cultured PIB groups and therefore the same trends were seen within the indigenous microbial community (Table S6). It should be noted that despite obtaining high colony counts on selective microbiological media for *Campylobacter* spp., 16S rRNA gene sequence analysis of some of these isolates suggested that none were *Campylobacter* spp. These data highlight the potential pitfalls of culture-based analyses of microbial taxa using a selective microbiological medium, and care must be taken when interpreting microbial culture counts. Despite this, the sequenced isolates of enterococci, *Vibrio* spp. and *E. coli* indicate that these media were selective for the desired bacterial target groups (Table S4). Little variation in temperature and salinity could be explained by the small geographical variation along each transect. There was no significant correlation between temperature and salinity and the abundance of PIB, with the exception of *Vibrio* spp. enumerated from the water column. Here, *Vibrio* spp. had a significant positive correlation with salinity and a significant negative correlation with temperature, supporting previous proposals that temperature and salinity have important implications for *Vibrio* spp. population dynamics [30]. The depth at which sediment samples were taken had no impact on the abundance of PIB enumerated from the sediments.

This study highlights the risk of periodic elevated FIB and other PIB in the water column due to the resuspension of microbial contaminated sediments. Furthermore, the risk of microbial pollution from the resuspension of sediments is not taken into consideration when assessing microbial pollution of recreational and shellfish harvesting waters. The time and place of sampling in addition to tidal and hydrodynamic conditions may impact upon FIB concentrations in the water column. Despite several reports on the importance of sediments as a reservoir for FIB [28,29], the enumeration of waterborne FIB has received much more attention. Quilliam *et al.*, (2011) revealed significant spatial variations in waterborne *E. coli* numbers on contrasting sides of the same four transverse transects of the Conwy estuary studied here, indicating that significantly different levels of microbial pollution were present in the east and west sides of the river, and the localised re-suspension of sediment associated FIB into the water column was one proposed explanation for such contrasting FIB counts in the water column [45]. Despite this, the dynamics of sediment transport and re-suspension in relation to PIB concentrations is poorly understood. The attachment of PIB to particulate matter in the water column provides a platform for transportation and downward flux to the bed sediment, cyclical changes in tidal flow and the salinity of the water will also impact the downward flow of particle-associated PIB to the bed sediments. Conversely, under certain hydrodynamic conditions (e.g. tidal cycles and storm flow events), bed stress and turbulence can also impart the resuspension of sediments and subsequent transportation and deposition of particle associated PIB to other areas of the estuary. Due to the hydrodynamics of an estuarine system, fine sediments are usually deposited around the banks of the basin [49] and our data support this trend, with finer particles detected in greater abundance in sediments on the east and west sides of the estuary and coarser sand deposited in the central channel. FIB levels in water can be affected by other factors such as temperature [33], exposure to sunlight and salinity [31], nutrient concentrations [5], predation by protozoan [50] and competition [51]. However, here we demonstrate that sediments also contribute to the distribution of FIB and other PIB in an estuarine environment.

Experimental results confirm that estuarine sediments harbour PIB, which may potentiate their prolonged persistence and survival in this environment. This study also identifies areas of high microbial contamination within the Conwy estuary, which highlights the risk of sediment and PIB resuspension back into the water column under turbulent hydrodynamic conditions that result in sediment bed stress and promote the erosion of sediments.

To our knowledge, this is the first comprehensive study of the co-occurrence of *E. coli*, coliforms, enterococci, *Salmonella* and *Vibrio* spp. (PIB) in relation to sediment composition. These data show that all PIB groups studied are strongly correlated with the presence of clay, silt and organic matter content in sediments. In addition, the presence of *E. coli* and total coliforms strongly correlates with the abundance of the other PIB tested, both allochthonous and autochthonous, suggesting that culture-based determinations of *E. coli* and coliform abundance in sediments represent a useful surrogate for the presence of other PIB groups.

**Conclusion**

Faecal contamination in aquatic environments is currently assessed by measuring culturable *E. coli* and enterococci counts in water samples only. Here, we demonstrate that sediment composition, specifically clay, silt and organic matter content, are linked with greater PIB abundance in sediments. The enhanced abundance of viable PIB in sediments therefore has implications for water quality and public health when considering the potential for resuspension of bed sediments, particularly finer particles such as clay and silt that we have demonstrated to contain higher concentrations of PIB. It may therefore be necessary to incorporate PIB loadings in bottom sediments into routine monitoring protocols and hydrodynamic models to adequately assess their risk to human health. The detection of spatial variations of PIB within sediments also highlights the necessity for further research on the interactions of pathogens with sediments and their role in the survival, persistence and transportation of PIB within environmental waters.
Supporting Information

Table S1 Selective media used to enumerate target bacterial groups.

Table S2 Sediment dry weight determined from 1 g wet weight.

Table S3 Details the depth at which the sediment samples were collected, salinity measurements taken for both water samples (0.2 m from the surface) and directly above the sediment samples, calculated as Practical Salinity Units (PSU). Temperature was recorded at a depth of 0.2 m from the surface and directly above the sediment.

Table S4 Identification of sequenced isolates.

Table S5 Bacteria counts, sediment (CFU/100 g) versus water (CFU/100 ml). Data shown as mean (n = 3, sample point 13 n = 2 for sediment).

Table S6 Correlation coefficient ($r_s$) matrix demonstrating the relationship between the abundance of each cultured bacterial group within estuarine sediments (n = 21).

Table S7 Correlation coefficient ($r_s$) matrix demonstrating the relationship between the abundance of each cultured bacterial group within estuarine sediments and sediment grain size (n = 21).

Table S8 Correlation coefficient ($r_s$) matrix demonstrating the relationship between estuarine sediment grain size (%) and organic matter content (%) (n = 21).

Table S9 Correlation coefficient ($r_s$) matrix demonstrating the relationship between the abundance of each cultured bacterial group within estuarine sediments and sediment organic matter content (n = 21).

Table S10 Correlation coefficient ($r_s$) matrix demonstrating the relationship between the abundance of each cultured bacterial group within estuarine sediments and physico-chemical parameters measured directly above the bottom sediments (n = 2).

Table S11 Correlation coefficient ($r_s$) matrix demonstrating the relationship between the abundance of each cultured bacterial group within estuarine sediments and physico-chemical parameters measured at 0.2 m depth (n = 21).

Acknowledgments

We are grateful to Ben Winterbourn, Gwynn Parry, Jones, Peter Hughes and Ben Powell for their assistance with estuarine sampling.

Author Contributions

Conceived and designed the experiments: TLP JHB CFJ DLJ SKM JEM. Performed the experiments: TLP KC JHB JEM. Analyzed the data: TLP KC JHB JEM. Contributed reagents/materials/analysis tools: JHB CFJ DLJ SKM JEM. Contributed to the writing of the manuscript: TLP KC JHB DLJ SKM JEM.

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11. JHB DLJ SKM JEM. Contributed to the writing of the manuscript: TLP KC JHB DLJ SKM JEM.

Table S8 Correlation coefficient ($r_s$) matrix demonstrating the relationship between estuarine sediment grain size (%) and organic matter content (%) (n = 21).

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Table S10 Correlation coefficient ($r_s$) matrix demonstrating the relationship between the abundance of each cultured bacterial group within estuarine sediments and physico-chemical parameters measured directly above the bottom sediments (n = 2).

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We are grateful to Ben Winterbourn, Gwynn Parry, Jones, Peter Hughes and Ben Powell for their assistance with estuarine sampling.

Author Contributions

Conceived and designed the experiments: TLP JHB CFJ DLJ SKM JEM. Performed the experiments: TLP KC JHB JEM. Analyzed the data: TLP KC JHB JEM. Contributed reagents/materials/analysis tools: JHB CFJ DLJ SKM JEM. Contributed to the writing of the manuscript: TLP KC JHB DLJ SKM JEM.

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