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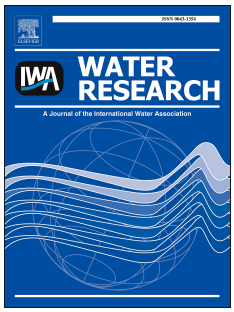

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Title

The role of zeta potential in the adhesion of *E. coli* to suspended intertidal sediments

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Running Title: *E.coli* adhesion to intertidal sediments

Abstract

The extent of pathogen transport to and within aquatic systems depends heavily on whether the bacterial cells are freely suspended or in association with suspended particles. The surface charge of both bacterial cells and suspended particles affects cell-particle adhesion and subsequent transport and exposure pathways through settling and resuspension cycles. This study investigated the adhesion of Faecal Indicator Organisms (FIOs) to natural suspended intertidal sediments over the salinity gradient encountered at the transition zone from freshwater to marine environments. Phenotypic characteristics of three *E. coli* strains, and the zeta potential (surface charge) of the *E. coli* strains and 3 physically different types of intertidal sediments was measured over a salinity gradient from 0 – 5 Practical Salinity Units (PSU). A batch adhesion microcosm experiment was constructed with each combination of *E. coli* strain, intertidal sediment and 0, 2, 3.5 and 5 PSU. The zeta potential profile of one *E. coli* strain had a low negative charge and did not change in response to an increase in salinity, and the remaining *E. coli* strains and the sediments exhibited a more

negative charge that decreased with an increase in salinity. Strain type was the most important factor in explaining cell-particle adhesion, however adhesion was also dependant on sediment type and salinity (2, 3.5 PSU > 0, 5 PSU). Contrary to traditional colloidal (Derjaguin, Landau, Vervey, and Overbeek (DLVO)) theory, zeta potential of strain or sediment did not correlate with cell-particle adhesion. *E. coli* strain characteristics were the defining factor in cell-particle adhesion, implying that diverse strain-specific transport and exposure pathways may exist. Further research applying these findings on a catchment scale is necessary to elucidate these pathways in order to improve accuracy of FIO fate and transport models.

Keywords: Pathogen, Adhesion, DLVO Theory, Zeta potential, Intertidal sediment

1. Introduction

In the early 2000s, it was predicted that local and foreign tourists spent two billion days each year at the coast worldwide (Shuval 2003), and an estimated 20 million people used the coast and inland waters each year in the UK (Pond 2005), with these numbers predicted to increase. Around this period, bathing at coastal sites caused an estimated 120 million cases of gastrointestinal illness worldwide (Shuval 2003), and bathing at English and Welsh beaches and bathing waters caused an estimated 1.75 million cases of gastrointestinal disease annually (Georgiou and Langford 2002). The most common disease associated with bathing in contaminated water is enteric illness with an associated risk of roughly 51/1000 bathers, and the risk of other respiratory, ear and eye disease between 20/1000 and 54/1000 bathers in water that contained <2000 faecal coliforms 100 ml^{-1} (Fleisher et al. 1998). The likelihood of gastrointestinal illness to sea-bathers compared to non-bathing beach goers increases 1.76 fold (Fleisher et al. 2010). However, risk is not solely associated with bathers, as an increase in enteric illnesses can be a direct result from increased contact with recreational beach sand (Heaney et al. 2012).

46 It is well established that survival of FIOs is greatly increased when in association with sediments
47 compared to the overlying water in both freshwater and marine systems (Gerba and Mcleod 1976;
48 Moore et al. 2003; Pachepsky and Shelton 2011). This is a result of many survival advantages
49 including increased nutrient availability (Burton et al. 1987) and protection from UV (Fujioka and
50 Yoneyama 2002) and protozoan grazing (England et al. 1993). Faecal Indicator Organisms gain these
51 survival advantages through adhering to particles in suspension, leading to incorporation of FIOs in
52 sediments as the particle is deposited (Davies et al. 1995; Geldreich 1970).

53 The transport and fate, and therefore spatial and temporal abundance, of faecal indicator organisms
54 (FIOs) within aquatic systems is heavily dependent on whether cells are freely suspended, or
55 associated with suspended particles (Bai and Lung 2005; Jeng et al. 2005; Muirhead et al. 2006b).
56 Particle association also governs FIO residence time through incorporation into the erosion,
57 transport, deposition and consolidation (ETDC cycle) of particles (Whitehouse 2000). The importance
58 of differentiating between these phases has been realised in recent modelling approaches
59 concerning FIO fate and transport on catchment scales (Cho et al. 2016b).

60 Free bacterial cells in the water column are maintained in suspension by Brownian motion but
61 become susceptible to sedimentation when in association with particles because of the increased
62 settling velocity. The mechanisms governing the adhesion of faecal bacteria to suspended particles
63 are complex and may be determined by a range of physical, and biological factors (Oliver et al.
64 2007). Derjaguin, Landau, Vervey, and Overbeek (DLVO) theory is known to serve as a basic model
65 for describing the initial adhesion of bacteria to suspended particles (Van Loosdrecht et al. 1990),
66 and has been since been improved upon for the prediction of cell adhesion with the extended DLVO
67 theory (xDLVO) (Perni et al. 2014)

68 Briefly, DLVO theory describes the interplay between electrostatic repulsion and the attraction of
69 Van der Waals forces between colloidal particles. The strength of the electrostatic repulsion can be
70 determined by measuring the particle charge, known as the zeta potential, of a colloidal suspension.

As zeta potential becomes more positive or negative, the larger the electrostatic repulsion between particles, the less likely they are to flocculate (Van Loosdrecht et al. 1987).

In this study, phenotyping assays and zeta potential analyses were followed by a microcosm experiment using natural intertidal sediments and river and seawater in order to investigate the role of strain and sediment characteristics and particle charge in the adhesion of *E. coli* to suspended particles. It was hypothesised that the less negative zeta potentials of cell and/or sediments induced by higher salinity would correlate with increased cell- particle adhesion.

2. Materials and Methods

2.1 *E. coli* strains

One of several wild-type *E. coli* strains isolated from intertidal sediment at the Ythan estuary, Scotland, UK (57°20'59.3" N 1°59'36.8" W) in May 2014 was selected for use and hereby referred to as Yth13 throughout. *E. coli* strains DSM 8698 and DSM 9034 were obtained from the Leibniz Institute DSMZ (Germany). Serological and isolation details are provided in Table 1. Unless otherwise stated, *E. coli* cultures were prepared from -80 °C stock cultures through overnight incubation in LB broth at 37 °C in a shaking incubator at 150 rpm. Cells were harvested by centrifugation at 3000 x g for 5 minutes, and washed three times in the appropriate dilution of seawater.

2.2 Bacterial strain characterisation

Swarm assays were performed using a similar method to that of Wolfe and Berg (1989). Cells from a single colony were stab-inoculated into the centre of a 0.4 % LB agar plates (LB broth, Merck Millipore, Darmstadt, Germany). Three replicate plates for each strain at each temperature treatment (15 ˚C and 25 ˚C) were sealed with laboratory film to reduce moisture loss, inverted and incubated. The swarm diameter was measured to the nearest mm every 24 hours.

Biofilm assays were performed using a similar method to that of Merritt et al. (2011). Briefly, *E. coli* cultures were grown overnight in 5 ml LB broth at 37 ˚C. Cultures were normalised to an OD600 of 0.5 using 70-850 µl disposable micro-cuvettes (Brand GMBH + CO KG, Germany) in a spectrophotometer (BioPhotometer 6131, Eppendorf). One µl of each normalised *E. coli* culture was inoculated into 4 replicate wells in a sterile 96 well cell culture plate (Costar, Corning, NY, USA) alongside 4 wells containing media only controls. One hundred µl of fresh sterile LB broth media was added to each well. The plate was covered with laboratory film to minimise evaporation and incubated at 25 °C for 48 hours. After incubation, the plate was washed twice with DI H_20, and 125 µl of 0.1% (w/v) crystal violet in DI H_2O was added to each well and the plate incubated for 10 minutes at room temperature. The plate was rinsed as above and left to air dry before 200 µl of 80:20 ethanol: acetone solution (O'Toole et al. 1999) was added to each well and the plate incubated for 15 minutes at room temperature. The contents of each well were mixed via pipetting, and 125 µl transferred to a micro-cuvette with the OD600 of each biofilm elution measured against a blank of the ethanol: acetone solution.

2.3 Sediment and Water Properties

Intertidal sediments with differing properties (Mud (M), Organic Mud (OM) and Mixed Sand (MS)) were collected from 3 locations within the Ythan estuary, Scotland, UK (Table 2). Bulk sediment for the microcosms was collected from the surface to 10 mm depth. Cores to a depth of 10 mm with a volume of 3.14 cm^3 were taken for measurement of bulk density. Sediments were dried at 105 ˚C for 24 hours, then ignited in a furnace at 450 ˚C for 12 hours to determine water content and organic content respectively. Laser particle size distribution was analysed using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK). Sediment surface area was measured by adsorption of nitrogen gas (Coulter SA 3100, Beckmann Coulter, UK) and calculated using the Brunauer, Emmett and Teller equation (Brunauer et al. 1938) using 10 points in the analysis. X-Ray Powder Diffraction (XRPD)

analysis was performed to determine sediment bulk mineralogy. Briefly, freeze-dried samples were hand ground, then wet milled in ethanol (McCrone Mill, McCrone, IL, USA) and spray dried. XRPD patterns were recorded from 3-70°2θ using Copper Kα radiation with a Panalytical Xpert Pro diffractometer equipped with an Xcelerator detector.

River water was collected from the River Ythan beyond the tidal range (57°21'48.5"N 2°04'23.9"W), and sea water collected from Collieston Harbour located 5 km Northeast of the estuary mouth (57°20'50.4"N 1°56'03.6"W). Soluble elemental analysis was performed using standard procedures on a Skalar SAN^{++} autoanalyser (Skalar Analytical B. V., Netherlands).

2.4 Microcosm preparation

River water (0.11 PSU) and sea water (34.5 PSU) were vacuum filtered through 0.45 µm filter paper (Merck Millipore, MA, USA). Solutions of river water (hereby referred to as 0 PSU), 1, 1.5, 2, 2.5, 3, 3.5, 4 and 5 PSU were created by mixing the stock waters whilst monitoring the salinity change. Salinity and pH were recorded at using a Hach HQ40d multi-probe (Hach Lange, UK). The equivalent of 50 g dry weight of each sediment type was weighed into 700 ml centrifuge vessels, suspended in 250 ml DI H_2O and centrifuged at 1450 x *g* for 15 minutes. The supernatant was removed, and the washing procedure repeated twice. Sediment was finally resuspended in 500 ml (1 g sediment: 10 ml water) of the appropriate salinity solution. Sediment suspensions were then sonicated for 5 minutes at 20 % power with the microtip attachment (600 W Ultrasonic Processor, Sonics and Materials Inc., CT, USA). The sediment was kept in suspension by magnetic stirring, and 20 ml suspension aliquoted into 3 replicate 50 ml falcon tubes for each *E. coli* strain/ salinity suspension. Overnight cultures of *E. coli* strains Yth13, DSM 8698 and DSM 9034 prepared as in Section 2.1 and inoculated into microcosms at a final inoculum concentration of 1 x 10^7 CFU ml^{-1}. Additional non-inoculated controls and non-sediment microcosms were prepared. Microcosms were incubated at 12 ˚C for 90 minutes, with a shaking speed of 300 rpm in order to keep sediment particles in suspension.

2.5 Quantification of adhered *E. coli*

Post-incubation, microcosms were centrifuged at 500 x *g* for 120 s at 12 ˚C to separate non-adhered *E. coli* from those adhered to particles > ~1.55 µm according to Stokes' law. Triplicate aliquots of 20 µl of supernatant were removed at a depth of 15 mm and serial dilutions made to 10^{-8}. Twenty µl of each serial dilution was pipetted from 10 mm onto HiCrome Coliform agar plates (Sigma Aldrich, UK) and, when dry, the plates inverted and incubated at 37 ˚C for 18 hours. Non-inoculated microcosms were analysed in order to enumerate *E. coli* pre-existing in each sediment. The inoculum culture and non-sediment microcosms were also analysed to establish the recovery of viable *E. coli* after the centrifugation and quantification process. Recovered *E. coli* values were normalised to an inoculum density of 1 x 10^7 CFU ml $^{-1}$. Non- recovered *E. coli* (non- sediment control microcosms) were added to the resulting value, and this subtracted from the inoculum density to give a final concentration of adhered *E. coli*.

2.6 Zeta potential measurements

Zeta potential measurements were made on *E. coli* cultures prepared as for microcosm inoculation, and non-inoculated sediment microcosms were left to settle for 15 minutes to reduce particle settling during the measurement. Measurements were performed using the Zetasizer Nano ZS (Malvern Instruments, UK) using the DTS 1070 cell at 12 ˚C using fresh samples for each of three replicates. In order to avoid electrode and sample degradation at the higher salinities (Malvern 2014), automatic voltage selection, monomodal analysis only, and a 60 s delay between measurements were applied. Zeta potential was calculated from the electrophoretic mobility of particles using the Smoluchowski model (Hiemenz 1977) with a fixed F(ka) value of 1.5.

2.7 Statistical analyses

Mean absorbance of the media-only controls in the biofilm assay was subtracted from each sample absorbance. Data was square root transformed to comply with the assumptions of ANOVA. Comparison of swarm diameter means at each time point was performed using ANOVA after Bonferroni correction of the family-wise significance threshold to $p = 0.0026$ to account for multiple comparisons. Zeta potential measurements were analysed using two-way ANOVA with salinity and either strain or sediment type as factors.

Any *E. coli* enumerated from the non-inoculated microcosms were subtracted from the counts from the relevant inoculated microcosms. Recovered *E. coli* counts from the microcosms were normalised to the same inoculum concentration adjusted according to non-recovered CFUs obtained from the non-sediment controls. Counts were then log transformed to meet the assumptions of ANOVA to produce the amount of $\log_{10}$ sediment-adhered *E. coli* CFU ml^{-1}. All statistics were performed using Genstat (GenStat Release 18.1 (PC/Windows); VSN International Ltd., 2015), and figures created using SigmaPlot (SigmaPlot 13.0, Systat Software Inc., CA, USA).

3. Results

3.1 Bacterial strain characteristics

There were large differences between the phenotypic characteristics of the three *E. coli* strains. The absorbance in the biofilm assay for DSM 8698 was over 4 times greater than the wild-type strain Yth13 (One-way ANOVA; F- statistic of strain effect= 30.093, total d.f.= 11, $p < 0.001$), which in turn was almost 4 times greater than DSM 9034 ($p < 0.001$) (Table 3). Growth rates of swarm colonies rates were higher for all strains at 25 ˚C than 15 ˚C (Table 3). At 15 ˚C the swarm colony diameter of DSM 9034 was significantly smaller than other strains from Day 3 (One-way ANOVA; F- statistic of

192 strain effect= 61.00, total d.f.= 8, $p < 0.001$), and Yth13 became significantly larger than DSM 8698
193 from Day 5 ((One-way ANOVA; F- statistic of strain effect= 127.00, total d.f.= 8, $p < 0.001$). At 25 ˚C
194 all replicates of DSM 8698 had reached the edge of the petri dish (85 mm) within 3 days, whereas
195 DSM 9034 and Yth13 demonstrated a more moderate increase in swarming rate compared to 15 ˚C
196 with the size of DSM 9034 significantly smaller than Yth13 again from Day 3 (One-way ANOVA; F-
197 statistic of strain effect= 25908.50, total d.f.= 8, $p < 0.001$).

198 The two-way interaction of strain and matrix salinity influenced the zeta potential of *E. coli* strains
199 (Two- way ANOVA; F- statistic of strain x sediment interaction= 18.044; total d.f.= 80; $p < 0.001$). The
200 zeta potential of DSM 9034 was much less negative than the other strains and changed minimally
201 across the salinity gradient (mean -3.23 ± SE 0.07 mV). DSM 8698 and Yth13 followed a similar trend,
202 remaining relatively stable at around -23 and -19 mV respectively between 0 and 2.5 PSU where
203 thereafter they became increasingly less negative with increasing matrix salinity before starting to
204 plateau between 4 and 5 PSU, following a sigmoidal trend with a point of inflection at roughly 3 PSU
205 (Fig. 1).

206

207 3.2 Sediment and water characteristics

208 Organic Mud (OM) and Mud (M) sediments had similar physical characteristics, whereas the Mixed
209 Sand (MS) had a higher bulk density, lower surface area, lower water and organic content and more
210 coarse particles (Table 2 and Supplementary Table 1). Mud had a slightly higher bulk density and
211 lower water and organic content than OM. X-Ray Powder Diffraction (XRPD) bulk mineralogy analysis
212 indicated a broadly similar abundance of minerals across all sediments, except MS contained a much
213 greater quartz content (Supplementary Table 2). Organic Mud also contained larger fractions of the
214 clay minerals kaolinite, trioctahedral clays and illite/muscovite, and smaller fractions of the
215 carbonate minerals aragonite and calcite than M. The river water collected beyond the tidal range
216 contained a lower NH_4 concentration than the sea water, but had a higher concentration of all other

components analysed (Supplementary Table 3). The chemistry of the river water used in this experiment was in line with the average levels for the Ythan river as reported by SEPA (SEPA 2013).

Sediment zeta potential generally became slightly less negative as matrix salinity increased (One-way ANOVA; F- statistic of salinity effect= 104.99; total d.f.= 35) (Fig. 2), with the magnitude of change less than *E. coli* strains DSM 8698 and Yth13. The mean zeta potential for all sediments was more negative at 2 PSU and below than 3.5 PSU and above ($p < 0.001$; 0 PSU: mean -21.38 ± SE 0.35 mV, 2 PSU: -20.83 ± 0.03 mV, 3.5 PSU: -17.03 ± 0.09 mV, 5 PSU: -16.11 ± 0.08 mV). The mean zeta potential over all salinities also differed with sediment type, where OM was less negative than M and MS sediments ($p < 0.001$; OM: -17.30 ± 0.05 mV, M: -19.30 ± 0.10 mV, MS: -19.91 ± 0.28 mV).

3.3 Adhesion of *E. coli* to sediments

There was no significant difference between *E. coli* numbers recovered from the different strain and salinity treatments of non-sediment control microcosms indicating negligible treatment effects, cell-cell adhesion or die-off during the incubation or centrifugation process.

E. coli adhesion to sediment particles increased with sediment type in the order OM > M > MS over all salinities and sediment types, however, only the difference between OM and MS was statistically significant (One-way ANOVA; F- statistic of sediment effect= 5.34, total d.f. 108; $p = 0.007$; Table 4). Adhesion of *E. coli* cells was significantly greater with strain DSM 8698 than both Yth13 and DSM 9034 (One-way ANOVA; F- statistic of strain effect= 43.38, total d.f. 108; $p < 0.001$). The adhesion of *E. coli* cells was significantly greater at 2 and 3.5 PSU than 0 and 5 PSU ($p < 0.001$).

DSM 8698 exhibited the greatest adhesion of all strains to M (Two-way ANOVA; F- statistic of sediment x strain effect= 25.28, total d.f. 108; $p < 0.001$) (Table 5; Fig. 3B). DSM 9034 adhered the least to M and demonstrated no major changes with salinity within treatments. In contrast, adhesion of DSM 8698 peaked at 2 PSU, and Yth13 peaked at 3.5 PSU to M. In OM and MS, these peaks of

adhesion for DSM 8698 and Yth13 were evident at the same salinities, and up to 2-fold higher than other salinities (Table 5, Fig. 3). With the exception of these peaks, in OM the strains exhibited broadly similar levels of adhesion across the salinity gradient (Fig. 3A). In MS, Yth13 adhered less than it did to other sediments, and to other strains to MS (Table 5, Fig. 3C).

Increase in zeta potential of the sediments significantly correlated with decreasing pH of sediment suspension as salinity increased. despite the narrow range of pH values observed (7.60 – 7.96) (n= 12, Adjusted R^2= 0.66, $p < 0.001$). However there was no significant relationship between sediment zeta potential and the number of adhered *E. coli.* Strain zeta potential explained little of the variation in adhesion of *E. coli* despite a significant relationship (n= 108, Adjusted R^2=0.09, $p < 0.001$).

4. Discussion

The zeta potential of all *E. coli* suspensions were negative, which is widely recognised to be the case for bacteria in natural aquatic systems (Rijnaarts et al. 1995; Sokolov et al. 2001; Van der Wal et al. 1997; Walker et al. 2004). The zeta potential profiles of *E. coli* strains in this study formed two distinct profile shapes; the stable profile of DSM 9034, and the sigmoidal curve of Yth13 and DSM 8698 with less negative zeta potential as ionic strength increased. The sigmoidal profile has been previously observed with different *E. coli* strains in increasing concentrations of NaCl and KCl (Chen and Walker 2012; Zhao et al. 2014). The decrease in zeta potential with increasing ionic strength is consistent with classic DLVO theory, resulting from charge screening by counter ions and increasing electrostatic double-layer compression (Kim et al. 2010; Walker et al. 2004). The limit of the decrease in zeta potential and subsequent plateau at higher electrolyte concentrations is explained

by a limit on the compression of the double layer of counterions surrounding the particle (Sharma et al. 1985). Instability of zeta potential similar to that exhibited by strains Yth13 and DSM 8698 between 0 and 2 PSU was previously observed in *E. coli* in low (10- 20 mM) concentrations of phosphate buffer (Carlsson 2012). The difference in the zeta potential profiles in this study are likely to be the result of differing cell surface morphology and lipopolysaccharide structures (Walker et al. 2004) and flagellar, fimbrae and curlin proteins, all of which are known to affect zeta potential (Feng et al. 2014).

Sediment zeta potential became less negative with increasing salinity and followed the same sigmoidal curve as the Yth13 and DSM 8698 *E. coli* strains. However, the magnitude of change was less for sediments than that of the *E. coli* strains, an observation made previously by Zhao et al (2014) who proposed it to be a result of the combination of variably and permanently charged minerals and organic matter present in soil colloids compared to pure samples. Similar sigmoidal trends have been observed for soil colloids at similar electrolyte concentrations with a plateau at 50 mM of KCl solution (~3.5 PSU) (Zhao et al. 2014), and for suspended estuarine particles with a plateau at 5- 7.5 PSU seawater (Hunter and Liss 1982). The zeta potential profiles are similar between sediment types despite the physical and mineralogical differences. This may be attributed to organic conditioning films, chiefly proteinaceous substances that cover particle surfaces and are found especially in highly productive environments such as estuaries, that can neutralise any physico-chemical features of the surface, including surface charge (Donlan 2001).

Despite large and significant differences in the zeta potentials of different bacterial strains and sediments over the salinity gradient, there was no significant correlation between zeta potential and adhesion of cells to suspended sediments. Visual inspection revealed decreased turbidity with increased salinity in microcosms post-centrifugation due to increased particle-particle adhesion and flocculation of sediment particles at higher salinities in line with DLVO theory) identified a critical salinity equivalent to ~1.22 PSU, below which *E. coli* cells became desorbed from a mixed sediment,

290 therefore it may be that adhesion between 0 and 3.5 PSU is in accordance with classic DLVO theory,
291 but this was inhibited or superseded at 5 PSU by stronger metabolic or physiological factors.
292 Increased salinity is well known to cause sub-lethal and lethal stress in *E. coli* (Anderson et al. 1979;
293 Carlucci and Pramer 1960), with *E. coli* growth rates observed to peak at intermediate
294 concentrations of NaCl and KCl (Abdulkarim et al. 2009), and seawater (Carlucci and Pramer 1960).
295 The metabolic response of *E. coli* to saline stress is complex, involving the accumulation of K^+ ions
296 and several osmosprotective and membrane-stabilizing endogenously synthesized organic solutes
297 (Sevin et al. 2016). Despite the relatively high saline tolerance of *E. coli* (Sevin et al. 2016), at the
298 higher osmotic stress of 5 PSU activation of stress response factors leading to alteration of cell wall
299 characteristics may be resulting in decreased adhesion to particles.

300 Trends in particle-cell adhesion observed here did not strictly follow DLVO theory, which supports
301 the conclusions of Rong et al. (2008) that non-electrostatic mechanisms rather than electrostatic
302 forces are more important in bacterial adhesion to particles. In the systems studied, it is postulated
303 that the turbulence simulated in the microcosms exceeded or obscured any electrostatic
304 interactions. Therefore, cell characteristics such as adhesins, fimbrae and flagella became more
305 significant than particle charge in successful adhesion under turbulence-mediated cell-particle
306 collisions. Future investigations into how cell surface morphology and characteristics effect adhesion
307 in natural systems are necessary in order to elucidate the mechanisms at work."

308 *E. coli* adhered better to sediments with larger proportions of fine particles (DSM 8698- M, Yth13-
309 OM, DSM 9034- MS, OM). This preferential adhesion by different strains has been observed by
310 Pachepsky et al. (2008) where the authors found 80 % of strains adhering to silt particles were not
311 found adhered to fine sand, with further strains found only to adhere to sands and not to silt or clay.
312 Similarly, several studies have observed greater adhesion of *E. coli* to soil particles of smaller size
313 fractions. However, Oliver et al. (2007) observed that although the greatest number of *E. coli* bound
314 to the 4-15 µm fraction of suspended soil particles, the 16-30 µm fraction had almost 4 times the

315 amount of adhered *E. coli* after adjustment for surface area. Muirhead et al. (2006) also found most
316 *E. coli* was being transported in overland flow in association with soil particles in the <20 µm
317 fraction.

318 The strains examined here exhibited very different phenotypic characteristics, however there was no
319 obvious correlation or trend between these and adhesion success despite strain type remaining the
320 most important factor determining adhesion extent. It has been established that there is strain
321 dependant preference of adhesion to suspended soils and sediments (Oliver et al. 2007; Pachepsky
322 et al. 2008), however the work presented here demonstrates that this preference is dependant on
323 sediment type and salinity. This is further complicated by the variation in the persistence between
324 strains that has been observed in freshwater microcosms (Anderson et al. 2005) and between soil
325 types (Ma et al. 2014).

326 Therefore the spatial variation in the abundance of strains of a single species in intertidal sediments
327 depends on strain input, salinity and suspended sediment characteristics. The highest adhesion of E.
328 coli to sediment particles was observed in this study at 2- 3.5 PSU. These salinities would be
329 encountered by FIOs being transported down-catchment at the tidal limits of estuaries and where
330 freshwater tributaries enter the estuary. Due to the dynamic hydrological regimes of estuaries, the
331 location of this transitional zone will vary spatially and temporally. Environmental conditions will
332 also affect where and when conditions allowing for increased adhesion will occur. For example,
333 increased rainfall increases FIO transport from land to surface waters (Guber et al. 2006; Muirhead
334 et al. 2004; Oliver et al. 2005) and will also push the tidal limit, where fresh and sea water mix,
335 further down the river channel resulting in higher adhesion and deposition of certain strains lower
336 down the estuary.

337 The variability in *E. coli* adhesion presented here introduces complications for catchment models of
338 FIO fate and transport that concern sediment-water exchange and sediment-fate of FIOs to
339 accurately predict disease risk. Firstly, it highlights the possible ecological discrepancies between

pathogen and non-pathogen strains of bacteria. The difference in adhesion of the different strains observed here are attributed to physiological differences independent of particle charge. Many physiological adaptations of pathogenic strains may be advantageous to cell-particle adhesion therefore there may be bias to how pathogen and non-pathogen strains are distributed in the environment. This is especially potent considering emerging evidence that sediments contribute a more significant proportion of FIOs to surface waters than previously thought through hyporheic exchange (Pachepsky et al. 2017). Since the use of FIOs such as *E. coli* are based upon correlation between total FIO loading and risk of disease, further work must be undertaken on a catchment scale to elucidate this relationship in order predict where pathogenic strains may be occurring in relatively high abundance to total FIOs. Secondly it is demonstrated here that, in addition to the known effects of salinity in faecal bacteria die-off that have been incorporated into recent FIO fate and transport models (Gao et al. 2015), salinity should also be considered in sediment-bacteria interaction sub models such as that of Gao et al. 2011. Finally, this study furthers the understanding of *E. coli* preference to sediment types as discussed above. This is especially important to recent modelling efforts that incorporate sediment-water column exchange of FIOs (Cho et al. 2016a; Feng et al. 2015).

It was hypothesised that where the zeta potential of cell or sediment suspension was less negative, the more cell-particle adhesion would occur. Adhesion instead varied greatly between strain type independent of zeta potential, was higher at 2 and 3.5 PSU than 0 and 5 PSU, and was slightly higher with smaller particle sediments with large surface area and high organic matter.

Conclusions

- *This study highlights the multiple drivers of cell-particle adhesion and its importance in respect to bacterial transport and fate.*

- *Despite large shifts in zeta potential for both cell and particle suspensions within the narrow range of salinities typical of the upper reaches of an estuary, zeta potential was a poor indicator of particle adhesion potential and therefore should be used with caution to inform upon bacterial transport pathways.*
- *Bacterial strain type was the most important factor in adhesion, highlighting the importance of understanding cell characteristics of pathogens in the environment.*
- E. coli *are likely to adhere to suspended sediments and subsequently be deposited at the tidal limit of estuaries where salinities of around 2- 3.5 PSU are encountered, and where sediments with small particle sizes and high organic matter are found.*

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Figure Legends

Table 1. Summary information of the *E. coli* strains used in this study including clade (Clermont et al. 2013) and MLST Complex (Wirth et al. 2006). DSMZ refers to the Leibniz Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen.

Table 2. Summary of bulk sediment characteristics for the 3 sediment types used.

387 **Table 3.** Extent of biofilm formation (n=4) and swarm diameter (n=3) for *E. coli* strains.

388 **Table 4.** General ANOVA summary table. F- statistics and *p*- values are for single factor effects.
389 Significantly different groups identified using Fisher's LSD test and are displayed in brackets under
390 the *p*-value.

391 **Table 5.** Summary of E. coli adhesion (log_{10} CFU ml^{-1} ± SE) for treatment interactions (n= 3).

392 **Figure 1.** Zeta potential measurements of cell suspensions of Yth13, DSM 8698 and DSM 9034 over a
393 salinity gradient (0- 5 PSU). Hollow circles- Yth13; solid triangles- DSM 8698; hollow triangles- DSM
394 9034. Error bars indicate SE (n= 3).

395 **Figure 2.** Zeta potential measurements of Organic Mud, Mud, Mixed Sand sediments at 0, 2, 3.5 and
396 5 PSU. Solid circles- Organic Mud; hollow circles- Mud; solid triangles- Mixed Sand. Error bars
397 indicate SE (n= 3).

398 **Figure 3.** Number of adhered *E. coli* CFUs to sediment particles (bars) and zeta potential
399 measurements of the sediment (solid circles) and 0, 2, 3.5 and 5 PSU. A- Organic Mud; B- Mud; C-
400 Mixed Sand. Solid fill bars- Yth13; dashed bars- DSM 8698; hatched bars- DSM 9034. Error bars
401 indicate SE (n= 3).

402 **Supplementary Table 1.** Particle size distribution of the Organic Mud (OM), Mud (M) and Mixed
403 Sand (MS) sediments. Modelled diameter parameters

404 **Supplementary Table 2.** Bulk mineralogical analysis of the Organic Mud (OM), Mud (M) and Mixed
405 Sand (MS) sediments.

406 **Supplementary Table 3.** Sampling location and water chemistry of the collected Ythan river water
407 and seawater.

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413 **References**

414 Abdulkarim SM, Fatimah AB, Anderson JG (2009) Effect of salt concentrations on the growth of heat-
415 stressed and unstressed *Escherichia coli*. Food, Agriculture and Environment 7(3&4):51-54
416 Anderson IC, Rhodes M, Kator H (1979) Sublethal stress in *Escherichia coli*: a function of salinity.
417 Applied and environmental microbiology 38(6):1147-1152
418 Anderson KL, Whitlock JE, Harwood VJ (2005) Persistence and differential survival of fecal indicator
419 bacteria in subtropical waters and sediments. Applied and environmental microbiology
420 71(6):3041-3048 doi:10.1128/aem.71.6.3041-3048.2005
421 Bai S, Lung WS (2005) Modeling sediment impact on the transport of fecal bacteria. Water Research
422 39(20):5232-40 doi:10.1016/j.watres.2005.10.013
423 Brunauer S, Emmett PH, Teller E (1938) Adsorption of gases in multimolecular layers. Journal of the
424 American Chemical Society 60(2):309-319 doi:10.1021/ja01269a023
425 Burton GA, Gunnison D, Lanza GR (1987) Survival of pathogenic bacteria in various freshwater
426 sediments. Applied and environmental microbiology 53(4):633- 638
427 Carlsson S (2012) Surface characterization of gram-negative bacteria and their vesicles. Thesis in
428 Chemistry, Umeå University
429 Carlucci AF, Pramer D (1960) An evaluation of factors affecting the survival of *Escherichia coli* in sea
430 water: II. Salinity, pH, and nutrients. Applied Microbiology 8(4):247-250
431 Chen G, Walker SL (2012) Fecal indicator bacteria transport and deposition in saturated and
432 unsaturated porous media. Environmental Science and Technology 46(16):8782-90
433 doi:10.1021/es301378q
434 Cho KH, Pachepsky YA, Kim M, Pyo J, Park M-H, Kim YM, Kim J-W, Kim JH (2016a) Modeling seasonal
435 variability of fecal coliform in natural surface waters using the modified SWAT. Journal of
436 Hydrology 535:377-385 doi:http://dx.doi.org/10.1016/j.jhydrol.2016.01.084
437 Cho KH, Pachepsky YA, Oliver DM, Muirhead RW, Park Y, Quilliam RS, Shelton DR (2016b) Modeling
438 fate and transport of fecally-derived microorganisms at the watershed scale: State of the
439 science and future opportunities. Water Research 100:38-56
440 doi:https://doi.org/10.1016/j.watres.2016.04.064
441 Clermont O, Christenson JK, Denamur E, Gordon DM (2013) The Clermont *Escherichia coli* phylo-
442 typing method revisited: improvement of specificity and detection of new phylo-groups.
443 Environmental Microbiology Reports 5(1):58-65 doi:10.1111/1758-2229.12019
444 Davies CM, Long JAH, Donald M, Ashbolt NJ (1995) Survival of fecal microorganisms in marine and
445 freshwater sediments. Applied and environmental microbiology 61(5):1888- 1896
446 Donlan RM (2001) Biofilm formation: A clinically relevant microbiological process. Clinical Infectious
447 Diseases 33(8):1387-1392 doi:10.1086/322972
448 England LS, Lee H, Trevors JT (1993) Bacterial survival in soil: effect of clays and protozoa. Soil
449 Biology and Biochemistry 25(5):525-531

Feng G, Cheng Y, Wang S-Y, Hsu LC, Feliz Y, Borca- Tasciuc DA, Worobo RW, Moraru CI (2014) Alumina surfaces with nanoscale topography reduce attachment and biofilm formation by *Escherichia coli* and *Listeria* spp. Biofouling 30(10):1253-1268

Feng Z, Reniers A, Haus BK, Solo-Gabriele HM, Wang JD, Fleming LE (2015) A predictive model for microbial counts on beaches where intertidal sand is the primary source. Marine Pollution Bulletin 94(0):37-47 doi:10.1016/j.marpolbul.2015.03.019

Fleisher JM, Fleming LE, Solo-Gabriele HM, Kish JK, Sinigalliano CD, Plano L, Elmir SM, Wang JD, Withum K, Shibata T, Gidley ML, Abdelzaher A, He G, Ortega C, Zhu X, Wright M, Hollenbeck J, Backer LC (2010) The BEACHES Study: health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. International Journal of Epidemiology 39(5):1291-8 doi:10.1093/ije/dyq084

Fleisher JM, Kay D, Wyer M, Godree AF (1998) Estimates of the severity of illnesses associated with bathing in marine waters contaminated with domestic sewage. International Journal of Epidemiology 27:722-726

Fujioka RS, Yoneyama BS (2002) Sunlight inactivation of human enteric viruses and fecal bacteria. Water Science and Technology 46:291- 295

Gao G, Falconer RA, Lin B (2011) Numerical Modelling Sediment-Bacteria Interaction Processes in the Severn Estuary. Journal of Water Resource and Protection 03(01):22-31 doi:10.4236/jwarp.2011.31003

Gao G, Falconer RA, Lin B (2015) Modelling the fate and transport of faecal bacteria in estuarine and coastal waters. Marine Pollution Bulletin 100(1):162-168 doi:https://doi.org/10.1016/j.marpolbul.2015.09.011

Geldreich EE (1970) APPLYING BACTERIOLOGICAL PARAMETERS TO RECREATIONAL WATER QUALITY. Journal (American Water Works Association) 62(2):113-120

Georgiou S, Langford IH (2002) Coastal bathing water quality and human health risks: a review of legislation, policy, and epidemiology, with an assessment of current uk water quality, proposed standards, and disease burden in England and Wales. CSERGE, Working Paper ECM 02-06, England

Gerba CP, Mcleod JS (1976) Effect of sediments on the survival of *Escherichia coli* in marine waters. Applied and environmental microbiology 32(1):114- 120

Guber AK, Shelton D, Pachepsky Y, Sadeghi AM, Sikora LJ (2006) Rainfall-induced release of fecal coliforms and other manure constituents: comparison and modeling. Applied and environmental microbiology 72(12):7531- 7539

Heaney CD, Sams E, Dufour AP, Brenner KP, Haugland RA, Chern E, Wing S, Marshall S, Love DC, Serre M, Noble R, Wade TJ (2012) Fecal indicators in sand, sand contact, and risk of enteric illness among beachgoers. Epidemiology 23(1):95-106

Hiemenz PC (1977) Electrophoresis and other electrokinetic phenomena. In: Lagowski JJ (ed) Principles of Colloid and Surface Chemistry. Marcel Dekker, New York, pp 452-487

Hunter KA, Liss PS (1982) Organic matter and the surface charge of suspended particles in estuarine waters Limnology and Oceanography 27(2):322- 335

Jeng HAC, Englande AJ, Bakeer RM, Bradford HB (2005) Impact of urban stormwater runoff on estuarine environmental quality. Estuarine, Coastal and Shelf Science 63(4):513-526 doi:10.1016/j.ecss.2004.11.024

Kauffmann F, Dupont A (1950) *Escherichia* strains from infantile epidemic gastro enteritis. Acta Pathologica Microbiologica Scandinavica 27(4):552-64

Kim HN, Walker SL, Bradford SA (2010) Macromolecule mediated transport and retention of Escherichia coli O157:H7 in saturated porous media. Water Research 44(4):1082-1093 doi:https://doi.org/10.1016/j.watres.2009.09.027

Ma J, Ibekwe MA, Crowley DE, Yang C-H (2014) Persistence of *Escherichia coli* O157 and non-O157 strains in agricultural soils. The Science of the total environment 490:822-829 doi:http://dx.doi.org/10.1016/j.scitotenv.2014.05.069

Malvern (2014) Technical note: Measuring the zeta potential of high conductivity samples using the Zetasizer Nano. In: Ltd MI (ed) UK.

Merritt JH, Kadouri DE, O'Toole GA (2011) Growing and analyzing static biofilms. Current Protocols in Microbiology:00:B:1B.1:1B.1.1–1B.1.17 doi:10.1002/9780471729259.mc01b01s22

Moore BC, Martinez E, Gay JM, Rice DH (2003) Survival of *Salmonella enterica* in freshwater and sediments and transmission by the aquatic midge *Chironomus tentans* (Chironomidae: Diptera). Applied and environmental microbiology 69(8):4556-4560 doi:10.1128/aem.69.8.4556-4560.2003

Muirhead RW, Collins RP, Bremer PJ (2006a) The association of *E. coli* and soil particles in overland flow. Water Science and Technology 54(3):153-9

Muirhead RW, Collins RP, Bremer PJ (2006b) Interaction of *Escherichia coli* and soil particles in runoff. Applied and environmental microbiology 72(5):3406- 3411

Muirhead RW, Davies-Colley RJ, Donnison AM, Nagels JW (2004) Faecal bacteria yields in artificial flood events: quantifying in-stream stores. Water Research 38(5):1215-24 doi:10.1016/j.watres.2003.12.010

O'Toole GA, Pratt LA, Watnick PI, Newman DK, Weaver VB, Kolter R (1999) Genetic approaches to study of biofilms. Methods in Enzymology 310:91-109

Oliver DM, Clegg CD, Haygarth PM, Heathwaite AL (2005) Assessing the potential for pathogen transfer from grassland soils to surface waters Advances in Agronomy. vol 85. Academic Press, pp 125-180

Oliver DM, Clegg CD, Heathwaite AL, Haygarth PM (2007) Preferential attachment of *Escherichia coli* to different particle size fractions of an agricultural grassland soil. Water, Air, and Soil Pollution 185(1-4):369-375 doi:10.1007/s11270-007-9451-8

Pachepsky Y, Stocker M, Saldaña MO, Shelton D (2017) Enrichment of stream water with fecal indicator organisms during baseflow periods. Environmental Monitoring and Assessment 189(2):51 doi:10.1007/s10661-016-5763-8

Pachepsky YA, Shelton DR (2011) *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. Critical Reviews in Environmental Science and Technology 41(12):1067-1110 doi:10.1080/10643380903392718

Pachepsky YA, Yu O, Karns JS, Shelton DR, Guber AK, van Kessel JS (2008) Strain-dependent variations in attachment of *E. coli* to soil particles of different sizes. International Agrophysics 22:61-66

Perni S, Preedy EC, Prokopovich P (2014) Success and failure of colloidal approaches in adhesion of microorganisms to surfaces. Advances in Colloid and Interface Science 206(Supplement C):265-274 doi:https://doi.org/10.1016/j.cis.2013.11.008

Pond K (2005) Water recreation and disease. Plausibility of associated infections: acute effects, sequelae and mortality. IWA Publishing in association with WHO, Geneva

Rijnaarts HHM, Norde W, Lyklema J, Zehnder AJB (1995) The isoelectric point of bacteria as an indicator for the presence of cell surface polymers that inhibit adhesion. Colloids and Surfaces B: Biointerfaces 4(4):191-197 doi:http://dx.doi.org/10.1016/0927-7765(94)01164-Z

Rong X, Huang Q, He X, Chen H, Cai P, Liang W (2008) Interaction of *Pseudomonas putida* with kaolinite and montmorillonite: a combination study by equilibrium adsorption, ITC, SEM and FTIR. Colloids and Surfaces B: Biointerfaces 64(1):49-55 doi:10.1016/j.colsurfb.2008.01.008

Roper MM, Marshall KC (1974) Modification of the interaction between *Escherichia coli* and bacteriophage in saline sediment. Microbial Ecology 1(1- 13)

Rowe B, Gross RJ, Woodroof DP (1977) Proposal to recognise seroovar 145/46 (Synonyms: 147, Shigella 13, *Shigella sofia*, and *Shigella manolovii*) as a new *Escherichia coli* 0 group, 0164. International Journal of Systematic Bacteriology 27(1):15-18

SEPA (2013) River Ythan 2011-2013 database. SEPA (© Scottish Environment Protection Agency and database right (2015) All rights reserved) (Accessed 2015)

551 Sevin DC, Stahlin JN, Pollak GR, Kuehne A, Sauer U (2016) Global Metabolic Responses to Salt Stress
552 in Fifteen Species. PloS one 11(2):e0148888 doi:10.1371/journal.pone.0148888
553 Sharma MM, Chang YI, Yen TF (1985) Reversible and irreversible surface charge modification of
554 bacteria for facilitating transport through porous media. Colloids and Surfaces 16:193-206
555 Shuval H (2003) Estimating the global burden of thalassogenic diseases: human infectious diseases
556 caused by wastewater pollution of the marine environment. Journal of water and health
557 1(2):53-64
558 Sokolov I, Smith DS, Henderson GS, Gorby YA, Ferris FG (2001) Cell surface electrochemical
559 heterogeneity of the Fe(III)-reducing bacteria *Shewanella putrefaciens*. Environmental
560 Science and Technology 35(2):341-347 doi:10.1021/es001258s
561 Van der Wal A, Minor M, Norde W, Zehnder AJB, Lyklema J (1997) Electrokinetic potential of
562 bacterial cells. Langmuir 13(2):165-171 doi:10.1021/la960386k
563 Van Loosdrecht MCM, Lyklema J, Norde W, Schraa G, Zehnder AJB (1987) Electrophoretic mobility
564 and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. Applied
565 and environmental microbiology 53(8):1898- 1901
566 Van Loosdrecht MCM, Norde W, Lyklema J, Zehnder AB (1990) Hydrophobic and electrostatic
567 parameters in bacterial adhesion. Aquatic Sciences 52(1):103-114 doi:10.1007/bf00878244
568 Walker SL, Redman JA, Elimelech M (2004) Role of cell surface lipopolysaccharides in *Escherichia coli*
569 K12 adhesion and transport. Langmuir 20:7736- 7746
570 Whitehouse R (2000) Dynamics of estuarine muds: A manual for practical applications. Thomas
571 Telford
572 Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H,
573 Achtman M (2006) Sex and virulence in *Escherichia coli*: an evolutionary perspective.
574 Molecular Microbiology 60(5):1136-1151 doi:10.1111/j.1365-2958.2006.05172.x
575 Wolfe AJ, Berg HC (1989) Migration of bacteria in semisolid agar. Proceedings of the National
576 Academy of Sciences of the United States of America 86(18):6973-7
577 Zhao W, Walker SL, Huang Q, Cai P (2014) Adhesion of bacterial pathogens to soil colloidal particles:
578 influences of cell type, natural organic matter, and solution chemistry. Water Research
579 53:35-46 doi:10.1016/j.watres.2014.01.009

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Strain	Serotype	Clade	MLST	Origin	Isolation	Details
Yth13	Unknown	B1	155	Ythan estuary, Scotland	2014	Isolated from a mixed mud sediment where salinity ~17 PSU.
DSM 8698	O111:H-	B1	20	DSMZ	1950	Enteropathogenic, isolated from human diarrhoea (Kauffmann and Dupont 1950)
DSM 9034	O164:H-	-	-	DSMZ	1947	Enteroinvasive, isolated from human diarrhoea (Rowe et al. 1977)

Sediment Type	UK Grid Reference (Lat., Lon.)	Bulk Density (g cm^{-3} ± SE)	Water Content (% core weight ± SE)	Organic Content (% dry weight ± SE)	Surface Area (sq.m g^{-1})
Organic Mud	57.359746, -2.017193	1.27 ± 0.01	65.42 ± 0.12	9.12 ± 0.12	7.101
Mud	57.334826, -2.004501	1.38 ± 0.07	62.82 ± 0.26	7.00 ± 0.12	6.014
Mixed Sand	57.313898, -1.993890	1.95 ± 0.03	23.66 ± 0.17	2.16 ± 0.02	1.136

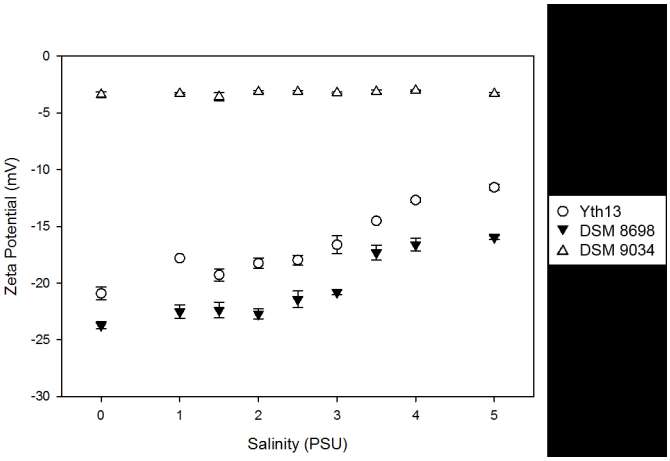
Strain	Biofilm (Relative adsorption at 600 nm ± SE)	Swarm Diameter (mm ± SE)						
		15 ˚C Day 3	15 ˚C Day 5	15 ˚C Day 10	15 ˚C Day 15	25 ˚C Day 1	25 ˚C Day 3	25 ˚C Day 6
Yth13	0.129 ± 0.029	7.00 ± 0.00	9.00 ± 0.00	12.67 ± 0.33	16.67 ± 0.67	5.00 ± 0.00	10.67 ± 0.33	17.33 ± 0.33
DSM 8698	0.546 ± 0.080	5.67 ± 0.33	7.00 ± 0.00	11.00 ± 0.58	13.00 ± 0.58	39.33 ± 11.68	> 85.00	> 85.00
DSM 9034	0.033 ± 0.012	4.00 ± 0.00	4.67 ± 0.33	5.33 ± 0.67	6.00 ± 1.15	4.00 ± 0.00	7.67 ± 0.33	10.33 ± 0.67

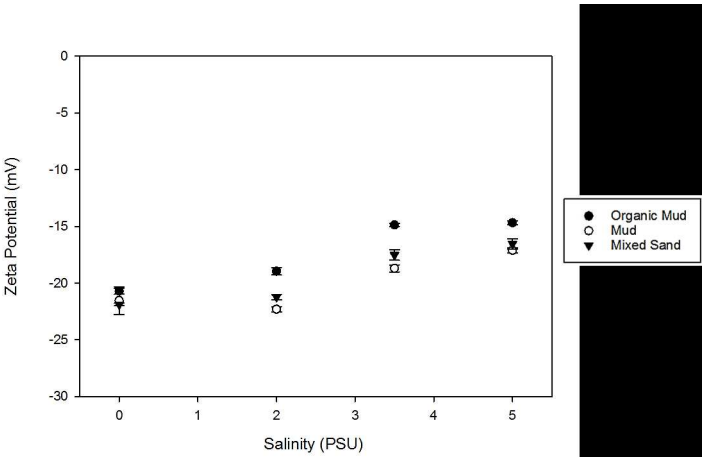
Sediment	**Organic Mud**	**Mud**	**Mixed Sand**	**F- statistic**	***p*- value**
Mean (log_{10} CFU ml^{-1} ± SE)	0.334 ± 0.020	0.299 ± 0.034	0.272 ± 0.029	5.34	0.007 (OM > MS)

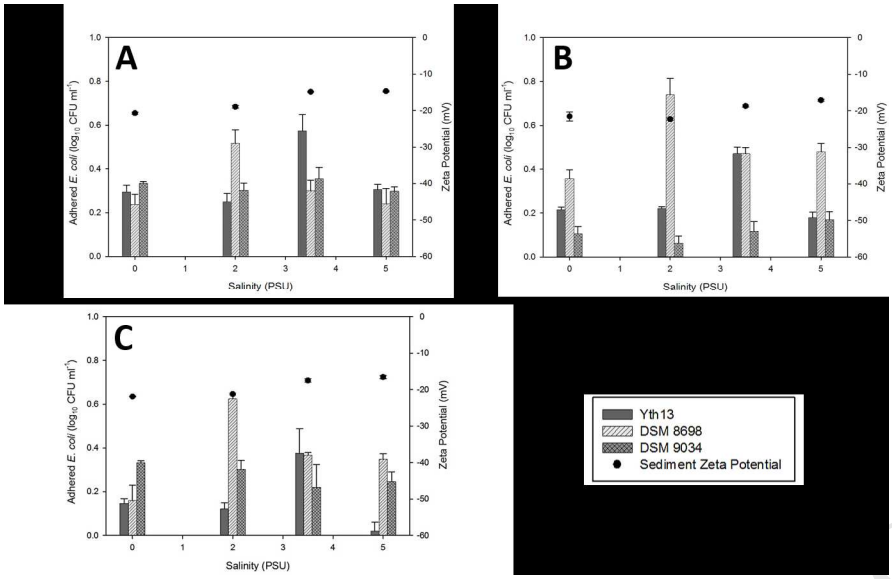
Strain	**Yth13**	**DSM 8698**	**DSM 9034**	**F- statistic**	***p*- value**
Mean (log_{10} CFU ml^{-1} ± SE)	0.265 ± 0.027	0.403 ± 0.030	0.238 ± 0.019	43.38	<0.001 (DSM 8698 > DSM 9034, Yth13)

Salinity (PSU)	**0**	**2**	**3.5**	**5**	**F- statistic**	***p*- value**
Mean (log_{10} CFU ml^{-1} ± SE)	0.243 ± 0.020	0.350 ± 0.044	0.361 ± 0.031	0.254 ± 0.026	15.87	<0.001 (2, 3.5 > 0, 5)

		Strain			Salinity (PSU)			
		Yth13	**DSM 8698**	**DSM 9034**	**0**	**2**	**3.5**	**5**
Sediment	**Organic Mud**	0.356 ± 0.043	0.323 ± 0.042	0.323 ± 0.015	0.289 ± 0.021	0.356 ± 0.047	0.410 ± 0.051	0.281 ± 0.024
	Mud	0.272 ± 0.036	0.512 ± 0.047	0.114 ± 0.019	0.226 ± 0.040	0.341 ± 0.105	0.354 ± 0.061	0.276 ± 0.054
	Mixed Sand	0.166 ± 0.047	0.374 ± 0.053	0.275 ± 0.029	0.213 ± 0.037	0.350 ± 0.075	0.321 ± 0.051	0.204 ± 0.052
Salinity (PSU)	**0**	0.219 ± 0.024	0.251 ± 0.040	0.258 ± 0.039				
	2	0.198 ± 0.024	0.627 ± 0.043	0.223 ± 0.043				
	3.5	0.474 ± 0.049	0.379 ± 0.030	0.231 ± 0.050				
	5	0.168 ± 0.044	0.356 ± 0.042	0.238 ± 0.025				







Highlights

- *E. coli* zeta potential profiles differ between strains over a salinity gradient
- Adhesion efficiency depended on strain > salinity > sediment
- Zeta potential did not influence adhesion efficiency
- Adhesion to sediments was greatest at moderate salinities tested (2 and 3.5 PSU)