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Title

Factors effecting the spatial and temporal distribution of *E. coli* in intertidal estuarine sediments

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Abstract

Microbiological water quality monitoring of bathing waters does not account for faecal indicator organisms in sediments. Intertidal deposits are a significant reservoir of FIOs and this indicates there is a substantial risk to bathers through direct contact with the sediment, or through the resuspension of bacteria to the water column. Recent modelling efforts include sediment as a secondary source of contamination, however, little is known about the driving factors behind spatial and temporal variation in FIO abundance. E. coli abundance, in conjunction with a wide range of measured variables, was used to construct models to explain E. coli abundance in intertidal sediments in two Scottish estuaries. E. coli concentrations up to 6 log10 CFU 100 g dry wt-1 were observed, with optimal models accounting for E. coli variation up to an adjusted R2 of 0.66. Introducing more complex models resulted in overfitting of models, detrimentally effected the transferability of models between datasets. Salinity was the most important single variable, with season, pH, colloidal carbohydrates, organic content, bulk density and maximum air temperature also featuring in optimal models. Transfer of models, using only lower cost variables, between systems explained an average deviance of 42 %. This study demonstrates the potential for cost-effective sediment characteristic monitoring to contribute to FIO fate and transport modelling and consequently the risk assessment of bathing water safety.

Keywords: FIO, estuaries, pathogens, bathing water quality, sediments

Introduction

Current bathing water quality monitoring, in accordance with microbial water quality regulations, does not take into account the presence of indicators of faecal contamination in sediments because samples are only taken from overlying waters. This is despite evidence that the resuspension or release of *Escherichia coli* (an organism regularly quantified in order to indicate the extent of recent faecal contamination, and therefore likelihood of pathogen occurrence) from underlying sediments, including that arising through anthropogenic disturbance, can increase concentrations in the water column (Grimes, 1975; Graczyk et al., 2010; Pachepsky and Shelton, 2011). Microbial contamination remains a significant concern to the public (Pouso et al., 2018) and legislative bodies worldwide (Oliver et al., 2016), therefore understanding the factors driving the distribution of faecal indicator organisms (FIOs), such as *E. coli*, in sediments is important to understanding the threat of microbial pollutants to aquaculture and public safety. Presently, there are no sediment 'microbial quality' classifications, and the current classifications for bathing water quality are unlikely to be directly transferrable due to differing human exposure and infection pathways for direct sediment contact, and resuspension mechanisms. Out of a total of 83 non-freshwater designated bathing waters in Scotland, UK, 55 have the potential to be affected by pollution through waterway contamination, and 50 are within an estuarine system or within 1 km of a significantly sized estuary (SEPA, 2018).

Sediments act as a reservoir for faecal bacteria, with freshwater and marine sediments observed to contain much higher densities of faecal bacteria than the overlying water (Davies et al., 1995; Hassard et al., 2017). Numbers of faecal bacteria can increase several-fold when associated with suspended particulates as compared to free- floating in surrounding waters (Droppo et al., 2009). Bacteria adhere to particles in the water column, and then become incorporated in sediment deposits as particles settle out of suspension (Davies et al., 1995). Rates of adhesion of faecal bacteria to particles in suspension have been demonstrated to be dependent on many factors, including water pH and

salinity, and characteristics of both the cell and suspended particles (Oliver et al., 2007; Pachepsky et al., 2008; Zhao et al., 2014; Wyness et al., 2018).

In order to understand FIO distribution in sediments and subsequently the risk of the resuspension of faecal bacteria, which can include human pathogens, further assessment of the ecology and survival of FIOs in sediments and sediment-associated transport is necessary in order to fully characterise the potential risks to water quality and public health (Halliday and Gast, 2011; Oliver et al., 2016). Whilst performing a full suite of sediment analyses is impractical for monitoring by regulatory bodies, the ability to pre-emptively identify areas and time periods of potentially high loading of FIOs using a limited number of easily accessible measurements will greatly facilitate risk management. A wide range of climatic, physical, chemical and biological variables relevant to both the sediment and interstitial water are known to affect the abundance and survival of faecal bacteria in estuarine sediments (Pachepsky and Shelton, 2011; Perkins et al., 2014; Hassard et al., 2016; Hassard et al., 2017; Schang et al., 2018). Many properties of intertidal sediments co-vary (Dale, 1974; Mayer et al., 1985; Flemming and Delafontaine, 2000; Paterson et al., 2000; Venkatramanan et al., 2011), for example, sediments with smaller particle sizes often having increased organic matter and nutrient content. Sediment characteristics that are known to affect FIO survival are not strictly limited to certain climates or geographical areas

For local regulatory bodies to monitor FIO abundance in intertidal sediments, direct enumeration of FIOs is time consuming and requires access to a microbiology laboratory. In order to assess dynamic systems such as estuaries, spatially and temporally intense monitoring would be necessary. The creation of a reliable model, based on easily measurable sediment characteristics and widely available online environmental data, to predict FIO abundance in intertidal sediments would allow for low-cost, fast-turnaround monitoring tool; one of the primary aims for catchment- based modelling of microbial pollutants. This, combined with mechanistic understanding of FIO flux between sediment and water

will allow for comprehensive assessment of water quality where only confirmatory culture-based enumeration would be necessary.

Recent efforts to predict microbial bathing water quality have successfully incorporated sediment-bacteria interactions. For example the inclusion of adsorption and desorption from suspended sediment particles and the inclusion of sediment transport processes (Kim et al., 2010; Cho et al., 2012; Cho et al., 2016b; Cho et al., 2016a; Huang et al., 2017). Whilst the importance of sediment characteristics has been noted (Pachepsky and Shelton, 2011; Palazón et al., 2017), predicting the concentration of *E. coli* released from sediment during a resuspension event is challenging as there is no current model accounting for the heterogeneous spatial distribution of *E. coli* within sediments and the variable rates of resuspension that results from it..

The aim of this study was to identify key driving factors that influence the spatial and temporal variation in the abundance of FIOs in intertidal sediments of two estuaries in order to subsequently improve microbial fate and transport models to ultimately improve prediction of bathing water quality. This was achieved through the culture-based enumeration of total coliforms (a common indicator of sanitary condition) and the FIO *E. coli* in conjunction with a wide range of physical, biogeochemical, biological and environmental variables over several sampling regimes. It was hypothesized that it would be possible to satisfactorily explain *E. coli* variation using measurements of sediment characteristics, and that explanatory models would be transferrable between both estuaries. Predictor variables were split into cost classes in order to investigate whether the addition of higher cost predictors improved the model power.

Experimental Procedures

Sampling design

Spatio-temporal samples were taken at the Ythan estuary (Aberdeenshire, Scotland, Lat. 57.343227, Lon. -2.000371) for an intensive sampling campaign to investigate variability for different sediment types on a metre scale. Transect samples were taken quarterly from the Ythan estuary and the Eden estuary (Fife, Scotland, Lat. 56.373028, Lon. -2.835975) to investigate variability across changing sediment types between the estuary head and mouth.

For the intensive sampling campaign, four areas of differing sediment types (Mud (M), Mixed Mud (MM), Mixed Sand (MS), Sand (S) (Fig. 5A)) were selected within the Ythan estuary. Within 15 x 15 metre areas at each sediment type 6 sampling sites no closer than 2 metres were marked with a pole, with all sample types collected within a 25 cm radius of the pole. Samples were taken every 4 weeks between December 2013- December 2014 with all samples collected within a 2-day period and all microbiological analyses performed within 24 hours of sample removal from the field. All samples collected in the field were returned to the laboratory in an insulated box where they were stored at 4 °C overnight before analysis or lyophilisation.

Sediment characteristics

Field sampling of sediments was performed by taking cores to a depth of 10 mm using a 20 ml BD Discardit™ Eccentric Luer-Slip Two-Piece plastic disposable syringe (Fisher Scientific, UK) cut just above the luer-slip tip (Lubarsky et al., 2010). Organic content, water content and bulk density were analysed following the procedures outlined in the HIMOM protocols (Brockmann et al., 2004). Several adjacent cores were combined before sieving through 2 mm mesh, and lyophilisation to form a larger sample for particle size, elemental and extracellular polymeric substance analysis. Laser particle size distribution analysis was performed using a Mastersizer 2000 (Malvern Instruments, UK). Interstitial

porewater was sampled in the field by compressing ~500 g wet sediment in 0.5 mm square aperture nylon mesh, and collecting the filtrate. The pH and salinity of the interstitial porewater sample was determined using a Hach HQ40d multi-probe (Hach, CO, USA). Environmental data (precipitation and temperatures) were obtained from the MIDAS database held at the British Atmospheric Data Centre (BADC). The boundary dates for seasons at the Ythan estuary (Zetsche et al., 2011) were used.

Culture-based enumeration of total coliforms and *E. coli*

To determine total coliform and *E. coli* concentration in sediments, 3 adjacent syringe cores (described above) were homogenised together to form a sample. Between 1 and 8 g were weighed into 100 ml sterile vessels. The quantity of sample depended on a visual analysis of grain size and therefore a coliform and *E. coli* abundance estimate; approximate quantities used were 1-3 g for fine grained mud, 2-6 g for mixed samples, and >6 g for sand. Seventy ml sterile Phosphate Buffered Solution (PBS) (137 mM NaCl (Fisherbrand, Fisher Scientific, UK), 2.7 mM KCl (BDH, UK), 4.3 mM $Na_2HPO_4.7H_2O$ (BDH, UK), and 1.4 mM KH_2PO_4 (BDH, UK)) was added, and the vessel reciprocally shaken at 30 cm and 120 rpm for 1 min. Vessels were left to settle for 1 h (Garzio-Hadzick et al., 2010) before 30 ml of supernatant was combined with 70 ml of fresh PBS and one snap-pack of IDEXX Colilert-18 reagent (IDEXX Laboratories, Westbrook, Maine, USA). The manufacturers' protocol for IDEXX Quanti-Tray/2000 was then followed and the results corrected to most probable number (MPN) per 100 g dry weight sediment.

Extracellular polymeric substances

Extracellular polymeric substances (EPS) constituents (colloidal protein and carbohydrates) were quantified by modifying the Lowry procedure (Raunkjaer et al., 1994) and Dubois assay (DuBois et al., 1956; Taylor and Paterson, 1998) respectively for use in 96-well plates. EPS extraction was performed by adding 600 µl DI H_2O to a lyophilised sediment sample in a 1.5 ml Eppendorf tube. The quantity of

sample depended on a visual analysis of grain size and therefore an EPS concentration estimate; approximate quantities used for each sediment type were 250 mg for fine grained mud, 300 mg for mixed samples, and 350 mg for sand. Samples with a larger particle size typically contained lower amounts of EPS. Samples were incubated for 30 mins at room temperature, vortexed thoroughly every 10 min. Samples were then centrifuged at 6200 x g for 10 min at room temperature (Eppendorf Minispin Plus, Eppendorf, Germany), and 400 µl of the supernatant containing the water soluble EPS was removed and stored in a clean 1.5 ml Eppendorf tube at 4 °C. Colloidal carbohydrates were quantified by combining, in triplicate in an optically clear 96 well plate (Corning, NY, USA), 35.7 µl of each sample supernatant or glucose standard, 35.7 µl of 5% w/v phenol (Sigma-Aldrich, UK) and 178.6 µl of concentrated sulphuric acid (BDH, UK) and mixed via pipetting. Plates were incubated for 35 min at 30 °C and absorbance determined at 486 nm using a 96-well spectrophotometer (SpectraMax 190 Microplate Reader, Molecular Devices, CA, USA). Colloidal proteins were quantified by combining a 143 mM NaOH (Fisher Scientific, UK), 270 mM Na_2CO_3 (BDH, UK) solution, with 57 mM $CuSO_4$ (Fisher Scientific, UK), and 124mM $Na_2C_4H_4O_6$ (Sigma-Aldrich, UK) in a 100:1:1 ratio. In triplicate in optically clear plates, 48 µl of each sample supernatant or standard was added to 48 µl of SDS 2% (Sigma-Aldrich, UK) (Gerbersdorf et al., 2008), and plates incubated for 15 min at room temperature in order to lineate the extracted proteins. After incubation, 134.5 µl of the working reagent was added to each well, followed by 19.5 µl of Folin and Ciocalteu's reagent (Sigma-Aldrich, UK) (diluted 5:6 with DI H_2O) and mixed via pipetting. The plates were then incubated for 45 min at 30 °C and absorbance determined at 750 nm. Standard curves were constructed for carbohydrates and proteins using glucose and bovine serum albumin respectively and are therefore expressed as µg g^{-1} equivalents per gram of dry sediment. EPS analysis can be performed efficiently for smaller numbers of samples using basic laboratory equipment, larger reagent volumes and a standard spectrometer as in the HIMOM protocols (Brockmann et al., 2004).

Sediment stability

Sediment bed stability was measured using a handheld shear vane (Geonor, Augusta, New Jersey, USA) with a 50 mm x 12 mm vane at a depth of 0- 50 mm.

Insert figure 1A & 1B here

For the transect sampling campaign, 14 sampling points were selected at approximately equal intervals down the river-ocean mixing zone of both the Ythan estuary (Fig. 1A) and the Eden estuary (Fig. 1B). The precise sampling site was established by calculating 20 % of the distance from the Median High Water Spring mark to the Median Low Water Spring mark using ArcGIS (ArcMap, ed. 10.2.1. ESRI, CA, USA). Samples were taken during February (Winter), May (Spring), August (Summer) and November (Autumn) in 2014. Sites 13 and 14 of both estuaries were relatively exposed beaches whilst still within the influence of the estuary, and sites 1 and 2 were close to the riverine reaches whilst remaining tidal and with saline influence.

Statistical analysis

Statistical analyses and graphics were produced using R (R_Core_Team, 2017), GenStat (VSN International, v17.1), SPSS (v.22, IBM corporations), and SigmaPlot (v.13.0, Systat Software Inc., CA, USA).

Generalised additive mixed models (GAMMs) were used to explore the variation in *E. coli* abundance in each dataset (Ythan intensive monthly sampling, Ythan transect, Eden transect). To account for additional correlation amongst sites, a random effect of site was included. A likelihood ratio test indicated that the application of a random effect improved model fit ($p < 0.001$). Continuous covariates were standardised to a mean of 0 and a standard deviation of 1. *E. coli* abundance was

log_{10} transformed prior to model fitting to meet assumptions of normality and homogeneity of variance. Diagnostic plots were checked for homogeneity of variance and residual normality. The Akaike information criterion with correction for small sample sizes (AICc) was used to select optimal GAMM models to reduce overfitting.

For each dataset, first only variables classified as cost Class 1 and Cost class 2 (see below) were used to provide predictions of *E. coli* at the lowest cost possible. Subsequently, cost class 3 variables were added to explore if the model predictive power and transferability to other datasets increased. Cost class 1 (using remote sensing/ publicly available data, <£1 per sample): minimum grass temperature (temperature 5 cm above ground level), minimum air temperature, maximum air temperature, antecedent precipitation the previous day, the previous 2 days, and previous 5 days, sea surface temperature (weather buoy at Lat. 57.2, Lon. 0.8) and season. Cost class 2 (<£10 per sample using standard laboratory and field equipment with no extra consumables cost): salinity, pH, organic content, water content, bulk density, and shear vane. Cost class 3 (£<20 per sample using specialised equipment with extra consumables cost: colloidal carbohydrate content, colloidal protein content, fine particle (<63 µm) proportion, median particle diameter and volume weighted mean particle diameter. Only the fixed effects from the GAMMs were used to make predictions to assess transferability. Model transferability was assessed by using the selected model from each dataset on the other two datasets. A Generalised Additive model was used to assess the relationship between the predicted values and the observations. The deviance and adjusted coefficient of determination (Adj R^2) was used to assess model fit.

Results

Spatial and temporal variation of sediment characteristics and FIOs

The four sites sampled every 4 weeks (intensive sites) were characterised according to their increasing median particle size: Mud (M), Mixed Mud (MM), Mixed Sand (MS) and Sand (S) (Fig. 1A). ANOVA summary tables can be found in the supplementary material for the one-way effects of site and season on all measured variables (Supplementary Tables 1 and 2). Briefly, organic content (loss on ignition) and water content (loss on drying), and proportion of fine particles (< 63 µm) were greater at M and MM than MS and S ($p < 0.001$). EPS constituents were greater at M and MM than MS and S ($p < 0.001$). Interstitial water pH was highest at S at 7.64 ± SE 0.04 ($p < 0.001$), with the remaining sites between 7.31 ± SE 0.03 and 7.42 ± SE 0.02. Salinity was highest near the mouth of the estuary at MS at 33.00 ± SE 0.68 PSU ($p < 0.001$), with the remaining sites between 17.72 ± SE 0.89 PSU and 21.85 ± SE 0.82 PSU. The magnitude of differences between sites were generally greater for the physical characteristics than biogeochemical characteristics (Supplementary Table 1).

Seasonal variation of sediment characteristics at the intensive sites was lower than between sites, however statistically significant differences were still present (Supplementary Table 2). Notably, the amount of EPS constituents in sediments were lowest during winter and highest in spring and summer (colloidal carbohydrates: 127.94 ± SE 6.47, 263.68 ± SE 13.26, 251.54 ± SE 23.24 µg g^{-1}; colloidal proteins: 176.77 ± SE 8.18, 340.10 ± SE 27.99, 319.54 ± SE 18.92 µg g^{-1} for winter, spring and summer respectively) ($p < 0.001$).. The pH of the interstitial water was most alkali in winter (7.58 ± SE 0.02) and most acidic in spring (7.22 ± 0.02) ($p < 0.001$), and salinity was lowest in winter (17.37 ± SE 0.83) and highest in spring (30.12 ± SE 0.59) ($p < 0.001$).

Insert figure 2 here

Coliform abundance at M and MM was significantly greater than S and MS ($p < 0.001$) (Fig. 2A). The mean *E. coli* abundance was approximately 1.2 log units less for each sediment type than total coliforms (Fig. 2B). Coliform abundance in all sediment types followed the order autumn > summer > winter > spring ($p < 0.001$). *E. coli* abundance followed a similar order of autumn > summer, winter > spring ($p < 0.001$). Coliform abundance for almost all individual S and MS sampling events were significantly lower than M and MM during all seasons, and those during autumn M and MM were significantly higher than all other sampling events except MM during summer. A similar pattern of *E. coli* abundance between treatments to that of total coliforms was observed, with MS and S frequently lower than M and MM with the exception of S during autumn being unusually high compared to other S and MS treatments, and M and MM during spring being lower than the general trend.

Insert figure 3 and 4 here

For the transects, *E. coli* abundance decreased progressively seawards from the head of the estuary (site 1) to the mouth (site 14) (Spearman's rho: Ythan N= 56, correlation coefficient= -.648, $p < 0.001$; Eden N= 56, correlation coefficient= -.744, $p < 0.001$), with lower abundances throughout both estuaries during spring than other seasons (ANOVA; residual d.f. = 105, F-statistic 8.791, $p < 0.001$) (Fig. 3 A-D and 4 A-D). Coliform abundance presented a similar trend (data not shown). Sediment characteristics followed a similar pattern between both estuaries; interstitial water salinity was lowest at the head of the estuaries (8.80 ± SE 1.83 PSU, sites 1 and 2), and increased seawards where Sites 13 and 14 were close to that of pure seawater (33.42 ± SE 0.86 PSU, sites 13 and 14). At the head of the estuaries (Sites 1-4), sediments contained the highest EPS constituent content, highest organic content and water content and the largest proportion of particles < 63 µm compared with sediments further down the estuary.

The most frequently occurring correlations between *E. coli* and the measured variables were an increase in *E. coli* abundance with an increase in EPS constituent content, organic and water content, proportion of fine particles, lower temperatures and lower salinity. Correlation coefficients between all data for sampling campaigns on both estuaries are included in the supplementary material (supplementary Figures 1-3).

Explanatory models

The best model explaining *E. coli* abundance on the Ythan intensive sampling campaign used salinity, organic content, bulk density, maximum air temperature, pH, and season, achieving an adjusted R^2 of 0.62 (Fig 5). Whereas the best model for the Ythan transect only used salinity and season (Fig 6), and for the Eden transect salinity and pH only (Fig 7), achieving an adjusted R^2 of 0.41 and 0.58 respectively (Table 1)

Insert table 1 here

Insert figs 5,6,7 here

Model transferability was best for the Eden transect model, with deviance explained of 45.2 % and 41.6 % for the Ythan transect and intensive datasets respectively (Table 2). The Ythan transect model was more successful at predicting the Ythan intensive dataset than the Eden transect (deviance explained of 51.9 % and 31.5 % respectively), and the Ythan intensive GAM transferred equally well to both transect datasets (38.3 % and 40.8 % for the Ythan and Eden transects respectively (Table 2).

Insert table 2 here

When including all available variables, the AICc did not improve for the Ythan intensive dataset. The Ythan transect model improved with a final adjusted R^2 of 0.59, and the Eden transect model improved to 0.66 (Table 3), both with addition of colloidal carbohydrates (Figs 8 and 9).

Insert table 3 here

Insert figs 8,9 here

Model transferability did not improve however with the addition of predictor variables (Table 4). The only exception was the Ythan transect model to the Eden transect dataset which increased the deviance explained from 31.5 % to 38.9 %. The Ythan transect to Ythan intensive prediction decreased from 51.9 % to 35.6 %, and the Eden transect model from 45.2 % to 29.1 %, and 41.6 % to 15.0 %for the Ythan intensive and Ythan transect datasets respectively.

Insert table 4 here

Discussion

Spatial and temporal variation of sediment characteristics

The range of observed sediment characteristics for the transect sampling campaigns were typical of estuaries (Dale, 1974; Flemming and Delafontaine, 2000; Paterson et al., 2000) and covered a wide range of sediment types from clay-heavy mudflats to sandy beaches. The intensive sampling campaign encapsulated significant differences in seasonal variation of sediment characteristics, in addition to the constant difference between sediment types. Therefore, explanatory models based upon the data collected can be reliably applied to the whole estuarine system. Many of the variables discussed here

as affecting *E. coli* abundance and distribution were similar to those affecting the spatial distribution of total bacteria in intertidal sediments (Dale, 1974; Kuwae and Hosokawa, 1999).

Interstitial pore-water salinity explained a large portion of the variability in all datasets. This likely resulted from good discrimination of salinity between sites primarily as a result of dilution of the seawater further up the estuary (Supplementary Tables 1 and 2). There was also significant discrimination between seasons with higher salinity in sediments in the warmer months than winter presumably caused by longer sunlight hours and higher temperatures increasing evaporation of surface water on the sediments, increasing the interstitial pore-water salinity. The interaction of salinity with *E. coli* abundance is therefore two-fold. The first is the effect of seawater (containing a relatively low *E. coli* load), diluting the *E. coli* load in the water column (Neill, 2004) and thereby reducing the number of *E. coli* able to settle out of suspension. The second is that *E. coli* persistence is reduced by high salinities (Carlucci and Pramer, 1960; Anderson et al., 1983; Mezriouri et al., 1995). All sediments contained an interstitial pore-water salinity higher than 8.75 PSU, reported to be the salinity above which *E. coli* persistence in seawater is significantly reduced (Carlucci and Pramer, 1960). In addition, an increase in salinity has been demonstrated to induce the 'viable but non culturable' (VBNC) state in *E. coli* (Barcina et al., 1990; Darcan et al., 2009), a reversible state where cells are not able to be recovered using traditional media-based enumeration. This results in an underestimate of numbers using culture-based methods. Therefore, the effects of salinity on the abundance of *E. coli* observed here may be underestimated (Williams et al., 2007; Hassard et al., 2017). Hyper-saline conditions resulting from surface water evaporation were observed occasionally during spring and summer at MS resulting in two of the lowest *E. coli* abundances throughout the year (data not shown). Low salinity at MM during summer highlighted the importance of salinity as this coincided with unusually high *E. coli* abundance for the season. Environmental conditions leading to

low salinity (e.g. rainfall as the tide recedes) during summer may therefore lead to higher risk of human exposure to faecal bacteria because it coincides with the bathing season.

Interstitial water pH also explained variability in *E. coli* abundance in the Eden transect dataset. Higher pH of interstitial water had a negative effect on *E. coli* abundance (Tables 4 and 5). A relationship that has been previously observed in both water and sediments (Alam and Zafar, 2013). The variability of incoming river water pH was relatively low; the river Ythan varied between 6.6 and 7.9 in 2012 – 2013 (SEPA, 2013), and between 7.5 and 8.2 in the river Eden (SEPA, 2015). However, pH variation in estuarine water can be a result of biological activity from different trophic groups (Howland et al., 2000), with photosynthesis by algal mats able to increase underlying sediment pH to around 10 during daylight hours, whilst respiration at night can decrease pH to around 5.5 (Montague, 1986).

Surprisingly, environmental variables such as temperature and rainfall did not feature frequently in the models, especially considering the seasonality of the observed data, and previous evidence of higher survival of FIOs at low temperatures in fresh, estuarine and marine waters and sediments (Hartke et al., 2005; Atwill et al., 2007; Blaustein et al., 2013). There were large differences in *E. coli* abundance between seasons, and season was used in both optimal GAMMs for the Ythan estuary, but the effect did not appear to be driven directly by variation in temperature, despite significant correlation of temperature variables and *E. coli* abundance (Supplementary Figures 1-3) suggesting other seasonal variation may be responsible such as increased rainfall (Muirhead et al., 2004; Oliver et al., 2005; Guber et al., 2006), bird migration and trends in land use. Waterborne FIO concentrations have also been demonstrated to be highly seasonal (Horman et al., 2004; Pachepsky and Shelton, 2011; Duris et al., 2013), however it is important to note the limited seasonality in enteric virus abundance (Farkas et al., 2018).

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Model performance

Generally, the models explaining *E. coli* abundance performed well with adjusted R^2 values up to 0.66. As discussed above, terms employed in the models often correlated highly with one or more other variables, such as salinity for grain size and organic matter, demonstrating that in order to predict *E. coli* abundance it is not necessary to measure many sediment characteristics, but select a few key variables that co-vary with others. Models which included more costly predictors improved on the models using cost class 1 and 2 variables only, however transferability decreased. This could be explained by having a correlation between the explanatory variable and E. coli in the data set that does not hold generally for other data sets (Wenger and Olden, 2012). The deviance explained by the transferred cost class 1 and 2 models was fairly consistent at roughly a deviance explained of 42 %. Further research on a larger variety of sampling sites is necessary to produce more consistent prediction, yet the work here demonstrates that predictive modelling of *E. coli* abundance in sediments using variables that cost less than monitoring of FIOs themselves is a useful and financially viable alternative.

Summary

The data presented here provide insight into the complex relationships between sediment characteristics and *E. coli* distribution that will aid future predictive modelling of *E. coli* abundance in estuarine sediments and their subsequent effects on water quality. The key driving factors influencing spatial and temporal variation in *E. coli* abundance were identified as salinity, pH, organic content,

bulk density, maximum air temperature, colloidal carbohydrates and season, with salinity being the most important individual variable. Combining these results with models concerning the resuspension and release of *E. coli* to the water column, accumulated risk scenarios, and subsequently with process-based models concerning FIO transport and fate would be a powerful tool in the regulation of bathing water safety, allowing for improved accuracy of real-time water quality prediction. The results from this study indicate it is feasible and relatively low cost to survey sediment characteristics in to inform on the spatial and temporal distribution of FIOs in estuaries.

Acknowledgments

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Table and Figure Legends

Figure 1 Sampling sites on the Ythan estuary (A) and the Eden estuary (B). Intensive sampling campaign sites M (Mud), MM (Mixed Mud), MS (Mixed Sand) and S (Sand) on the Ythan estuary. Grey filled area- river channel at low tide; dotted area- intertidal mudflat.

Figure 2 Mean values of total coliform (A) and *E. coli* (B) abundance at the four intensively sampled sediment types for each season. Black- Mud (M); light-grey dashed- Mixed Mud (MM); dark-grey dashed- Mixed Sand (MS); hatched light grey- Sand (S). Error bars indicate standard error from the mean (n= 18, 18, 12, 30 for all sites at Spring, Summer, Autumn, Winter).

Figure 3 Mean values of *E. coli* abundance for the transect sampling campaign over during Spring (A), Summer (B), Autumn (C) and Winter (D) at the Ythan estuary, with sites 1 and 14 at the head and mouth of the estuary respectively. Error bars indicate standard error from the mean (n= 3).

Figure 4 Mean values of *E. coli* abundance for the transect sampling campaign over during Spring (A), Summer (B), Autumn (C) and Winter (D) at the Eden estuary, with sites 1 and 14 at the head and mouth of the estuary respectively. Error bars indicate standard error from the mean (n= 3).

Figure 5 Partial GAM plots the selected model for the standardised Ythan intensive dataset using cost class 1 and 2 variables only. Plots represent variable response to *E. coli* abundance independent of other variables. Degree of smoothing is indicated in the y-axis label. Dotted lines represent 95 % confidence intervals. For season, 1= Winter, 2= Spring, 3= Summer, 4= Autumn.

Figure 6 Partial GAM plots the selected model for the standardised Ythan transect dataset using cost class 1 and 2 variables only. Plots represent variable response to *E. coli* abundance independent of other variables. Degree of smoothing is indicated in the y-axis label. Dotted lines represent 95 % confidence intervals. For season, 1= Winter, 2= Spring, 3= Summer, 4= Autumn.

Figure 7 Partial GAM plots the selected model for the standardised Eden transect dataset using cost class 1 and 2 variables only. Plots represent variable response to *E. coli* abundance independent of other variables. Degree of smoothing is indicated in the y-axis label. Dotted lines represent 95 % confidence intervals.

Figure 8 Partial GAM plots the selected model for the standardised Ythan transect dataset using all cost class variables. Plots represent variable response to *E. coli* abundance independent of other variables. Degree of smoothing is indicated in the y-axis label. Dotted lines represent 95 % confidence intervals. For season, 1= Winter, 2= Spring, 3= Summer, 4= Autumn.

Figure 9 Partial GAM plots the selected model for the standardised Eden transect dataset using all cost class variables. Plots represent variable response to *E. coli* abundance independent of other variables. Degree of smoothing is indicated in the y-axis label. Dotted lines represent 95 % confidence intervals.

Table 1 Selected models explaining *E. coli* abundance for the three datasets using cost class 1 and 2 variables only. Deviance explained, adjusted R2 and p value from fitted GAMMs.

Table 2 Model transferability between the three datasets using cost class 1 and 2 variables only. Deviance explained, adjusted R2 and p value from fitted GAMs.

Table 3 Selected models explaining *E. coli* abundance for the three datasets using all variables. Deviance explained, adjusted R2 and p value from fitted GAMMs.

Table 4 Model transferability between the two transect datasets using all variables. Models are GAMs using site as a random effect. Deviance explained, adjusted R2 and p value from fitted GAMs.

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Table 1

Modelled dataset	Salinity	Organic content	Bulk density	Max. air temperature	pH	Season	AICc	Adjusted R^2
Ythan intensive	X	X	X	X		X	430.46	0.62
Ythan transect	X					X	130.14	0.41
Eden transect	X				X		146.42	0.58

Table 2

Modelled dataset	Tested dataset	Deviance explained	Adjusted R^2	*p* value
Ythan intensive	Ythan transect	38.3	0.37	< 0.001
Ythan intensive	Eden transect	40.8	0.39	< 0.001
Ythan transect	Ythan intensive	51.9	0.52	< 0.001
Ythan transect	Eden transect	31.5	0.30	< 0.001
Eden transect	Ythan transect	45.2	0.43	< 0.001
Eden transect	Ythan intensive	41.6	0.41	< 0.001

Table 3

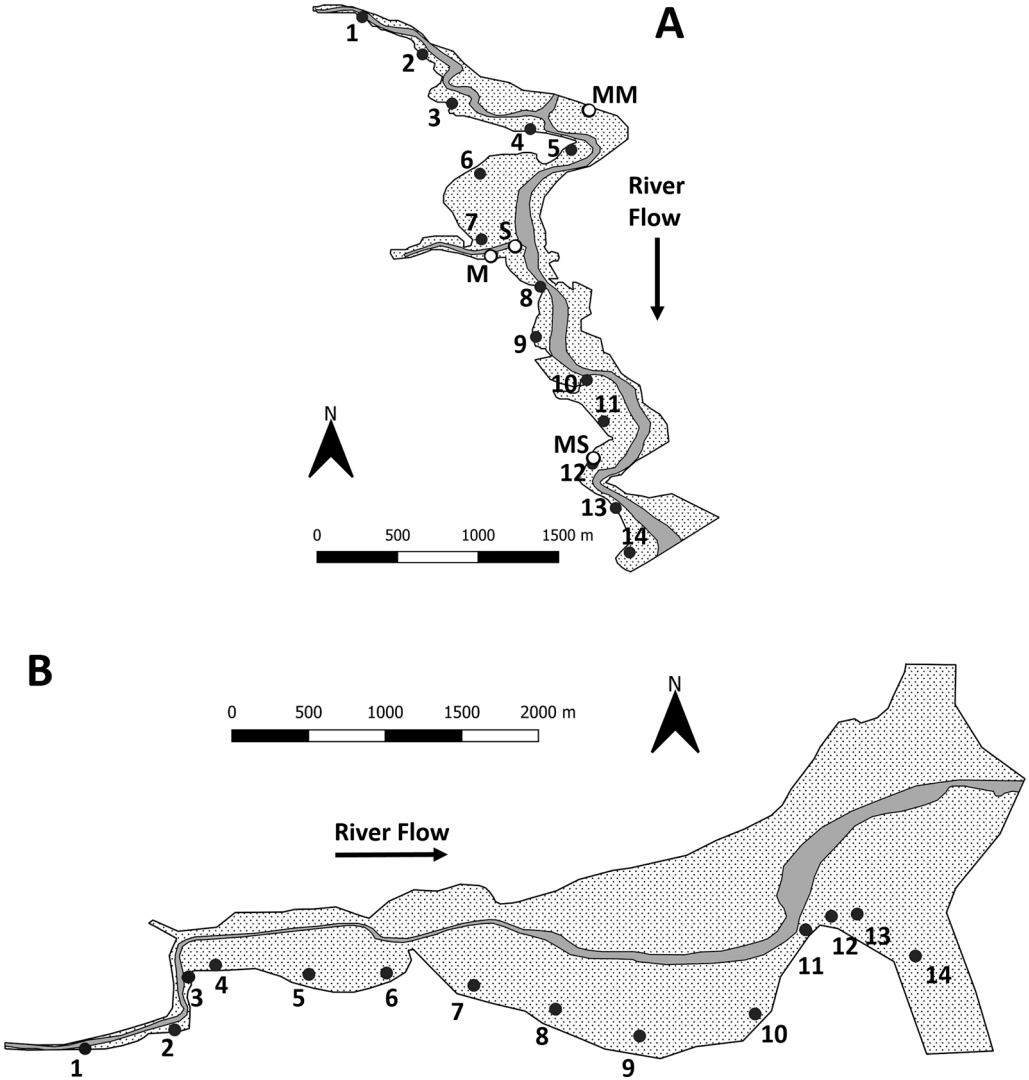
Modelled dataset	Salinity	Colloidal carbohydrates	pH	Season	AICc	Adjusted R^2
Ythan transect	X	X		X	124.82	0.59
Eden transect	X	X	X		136.60	0.66

Table 4

Modelled dataset	Tested dataset	Deviance explained	Adjusted R^2	*p* value
Ythan transect	Ythan intensive	35.6	0.35	< 0.001
Ythan transect	Eden transect	38.9	0.38	< 0.001
Eden transect	Ythan transect	39.1	0.37	< 0.001
Eden transect	Ythan intensive	15.0	0.15	< 0.001

Highlights

- *E. coli* concentrations up to 6 $\log_{10}$ CFU 100 g dry wt^{-1} were observed in sediments
- Sediment characteristics explained *E. coli* abundance to an Adj-R^2 of 0.66
- Higher cost predictor variables did not increase model transferability
- Model transferability between datasets explained up to 51.9 % of deviance
- Salinity and season were the most important predictor variables


A
1
2
3
4
5
6
7
S
M
MM
8
9
10
11
MS
12
13
14
River
Flow
N
0
500
1000
1500 m
B
0
500
1000
1500
2000 m
N
River Flow
1
2
3
4
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6
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11
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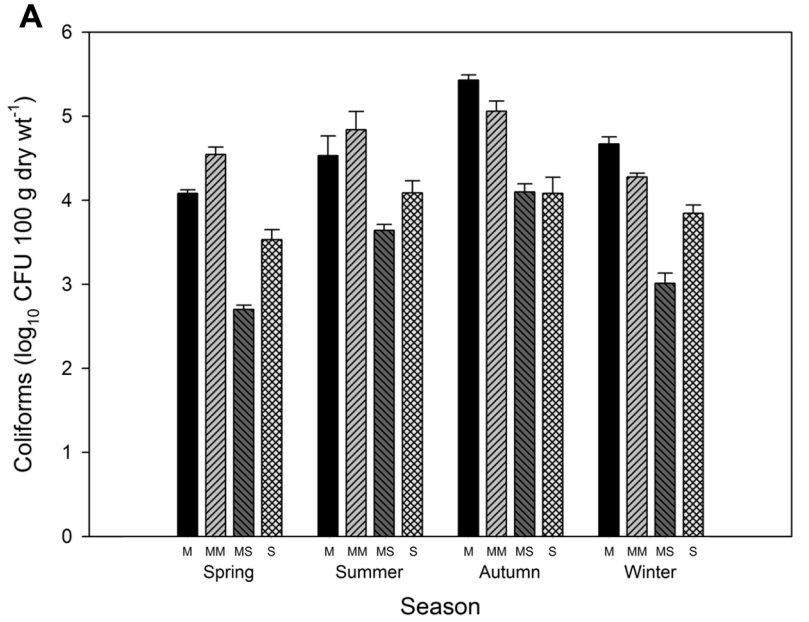
14

Figure 1


A
Coliforms (log_{10} CFU 100 g dry wt^{-1})
0
1
2
3
4
5
6
M MM MS S
Spring
Summer
Autumn
Winter
Season

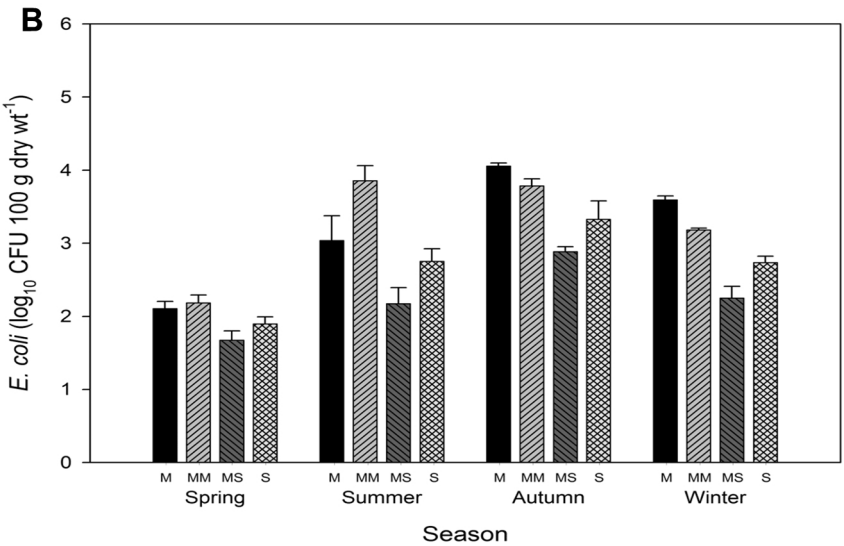

B
E. coli (log_{10} CFU 100 g dry wt^{-1})
0
1
2
3
4
5
6
M MM MS S
Spring
Summer
Autumn
Winter
Season

Figure 2

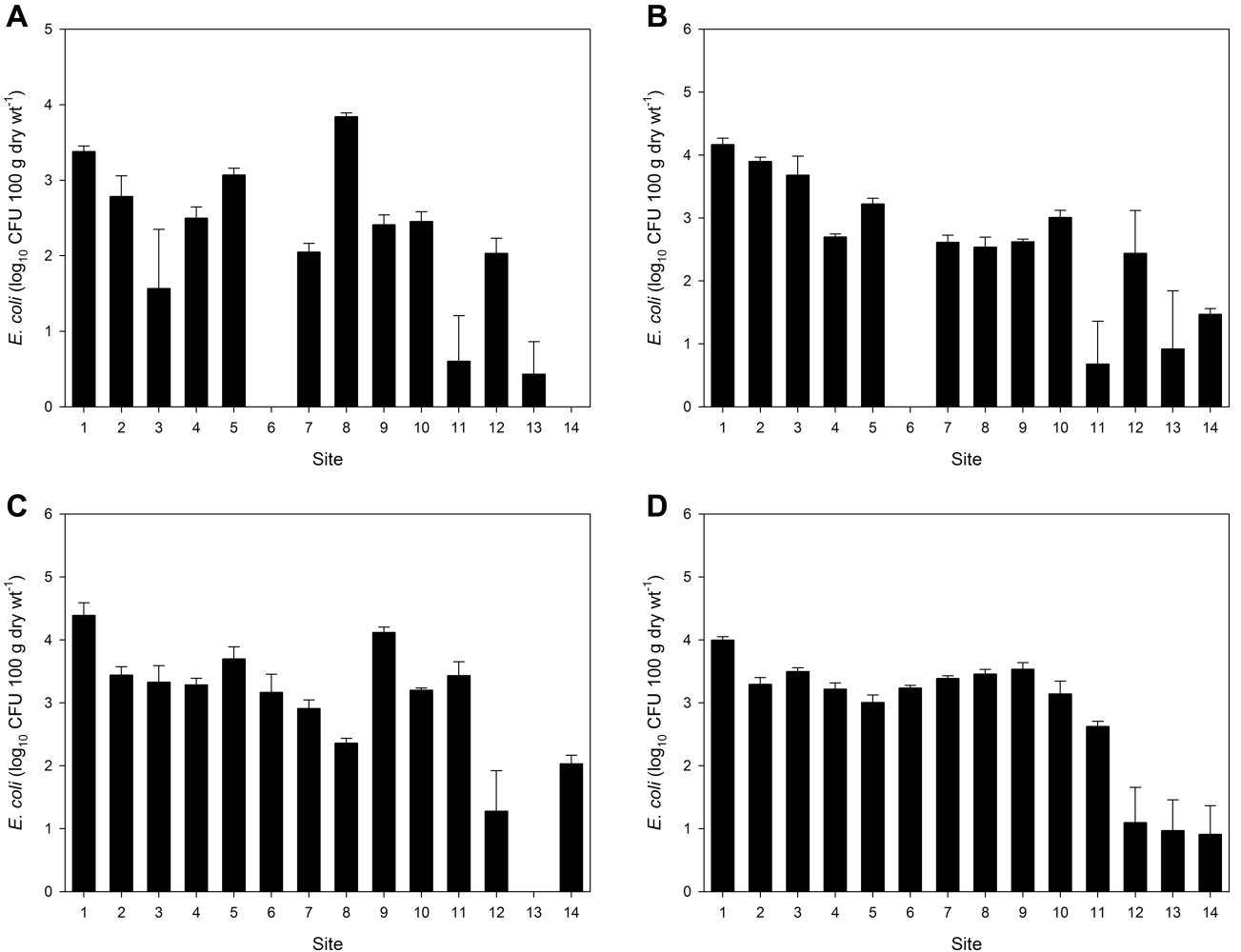
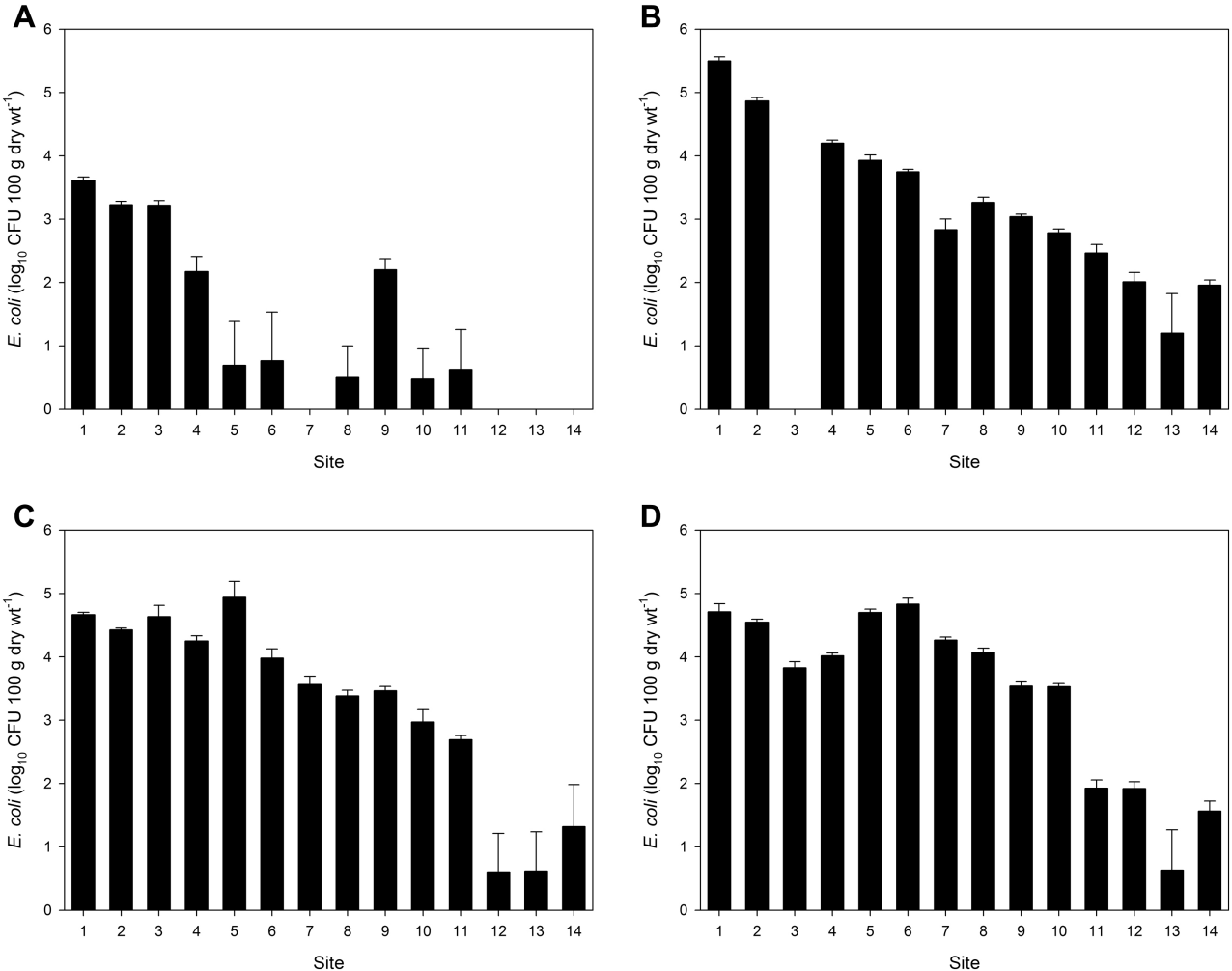

A
B
C
D
E. coli (log10 CFU 100 g dry wt-1)
Site
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 3


A
B
C
D
E. coli (log10 CFU 100 g dry wt-1)
Site
0
1
2
3
4
5
6
1
2
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7
8
9
10
11
12
13
14

Figure 4

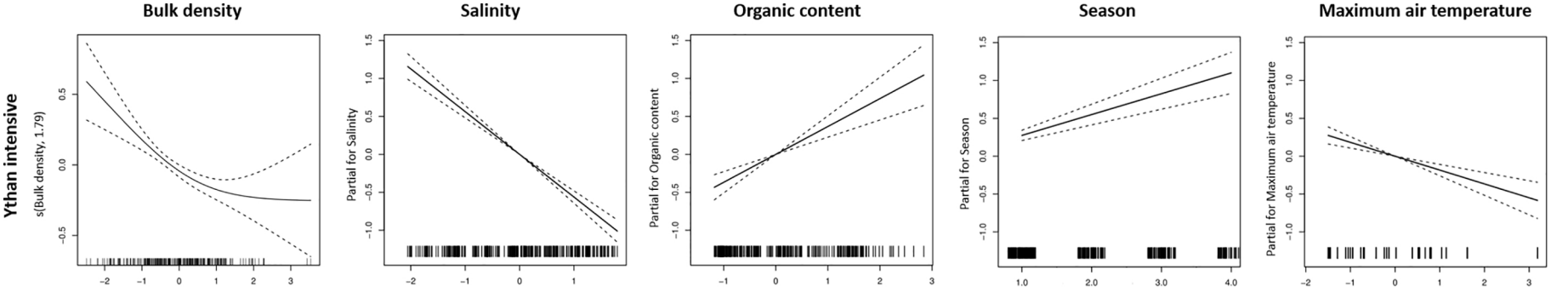
Ythan intensive
Bulk density
s(Bulk density, 1.79)
Salinity
Partial for Salinity
Organic content
Partial for Organic content
Season
Partial for Season
Maximum air temperature
Partial for Maximum air temperature

Figure 5

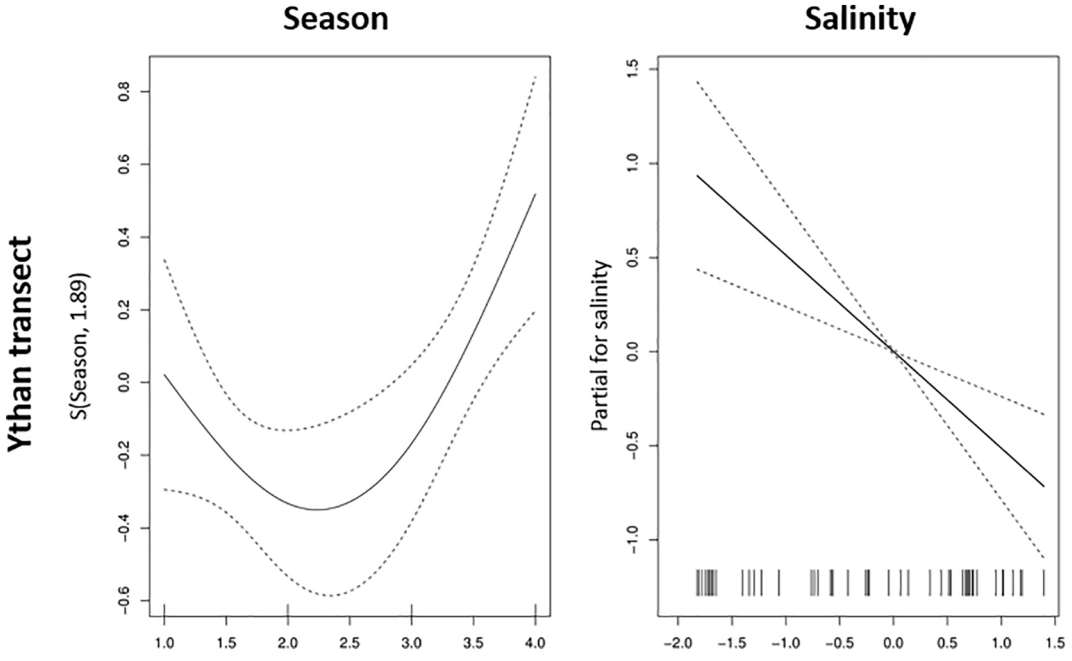
Ythan transect
Season
S(Season, 1.89)
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Salinity
Partial for salinity
1.5
1.0
0.5
0.0
−0.5
−1.0
−2.0
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5

Figure 6

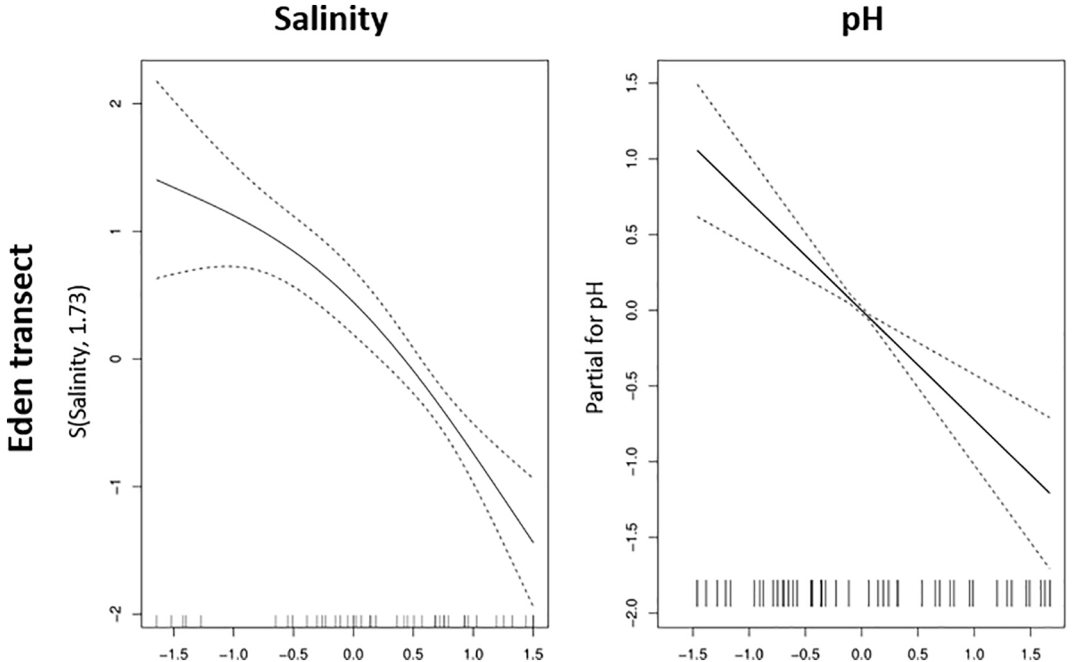

Figure 7

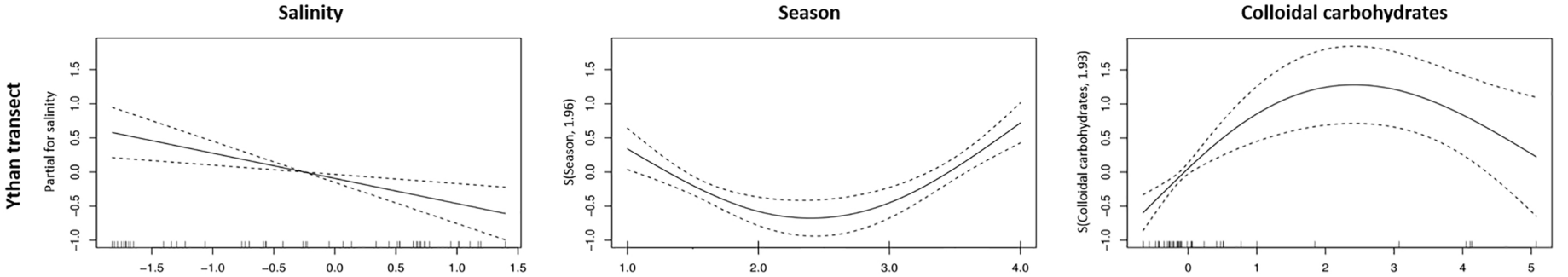
Ythan transect
Salinity
Partial for salinity
Season
S(Season, 1.96)
Colloidal carbohydrates
S(Colloidal carbohydrates, 1.93)

Figure 8

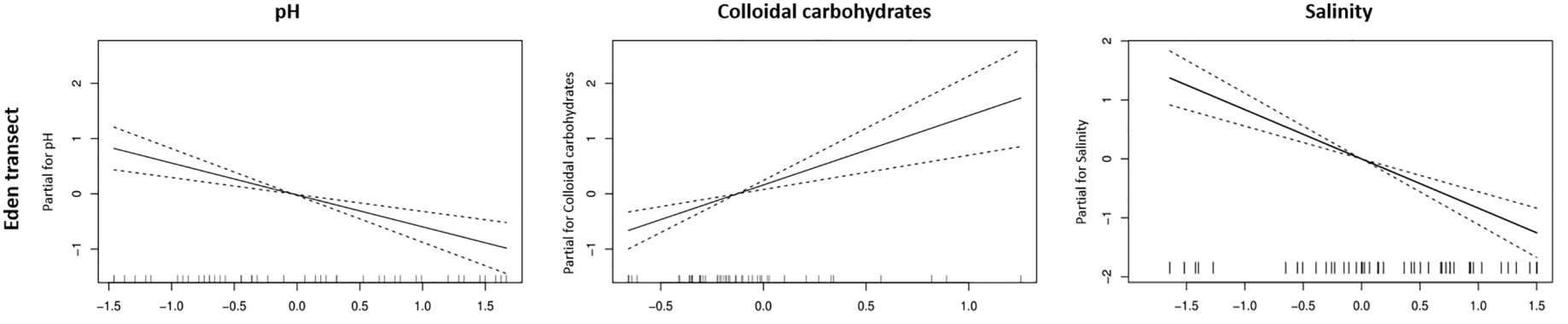
Eden transect
pH
Partial for pH
Colloidal carbohydrates
Partial for Colloidal carbohydrates
Salinity
Partial for Salinity

Figure 9