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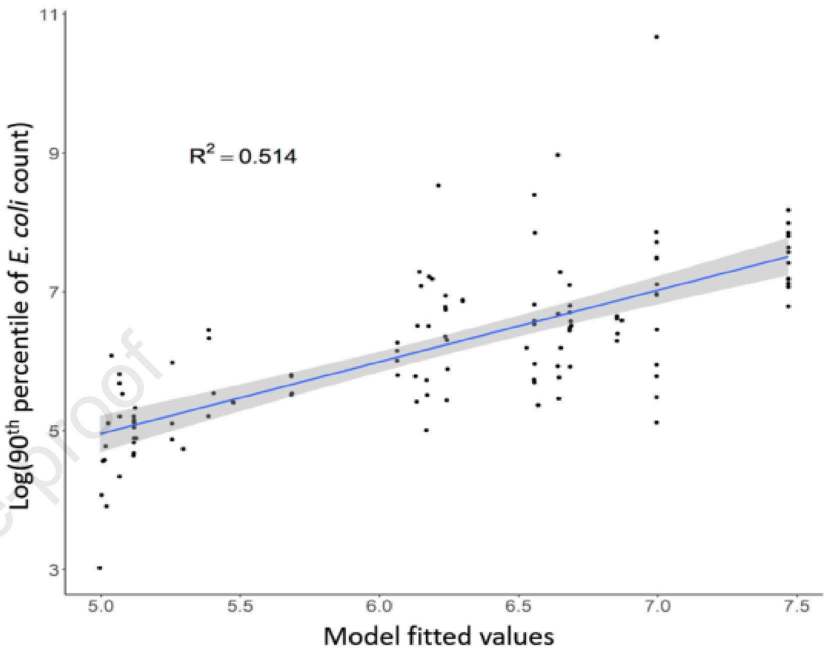
In oysters and mussels …

Images from Pixabay



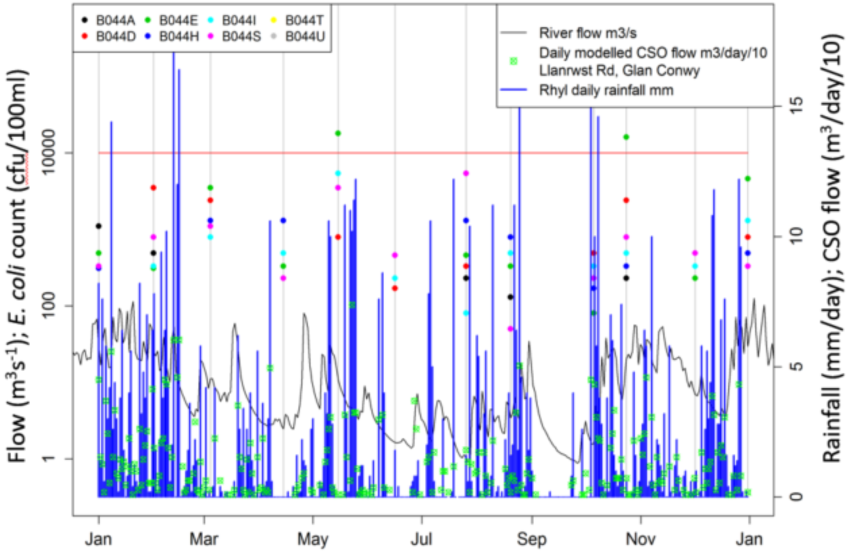
… catchment characteristics predict risk of *E. coli* in shellfish flesh:

-River flow

-River nitrate

-River turbidity

… CSO operation was associated with risk of contamination



Multi-scale influences on *Escherichia coli* concentrations in shellfish: from catchment to estuary

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Highlights

- Catchment models predict estuary risk but not levels of shellfish bed *E. coli*
- High estuary *E. coli* correlates with high river flow, nitrate and turbidity
- 64% of beds show a link between river flow and *E. coli* with 1 day lag
- Combined sewer overflows (CSO) associate with high *E. coli* in shellfish

- Highly variable *E. coli* across and within estuaries prevents bed-level prediction

Abstract

Sustainability of bivalve shellfish farming relies on clean coastal waters, however, high levels of faecal indicator organisms (FIOs, e.g. *Escherichia coli*) in shellfish results in temporary closure of shellfish harvesting beds to protect human health, but with economic consequences for the shellfish industry. Active Management Systems which can predict FIO contamination may help reduce shellfishery closures. This study evaluated predictors of *E. coli* concentrations in two shellfish species, the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*), at different spatial and temporal scales, within 12 estuaries in England and Wales. We aimed to: (i) identify consistent catchment-scale or within-estuary predictors of elevated *E. coli* levels in shellfish, (ii) evaluate whether high river flows associated with rainfall events were a significant predictor of shellfish *E. coli* concentrations, and the time lag between these events and *E. coli* accumulation, and (iii) whether operation of Combined Sewer Overflows (CSO) is associated with higher *E. coli* concentrations in shellfish. A cross-catchment analysis gave a good predictive model for contamination management ($R^2 = 0.514$), with positive relationships between *E. coli* concentrations and river flow (p=0.001), turbidity (p=0.002) and nitrate (p=0.042). No effect was observed for catchment area, the number of point source discharges, or agricultural land use type. 64% of all shellfish beds showed a significant relationship between *E. coli* and river flow, with typical lag-times of 1-3 days. Detailed analysis of the Conwy estuary indicated that *E. coli* counts were consistently higher when the CSO had been active the previous week. In conclusion, we demonstrate that real-time river flow and water quality data may be used to predict potential risk of *E. coli* contamination in shellfish at the catchment level, however, further refinement (coupling to fine-scale hydrodynamic models) is needed to make accurate predictions for individual shellfish beds within estuaries.

Keywords: Active management system; Public health risk; Sewage discharges; Shellfish contamination; Water quality

1 Introduction

Bivalve shellfish aquaculture is considered a sustainable source of dietary protein and the industry continues to expand globally (Suplicy, 2020; Naylor et al., 2021; Krause et al., 2022;). Within the European Union, ca. 0.5 million tonnes of mussels and oysters are harvested per year with an estimated economic value of ca. €1 billion (EUMOFA, 2022). The industry, however, faces a number of interlinked threats to its sustainability including climate change, water pollution, loss of habitat, overharvesting, invasive species and shifting markets (Brown et al., 2020; Webber et al., 2021). Bivalve aquaculture farms are commonly located in sheltered estuaries and coasts, where the organically enriched waters provide an ideal food source for shellfish. However, increasing urbanisation and agriculture within coastal areas results in increased domestic wastewater discharge and surface runoff (eg agricultural pollution) to coastal water bodies, potentially containing high loads of faecal bacteria and pathogenic viruses which pose a risk to human health (Malham et al., 2014; Manini et al., 2022). Similarly, the impact of diffuse and point source pollution affects the shellfish industry and has socio-economic implications including potential loss of revenue and employment (Clements et al., 2015). Because bivalves are filter feeders, shellfish may bioaccumulate pathogenic micro-organisms from the surrounding environment which may ultimately enter the food chain and cause disease outbreaks (Potasman et al., 2002; Lee and Morgan 2003; Teplitski et al., 2009; Webber et al., 2021). Being able to predict in advance when the greatest risk of shellfish contamination with faecal organisms will occur therefore represents a major goal for the industry (Schmidt et al., 2018).

Faecal indicator organisms (FIOs), such as *Escherichia coli*, typically enter the aquatic environment via human and animal faeces originating from urban wastewater discharges and agricultural runoff (Olivier et al., 2016; Malham et al., 2014). Although *E. coli* in humans can be considered relatively harmless, there are several strains which can be pathogenic to humans (Vásquez-García et al., 2019). Once in the water column FIOs can attach to flocculated suspended sediment, organic material (Jago et al 2024) and plastics providing physical and chemical protection from biotic and abiotic stresses and increasing their likelihood of reaching shellfish areas (Oberbeckmann et al., 2014; Hassard et al., 2016; Garcia-Aljaro et al., 2017; Jago et al., 2024). FIO persistence and survival in estuarine and coastal areas is also dependent on the type of FIO strain and the physico-chemical properties of the environment, such as hydrodynamic flow regime, temperature, pH, turbidity, UV irradiation and salinity, as previously reviewed (Hassard et al., 2017). This inherent complexity makes prediction of FIO persistence in the environment and potential shellfish contamination difficult to achieve.

Currently, public health protection monitoring for shellfish destined for human consumption in many countries is based on routine monthly sampling from specific points on the shellfish bed. The samples are tested for levels of the faecal indicator bacterium *E. coli* and faecal coliforms (FC) (Schmidt et al., 2018; Pinn and LeVay, 2023). The fixed monthly nature of the sampling regime carries risks both to human health and to viability of the shellfish industry. Due to short-term temporal and spatial variation in FIO presence in the coastal zone, monthly routine regulatory spot-sampling for FIOs may fail to capture episodes of high *E. coli* concentrations, thereby providing inadequate human health protection. The infrequent monthly repeat sampling regime may also extend closure periods unnecessarily, resulting in losses to the industry. These health and economic risks could be reduced by implementing an intelligent and reactive monitoring system which predicts likely episodes of high *E. coli* concentrations and predicts when concentrations are likely to reduce to a safe level (Qin et al., 2022; Campos et al., 2023).

Early warning systems aim to use real-time data for risk management using statistical or deterministic models based on either simple relationships (e.g. rainfall and *E. coli* counts) or complex models (e.g. transport processes) (Gourmelon et al., 2010). Agencies in Australia, New Zealand, Canada and the USA utilise early warning systems, however, there is no standardised approach to detecting high FIO loads, with systems implemented differently both within and between countries (Pinn and LeVay, 2023). In New Zealand for example, rainfall, river discharge and salinity are used to indicate potential faecal contamination of water using real time data collected in the catchment and compared to pre-determined criteria (Gourmelon et al., 2010). Such approaches have been primarily applied to prediction of bathing water quality on beaches, while prediction of *E. coli* concentrations in shellfish flesh (rather than water, e.g. Zimmer-Faust et al., 2018) and within estuaries is more challenging. Relationships between *E. coli* concentrations at shellfish beds and rainfall can be highly contingent on catchment and estuary characteristics (Robins et al., 2018), and location of shellfish beds (Campos et al., 2012), as well as season and tidal cycles (Lee and Morgan 2003). Estuary size, the speed that rainfall will traverse from land to sea, the presence and location of point sources (Sewage Treatment Works (STWs) and Combined Sewer Overflows (CSOs)) in addition to diffuse sources within the catchment, alongside seasonal aspects of catchment management, particularly livestock management, throughout the year, all contribute to variability in *E. coli* levels (Suslovaite et al., 2024; Younger et al., 2022; Hassard et al., 2016; Malham et al., 2014; Bougeard et al., 2011). Nonetheless, such predictions are possible for contaminants in shellfish. For example, Riou et al. (2007) show that viral contamination in shellfish can potentially be predicted from weather parameters and viral disease outbreaks in the human population.

The aim of this study was to use a multi-scale approach to evaluate predictors of *E. coli* concentration in shellfish flesh of two species, the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*), at different spatial and temporal scales. Using a combination of long-term monitoring data and variables for diffuse and point sources and catchment characteristics across twelve estuaries in the UK, our aims were to: (i) identify consistent catchment-scale or within-estuary predictors of elevated *E. coli* levels in shellfish; (ii) evaluate whether high river flows associated with rainfall events are a significant predictor of *E. coli* concentrations in shellfish, and determine the time lags between these events and shellfish *E.*

coli accumulation, and (iii) whether operation of Combined Sewer Overflows (CSO) are associated with higher *E. coli* concentrations in shellfish.

2. Methods

2.1.Site selection

Twelve river catchments and associated estuaries were selected to investigate the between- and within-estuary factors which influence *E. coli* levels in shellfish. All sites had commercial shellfishery operations and encompassed a range of geographic locations around England and Wales (Fig 1). The sites also encompassed a wide variation in catchment size and agricultural land use (Table 1). Specifically, the amount of improved grassland was deemed important as this represents the main land cover category for cattle and sheep grazing stock which are major contributors of diffuse catchment sources of *E. coli* (Kay et al., 2008). Estuaries with shellfish beds of hygiene class B and class C classifications (EC, 2015, 2019) were prioritised for selection as these reflect sites with historical issues of *E. coli* contamination, and are the areas where Active Management Systems would have the greatest benefit for the industry, and comprise ~85% of the monitored shellfish beds in Great Britain. However, some class A areas and some Prohibited areas were also included to ensure sufficient gradient in *E. coli* concentrations for analysis. The classification criteria for shellfish beds in England and Wales are: Class A (80% of sample results must be less than or equal to 230 *E. coli* per 100 g flesh; AND no results may exceed 700 *E. coli* per 100 g flesh), Class B (90% of samples must be ≤ 4600 *E. coli* per 100 g flesh; AND all samples must be less than 46000 *E. coli* per 100 g flesh), Class C (≤ 46000 *E. coli* per 100 g flesh), and Prohibited (> 46000 *E. coli* per 100 g flesh) (EC, 2015, 2019; Malham et al., 2017; Ciccarelli et al., 2022). If *E. coli* levels exceed the threshold concentration of Class C, the bed is shut until levels drop below the regulation threshold for two subsequent months. A value of 10,000 *E. coli*/100 g shellfish flesh is the trigger value for formal investigations with the B class classification. Other risk factors for *E.*

coli loadings were considered, based on the literature, including rainfall and river flow, which are factors governing *E. coli* transport into river systems (Campos et al., 2013), and water chemistry variables (NO_3^- concentrations, turbidity) which are implicated in the persistence and survival of *E. coli* in the environment (Campos et al., 2013; Malham et al., 2014; Malham et al., 2017).

2.2. Data on E. coli concentration in shellfish flesh

Data on *E. coli* concentrations in shellfish flesh (Maximum Probable Number (MPN), measured as *E. coli* per 100 g shellfish flesh; Walker et al., 2018) were collated from the routine monthly monitoring for regulatory sampling at Representative Monitoring Points (RMPs) on designated shellfish beds in the twelve estuaries. Data were collated for the eight-year period 2010-2017 for *E. coli* levels in shellfish recorded by the national reference laboratory for shellfish hygiene (CEFAS, Weymouth, UK). The study focused on the two main shellfish species of commercial value, namely Blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*). There were 131 beds sampled overall across the 12 sites (Fig. 1). The 90th percentile of *E. coli* counts in each year were calculated for each bed for the eight-year period between 2010 and 2017 (Mok et al., 2018; Tiwari et al., 2021; Suslovaite et al., 2024).

2.3. Catchment, estuary and river characteristics

Catchment areas were taken from the Water Framework Directive data held by Welsh Government for the sites in Wales and by the Department for Environment, Food and Rural Affairs for the sites in England. Catchments included the entire contributing catchment for the estuary, not just the catchment for the dominant river. Initial analysis of catchment characteristics considered the proportion of three key land cover types, obtained from CEH Landcover 2007 (Morton et al., 2011). Improved grassland was used as a proxy for cattle grazing, unimproved grassland was used as a proxy for sheep grazing, arable was used as a proxy for sediment and fertiliser nutrient input into rivers. Following initial analysis, only improved grassland was retained as an explanatory variable. Potential urban and industrial sources were assumed to be captured in the variable ‘loading risk’ focusing on permitted discharges (see next section). Mean annual rainfall was taken from the nearest meteorological office rain gauge in the catchment. Annual river flows for the main rivers flowing into each estuary were obtained from the Environment Agency and Natural Resources Wales, and daily river flow data was obtained from the CEH National River Flow Archive (NRFA) database (https://nrfa.ceh.ac.uk/). The annual data on river flow were averaged to create a long-term 30-year annual average flow, for comparison across estuaries. In addition, daily flow data were extracted for specific time periods corresponding to the monthly *E. coli* monitoring dates within the period 2010 – 2017 for analysis of *E. coli* concentrations relative to lagged daily river flow. Water quality data were obtained from the Environment Agency WIMS database which curates the Historic UK Water Quality Sampling Harmonised Monitoring Scheme, for nitrate-N and turbidity. Water quality data for the river Stour in the north Kent estuary was not available in the WIMS database, therefore data for Swalecliffe Brook was used as a proxy. While river-specific data is preferable, water courses draining neighbouring catchments in areas where land-use cover and type is similar, particularly with respect to likely sources of *E. coli*, physical and chemical characteristics of the catchment, and consequent in-stream transport and processing, the additional uncertainty introduced is not likely to substantially alter the findings. Site characteristics are summarised in Table 1.

2.4. Within-estuary point sources of E. coli

Two variables were calculated to assess the influence of within-estuary point source inputs of *E. coli*. The first, ‘Loading risk’ was based on point sources for which permitted bacterial discharge loadings were available (e.g. urban sewage treatment works). The second, 'Source count' summarised the number of potential point sources, including those where bacterial discharge loads were not known. Data for both variables were obtained from the sanitary surveys for each estuary (CEFAS sanitary surveys https://www.cefas.co.uk/data-and-publications/sanitary-surveys/). Loading risk was calculated for each RMP as an inverse distance-weighted loading from all continuous sewage treatment works (STWs) with known loading rates. The 'loading risk' (LR) was calculated as:

$$LR = \sum_i \frac{n_{bacteria}}{d^2} \qquad \text{(Eqn. 1)}$$

where $n_{bacteria}$ is the estimated bacterial loading (cfu day^{-1}) at a given STW, and d is the linear distance (m) between that STW and the RMP. All STWs in a single estuary that had an estimated bacterial loading were used to calculate the 'cumulative risk factor'. Where inland STWs were known to discharge to the estuary, but the exact discharge point was unknown, this was estimated based on the most likely position.

Source count was calculated as the number of all potential sewage outflow points (continuous, intermittent and private sources, including Combined Sewer Overflows (CSOs)), within a 1 km radius of each RMP.

2.5. Time-series data

For the time-series analysis of river flow for each estuary, data for the main gauged river entering each estuary was used. Where river gauge data was not available, flow data for a similar nearby gauged river was used, on the assumption that rainfall patterns are broadly consistent geographically.

2.6. Influence of CSO discharges on shellfish E. coli levels (Conwy catchment case study)

For analysis of the Conwy catchment case study, rainfall data for the Conwy estuary was taken from the nearest UK Meteorological Office station at Rhyl, situated at sea level and 25 km east of Conwy. River flow data was taken from the Llanrwst gauge. Welsh Water/Dŵr

Cymru provided the locations of CSOs in the Conwy estuary, and the timing of their operation from Event Monitoring Data which records when CSOs are releasing sewage. Welsh Water/Dŵr Cymru also provided estimates of CSO discharge volumes while operating. These estimates are derived from the InfoWorks ICM sewer model (Autodesk Inc., San Francisco, CA), run by the consultancy Arup Ltd, London, UK. Analysis focused on two of the 35 CSOs which might be considered as possible influences on *E. coli* numbers in mussels in the Conwy estuary, based on their position in the catchment (closest to the mouth of the estuary where the shellfish beds are located).

2.7. Data preparation and analysis

All statistical analyses were carried out using the program R version 3.3.1 (R Development Core Team 2016). Statistical analysis was undertaken on the 12 selected catchments and associated 131 nearshore mussel and oyster beds. To test for the effects of between-estuary and within-estuary factors, annual *E. coli* data were averaged over the eight years to give an average annual 90th percentile *E. coli* value for each bed. Values were log-transformed to reduce the influence of outlier values. A linear mixed effects model (LMM) was used to test for significant effects, including both catchment-level characteristics and within-estuary variables. Estuary was included as a random effect to account for nesting of 'bed' within 'estuary'. Initial explanatory and response variable data were assessed for outliers in the response and collinearity among the explanatory variables. The LMM was fitted by first scaling the selected explanatory variables. Significant relationships between each of the main effects on *E. coli* log abundances was assessed via permutation tests (Chihara et al., 2018). The full model is: log(bac90) ~ catchment area + improved grassland + flow + turbidity + nitrate + loading risk + source count + species + (1|estuary). Each permutation test consisted of first calculating the log-likelihood ratio between the model with the main effect (full model) and the model with the main effect removed (reduced model). We then compared this ratio to the respective null distribution, which was determined by permuting the main effect N=1000 times. If the log-likelihood ratio for full model vs reduced model was greater than the log-likelihood of the permuted model vs reduced model for at least 95% (i.e. $p \leq 0.05$) of the permutation outcomes the main effect was deemed significant. This modelling approach was used to develop a predictive relationship for *E. coli* concentrations in shellfish flesh based on catchment characteristics.

In a separate analysis, possible lagged flow effects on *E. coli* counts were assessed, using 2012 as an example year. Regression analyses of logged *E. coli* counts on logged daily river flows included 'no lag' and lag periods of 1 to 7 days. Paired plots were used to examine within-estuary variability of *E. coli* concentrations between RMP monitoring locations. Plotted pairs correspond to a day on which a sample was taken at each of the sites being compared. In some cases, there was no overlap in sampling days so no basis for a paired plot. The paired plots also include comparison of concentrations with daily mean flow in the associated river. Time-series data for the Conwy were analysed for relationships of *E. coli* with river flow, and with CSO operation. Analysis of variance was used to compare *E. coli* counts when there was no CSO activity at Deganwy pumping station or Llanrwst Road, with counts for periods when the CSO had been active during the previous week.

3. Results

Median values of the 90th percentile of *E. coli* concentrations in shellfish flesh in each year show considerable variation among the 12 estuaries (Fig. 2). The Fal and the Taw sites, both in south-west England show the highest concentrations of *E. coli* in shellfish flesh (ca. 500-3000 *E. coli*/100 g). The lowest levels were in the Blackwater estuary in eastern England and Menai in Wales (<250 *E. coli*/100 g). However, there is no consistent national geographical pattern to the variation in shellfish *E. coli* concentrations with the results appearing to be highly estuary-specific.

Analysis of the factors contributing to high shellfish *E. coli* concentrations across the 12 estuaries revealed a significant positive relationship with increasing river flow ($p \leq 0.001$), river water nitrate concentration ($p = 0.042$) and turbidity ($p = 0.002$). In addition, *E. coli* concentrations were also found to be significantly greater in mussels compared to oysters ($p < 0.05$). We found no significant effect of the proportion of improved grassland (i.e. livestock areas) in the catchment ($p = 0.178$), or catchment area ($p = 0.207$), or of variables summarising risk from within-estuary sources: loading risk ($p = 0.542$), source count ($p = 0.232$) and *E. coli* levels in shellfish. Overall, the predictive model of shellfish *E. coli* concentrations performed well against our observed data, with an adjusted R^2 value of 0.514 (Fig. 3)

The model described above tested if significant general relationships existed across catchments with long-term river flow. In addition, relationships with river flow for multiple mussel bed sites in each estuary were also tested. Across the twelve estuaries, this analysis showed that 64% of all beds showed a significant relationship with river flow in the preceding week. For most significant relationships, there was a time lag in the response, with a one-day lag being the most common, with the next most common being no time lag between high river flow events and shellfish contamination (Fig. 4). Some beds also showed significant relationships at a range of lag times (Table S1) illustrating that relationships with river flow are not straightforward. Lag times up to seven days between high flow and *E. coli* contamination were tested, however, we found no significant relationships with time lags greater than 3 days. Only the Crouch and the Wash estuaries, both on the east coast of England, showed a significant lag at three days, and even then, it was only observed for some shellfish beds. There was often a considerable variation in lag times among beds in the same estuary, including beds showing no relationship with flow (Table S1). In addition, in no estuary was there a consistent response of all beds to flow (Table S1).

Paired plot analysis among shellfish beds within each estuary demonstrated a weak relationship between flow and *E. coli* concentration in mussels (Fig 5). Further analysis of the log scaled data indicated a relationship at low to medium river flow and low to medium *E. coli* counts but little association between high river flows and high bacterial counts. Analysis of variation of *E. coli* levels within estuaries showed that at a particular point in time beds often had widely varying *E. coli* concentrations in shellfish flesh. Figure 5 shows these relationships for six selected estuaries. Barrow estuary in north-west England and the Wash estuary in eastern England both showed weak correlations of *E. coli* levels among beds within the estuary. By contrast, the Taw estuary in southwest England and the Conwy estuary in north Wales showed reasonably strong correlations in values among beds, while the Frome estuary in the south and the Fal estuary in southwest England, showed a mix of strong and weak correlations among beds.

Analysis of time series data for mussel beds in the Conwy estuary showed complex relationships with daily mean flows (see example year 2014 in Fig. 6). Modelled CSO release did not always coincide with high rainfall, and individual beds did not show consistently high *E. coli* counts. Nevertheless, when split into periods before and outside of a modelled CSO event, *E. coli* counts were consistently higher when the CSO had been active the previous week (Fig.7).

4. Discussion

4.1. Development of predictive models for E. coli contamination in shellfisheries

Our findings suggest that catchment level characteristics can be used to predict the type of estuaries in England and Wales and prevailing factors under which shellfish may be at greater risk of high *E. coli* loads and subsequent contamination. Estuaries at greater risk are those containing rivers with high flow volumes, high nitrate, and high turbidity. Local permitted discharges within estuaries (e.g. from wastewater treatment plants) do not appear to be a risk factor when they are operating normally. However, a more detailed analysis of one estuary with more extensive data suggests that there is an association between operation of CSOs which release untreated sewage and high *E. coli* levels in shellfish. We note that this is not necessarily causal and further work would be required to validate this. In all the estuaries studied here, we observed a high variability in shellfish *E. coli* levels among individual beds within an estuary. This highlights the challenge in developing predictive models for *E. coli* contamination for shellfisheries and probably reflects differences in hydrodynamic flow which can change both seasonally, across tidal cycles and in response to lateral and longitudinal shifts in sediment dynamics (Dunn et al., 2015; Matte et al., 2017; Robins et al., 2019). Similarly, we found different time lags for relationships with river flow and *E. coli* accumulation in shellfish, even within the same estuary. Hence, the use of fine-scale hydrodynamic and sediment transport catchment-to-coast models could be applied to improve *E. coli* predictions (e.g., Bashawri et al 2020; Huang et al. 2022). Whilst such models capture estuarine tidal and density-driven circulation and sediment transport (e.g. Huybrechts et al 2022), advancements in this field will lead to improved model simulations of turbulent mixing, various aggregation and settling processes including flocculation of organic material (Bi et al. 2020) and binding of suspended materials with bacteria (Shen et al. 2024), bottom boundary layer dynamics in tidal settings (Davies et al. 2023), and the response of bacteria to fluctuating environmental conditions such as water temperature, salinity, turbidity, and sunlight (Carneiro et al. 2018; Garcia-Garcie et al. 2021).

4.2. Prediction of E. coli contamination risk at a catchment level

The catchment characteristics which were predictors for long-term high *E. coli* loadings in shellfish for an individual estuary reflect the range of factors which broadly contribute to elevated risk. For example, livestock and particularly dairy cattle, are a known source of microbial contaminants to water courses (Vinten et al., 2004; Oyafuso et al., 2015). The significant relationship between high nitrate levels and bacterial loadings suggests that runoff from intensively used agricultural land may have been a source of *E. coli*. Sustained turbidity, as opposed to episodic high turbidity levels during storm events, may be a feature of catchments with relatively little riparian vegetation or bank protection allowing the easy transport of soil and agricultural waste into water courses (Cole et al., 1999). High levels of suspended particulate matter also acts as surfaces for adherence of faecal coliforms (Perkins et al., 2016; Hassard et al., 2017) and can increase their survival time in the environment (Poomepuy et al., 1992; Alkan et al., 1995). Hence, pathogens in turbid water are likely to be more persistent and to be transported further than they would in less turbid conditions (Fries et al., 2008). Land use type was not a good predictor of *E. coli* in shellfish. Future models should therefore focus on more direct measures of livestock contributions of *E. coli* in watercourses, including stocking density, distance of livestock to watercourses and farm waste management practices (e.g. slurry spreading) (Oliver et al., 2018). There are some potential limitations to the modelling. With only twelve estuaries, it was not possible to test all possible factors. For example, urban-dominated catchments might differ from rural-dominated catchments due to the different balance of point vs diffuse sources. This aspect could be explored in further work. Future models could also test a range of modelling approaches, including GAMs and machine learning.

4.3. Relationships between E. coli in shellfish and river flow

Relationships with river flow in this study were complex. While other studies have shown strong relationships with flow volume (Campos et al., 2011; Campos et al., 2015), the time series analysis here showed only weak relationships, and mainly at intermediate rather than high flows. The first flush phenomenon may explain why high flow alone is not a good predictor of FIO loading. The first flush of heavy rainfall washes surface material including livestock faeces and other contaminants into waterways, but subsequent rainfall which maintains high river flows will carry much lower sediment and contaminant load (Bach et al., 2010). It should also be noted that many previous modelling studies have had a focus on water quality for bathing (Huang et al., 2017), and the shellfish hygiene aspect has had less coverage (Bougeard et al., 2011; Schmidt et al., 2018). In this multi-estuary analysis we found the most common lag time with river flow was only 1 day, or there was no lag. Our results broadly confirm the range of lag times of river flow or rainfall with *E. coli* response reported by other authors, from 0 to 3 days (Campos et al., 2011; Schmidt et al., 2018). There are also temporal delays in peak *E. coli* loads in shellfish compared with *E. coli* loads in water due to timescales of accumulation and depuration *in situ* (Campos et al., 2011; Sharp et al., 2021), as well as effects mediated by longer-term persistence within the estuary and in sediment (Campos et al., 2013) after high load events. It is also possible that different species may exhibit varying lag times of response due to their different feeding behaviours and filtration rates.

4.4. Relationship of E. coli in shellfish and the release of sewage from CSOs and other point sources

The within-estuary routine discharges from permitted effluent sources under normal operating conditions do not appear to be a predictor of high *E. coli*. Either these sources do not release large quantities of *E. coli*, or they are sufficiently diluted by mixing in seawater, or the degree of pre-treatment of any released effluent is sufficient to reduce the risk of accumulation in shellfish flesh in the study estuaries. However, the case-study analysis suggested a strong association with CSO operation, and this is potentially a much greater source since these flows are untreated. This is consistent with other UK studies (Campos et al., 2013; Garcia-Garcia et al., 2021), but does not necessarily indicate causality. While it is certainly possible that CSOs are a major source, the data provided for use in this study are outputs of sewerage network modelling of the conditions likely to lead to CSO. Combined sewage overflow events also correspond with when contaminants are most likely to be washed off agricultural land and into watercourses, making direct causal inference difficult. There is also the possibility that unrecorded discharges from CSOs may be contributing to *E. coli* in coastal waters (Hammond et al., 2021).

4.5. Within estuary variability in shellfish E. coli contamination

The high variability of *E. coli* concentrations among individual shellfish beds within estuaries, and the variability in lag times, or complete lack of relationship with river flow represents a major problem for predictive modelling. These findings suggest that individual shellfish beds may be highly context-dependent, with very specific local sources, or that the patterns and timings of water movement within estuaries are highly complex (Van Niekerk et al., 2019; Alabyan et al., 2022). Taken together, these make the prediction of water quality for shellfish hygiene more challenging than for bathing water quality. Water movement within estuaries is influenced by tidal cycles, wind speed and direction, river flows, and estuary morphology (Garcia-Garcia et al., 2021; Chao, 1990; Burningham, 2008). Hydrodynamic modelling could therefore greatly help understand how risk from different contaminant sources will affect individual beds and support and refine the models developed here (de Brauwere et al., 2011; Robins et al., 2019). In addition, there is high variability in the measurement technique of *E. coli* using the MPN method (Lee and Murray, 2010; Walker et al., 2018), and this large uncertainty gives a low signal-to-noise ratio, reducing the accuracy of predictive modelling. Analysis of the statistical properties of the MPN method and an additional ISO accredited method, the Pour Plate method demonstrated differences in the statistical properties of the two methods, with the pour plate method exhibiting lower intrinsic variability, further tested using a spiking experiment. Overall, the Pour Plate method was more reliable over crucial classification boundaries (Cooper et al., 2024). The use of Pour Plate data for regulatory testing may improve accuracy of the classification system, in turn improving the explanatory power of predictive models. Further research is required into its potential use in Active Management programmes.

5. Conclusions

Utilising statutory reporting data collected from shellfish classification areas and from environmental databases it was possible to predict risk at an estuary level , with reasonably good model fit (R^2 value = 0.514). Significant positive explanatory variables included river flow, river water nitrate and turbidity. Under normal operating conditions, consented discharges from sewage treatment works within estuaries did not appear to be a major source of *E. coli* in shellfish. However, the case-study analysis suggests that the operation of CSOs within the Conwy catchment was associated with an elevated risk of *E. coli* in shellfish. This association does not indicate causality, since common factors can lead to both CSO operation and overland flow potentially confounding attempts to apportion sources to the FIOs detected. For example, high intensity rainfall during summer storms, particularly when falling onto saturated ground, would be associated with surface runoff which could flush *E. coli* into watercourses, and would lead to surface drainage in urban systems which would overload sewerage systems and trigger CSO operation. Further analysis would be required to determine whether this association is found in other areas and where the attribution lies.

The data analysed in this study across multiple estuaries indicate that the relationships with environmental factors and *E. coli* concentrations appear to be estuary-specific, and indeed shellfish bed-specific, and exhibit a high level of both spatial and temporal variation. Therefore, predicting risk at the level of the shellfish bed still represents a major problem for the industry. Although this study revealed no simple risk factors underlying shellfish contamination, the findings suggest that a focus on catchment locations, hydrological conditions and their interactions with meteorology i.e. the factors which govern rainfall-induced runoff or discharge into river systems would be more fruitful than a focus on permitted discharges. Therefore, it may be possible using a combination of higher frequency data collection under a range of rainfall and tidal conditions, and modelling approaches including hydrodynamic modelling within an estuary, to develop an effective predictive tool at shellfish bed-level. with sufficient accuracy to underpin an Active Management System. Installing telemetered sensors at key locations, allowing both real-time monitoring and linking to meteorological forecasting would facilitate development of a predictive warning system.

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Author contributions

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Table 1. Characteristics of the 12 estuaries across England and Wales used in the analysis of *E. coli* concentrations in shellfish. River flow, water turbidity and nitrate-N concentrations are average values for the main river entering each estuary. Loading risk and Source count are averaged for all Representative Monitoring Points (RMPs) within each estuary. # Data presented in this table for Loading risk, Source count and RMP *E. coli* are averages across beds within an estuary (see Section 2.4 for more details). ## Helford shares flow and water chemistry data with the river Fal since the catchments adjoin, have similar land use, and only the Fal is flow gauged.

Estuary name	Catchment area (km^2)	Improved grassland (%)	Flow ($m^3 s^{-1}$)	Turbidity (NTU)	NO_3-N ($mg L^{-1}$)	Loading risk#	Source count# (within 1 km)	RMP *E. coli*# (90^{th} percentile)
Barrow	1,296	38.7	5.2	1.39	0.64	5,269,860	2.8	413
Blackwater	1,263	17.6	1.4	6.89	6.98	3,610,051	0.5	1,319
Burry	486	38.5	2.2	13.07	1.00	499,274	1.5	1,175
Conwy	672	27.9	19.8	1.67	0.51	595,772	2.4	1,281
Crouch	370	23.4	0.3	12.28	4.11	623,874	0.3	1,492
Fal	701	27.3	2.0	20.30	3.93	162,192	0.5	19,672
Helford##	147	29.3	2.0	20.30	3.93	425,059	1.0	1,578
Kent	193	21.1	3.1	5.60	2.76	686,675	0.8	1,049
Menai	577	33.4	4.7	1.70	0.48	839,536	2.0	253
Poole	826	31.2	6.6	8.25	5.72	509,953	0.4	1,379
Taw	2,107	50.5	18.3	8.26	2.14	29,888	1.4	4,084
Wash	15,992	14.8	3.4	8.88	5.95	6,742,788	0.1	954

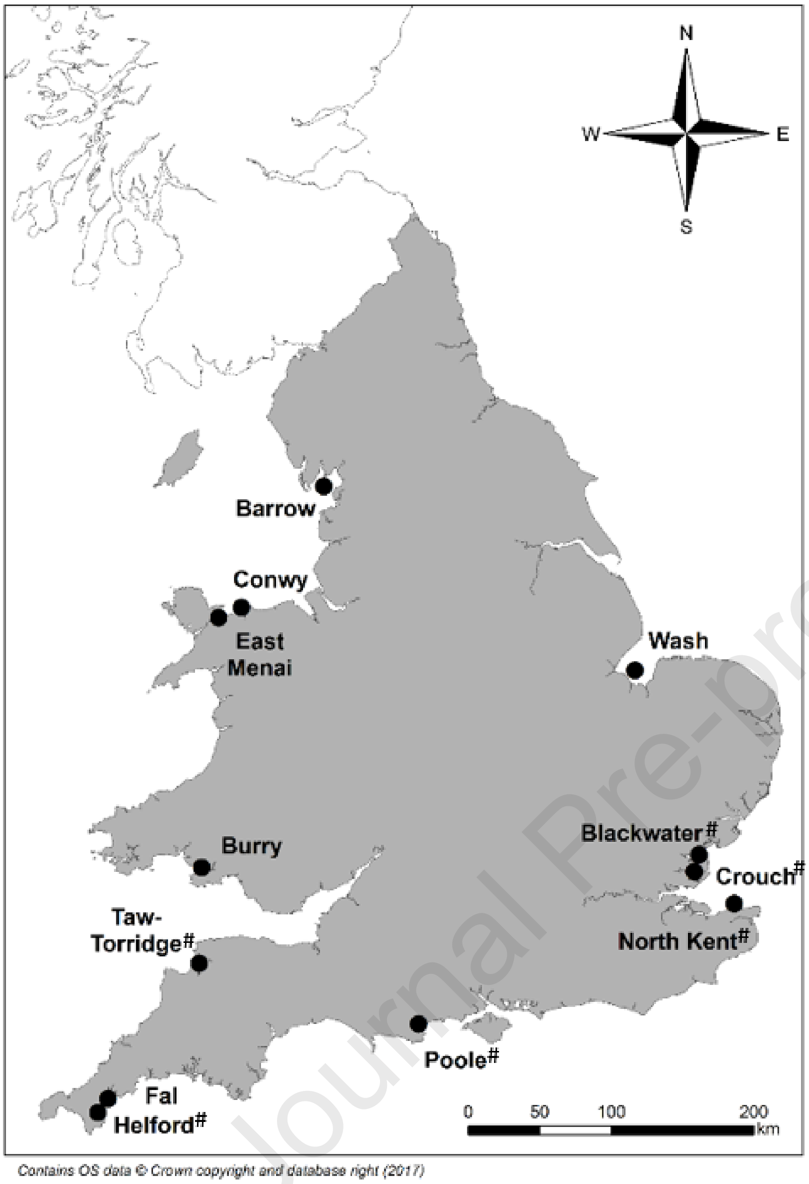

Fig. 1. Map showing the location of the 12 estuaries across England and Wales used in the analysis of catchment-scale or within-estuary predictors for elevated *E. coli* concentrations in shellfish. All sites have mussel beds, site names followed by # contain oyster beds in addition to mussels.

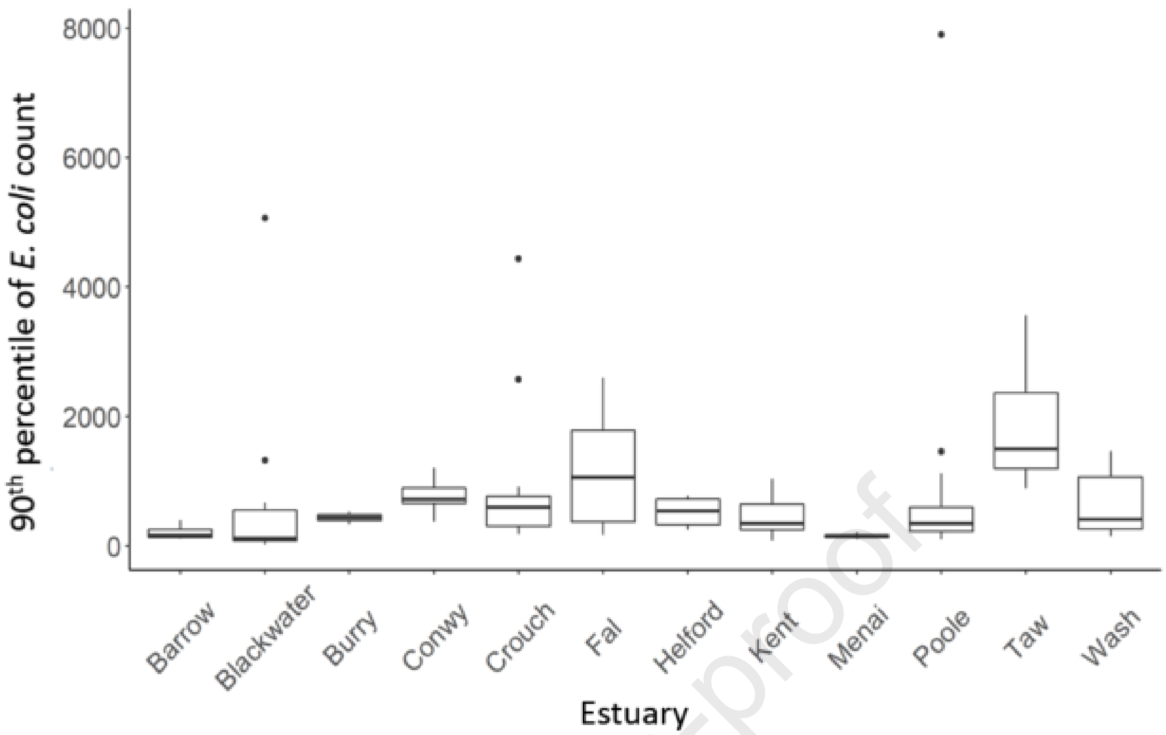

Fig. 2. Boxplots showing the 90th percentile *E. coli* values (*E. coli*/100 g) in shellfish (mussels and oysters) from 12 different estuaries in England and Wales. Note: 3 extreme outlier values of 190,000 and 43,097 in the Fal, and 15,000 in the Taw are not presented. Each box plot shows the Bac90 distribution for each estuary with the whiskers representing the 1st and 3rd quartile range and the points showing points outside this range.

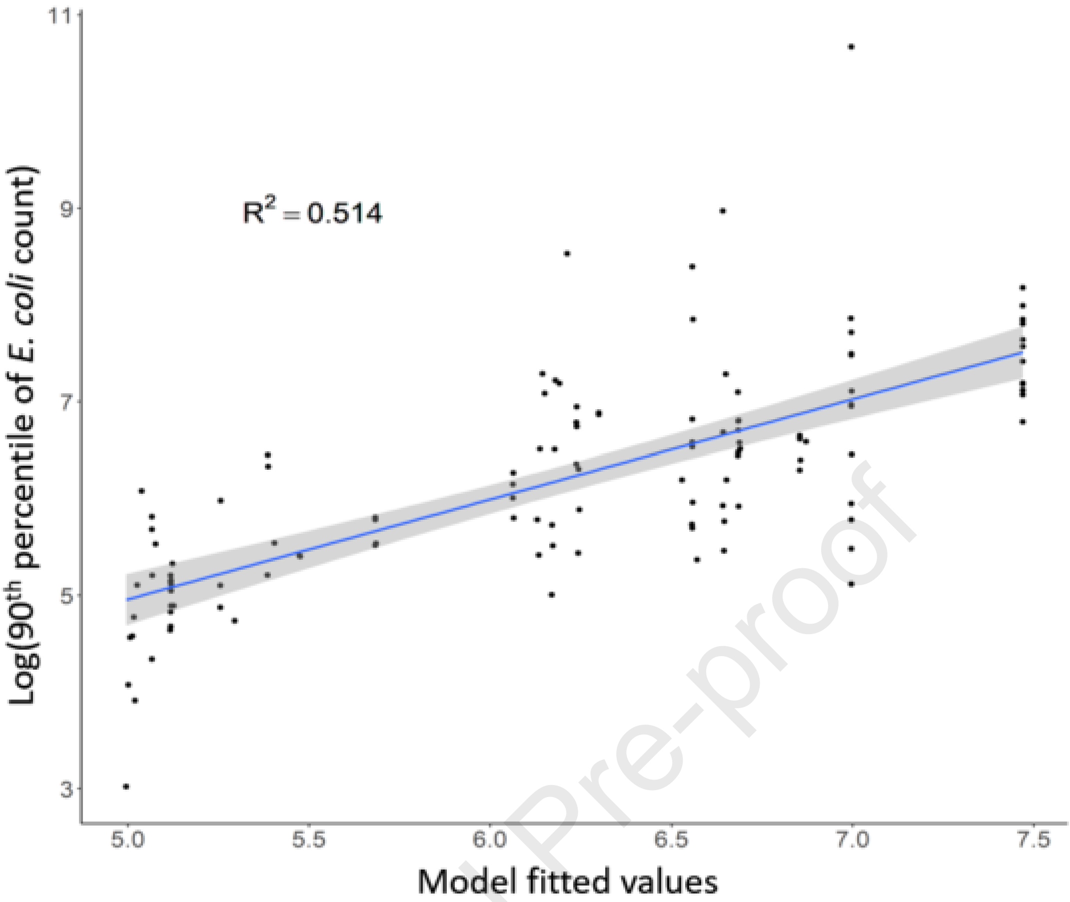

Fig. 3. Log(observed 90th percentile) actual measures of *E. coli* concentrations in shellfish (oysters and mussels) versus model fitted values for *E. coli* loads in 12 different estuaries in England and Wales. The line is the fitted linear model with the shaded area showing the 95% CI.

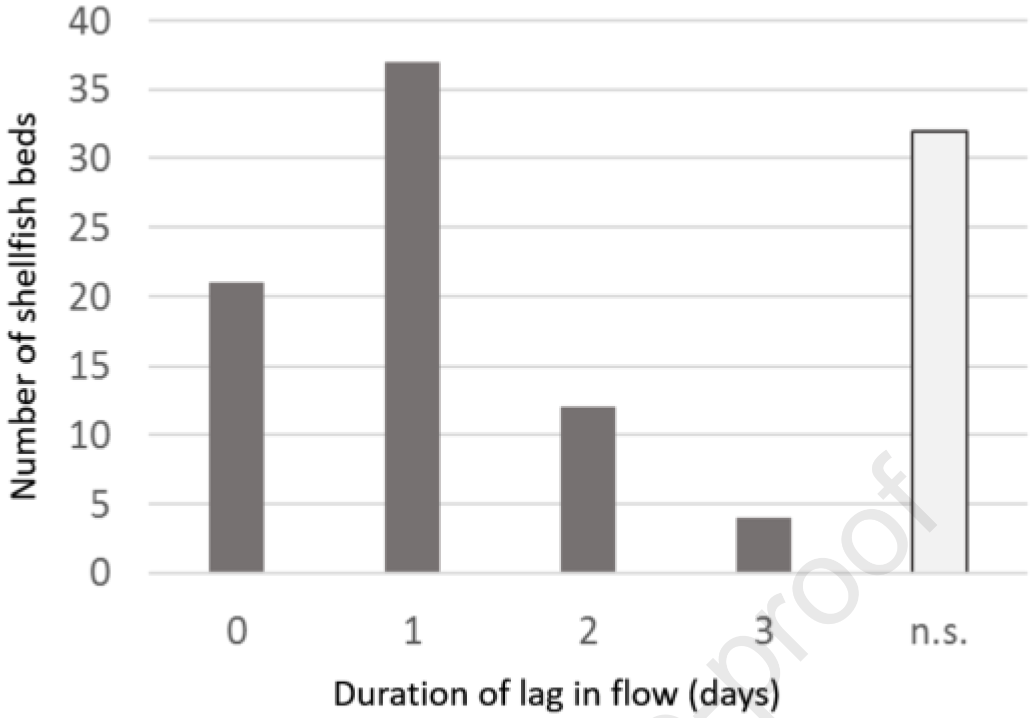

Fig. 4. Number of shellfish beds (RMPs) where *E. coli* concentrations in shellfish (mussels only) were significantly related to river flow, at varying lag times (days) since high flows. Some RMPs showed significant lags at more than one interval. Lags were tested to 7 days, but none were significant beyond 3 days. n.s. = RMPs with no significant lags. See Table S1 in Supplementary Material for more detail.

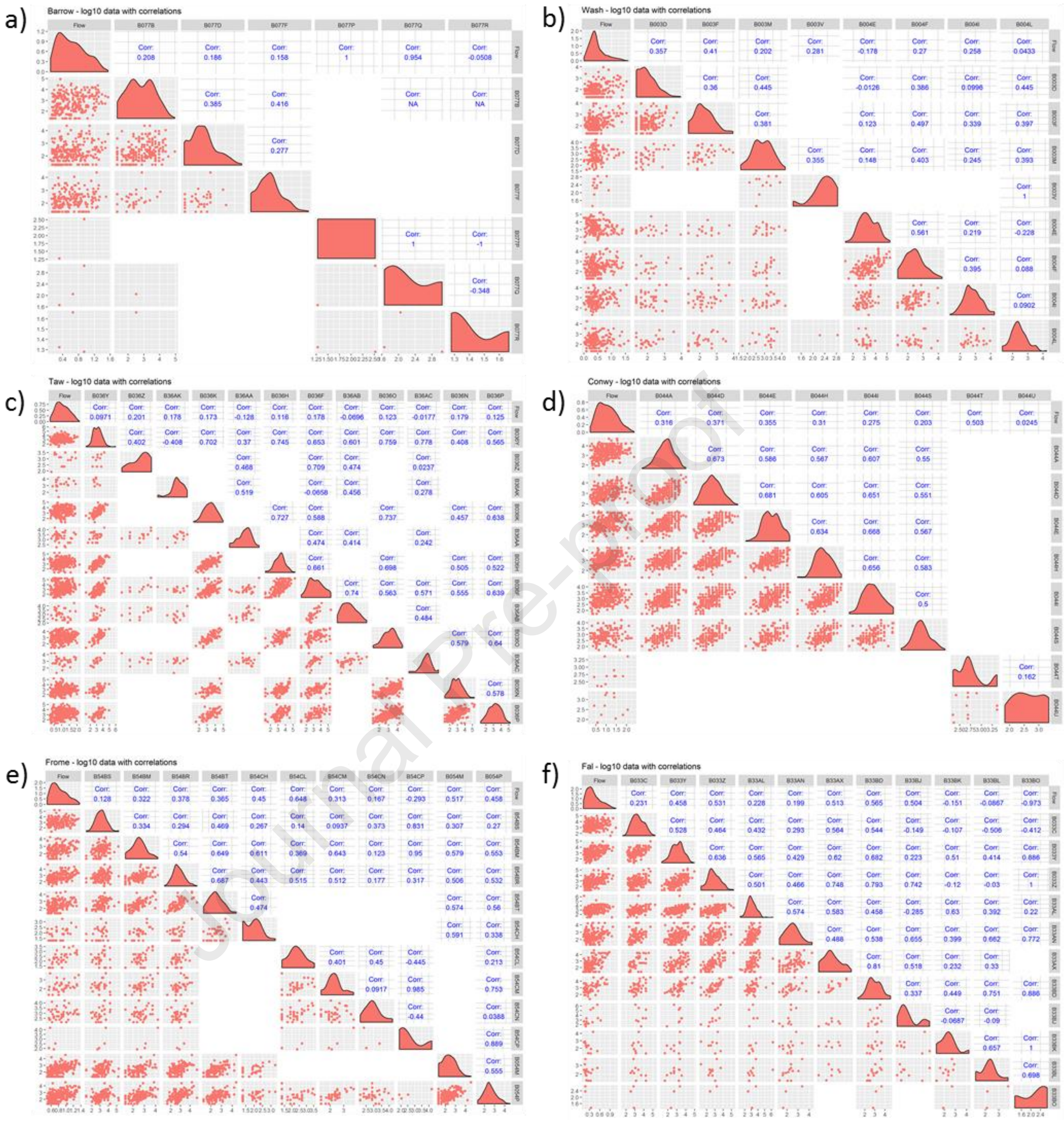

Fig. 5. Correlations among *E. coli* concentrations in shellfish at representative monitoring points (RMPs) within shellfish beds (mussels only) in 6 different estuaries across England and Wales: (a) Barrow, (b) Wash, (c) Taw, (d) Conwy, (e) Frome/Poole, (f) Fal. First column/row shows flow, subsequent columns/rows are RMP locations within each estuary.

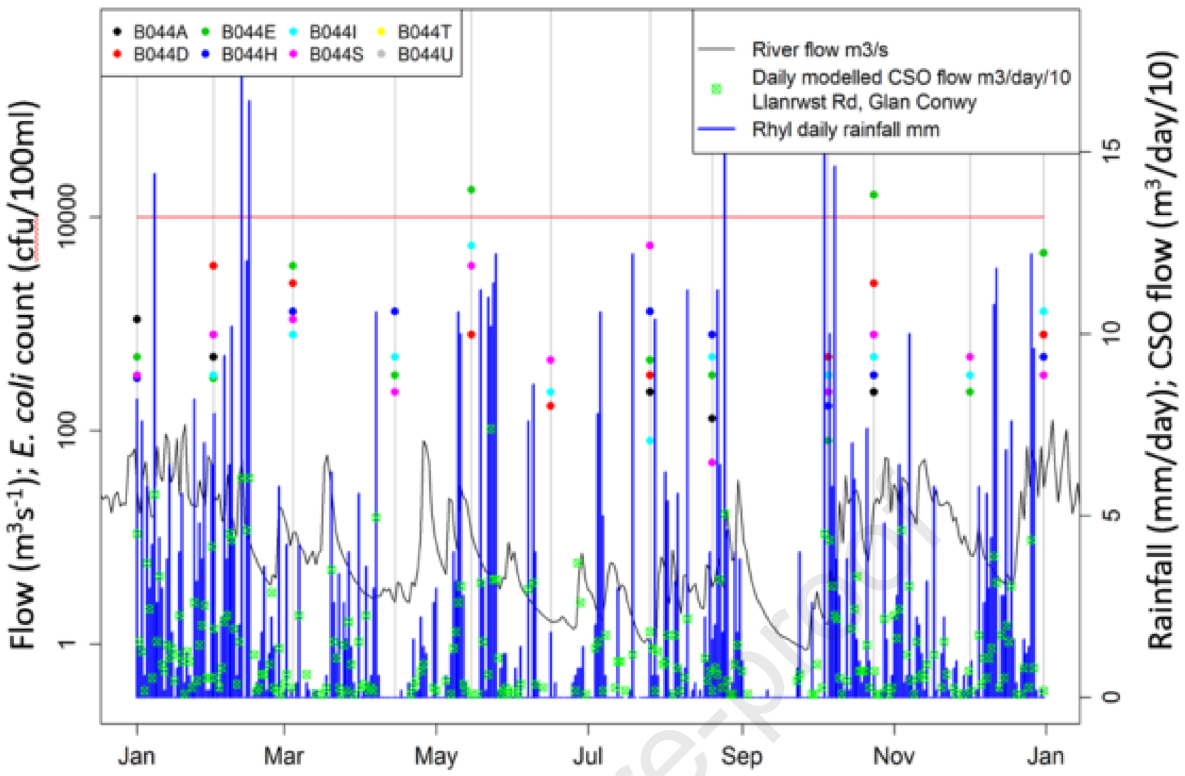

Fig. 6. Annual time series of *E. coli* counts in mussel flesh in the Conwy estuary, daily flow in the river Conwy, daily rainfall at Rhyl, and daily combined sewer overflow (CSO) discharge at Llanrwst (approximately 20 km upstream from the mussel beds), for 2014. Coloured dots represent data from individual beds; red line represents 10,000 cfu trigger for Class B investigations.

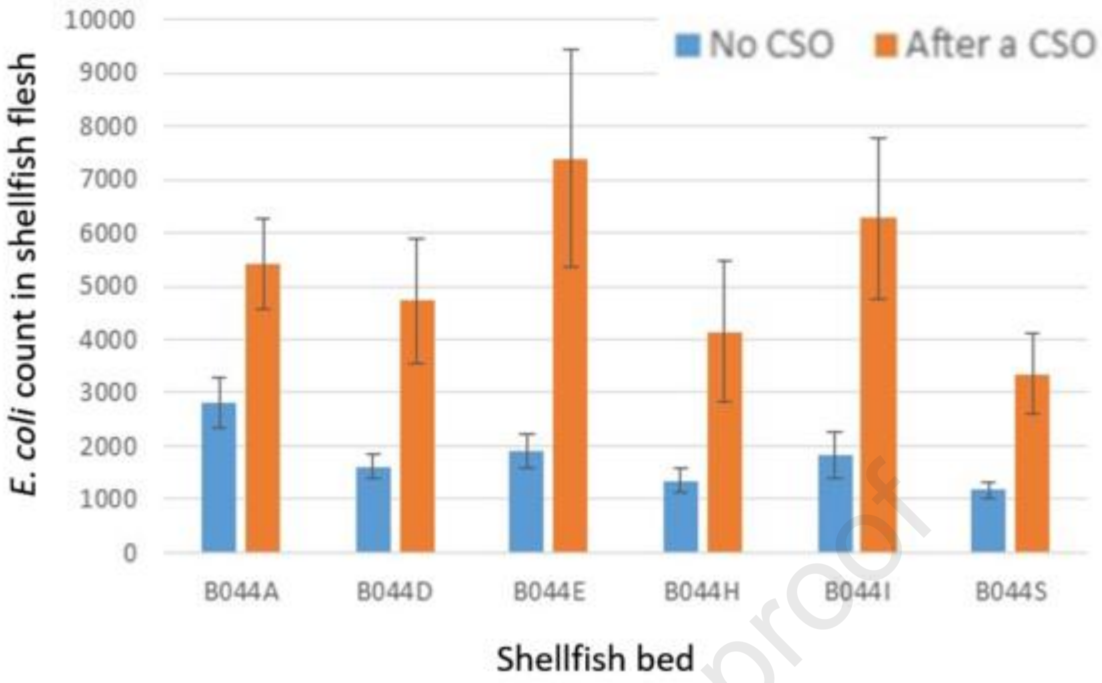

Fig. 7. Average annual values for *E. coli* concentrations in mussels at representative monitoring points (RMPs) during periods with no combined sewer overflow (CSO) release (blue bars) and during the week after a CSO release in the Conwy estuary (orange bars).

Highlights

- Catchment models predict estuary risk but not levels of shellfish bed *E. coli*
- High estuary *E. coli* correlates with high river flow, nitrate and turbidity
- 64% of beds show a link between river flow and *E. coli* with 1 day lag
- Combined sewer overflows (CSO) associate with high *E. coli* in shellfish
- Highly variable *E. coli* across and within estuaries prevents bed-level prediction

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: