Spatial variation of waterborne *Escherichia coli* – implications for routine water quality monitoring

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ABSTRACT

Escherichia coli are often used as faecal indicator bacteria (FIB) to provide a measure of microbial pollution in recreational and shellfish harvesting waters. However, although model forecasts for predicting the concentrations of FIB in surface waters are becoming more robust, they suffer from an inconsistency in quantification methods and an understanding of the spatial variation of FIB within a water course. The aim of this study was to investigate the transverse spatial variation in *E. coli* numbers (as an indicator of faecal pollution) across the estuary of the River Conwy, UK. Water samples were collected from four transverse transects across the estuary. Spatial variation of *E. coli* was significantly different from one side of the river to the other, although was not correlated with depth or the physiochemical properties of the water. Subsequently, microbial water quality classifications on the two opposite banks suggested very different levels of pollution coming down the river. This work has shown that the side of the river that routine water monitoring samples are taken from can make a significant difference to the classification of microbial water quality. This has important implications for sampling strategies and the use of microbial source tracking (MST) techniques.

Key words | faecal coliforms, faecal indicator bacteria (FIB), microbial pollution, quantitative microbial risk assessment (QMRA), water framework directive

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INTRODUCTION

Levels of faecal indicator bacteria (FIB), such as *Escherichia coli*, are often used as a measure of microbial pollution in recreational and shellfish harvesting waters. Although *E. coli* is now considered a poor surrogate for most pathogenic bacteria, viruses and protozoa (Brookes *et al*. 2005; Savichtcheva & Okabe 2006), its presence is still widely accepted as being an important indicator for faecal contamination. Furthermore, compared with quantifying individual waterborne pathogens (Quilliam *et al*. 2011), the measurement of *E. coli* (either as MPN or CFU) is relatively straightforward. Epidemiological studies have established that exposures to FIB in recreational waters is significantly linked to a decrease in public

health (Wade *et al*. 2003; Wiedenmann *et al*. 2006), and maintaining and improving the microbial quality of freshwaters has resulted in legislative pressures through implementation of the Drinking Water (98/83/EC) and Water Framework (2000/60/EC) Directives (E.C. 1998, 2000). As a consequence, model forecasts for predicting the concentrations of FIB in surface waters are becoming an increasingly important management decision tool (Hellweger & Masopust 2008; Gronewold *et al*. 2009). However, despite two-dimensional models combining data on the rate of mixing and die-off (Smith & Putz 1993; Vandenberg *et al*. 2005) together with depth and temporal variability (Kashefipour *et al*. 2002; Li *et al*. 2008;

Parks & van Briesen 2009; Pote *et al.* 2009), an inconsistency in quantification methods and a lack of understanding of the spatial variation of FIB within a water course can hinder the robustness of such models. Ultimately this could lead to the unnecessary closure of public beaches or the restricted harvesting of shellfish.

The aim of this study therefore, was to determine the spatial transverse variation in *E. coli* numbers across the estuary of the River Conwy in North Wales, UK. This area is important for the commercial harvesting of shellfish, and has several public beaches with designated EC bathing waters (Bathing Water Directive, 76/1160/EEC) (E.C. 1976). There is a dynamic deposition of sediments within this estuary that results in heterogeneously dispersed banks of mud and sand. This provides contrasting habitats for coliforms (Howell *et al.* 1996) and although progress is being made to incorporate sediment reservoirs into mathematical models (Jamieson *et al.* 2005; Badgley *et al.* 2011), the spatial re-suspension of sediment-associated *E. coli* due to tidal movements and storm events is still poorly understood. We envisage that our results will have a significant impact on future sampling strategies for routine water quality monitoring. In addition, this work will facilitate further developments in microbial source tracking (MST) techniques and

contribute to the improvement of hydrodynamic and water quality models.

MATERIALS AND METHODS

Boat sampling was carried out in the estuary of the River Conwy during the first week of October, 2010, in an area that did not contain any large point sources. Four transverse transects were conducted on the same day (Figure 1), with four replicate water samples collected from each point in the transect. Samples were taken approximately 1 m below the surface with sterile 1 L plastic bottles. Following EU guidelines, all samples were stored at 4 °C and processed within 6 h of collection. Each water sample was briefly shaken and 25 mL was vacuum-filtrated through a 0.2 μm cellulose acetate membrane (Sartorius Stedim Biotech., Gottingen, Germany). The membrane was aseptically transferred to the surface of a plate containing M-endo agar LES (Oxoid Ltd., Basingstoke, UK); the plate was inverted and incubated at 37 °C and enumerated 24 h later. Turbidity was measured with a T-100 Turbidimeter, and electrical conductivity (EC), salinity and pH were measured directly using standard electrodes.
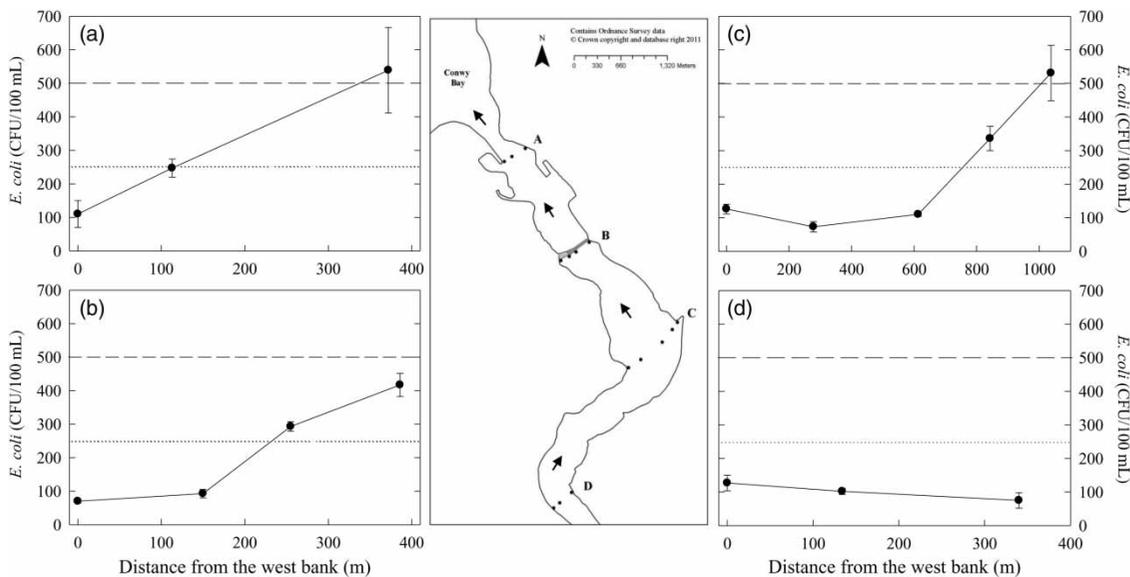
Figure 1 | Tranverse transects across the River Conwy. The locations of the four transects and approximate sampling points are shown on the map and all have been presented as distance from the west bank. Arrows show direction of river flow. The Revised Bathing Water Directive (2006/7/EC) classifications of 'excellent quality' (250 *E. coli* CFU/100 mL) and 'good' or 'sufficient' quality (500 CFU/100 mL) are marked on graphs a–d as a dotted and dashed line respectively. Data points represent the mean of 4 replicates ± SEM.

RESULTS AND DISCUSSION

Although there were no significant differences in pH, EC, turbidity, temperature or salinity across each transect ($P >$ 0.05), there was significant spatial variation in *E. coli* numbers in three of the transects ($P < 0.001$), with approximately five times more CFU on the east side of the river compared to the west side (Figure 1(a)–(c)). We believe that this result has important implications for sampling strategies, for example interpreting the Revised Bathing Water Directive (2006/7/EC) (E.C. 2006), which classifies a concentration of 250 *E. coli* CFU/100 mL as 'excellent quality' while 500 CFU/100 mL is only classified as 'good' or 'sufficient' depending upon the percentile evaluation. Although this stretch of water is not specifically designated as 'bathing water', it does have a direct impact on several public beaches within the catchment, and drains directly into EC bathing waters and commercial shellfish harvesting areas. It is clear that the side of the river that water samples are taken from does make a significant difference to the concentration of indicator bacteria. Classifications on the two opposite banks suggest very different levels of pollution coming down the river, which will have important implications for public health and the management of bathing waters. Although the water on the west side of the transverse transects was deeper, this was not significantly correlated with the concentration of *E. coli* ($P = 0.254$). Sampling bias may be introduced however, as the shallower east side of the river has much easier access for sampling whilst fulfilling the minimum 1 m depth requirement for sampling water for microbial monitoring.

Over the last two decades a number of MST methods have been developed, with the aim of pinpointing exact sources of microbial pollution (Simpson *et al.* 2002). One of the major limitations associated with MST methods is the complexity associated with the persistence and survival of indicator species within the environment, together with the spatial and temporal heterogeneity within these different environmental matrices. Such spatial heterogeneity can confuse attempts at identifying the cause of microbial pollution, particularly when water bodies are not well mixed. Although the physiochemical variables measured here implied that the water was well mixed across the

transect, the variation in *E. coli* numbers suggests that localised re-suspension from the sediment may significantly affect spatial concentrations of FIB. Our results have demonstrated the importance of the sampling location within a watercourse for routine water quality monitoring and the effect this can have on interpreting data used for MST.

ACKNOWLEDGEMENTS

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