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Spatial and temporal heterogeneity of bacteria across an intertidal shellfish bed: Implications for regulatory monitoring of faecal indicator organisms

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HIGHLIGHTS

- Spatial/temporal patterns of faecal indicator organisms (FIO) was investigated.
- No relationship between FIO and autochthonous bacteria were observed.
- FIO concentrations and sediment physico-chemical properties were not correlated.
- Current FIO monitoring may not accurately represent microbial contamination levels.

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ABSTRACT

Routine bacterial monitoring of shellfish beds using indicator species is a common global practice designed to prevent human consumption of contaminated shellfish products. However, current bacteriological monitoring procedures which focus on the quantification of faecal indicator organisms (FIOs) as a proxy for microbial pollution may not be representative of total bacterial contamination levels present in shellfish harvesting areas. The objective of this study was to critically assess the accuracy of current monitoring strategies by quantifying the spatial (lateral and longitudinal distance) and temporal (seasonality and tidal state) concentrations of FIOs (*Escherichia coli* and total coliforms) within a single intertidal commercially harvested shellfish bed. Spatial and temporal FIO dynamics, including the effects of tidal state and seasonality, were quantified in mussel flesh and sediment samples from a single intertidal mussel (*Mytilus edulis*) bed. Our results confirmed that FIO concentrations across a shellfish bed were heterogeneous over larger spatial and temporal scales, but showed no relation to the concentrations of autochthonous bacteria, such as *Vibrio* spp., or the physico-chemical parameters of the sediment. These results have important implications for both public health and the economic prosperity of the shellfish industry, and demonstrate the importance of accommodating both spatial and temporal fluctuations in routine bacteriological monitoring protocols. We conclude that current FIO monitoring procedures may not accurately represent levels of microbial contamination within shellfish harvesting areas and that more robust microbiological testing procedures need developing.

1. Introduction

The global demand for seafood products has risen dramatically over the previous three decades. In 2009, it was reported that 3 billion people globally rely on fish or other seafood products to supply approximately 20% of their intake of animal protein (FAO, 2012). The demand for shellfish products is expected to increase as they become more widely recognised as a relatively cheap and nutritious food source (Gjedrem et al., 2012). However, the increase in shellfish consumption is accompanied by an increase in shellfish-vectored illness in humans (Potasman et al., 2002). Thus, the challenge for the shellfish industry is to supply a good quality product that is safe for human consumption (Lee and Younger, 2002).

Shellfish are commonly cultivated in sheltered waters which are vulnerable to microbial contamination from both point-source

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pollution, e.g. sewage outflow, and diffuse pollution, e.g. agricultural runoff (Oliveira et al., 2011). The potential for contamination of shellfish harvesting waters by bacterial pathogens such as *Escherichia coli* O157 (Riou et al., 2007) and viral pathogens such as norovirus (Lees, 2000) is increased during storm events when sewage treatment facilities exceed capacity, leading to the release of untreated sewage into the sea (Lessard and Beck, 1990). Sewage discharge not only increases the microbiological loading into shellfish harvesting waters, but also raises nutrient levels, which in turn can alter indigenous microbial community dynamics. Bivalve shellfish such as mussels (*Mytilus edulis*), can bioaccumulate potentially pathogenic micro-organisms from the surrounding water by concentrating them within their somatic tissues (Teplitski et al., 2009); as some shellfish are traditionally consumed either raw or lightly cooked, they are capable of transferring pathogenic micro-organisms into the human food chain (Potasman et al., 2002). Outbreaks of gastroenteritis are often attributed to shellfish consumption, e.g. norovirus and oysters (Ang, 1998; Lee and Younger, 2002; Rippey, 1994), which can reduce consumer trust and challenge the promotion of shellfish as a safe and healthy food source.

To safeguard against contaminated shellfish products entering the human food chain, many countries have stringent legislation in place to regulate all aspects of the shellfish industry, i.e. from classifying shellfish harvesting areas to controlling post-harvest treatment and processing. In the European Union, shellfish quality assurance is currently governed under EC/854/2004 (EU, 2004a) and EC/853/2004 (EU, 2004b) where the hygiene status of shellfish is monitored via the use of faecal indicator organisms (FIOs) specifically, *E. coli*. The presence of *E. coli* is widely recognised as being an important indicator for faecal contamination, although there remains controversy over its relevance as an indicator of viruses (e.g. hepatitis A, norovirus) or naturally occurring pathogenic bacterial strains such as *Vibrio parahaemolyticus* and *Vibrio vulnificus* (Muniain-Mujika et al., 2002; Marino et al., 2005; Romalde et al., 2002).

In Europe, shellfish beds are assigned a classification grade under EC/854/2004 (EU, 2004a) based upon *E. coli* concentrations within shellfish flesh. These controls apply to both EU member states and other countries applying similar controls. Subsequently, the classification grade assigned to an individual shellfish bed impacts not only consumers, but also the shellfish industry, as it dictates the level of post-harvest treatment required for shellfish products at each classification grading, and could promote either a change in management practice or a temporary closure of the harvesting area. Previous research has shown that environmental factors such as seasonality, tidal state and rainfall events may alter concentrations of *E. coli* detected within shellfish tissues and hence affect the classification assigned to a harvesting area (Stapleton et al., 2007; Riou et al., 2007; Kay et al., 2008a). Subsequently, the classification assigned to each shellfish harvesting area has substantial socio-economic implications (e.g. the downgrading in classification of shellfish harvesting areas leading to a direct loss of revenue for the local shellfish industry coupled with potential loss of employment for both fishermen and employees involved in various post-harvest processes). However, the spatial variation of *E. coli* within single mussel beds and the implications of this potential heterogeneity on shellfish quality monitoring have received little consideration.

The overarching aim of this study was to critically assess the spatial and temporal concentrations of FIOs within mussel tissues across a single, commercially harvested mussel (*M. edulis*) bed. In order to do this, we have quantified mussel flesh FIO concentrations in longitudinal and transverse transects across the mussel bed during different seasons and tidal states. In addition, to assess the suitability of *E. coli* as an indicator of microbial quality, we simultaneously quantified the concentration of naturally occurring bacteria such as *Vibrio* spp. within mussel tissues. Finally, we examined whether the sediment of the mussel bed was providing a dynamic reservoir for FIOs, and whether this was regulated by nutrient levels or physico-chemical parameters.

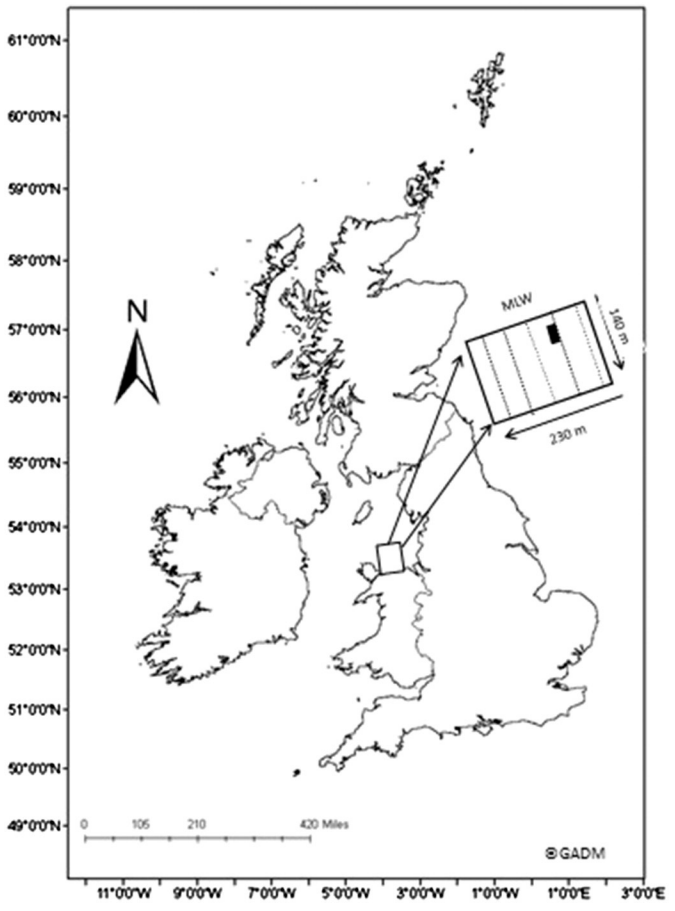

Fig. 1. Map showing the study sampling location. Inset diagram shows the location and approximate positions of the five transects across the shellfish bed. The approximate intensive sampling location is shown in the black highlighted area. Inset panel not to scale.

2. Materials and methods

2.1. Sampling location and large-scale transects

Sampling was conducted on a commercial intertidal mussel bed at Conwy Morfa (53.298015 N, 3.854535 W) in North Wales, UK, which is currently classified according to EC 854/2004 (EU, 2004a) as 'class B' containing between 230 and 4600 *E. coli* 100 g^{-1} of mussel flesh. This mussel bed was surveyed and mapped using GPS and was estimated to be approximately 231 m in length (north–south) and 140 m in length (east–west). Five vertical transects running east–west were used to survey the entire mussel bed from the mean low water mark (MLW) to the upper limit of marketable-size mussels (140 m from MLW). All transects were evenly spaced 57.5 m apart from each another and approximately 10 individual mussels were collected at 10 m intervals along each transect, beginning from MLW (0 m) to the upper limit at 140 m (Fig. 1). One transect was surveyed per day, over five consecutive days 1 h either side of low water. Only mussels of marketable size (>45 mm) were included in the sampling strategy, areas of smaller 'seed mussels' were excluded. At each sample point, three replicate mussel samples (ca. 10 animals per sample), and four sediment samples (0–5 cm depth) were collected, stored at 4 °C and processed within 12 h of collection.

2.2. Intensive spatial sampling

An adaptive cluster sampling strategy was utilised to provide a measure of variability over spatial scales smaller than the 10 m intervals described above. Sampling took place at low water, one month after the