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Determining the zone of impact of norovirus contamination in shellfish production areas through microbiological monitoring and hydrographic analysis

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ABSTRACT

Norovirus (NoV) contamination of filter feeding bivalve shellfish is a well-recognised human health threat when shellfish are grown in sewage polluted waters. To date, the identification of high risk zones around sewage discharges in shellfish production areas (SPAs) has not been based on NoV data. This study utilised molecular methods for NoV analysis, combined with hydrographic studies, to determine the relationship between NoV concentrations in shellfish and sewage effluent dilution. Cages with mussels and oysters were placed at different distances downstream of sewage discharges in two coastal sites in England. The shellfish were tested for concentrations of NoV (genogroups I and II) and *E. coli*. Drogue tracking and dye tracing studies were conducted to quantify the dispersion and dilution of sewage effluent in the SPAs. Significant negative associations were found between both total concentrations of NoV (GI + GII) and *E. coli* and sewage effluent dilution in the SPAs. The total NoV concentrations predicted by the model at 300:1, 1000:1 and 5000:1 ratios of estuarine water to sewage effluent were 1200; 600; and 200 copies/g, respectively. The estimated area of NoV contamination varied according with local pollution source impacts and hydrographic characteristics. The results help to inform the derivation of sewage discharge buffer zones as a control measure for mitigating risk from human NoV contamination in SPAs.

1. Introduction

The consumption of raw or lightly cooked bivalve shellfish (oysters, clams, mussels) can result in illness if these shellfish are harvested from coastal areas impacted by sewage discharges. The epidemiological evidence indicates that most cases of shellfish-related illness are gastroenteritis caused by viruses, particularly human norovirus (NoV) (genogroups I (GI) and II (GII)) (Lees, 2000; Iwamoto et al., 2010). While most cases of NoV illness are attributed to person-to-person transmission, or to contamination of foods during food handling (Wallace et al., 1999; Hall et al., 2014), many outbreaks associated with shellfish harvested from waters contaminated with sewage pollution have also been reported in the literature (Huppatz et al., 2008; Wall et al., 2011; Fitzgerald et al., 2014).

Internationally, shellfish hygiene controls aimed at preventing shellfish-related illness prescribe standards based on faecal indicator organisms (FIOs) (*Escherichia coli* in the European Union and faecal (or total) coliforms in the USA) in both growing areas during primary production and for end-products at the point of sale (European Commission, 2005; European Parliament and Council of the European Union, 2004; USFDA and ISSC, 2015). While these standards provide a measure of the sanitary quality of the shellfish production areas, they do not reliably index the presence of viral pathogens because of several factors including differential inactivation in the environment outside the human host, different inactivation/removal rates during sewage treatment, and different uptake and elimination kinetics in shellfish (Lees, 2000; Campos and Lees, 2014). Furthermore, NoV GI and GII are exclusively associated with human faecal pollution, and varies considerably

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both by season and year (Ahmed et al., 2013), while *E. coli* is associated with both human and animal sources of pollution (Campos et al., 2013).

To reduce the risk of shellfish-related illness of viral origin, many countries have implemented additional precautionary control measures for shellfish production areas (SPAs). In the USA, the Shellfish Sanitation Program recommends that all SPAs impacted by a sewage treatment works (STW) outfall or other point source outfall of public health significance within or adjacent to the production area be prohibited to shellfish harvest (USFDA and ISSC, 2015). The recommended minimum size of the prohibited area (buffer zone) for SPAs that are closed to harvest due to episodic poor water quality following discharges of sewage effluent is 1000:1 dilution of estuarine water to treated effluent (Goblick et al., 2011). In Europe, there is no direct parallel legislation although some EU Member States have implemented "closure areas" around sewage outfalls, harbours and rivers (Cefas, 2013). This is consistent with Regulation (EC) No 854/2004 which requires the authorities to close the production area when there is a risk to human health (European Parliament and Council of the European Union, 2004). However, the legislation does not specify how to define the area that should be closed. The European Food Safety Authority Panel on Biological Hazards has reviewed this hazard and has also determined the need to restrict shellfish harvesting from areas contaminated with faecal pollution (EFSA, 2012).

Shellfish production areas usually occur in nearshore shallow waters with highly dynamic contaminant fluxes derived from land-based and marine sources of pollution. Consequently, the implementation of buffer zones in these areas requires a precise understanding of the dispersion and dilution of sewage effluents. Hydrodynamic models that simulate the fate of microbial concentrations in nearshore waters have been increasingly used in the context of risk management of SPAs. However, they often lack robust calibration data and difficulties with the parameterisation of bioaccumulation and elimination dynamics in shellfish - particularly for viruses - constrain the use of these models in regulatory controls (Gourmelon et al., 2010; Kay and Rees, 2010). Alternatively, dye tracing studies offer significant advantages over computer modelling since they do not require measurement of the hydraulic parameters of the receiving waters (Kilpatrick and Cummings, 1972). Dye studies have been successfully used to simulate the near field characteristics of sewage effluents (Hunt et al., 2010) and to demonstrate linkage between pollution sources and levels of contamination in shallow coastal environments (Grant et al., 2005; Wyer et al., 2010; Habteselassie et al., 2011). This study applied a protocol that combined microbiological monitoring with dye tracing and drogue tracking studies to establish the relationship between NoV contamination in shellfish and effluent dilution/dispersion in SPAs. This information can assist risk managers to determine the size of sewage discharge buffer zones necessary to mitigate NoV risk. The approach was tested in two coastal sites with different characteristics relating to pollution sources and hydrodynamics. The sites were a deep coastal embayment impacted by a marine long sea outfall (LSO) (site A); and a shallow estuary principally impacted by a large discharge at the head of the estuary (site B). Both sites contained areas classified under European regulations for commercial production. Concentrations of NoV and *E. coli* were quantified in mussels and/or oysters placed in cages which were deployed at different distances from the sewage outfalls in the production areas.

Monitoring of *E. coli* was included in the study protocol to assess the relevance of the study results in relation to legislative standards. The tracer studies involved injection of Rhodamine WT dye into STW outfalls and monitoring the dye-tagged effluent to characterise dilution, dispersion and build-up of the sewage effluents in the receiving waters. In site A, a drogue tracking study was also conducted to characterise the surface water movements in the bay.

Results from the field studies were analysed to determine the relationship between sewage effluent dilution/dispersion and NoV contamination levels in shellfish and consequentially the implications for determination of NoV buffer zones in SPAs.

2. Study sites

The study site A is an open coast embayment approximately 6 km wide and recessed by 3.5 km. The bathymetry gently slopes from about 5 m (Chart Datum; CD) near the shore down to about 15 m (CD) in the centre of the bay. The western part of the bay is deeper than the eastern part. The tidal range is moderately large (mean spring range = 4.5 m). In the approaches to the bay, tidal currents often exceed 0.25 m/s. Inside the bay, average tidal currents are small (0.02 m/s). The main source of sewage pollution impacting the bay is a STW that discharges secondary-treated effluent from a population equivalent of 22,140 (average daily flow = 10,517 m^3) via a 1.25 km LSO at 12 m depth (CD). The bay also receives freshwater inputs from a river flowing from its source at the northern boundary of the catchment (110 m above ordnance datum) to the tidal limit on the north-eastern part of the bay. Commercial common mussels (*Mytilus* spp.) are grown on ropes suspended from longlines in two areas on the south-western part of the bay. The commercial production area is class B status under Regulation (EC) No 854/2004.

The study site B is a shallow semi-enclosed tidal inlet with four confluent channels. The study focused on the main channel which is about 13 km in length and meanders from the mouth of the estuary. The mean spring tidal range in the estuary is 4.2 m. Tidal currents tend to follow the morphology of the channels parallel to the banks. The main source of sewage pollution impacting the main estuary channel is a STW which continuously discharges UV-disinfected effluent and intermittently discharges of settled sewage overflows from a population equivalent of 36,300 (average daily flow = 18,072 m^3). There are other relatively minor sewage discharges on the shoreline. The main sources of freshwater into the estuary are a river flowing on the eastern part of the catchment to the tidal limit in the main meandering channel and a smaller river flowing in the central part of the catchment which discharges to a confluent channel. Wild beds of commercially harvested native oysters (*Ostrea edulis*) are dredged from several areas in the estuary including the main river channel. The commercial production areas are classified as class B and C status under EU Regulation 854/2004.

3. Methods

3.1. Shellfish sampling

At site A, approximately 15 kg of Pacific oysters (*Crassostrea gigas*) and common mussels (*Mytilus* spp.) were placed in each of nine cages for sampling. The cages were placed at nine locations (each cage was at 3 m depth) forming a grid of stations around the LSO. The water depths at the sampling stations ranged from 5 m to 16 m. The shellfish were allowed to acclimatise to the surrounding water quality over 10 days before the first sampling event.

At site B, approximately 10 kg of native oysters were placed in each of four mesh bags which were deployed on the bottom of the estuary at different distances downstream of the STW outfall to represent the anticipated path of sewage effluent in the meandering channel.

The shellfish used in the experiments were subject to purification treatments (depuration) at an approved plant prior to placing the cages/bags in the production areas. The batches of shellfish were tested for NoV and were positive for the virus at