Sewage impact on shellfish microbial contamination


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Abstract Coastal areas are frequently contaminated by microorganisms of human origin, due to high population density and low seawater renewal. To evaluate the impact of wastewater input on shellfish quality, a study was conducted in Brittany (France) over a period of 20 months. A hydrodynamic model was used to simulate wastewater impact on microbial water quality. To validate the model, wastewater from the three main sewage treatment plants and shellfish from three sites were sampled monthly. Bacterial indicators (E. coli), F-RNA phages were searched for by culture and noroviruses by RT–PCR and hybridisation. These microorganisms were detected in the three effluents and clams, with no marked seasonal variation. The microbial concentrations in the two oyster beds, distant from the effluent outfall, were low, and only three of the samples were positive for norovirus. For simulation, the winter wastewater inputs of E. coli and phages were calculated and an estimation for norovirus flux was made from the epidemic situation in the population. The microbial behaviour was included in the model by a decay-rate factor. Results from the model calculations were found to be very similar to E. coli and phage concentrations observed in shellfish. For noroviruses, the model indicated that shellfish distant from the wastewater input were under the detection limit of the RT–PCR method. This study demonstrated the use of modelisation to interpret norovirus contamination in various areas.

Keywords E. coli; F-RNA bacteriophages; modelisation; norovirus; seawater; shellfish

Introduction

Despite major improvements to increase sewage treatment plant performance, some pathogenic microorganisms are still discharged into the marine environment. This is of particular concern in coastal areas devoted to shellfish production, as bivalve molluscs can concentrate microorganisms. To protect the consumer, regulations based on E. coli counts have been established, leading to a decrease of bacterial infectious diseases. However, at present, the greatest risk for shellfish consumption is gastroenteritis viruses such as noroviruses (formerly Norwalk-like viruses). The occurrence of pathogenic viruses in shellfish is not well characterised and only a few studies have been published (Chung et al., 1998; Le Guyader et al., 2000; Formiga-Cruz et al., 2002; Hernroth et al., 2002). Some of the most important parameters responsible for shellfish contamination seem to be virus circulation in the population and climatic events (Miossec et al., 1998; Crowther et al., 2001; Griffin et al., 2003). Controlling viral contamination is difficult as there is no standardised method for their detection and no reliable indicators (Formiga-Cruz et al., 2003).

The innovative point of our study was to use a mathematical model developed for bacteria. Bi-dimensional numerical models include physical and biological processes involved in coastal areas or time-varying inputs (Salomon and Pommepuy, 1990; Bell et al., 1992). Recently, modelling applications have been done for the bathing-water compliance of an estuarine basin (Kashefiopour et al., 2002) or to quantify the impact of bacterial inputs into a Mediterranean lagoon devoted to shellfish production (Fiandrino et al., 2003). To our knowledge, this has never been done to study the impact of a viral outbreak in a coastal
area. This study presents the feasibility of a hydrodynamic model to interpret the microbial contamination of a harvesting area subjected to sewage inputs.

Material and methods

Study area
The selected area (Figure 1) was located on the southern coast of Brittany (Atlantic Coast, Golfe du Morbihan). This area was a bay (115 km²) connected to the sea by a southern narrow mouth (about 1 km). The sea tide is mainly semi-diurnal, with a maximum range of about 5 m during spring tide. Oyster (Crassostrea gigas) leases produced 5,000 tons/year, and natural clam beds (Ruditapes philippinarum) 1,500 tons/year. Most of the oyster and clam leases are class A and B, according to the sanitary classification of harvesting area for bivalve molluscs based on E. coli counts (EC Directive 91/492, EEC). The main microbial inputs were three sewage treatment plants (STP), located in the northern-eastern part of the bay, equipped with activated sludge treatments: Vannes-Tohannic (60,000 population equivalents, Peq), Arradon (2,500 Peq) and Séné-Le Ranquin (1,500 Peq).

Environmental sampling
Treated wastewater (1 L) was sampled monthly and water flow was monitored at the three STP outfalls between October 1999 and May 2001. Shellfish samples were collected on the same day as wastewater samples. Each shellfish sample was composed of at least 20 oysters and 35 clams. Three sites were selected based on their levels of bacterial contamination: a clam bed (site 1) close to the STP outfall, was banned for harvesting and consumption, and two oyster beds (sites 3 and 4) were occasionally contaminated under exceptional events (EC class A). Shellfish and water samples were stored at 4°C during shipment and analysed within 48 h.

Shellfish microbial analysis
On arrival, shellfish were washed, shucked and weighed. For E. coli detection, tissue and liquor were homogenised using a Waring blender with one volume of a 10% NaCl solution. E. coli concentrations were determined by conductance measurement (NF-VO8-106).
F-RNA phages were detected according to the European norm (ISO 10705-1, 1995). Viral analyses were performed on dissected tissues (stomach and digestive gland frozen until analysis). Viruses were concentrated by PEG precipitation after homogenisation and viral elution using chloroform-butanol. For norovirus detection, nucleic acids (NA) were extracted and purified as previously described (Le Guyader et al., 2000). All the NA extracts were tested for inhibitory compounds using an RNA internal control. Detection was done by RT–PCR using a panel of primer sets from the polymerase or the capsid coding regions, and confirmed by hybridisation (Le Guyader et al., 2000).

**Water microbial analyses**

*E. coli* and F-RNA phages analyses were directly performed from water by conductimetry and culture (ISO 10705-1, 1995) respectively. Norovirus detection was performed after PEG precipitation and trizol extraction (Vilaginès et al., 1997), using the shellfish primer sets.

**Hydrodynamic model**

The technique of modelisation was based on a set of several nested numerical models (grid sizes from 5 km down to 300 m). A local detailed model (80 m) nested in the previous one, was built to take the bay morphology (narrow channels, extensive tidal flats), as well as the microbial behaviour, into account. The models were two-dimensional in the horizontal plan (2DH) and solved the mathematical equations for hydrodynamics (Saint-Venant), describing, at each point, a depth-averaged current. Open boundary conditions required to run the model are the tidal elevation (given by the world model FES 99) and the water discharged from the main rivers and sewage (IFREMER data). The detailed model (80 m, Mars 2DH, IFREMER) was a compromise between the need to remain within acceptable computational costs and the need for accuracy, especially for the internal river beds. The dispersion/advection model was similar to the one described in Salomon and Pommepuy (1990). The microbial model integrated a term summarising all biological aspects of microorganisms. The behaviour of these organisms could be described by a simple first-order reaction, which would be efficient enough to obtain a reasonable level of accuracy. Equations describing the fate of bacteria or viruses were the same for any dissolved constituent, except for the T90 (time needed for a one log decrease), which takes into account the behaviour aspect of these living organisms (Salomon and Pommepuy, 1990). The Saint Venant equations were:

\[
\begin{align*}
\frac{\partial u}{\partial t} + \frac{u}{R \cos \phi} \frac{\partial u}{\partial \phi} + \frac{v}{R \cos \phi} \frac{\partial u}{\partial \theta} - \frac{uv}{R} \frac{\partial}{\partial \phi} &= 2 \Omega v \sin \phi + \frac{\sqrt{u^2 + v^2}}{K^2 H^{4/3}} + \frac{g}{R \cos \phi} \frac{\partial \xi}{\partial \lambda} - \\
\varepsilon \nabla^2 u + \frac{\tau_\phi}{\rho H} + \frac{\epsilon g H}{2} \frac{\partial S}{R \cos \phi \lambda} + \frac{\partial P_a}{\rho R \cos \phi \lambda} &= 0
\end{align*}
\]

\[
\begin{align*}
\frac{\partial v}{\partial t} + \frac{u}{R \cos \phi} \frac{\partial v}{\partial \phi} + \frac{v}{R \cos \phi} \frac{\partial v}{\partial \theta} + \frac{u^2}{R} \frac{\partial}{\partial \phi} &= 2 \Omega u \sin \phi + \frac{\sqrt{u^2 + v^2}}{K^2 H^{4/3}} + \frac{g}{R} \frac{\partial \xi}{\partial \phi} - \\
\varepsilon \nabla^2 v + \frac{\tau_\phi}{\rho H} + \frac{\epsilon g H}{2} \frac{\partial S}{R \cos \phi \lambda} + \frac{\partial P_a}{\rho R \cos \phi \lambda} &= 0
\end{align*}
\]

\[
\begin{align*}
\frac{\partial \xi}{\partial t} + \frac{1}{R \cos \phi} \frac{\partial}{\partial \lambda} + \frac{\partial}{\partial \phi} - \frac{H v}{R} \frac{\partial \xi}{\partial \phi} &= 0
\end{align*}
\]

\[
\begin{align*}
\frac{\partial H C}{\partial t} + \frac{1}{R \cos \phi} \frac{\partial}{\partial \lambda} + \frac{\partial (H(u C - K_\lambda \frac{\partial C}{R \cos \phi \lambda}))}{\partial \phi} + \frac{\partial (H v - K_\phi \frac{\partial C}{R \cos \phi \lambda})}{\partial \phi} - \frac{H v C}{R} \frac{\partial \xi}{\partial \phi} &= \text{decay}
\end{align*}
\]
where: \( u, v \): current components eastward and northward; \( \zeta \): sea surface elevation; \( H \): water column height; \( \phi, \lambda \): latitudes and longitude respectively; \( g \): gravity; \( R \): earth mean radius; \( K_\phi, K_\lambda \): diffusion coefficient in both (eastward and northward) directions; \( K_r \): Strikler coefficient; \( P_a \): atmospheric pressure; \( \tau_f \): bottom friction. Decay was the sinking term for \( c \); \( \text{Decay} = K_c \), where \( K \) could directly be related to \( T_{90} \) (time after which 90% of an initial concentration has disappeared). The \( T_{90} \) used for \( E. coli \), bacteriophages and norovirus were 1 d, 7 d and 30 d respectively.

**Results and discussion**

**Wastewater analyses**

A total of 58 wastewater samples were collected and analysed. \( E. coli \) and bacteriophage results are presented in Table 1. Noroviruses were detected in October and November 1999 and March 2000 samples.

**Shellfish analyses**

Shellfish samples (60) were collected and analysed: 20 clam samples from sites 1 and 40 oyster samples from sites 3 and 4. The bacterial concentration of 60% of the clam samples (site 1) was <4,600 \( E. coli/100 \) g with 10% <46,000 \( E. coli/100 \) g; 70% of these samples contained >1,000 phages/100 g and 84% <10,000 phages/100 g. These results corresponded with the sanitary classification which prohibits collecting and exploitation of clams in this area. At site 3, 95% of oyster samples contained <230 \( E. coli/100 \) g, while all oyster samples from site 4 contained <230 \( E. coli/100 \) g; F-RNA phage concentrations were below the detection limit (100 phages/100 g) in 90% and 94% of the samples from sites 3 and 4 respectively. Noroviruses were detected in 10% of the samples from each site.

**Input simulation**

The microbial model was run under winter conditions. Sewage treatment plant fluxes for \( E. coli \) and F-RNA phages were calculated from collected data and analyses (Table 2). Norovirus fluxes were estimated from the local population impacting the area (64,000 inhabitants) and from an epidemiological event recorded during the winter season (data from “Réseau Sentinelle”: www.b3e.jussieu.fr/sentiweb/) with a gastroenteritis illness attack rate of about 6% of the population. Based on published virus concentrations (\( 10^5 \text{–} 10^{10} \) viral particles/g stool) an average of \( 10^5 \) particles/g was selected for a period of 20 d of the outbreak duration (Yuen et al., 2001; Kageyama et al., 2003). After this date,

**Table 1** \( E. coli \) and F-RNA phage levels at the three sewage treatment plants

<table>
<thead>
<tr>
<th>STP</th>
<th>Mean (range) values for</th>
<th>F-RNA phages (PFU/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( E. coli ) (CFU/100 mL)</td>
<td></td>
</tr>
<tr>
<td>Tohannic</td>
<td>( 1.9 \times 10^5 ) (1.7 \times 10^4 \text{–} 8.0 \times 10^5)</td>
<td>( 2.8 \times 10^3 ) (2.0 \times 10^2 \text{–} 1.0 \times 10^4)</td>
</tr>
<tr>
<td>Arradon</td>
<td>( 8.4 \times 10^4 ) (1.9 \times 10^3 \text{–} 5.4 \times 10^5)</td>
<td>( 6.8 \times 10^3 ) (7.0 \times 10^1 \text{–} 8.3 \times 10^4)</td>
</tr>
<tr>
<td>Le Ranquin</td>
<td>( 8.0 \times 10^5 ) (1.7 \times 10^4 \text{–} 1.0 \times 10^6)</td>
<td>( 5.4 \times 10^3 ) (4.0 \times 10^2 \text{–} 2.4 \times 10^4)</td>
</tr>
</tbody>
</table>

**Table 2** Calculated fluxes for \( E. coli \) and F-RNA and estimated norovirus fluxes in the STPs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tohannic</th>
<th>Arradon</th>
<th>Le Ranquin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population equivalent</td>
<td>60,000</td>
<td>2,500</td>
<td>1,500</td>
</tr>
<tr>
<td>Water flow rate m³/d</td>
<td>9,500</td>
<td>373</td>
<td>500</td>
</tr>
<tr>
<td>( E. coli/s )</td>
<td>( 2.3 \times 10^8 )</td>
<td>( 8.0 \times 10^7 )</td>
<td>( 3.5 \times 10^7 )</td>
</tr>
<tr>
<td>F-RNA/s</td>
<td>( 3.0 \times 10^6 )</td>
<td>( 8.0 \times 10^6 )</td>
<td>( 1.6 \times 10^5 )</td>
</tr>
<tr>
<td>Norovirus/s</td>
<td>( 1.0 \times 10^6 )</td>
<td>( 4.0 \times 10^4 )</td>
<td>( 2.0 \times 10^4 )</td>
</tr>
</tbody>
</table>
considering that viral input was over, the model for viruses only ran with the viral decay rate.

Water-calculated concentrations are a vertical average on each point after a continuous microbial input, and included the biological decay rate effect. T90 (time needed for a one log decrease) was equivalent to 1d for *E. coli* (Pommepuy *et al.*, 1992), to 7 d for phages (Girones *et al.*, 1989; Wait and Sobsey, 2001) while for norovirus, based on data published for other human enteric viruses, we estimated the T90 to be about 30 d ( Arnal *et al.*, 1998; Wait and Sobsey, 2001).

**Simulation results**

The variation of viral concentrations from d 0 (starting date of the outbreak) to d 50 (when the simulation was stopped) is reported in Figure 2 (sites 1, 3 and 4).

An increase of the contamination was observed during the first days corresponding to the viral dispersion in the bay. After 2 d and 7 d (sites 1 and 4 respectively), concentrations were well established and were only dependent on the tide and the decay-rate. The effect of the semi-diurnal tide (5 m during spring tide, 3 m during neap tide) was well modelised. The maximal concentration was observed every 12 h at low tide. The viral water concentration was highly variable, depending on the tide. In this simulation, the maximal water concentration was equivalent to 100 viruses/100 mL (site 1), 3 viruses/100 mL (site 3) and 0.1/100 mL (site 4) (Table 3). After 20 days, the viral outbreak was considered to be over and the input was stopped. Thus, viral concentration affected only by the dispersion and the T90, decreased slowly.

The *E. coli*, phages, and norovirus concentrations were calculated by the model after 20 days of sewage discharge (Table 3). *E. coli* water concentrations varied from 5,000 CFU/100 ml (site 1, directly under the STP outfall influence) to 0.1 CFU/100 ml (site 4).

![Figure 2](https://iwaponline.com/wst/article-pdf/50/1/117/421367/117.pdf)

**Figure 2** Simulation of the impact of a viral outbreak on microbial seawater quality in the Golfe du Morbihan (sites 1, 3 and 4). (Hypothesis of an outbreak duration 20 d. The viral decay rate (T90) was 30 d.)

**Table 3** Water concentrations obtained by modelisation

<table>
<thead>
<tr>
<th>Site</th>
<th>Minimum and maximum calculated concentrations of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em> (CFU/100 mL)</td>
</tr>
<tr>
<td>1</td>
<td>158–5,000</td>
</tr>
<tr>
<td>3</td>
<td>6–32</td>
</tr>
<tr>
<td>4</td>
<td>0.1–10</td>
</tr>
</tbody>
</table>
Phage concentrations varied from 10,000 to 0.3 PFU/100 ml within the three sites. The difference in decay rate for *E. coli* and phages is clearly demonstrated by their concentration ratio equivalent to 100 in wastewater (calculated from Table 1) and to 1 in site 4 (calculated from Table 3).

To achieve shellfish concentrations, the concentration rate of eight observed in oysters compared to surrounding waters (Lees *et al.*, 1995) was extended for F-RNA phages and norovirus. *E. coli* results (Table 4) obtained from the simulation were in accordance with those obtained from the current analyses (sites 1, 3 and 4) and from the surveillance network established for 15 years in this area (REMI, IFREMER). The F-RNA phage calculated concentrations and those obtained by analysis were in accordance, with very low concentrations in site 4 (<100 PFU/100 mL).

The model indicated minimal and maximal concentration for the three sites and for the different microorganisms. For example, at site 1, all the *E. coli* results obtained by analysis were in accordance with the model, as no sample was found to be contaminated by more than 38,000 CFU/100 g. For F-RNA phages, all the results obtained fitted the values calculated with the model. Norovirus concentrations obtained in shellfish with the model were <27 viruses/100g (sites 3, 4) and 70–7,000 viruses/100 g near the sewage input (site 1), indicating that a low viral concentration could be expected at sites 3 and 4.

**Discussion and conclusion**

The impact of virus discharge in the environment on shellfish quality is difficult to evaluate. Some studies have shown a high frequency of human enteric viruses in different areas, and several gastroenteritis outbreaks linked to shellfish consumption have been described (Lees, 2000). If we want to improve shellfish quality, one has to understand the way that shellfish become contaminated. Two main factors are responsible for the occurrence of shellfish contamination: (a) the prevalence of the virus in the population and (b) the accumulation and behaviour of virus in shellfish. Mathematical models were previously shown to be efficient to evaluate sewage impact on bacterial contamination (Bell *et al.*, 1992; Kashefiipour *et al.*, 2002). The innovative point of this study was to use a rough modelling application to study the behaviour of viral input on shellfish quality.

After validation of the model with *E. coli* and phages, norovirus input linked to the winter outbreak in the surrounding population was simulated. Even if several parameters were arbitrarily selected, the results obtained were consistent with those obtained by shellfish analyses. Sensitivity threshold was difficult to assess in naturally contaminated shellfish, especially for norovirus detection; however, it is unlikely that we can detect <2 viruses/100 g of shellfish as predicted by the model for two sites. Norovirus approximated fluxes calculations were based on hypothesis; however, very few positive norovirus samples were detected during our study at sites 3 and 4. The dilution mechanisms in this area were very important and could be the origin of the low viral contamination (under the detection limit threshold).

### Table 4 Shellfish contamination: comparison between calculated concentrations (model) and analyses

<table>
<thead>
<tr>
<th>Site</th>
<th><em>E. coli</em></th>
<th>F-RNA</th>
<th>Norovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model</td>
<td>Analysis</td>
<td>Model</td>
</tr>
<tr>
<td>1</td>
<td>Min.</td>
<td>1,200</td>
<td>21%; &lt;1,200</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>38,000</td>
<td>0%; &gt;38,000</td>
</tr>
<tr>
<td>3</td>
<td>Min.</td>
<td>48</td>
<td>60%; &lt;48</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>243</td>
<td>5%; &gt;243</td>
</tr>
<tr>
<td>4</td>
<td>Min.</td>
<td>0.8</td>
<td>0%; &lt;0.8</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>80</td>
<td>10%; &gt;80</td>
</tr>
</tbody>
</table>

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The use of mathematical modelling could help to interpret the large variations observed between viral occurrences among studies made in different European countries – 2% in Greece and up to 41% in Sweden (Formiga-Cruz et al., 2002). However, some hypotheses have to be specified, and real-time PCR data are required to precisely determine the fluxes and viral die-off rate. We also need to simulate other accumulation rates for viruses in shellfish, as some variation may be observed during the year (Burkhardt and Calci, 2000). Hydrodynamic models are now available and currently used to assess bacterial contamination in shellfish-growing areas (Fiandrino et al., 2003). Such models, in correlation with quantitative approach development, will help to understand the distribution of norovirus in the environment, and thus may lead to an extension of a public health protection level for shellfish quality.

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References


