REDRISK: Reduction of the virus risk in shellfish harvesting areas.

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Introduction:

Filter feeding bivalve shellfish can accumulate human pathogenic bacteria and viruses if grown in sewage-contaminated waters. Current consumer protection legislation relies on classification of harvesting areas based on their sanitary quality, using *E coli* as an indicator of sewage contamination. Advances in viral monitoring have shown that *E coli* can underestimate the extent of the contamination.

The most common cause of gastroenteritis associated with shellfish is norovirus, commonly known as winter vomiting virus. The REDRISK project was undertaken to investigate the main environmental factors that cause viral contamination in shellfish. The REDRISK project is part of a EU research pillar with parallel research being undertaken in the UK, France and Spain. A recently developed technique to quantify norovirus in shellfish, real-time PCR, has been used in the REDRISK project.

Clew Bay, in Co. Mayo was chosen as the study area in Ireland. The bay is generally considered to have good water quality but with certain areas subject to intermittent sewage contamination. The cooperation of local producers and organisations such as the Clew Bay Marine Forum and the Native Oyster Co-op greatly helped the project. The project was divided into a two-phased approach. Phase one involved the identification of contamination sources impacting the bay through a sanitary survey and selection of appropriate sites for further study. Results of the first phase of this study were presented previously at this forum (Keaveney, et al 2006) and the characteristics of the sites selected for study and locations within the bay are shown in table 1 and figure 1 respectively. The second phase of the project focused on monitoring environmental conditions and microbiological levels in shellfish to identify environmental conditions leading to viral contamination. This paper reports the finding of this monitoring.

Material and methods

Samples were collected from the sites on 40 occasions between August 2005 and July 2006. On each occasion 24 Pacific oysters (*Crassostrea gigas*) were collected from each site. Samples were then sent to the laboratory within 30 hours under chilled condition (<15°C) for *E coli*, FRNA bacteriophage and Norovirus analysis. Concurrent measurements of riverflow, rainfall, outflow volumes from the wastewater treatment plant, as well as salinity on site, were also recorded. On receipt in the laboratory oysters were cleaned and scrubbed under running potable water. A minimum of 10 oysters were shucked and homogenised for *E. coli* and FRNA bacteriophage analysis. Homogenates were analysed for *E. coli* using a standard ISO procedure (ISO/TS 16649-3). The same homogenate was centrifuged at 2000 x g and supernatant analysed for FRNA bacteriophage using a standard ISO method (ISO method 10705 – 1). Hepatopancreas was dissected from a further 6 oysters and analysed for norovirus using an established real-time PCR assay (Jothikumar, et al 2005).

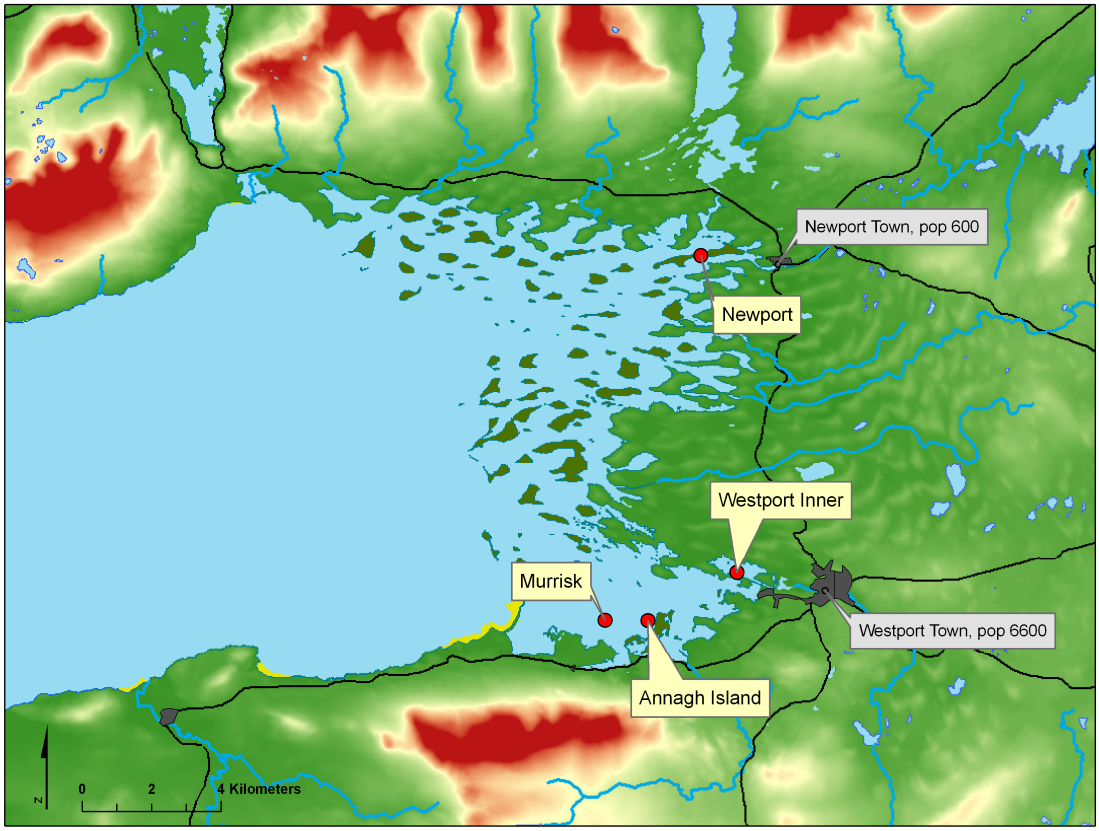

Figure 1: *Indicating the 4 experimental sample sites chosen to monitor microbiological contamination. Also indicated are the 2 main towns in the bay and the main rivers.*

Site	**1**	**2**	**3**	**4**
Classification	Category B	On cat. A/B boundary	Category A	Category B
Previous *E. coli* results:	None available	24/26 Cat A results	178/193 Cat A results	7/13 Cat A results
Distance to nearest WWTP* outfall	300m	3500m	4500m	1500m
Local population	6600	Minimal	Low	600
Freshwater input	River A av. flow 0.96 m^3/s)	River B (av. flow 1.5 m^3/s)	Very little freshwater input	River C (av. flow 5.2 m^3/s)
Animal population	1300 sheep and 1200	Some local animals	Some local sheep farming	3200 sheep and 1900 cattle
Potential risk of virus	**High**	**Medium**	**Low**	**High**

*WWTP-Waste Water Treatment Plant

Table 1: *Key characteristics for each sampling informing potential risk of viral contamination.*

Results:

The microbiological results are presented for each of the individual sites in figures 2 to 7. A high frequency of norovirus positive results were observed at the Westport site (fig. 3). Although norovirus contamination was present for most of the year (fig. 3.) levels showed a clear seasonal trend with peak PCR unit levels observed during the winter period and in particular January and February of 2006. Despite high norovirus levels at the site, *E. coli* levels were consistent with a category B classification throughout the year.

At Annagh Island both frequency of norovirus contamination and levels were significantly lower than at Westport. Norovirus was absent for much of the time (fig 5). Norovirus occurrence in oysters at the site appeared to be linked to periods of peak WWTP influent flow causing overflows of untreated sewage. The initial occurrence of norovirus at the site coincided with a storm event in October of 2005 resulting in sewage overflowing at the WWTP site. A sewage overflow event in January 2006 also appeared to coincide with a prolonged spell of norovirus contamination at the site (fig 5). This contamination appeared to last through until the middle of February. During this period despite no further sewage overflows, levels of norovirus GII appeared to increase in oysters at the site. This may be a genuine increase in virus contamination at the site at this time caused by further unidentified contamination. Alternatively it may be a feature of the low virus levels observed during the period. Apparent differences in norovirus levels in the shellfish sampled at this time may in fact be an artefact of the accuracy of the relative quantitative aspects of the assay at this level.

Norovirus contamination at the Murrisk site was observed only rarely throughout the study period (fig. 7). When norovirus contamination was observed this was at very low levels which equate to the limit of detection of the assay. In the site norovirus contamination again appeared to coincide with sewage overflow events related to increased influent levels at the WWTP in October 2005 and January 2006.

Untreated sewage also overflowed from the WWTP on two further occasions during the study period, once in August 2005 and again in May 2006. No norovirus contamination in the Annagh island and Murrisk sites were observed during these two events. This would coincide with the fact that norovirus associated illness in the population at this time would be lower at this time of year given the usual seasonal course of infection in community. Therefore levels of norovirus in sewage effluent at this period would be considerably reduced compared with levels during the winter period.

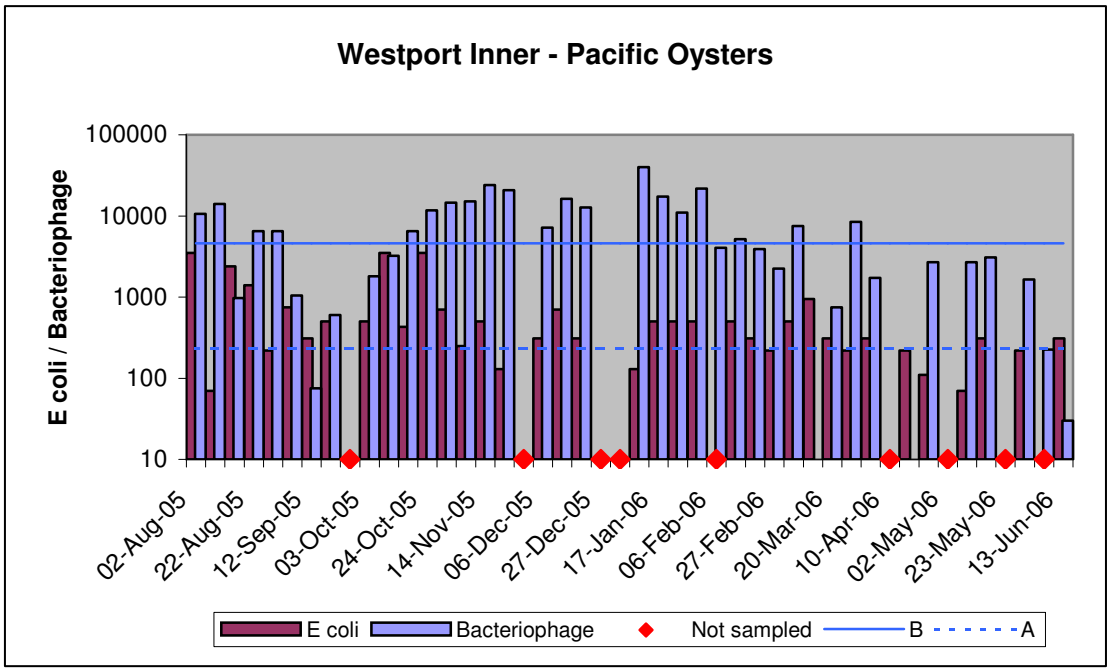

Fig 2 *E. coli (MPN 100g^{-1}) and bacteriophage (pfu 100g^{-1}) levels in pacific oysters from Westport Inner. The category A and B classification limits are indicated. The weeks not sampled are indicated.*

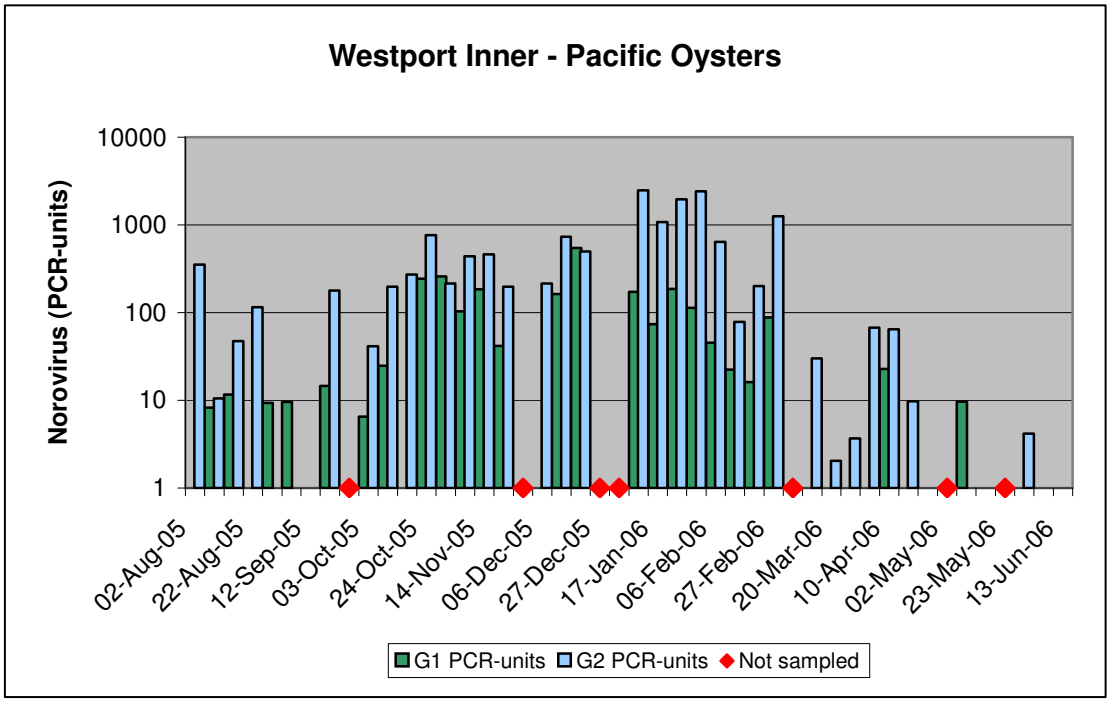

Fig 3 *Norovirus GI and GII levels (PCR units) in Pacific oysters at Westport Inner. The weeks not sampled are indicated.*

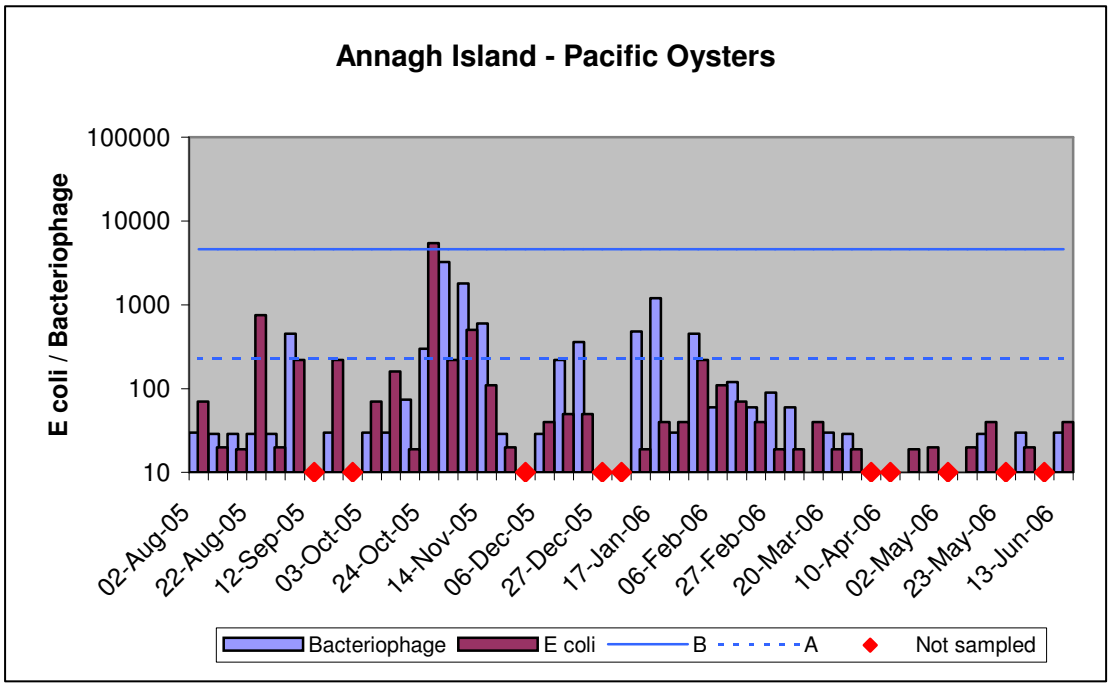

Fig 4 *E. coli (MPN 100g^{-1}) and bacteriophage (pfu 100g^{-1}) levels in pacific oysters from Annagh Island. The category A and B classification limits are indicated. The weeks not sampled are indicated.*

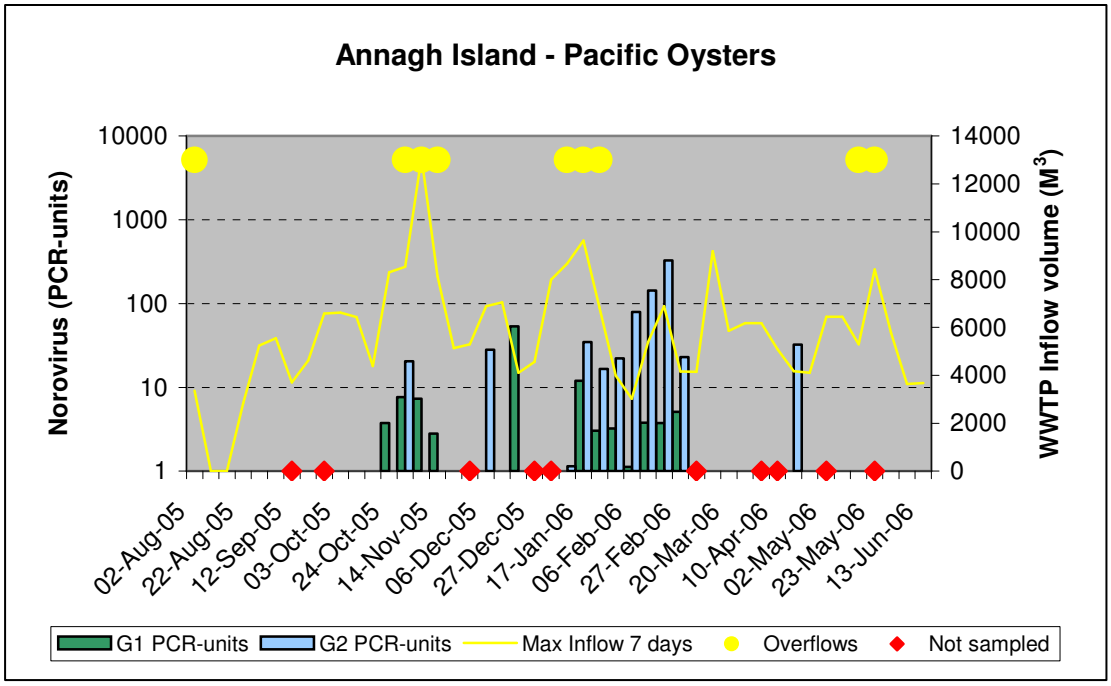

Fig 5 *Norovirus GI and GII levels (PCR units) in Pacific oysters from Annagh Island. The weeks not sampled are indicated. The inflow volume to the WWTP and the periods of overflow are indicated.*

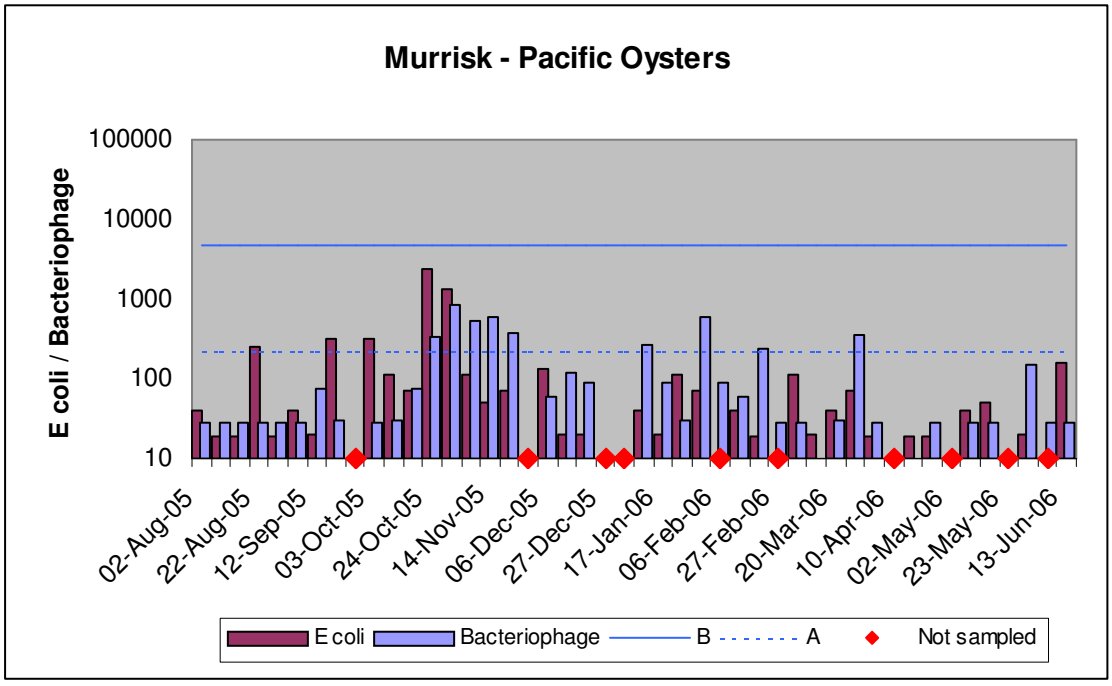

Fig 6 *E. coli (MPN $100g^{-1}$) and bacteriophage (pfu $100g^{-1}$) levels in pacific oysters from Murrisk. The category A and B classification limits are indicated. The weeks not sampled are indicated.*

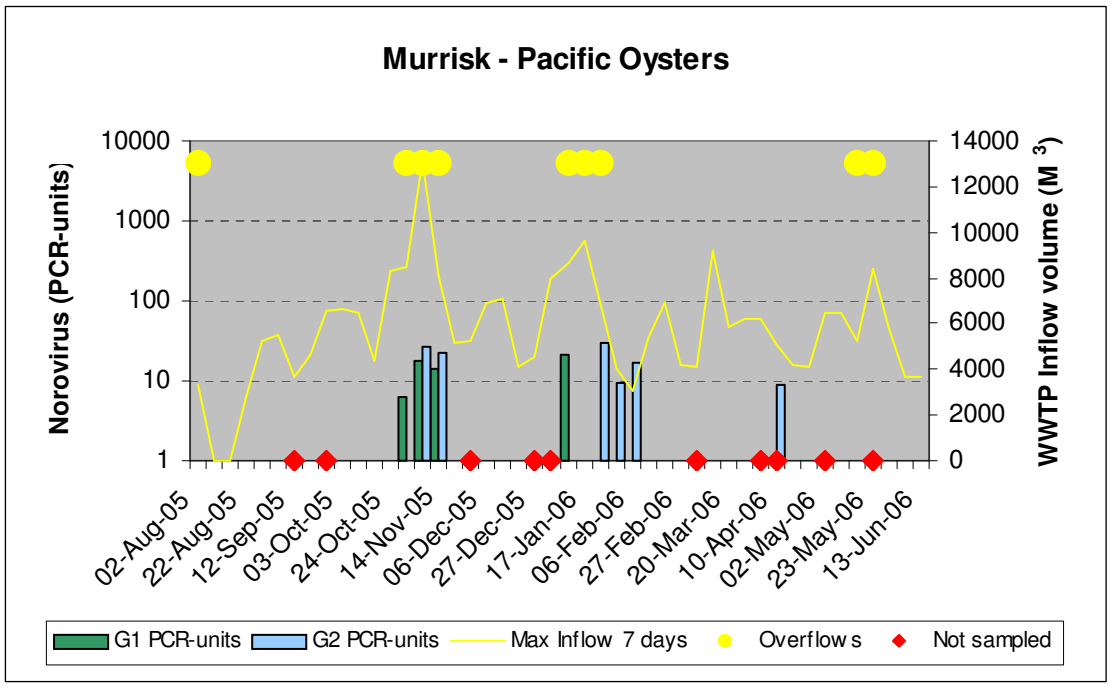

Fig 7 *Norovirus for GI and GII levels (PCR units) in Pacific oysters from Murrisk. The weeks not sampled are indicated. The inflow volume to the WWTP and the periods of overflow are indicated.*

Discussion:

Although, long-term, the most effective way forward to control the health risks associated with shellfish consumption is reduction of levels of sewage initially impacting shellfisheries at source (Pommepuy et al, 2004), there is an immediate need to implement active risk management procedures. In moving towards developing active risk management procedures this study demonstrates that the identification of factors leading to norovirus contamination in shellfish is possible using new real-time PCR methods. This information can be used to determine when intervention measures should be introduced to limit the exposure of contaminated shellfish to consumers. However, as demonstrated at the Westport site in this study, the almost continuous incidence of norovirus contamination at some sites (despite compliance with the existing *E. coli* standard) preclude the suitable introduction of intervention controls. Therefore a pre-requisite for the use of active risk management procedures is that shellfish harvesting areas should be relatively free from sewage pollution and subject to only intermittent norovirus contamination. Therefore a first step in developing site-specific risk management procedures is to extensively characterise the shellfishery in question. In this study the sanitary survey successfully identified sites at less risk of norovirus contamination. Sanitary surveys in other area could also be used to determine areas likely to be impacted by intermittent contamination.

Where intermittent norovirus contamination was observed this was closely linked to discharge of untreated sewage as a result of storm events. Procedures for rapid identification of these events and communication to relevant shellfish producers and risk managers is a key step in identifying high risk periods requiring intervention to manage the risk. Developing these links represents a major challenge requiring resource and commitment from all parties. The adoption of appropriate management options in each area will depend on local circumstances and the level of viral contamination.

The introduction of real-time PCR procedures allow the effectiveness of the control measures in preventing significant norovirus levels reaching consumers to be monitored as well as how long the extra procedures should be in place. However, further work is required to relate the risk of viral illness to virus levels found in shellfish to determine whether complete removal of virus is required to provide a safe product or whether there is an acceptable virus level which can be considered to present an acceptable level of risk.

Conclusions:

1. The sanitary survey accurately predicted the relative risk of norovirus contamination in oysters at each site within the study area.
2. The three major factors influencing norovirus contamination were proximity to sewage input, season, with winter representing a higher risk, and the influence of untreated sewage inputs as the result of overflows from the WWTP.
3. The introduction of active risk management procedures is only appropriate in areas subjected to intermittent contamination. Sanitary surveys can provide an initial assessment of the likely risk of norovirus contamination and determine

the suitability of a shellfish production area for the application of active risk management procedures.

4. One site was shown to be almost continuously contaminated with norovirus through the study period and was considered unsuitable for shellfish harvesting. This was despite complying with European hygiene regulations for shellfish harvesting as judged by *E. coli* data.
5. Intermittent norovirus contamination in two sites appeared to be associated with untreated sewage from overflows. These events could be used to trigger management action at those sites. Close links between WWTP managers, shellfish producers and risk managers should be developed.
6. The highest incidence and levels of norovirus contamination in shellfish occurred during the winter months. Closer links between health professionals and shellfish risk managers should be developed to more accurately determine high-risk periods from the surveillance of outbreak data.
7. Further studies are urgently required to establish the link between norovirus levels observed in shellfish and health risk in consumers. Such studies will indicate the level of management and treatment required to provide an acceptable risk in shellfish.

References

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