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Introduction

Sewage contaminated bivalve molluscan shellfish present a considerable public health risk when consumed raw or lightly cooked. Treatment of sewage outfall effluent entering the banks of clean seawater (depuration) has been employed extensively to reduce this risk (Richards, 1988). Despite this, outbreaks of viral illness following the consumption of depurated oysters continue (Chalmers and McMillan, 1995; Kohn et al., 1995) and viruses have been shown to persist in depurated oysters in the absence of E. coli, over the traditional indicator of the sanitary quality of shellfish. The principle illness associated with the consumption of depurated oysters in the UK is gastro-enteritis caused by Norwalk-Like-Viruses (NLVs). Difficulty in detecting these viruses has hindered research into viral elimination during depuration.

Male-specific RNA (FRNA) bacteriophages have similar physical and genetic characteristics as the NVLs and are commonly found in sewage. Because of this and their ease of analysis they have been proposed both as an indicator of the behaviour of viruses in shellfish (Doré and Lees, 1995) and as an index organism for the viral risk associated with shellfish (Doré et al., 2000). Investigations by this laboratory have demonstrated that depurated oysters associated with outbreaks of gastro-enteritis and containing NLVs also contain all levels of FRNA bacteriophages even in the absence of E. coli. Conversely, at date, depurated oysters which have been shown to be free of FRNA bacteriophages have also been shown to be free of NLVs. It is considered that if depuration procedures were developed which produced oysters free from FRNA bacteriophage such procedures would also be likely to produce oysters free from NLVs and other human viruses. In this study we investigated the effect of temperature on the elimination of viruses from oysters (Crassostrea gigas) during depuration using FRNA bacteriophage as an indicator organism.

Materials and Methods

Contamination of Oysters

Pacific oysters (Crassostrea gigas), obtained from a commercial harvesting area, were contaminated by relaying approximately 800 m from a sewage outfall for a minimum of two weeks. The sewage outfall discharged sewage at a rate of 1200 l/min and sterilised by ozonation at a 15 mg/L ozone demand (type 15/3p UVAQ Ltd., Sudbury, UK). Temperature was maintained above 80% saturation by the use of a spray bar for 0.5 h. Temperature was maintained by the external temperature controller of the system. Average rates of elimination were determined as 0.14, 0.2 and 0.36 for 8, 14 and 20°C respectively.

Depuration

Experimental systems had tanks with dimensions of 1.050 m (length) by 550mm (width) by 450mm (height) with a working volume of 180 l. Seawater (30±3°C) salinity, which was subject to ozonation, was maintained at a rate of 1200 l/min and sterilised by ozonation at a 15 mg/L ozone demand (type 15/3p UVAQ Ltd., Sudbury, UK). Temperature was maintained above 80% saturation by the use of aquarium heaters (salternated temperature) or by placing the whole tank in controlled temperature room (low-temperature tank). Dissolved oxygen was maintained above 80% saturation by the use of a recirculating water. Shelf-life work was performed in plastic must boxes (30 l; 41042, Samon Products Ltd, UK). Significant staining of the sides of the must boxes was observed but the main contamination was the result of a relatively low contamination rate of around 2 ml/min. Boxed seawater was introduced into each tank at a rate of 1200 l/min through a 150 mm diameter venturi nozzle (41042, Samon Products Ltd, UK). The water was continuously recirculated at a rate of 1200 l/min and sterilised by ozonation at a 15 mg/L ozone demand. The system was designed and constructed in a single pass through the system. Water from each tank was recirculated through a 1200 l/min steriliser and sterilised by ozonation at a 15 mg/L ozone demand. The system was designed and constructed in a single pass through the system. Water from each tank was recirculated through a 1200 l/min steriliser and sterilised by ozonation at a 15 mg/L ozone demand. The system was designed and constructed in a single pass through the system. Using this technique, the average temperature in the tanks was maintained at 18°C with great accuracy. Collection and transport of shellfish was similar to our standard technique. Samples were collected from 24-hour collection of the tanks. Samples were assayed for FRNA bacteriophages using methods described previously (Doré et al., 2000).

Sampling and analysis

A minimum of 30 samples of 4-6 oysters were taken from all experiments on each day of depuration over a minimum period of depuration of 5 days. Samples were assayed for FRNA bacteriophages within 24 hours of collection. Samples were assayed for FRNA bacteriophages using methods described previously (Doré et al., 2000)

Results

Temperature was found to have a significant effect on the rate of elimination of FRNA bacteriophage with temperatures increasing with decreasing temperature. Figure 1 shows the effect of temperature on the elimination of FRNA bacteriophage. Levels of FRNA bacteriophage in oysters depurated at 20°C for 5 days were reduced to levels ranging between 0.6 and 42.5% (p<0.05) of initial contamination levels whereas levels in oysters depurated at 14°C were reduced to levels ranging between 0.3 and 3.8% of initial levels in the same time.

Using the elimination rate values determined above it is possible to predict the level of FRNA bacteriophage remaining at any time during depuration at any temperature for any starting level. Table 3 shows the predicted levels of FRNA bacteriophage remaining at 8°C, 14°C and 20°C respectively. In preliminary experiments conducted at the Centre for Environment, Fisheries & Aquaculture Science (CEFAS), Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK. http://www.cefas.co.uk

Discussion

From this study it appears that the use of elevated temperature during depuration would significantly increase viral elimination during depuration as judged by FRNA bacteriophage removal. The fact that the effect of increased temperature on viral elimination is consistent regardless of species or harvest area temperature (temperature compensated) suggests that a consequence elevated temperature could be applied in the winter months when incidence of viral illness associated with consumption of depurated oysters is greatest. It is possible therefore that the application of elevated depuration temperatures during this period could significantly reduce the viral risk associated with the consumption of shellfish. Further work is required to establish the viral risk associated with FRNA bacteriophage elimination and NLV elimination. Similarly further work is required to establish any effect on the shelf-life or quality of the product.

FRNA bacteriophages have been proposed as a potential index organism for the viral risk associated with shellfish consumption and clearly the potential for saving an end product standard exists. In this study, the calculation of FRNA bacteriophage elimination rates during depuration for Pacific oysters allows an upper level of FRNA bacteriophage which can be eliminated by a green depuration regime (temperature and temperature) to be determined. This study therefore provides a basis for assessing and setting depuration parameters for Pacific oysters which could be used to successfully reach a target shelf-life or quality of the product.

References


