



# Viral Contamination in Bivalve Shellfish from the North of Portugal



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## ABSTRACT

In this study we aim to assess the level of enteric viral contamination in two growing areas of *Mytilus edulis* located in the North of Portugal: Rio Lima Estuary and Carreço (Viana do Castelo), using F-specific RNA bacteriophages as indicators. Samples of *Mytilus edulis* were harvested and water collected once a month from July to December of 2004 from each study area. Water samples were processed in order to determine salinity, temperature and pH. Detection and enumeration of FRNA bacteriophages in bivalve were determined using the double-agar-layer method. *Escherichia coli* levels were also enumerated using the method described by Donovan et al (1998). The results indicate a direct variation between the FRNA and *E. coli* levels in the Rio Lima Estuary. In Carreço the data appear to be more stable throughout the entire study. In conclusion the level of viral and faecal contamination in the Rio Lima Estuary is higher than in Carreço.

## INTRODUCTION

The level of viral contamination in the North of Portugal is unknown. There are more than 100 different types of viruses present in the residual sewage, whose survival and mode of transmission are recognized as a potential public health problem [1]. They are able to survive in the environment, either suspended in the water column, associated with organic matter or in sediments, for weeks to months.

Shellfish in their adult stage are sedentary, forming large natural beds either in rivers, estuaries or coastal areas. They have a filter-feeding process, which results in the accumulation of large quantities of micro-organisms (bacteria, virus and others), reaching values around a hundred times superior to that encountered in the surrounding aquatic environment [2].

For several decades, bivalves have been implicated as the source of transmission of enteric viral diseases, such as hepatitis A, E and gastroenteritis.

Male or F-specific RNA bacteriophages are bacterial viruses which have a similar morphology and genetic structure to human pathogenic viruses. Their abundance in sewage and ease of enumeration make them a good indicator of viral contamination in the marine environment [3]. Their presence in shellfish is indicative of sewage pollution and potential contamination by human pathogenic viruses [3]. However, traditionally faecal coliforms have been used as indicators in the sanitary control of bivalve molluscs.

With this study we've aimed to assess the level of viral contamination in two natural shellfish-growing areas, located in the North of Portugal using F-specific RNA bacteriophages as indicators.

## MATERIALS & METHODS

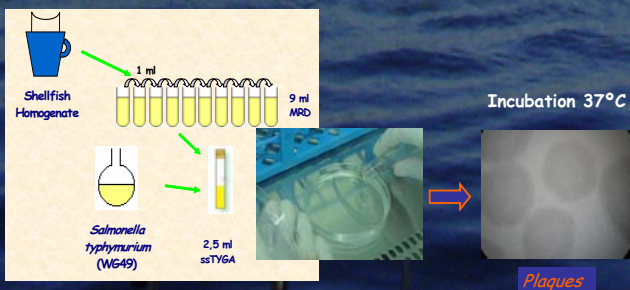
**Animals and sampling.** *Mytilus edulis* individuals and water were monthly sampled between July and December of 2004. Mussels were processed for *Escherichia coli* and F-specific bacteriophages and water was assessed to determine its physical and chemical properties: temperature, pH, salinity and conductivity.



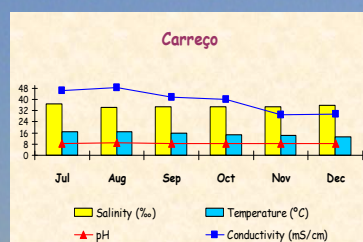
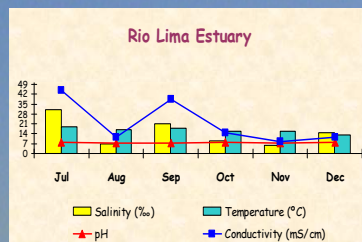
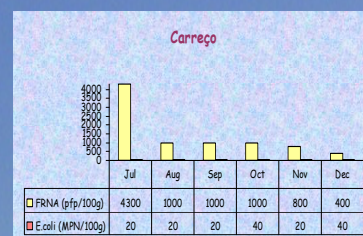
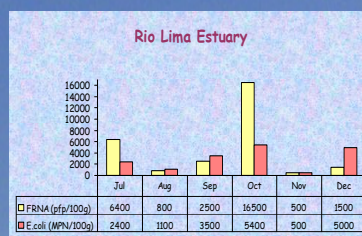
Sampling Sites	
<b>Rio Lima Estuary</b>	
41° 41, 41' N	
08° 47, 06' W	
<b>Coast of Viana do Castelo (Carreço)</b>	
41° 44, 34' N	
08° 52, 42' W	

**Shellfish Processing and *E. coli* Analysis.** Shellfish homogenate obtained in Maximum Recovery Diluent was used for detection of *E. coli* using the method described by Donovan et al [4]. This consisted in a two-stage, five-tube, and three-dilution most probable number (MPN) method.

**F-specific RNA Bacteriophage Enumeration.** The supernatant of Mussel homogenate was used to quantify the viruses using the double-agar-layer method described by ISO 10705-1 [3]. After overnight incubation at 37°C, the number of plaques formed was enumerated.



## RESULTS



## CONCLUSIONS

The level of viral contamination in the North of Portugal was until now unknown. With this study we intended to determine this, using two different ecosystems, with distinct salinity, temperature and pH values.

Once more it was proved that *E. coli* isn't a good indicator in the detection of enteric viruses.

When comparing the results obtained for both *E. coli* and F-specific RNA bacteriophages, we can conclude that, the degree of viral contamination in Carreço, is inferior to Rio Lima Estuary. We've also observed, that the obtained values for FRNA in Rio Lima Estuary reported a higher variation throughout the entire sampling period in contrast with Carreço. This could be due to the winter weather typical conditions, the number of *E. coli*, the natural FRNA hosts, and the presence of two waste water treatment plants present near the sampling sites that only possess primary and secondary treatment.

The results obtained in Rio Lima Estuary indicate a direct variation between the *E. coli* and FRNA, in contrast with Carreço.

## ACKNOWLEDGEMENTS

Dr<sup>a</sup> Filomena Pombal for collecting the samples and transportation to the Laboratory. Prof. Doutora Adelaide Almeida for the supply of the bacterial strains.

## BIBLIOGRAPHY

- [1] Tartera, C. and Jofre, J. (1987). Bacteriophages Active against *Bacteroides fragilis* in Sewage-Polluted Waters. *Applied and Environmental Microbiology*, 53(7): 1632-1637.
- [2] Doré, W.J. and Lees, D.N. (1995). Behavior of *Escherichia coli* and Male-Specific Bacteriophage in Environmentally Contaminated Bivalve Molluscs before and after Depuration. *Applied and Environmental Microbiology*, 61(8): 2830-2834.
- [3] ISO 10705-1:1995 (E). Water Quality and enumeration of bacteriophages - Part 1: Enumeration of F-specific RNA bacteriophages.
- [4] Donovan, T.J. et al. (1998). Modification of the standard method used in the United Kingdom for counting *Escherichia coli* in live bivalve molluscs. *Communicable Disease and Public Health*, 1(3): 188-196.