



THE SHELLFISH CENTRE  
CANOLFAN PYSGOD CREGYN



# Scientific Understanding and Proposed Regulation for Norovirus in Shellfish

Workshop Report

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## Introduction

On 14<sup>th</sup> October 2020, the Shellfish Centre at Bangor University, supported by Seafish and the Shellfish Association of Great Britain, hosted a digital workshop entitled 'Scientific Understanding and Proposed Regulation for Norovirus in Shellfish'. The online workshop was opened by Prof. Lewis LeVay, Director of the Centre for Applied Marine Sciences at Bangor University.

The workshop brought together representatives from the shellfish industry, EU regulatory agencies and researchers in the field of shellfish and human health to exchange information and views around approaches to regulation of shellfish microbial quality and shellfish production. A primary motivation for convening the workshop was development of proposals for introducing Norovirus (NoV) controls into EU regulations for shellfish for human consumption. Discussions focused on protecting consumer safety through dealing with the risk of NoV contamination in shellfish in a more effective, informed, and comprehensive way. It brought together recent industry experience and scientific updates to inform discussion of the potential for new regulatory approaches to NoV in the environment and shellfish.

## Key Findings

- Proposed revisions of EU Regulation 853/2004 Annex III to include testing of 10% of live shellfish destined for raw consumption with a NoV threshold of 500 genome copies per g, were considered to be potentially a significant cost burden on producers, while the proposed use of the current testing regime risks over-representation of risk with consequent potential for disproportionate rejection of batches.
- The current method for NoV testing does not differentiate between viable and non-viable virus and remains a major obstacle to determining appropriate regulatory control thresholds for contamination. Developing an accurate NoV test that can discriminate between viable and non-viable virus and determine the level of NoV infectivity, should be a joint priority among industry, scientists and regulators.
- New approaches such as the F-specific RNA bacteriophage (FRNAPH) approach could yield potential advancements for estimating the infectious NoV hazard in shellfish.
- A risk-based approach, which could include wastewater and pollution-source testing combined with weather event and public health alerts, may be a more cost-effective and preventative approach to management of NoV in shellfish for human consumption.
- Surveillance of wastewater, similar to that adopted in response to the Covid-19 pandemic, has the potential to improve monitoring for both emerging and existing viruses, such as NoV, and could increase the effectiveness of a risk-based approach to harvest controls.
- Risk-based management should be focused on producers, providing them with improved control and better business security.
- Clear and transparent communication is needed to convey the risks of eating shellfish to consumers in comparison to other foodstuffs.
- Improving environmental water quality is the best long-term solution to NoV prevalence in shellfish. Governments should be encouraged to implement policies for the protection of water quality, to ensure wastewater companies practise transparent reporting and meet their obligation to deliver clean water.

## Presentations

### Iain Shepherd – European Commission

#### ‘Shellfish and the Green Deal’

Iain Shepherd, from the Directorate General for Maritime Affairs and Fisheries at the European Commission, explained how the development of shellfish aquaculture fits into the Commission’s flagship Green Deal policy. He said the Green Deal is “at the heart of the EU’s mission to be the first climate-neutral continent by 2050” and sustainable fish and algae production are set to play a part in this. He told the workshop how shellfish production in the EU has stagnated in recent years, shifting towards fish production, but emphasised that the EU has potential to produce more shellfish through co-existence with the offshore wind industry. Shepherd added that the Green Deal’s Circular Economy Action Plan highlights the role that shellfish could provide as a natural aid to reducing nitrification in Europe’s seas. He concluded by sharing the details of a new study, due to commence in early 2021, to explore the growth capacity of shellfish and algae aquaculture and the associated impacts on nutrient cycling.

### Professor Sarah O’Brien – Newcastle University

#### ‘Norovirus Attribution Study’

Prof. Sarah O’Brien shared her research on the NoV Attribution Study, which investigated how and in what quantities NoV are transmitted through contaminated food. She detailed that the research was conducted through six inter-disciplinary work packages (WP) and summarised the findings of each one. WP 1 provided a literature review of the evidence for foodborne transmission of NoV. A review of scientific and grey literature implicated that 57% of foodborne outbreaks were in Europe, with seafood being the most common food vehicle. However, the literature was dominated by studies involving oysters and seafood, and this WP assessed whether this was a genuine food-related effect or whether there were other explanations like investigator and publication bias, viral load and other factors. Conclusions were that representation of oysters in the literature may not accurately reflect their contribution to causing outbreaks. WP 2 sought to differentiate between infectious and non-infectious NoV using a capsid integrity assay. Whilst the technique proved successful for salad, vegetables and fruit, it was incompatible with oysters. WP 3 investigated NoV contamination levels in oysters at retail and found oyster contamination to be year-round, but with strong seasonality. WP 4 explored contamination in salad and soft-berry fruits. The results showed that NoV was detected at 5% or less in lettuce and both fresh and frozen raspberries. WP 5 investigated the prevalence of NoV in catering environments and found that 11% of catering premises were surveyed overall. WP 6 provided an overall assessment of food-related NoV using two methods – modified quantitative microbiological risk assessment (QMRA) and individual-based modelling. O’Brien concluded that between a fifth and a third of all NoV could be attributed to foodborne transmission, with nearly 75% of those instances occurring through contaminated catered food, 20% through lettuce, and 3% through berries and oysters respectively.

### Audrey Lainé – Comité National De La Conchyliculture

#### ‘French shellfish sector perspective’

Audrey Lainé offered the workshop the French shellfish sector perspective, stating that France is one of the top five shellfish producer countries in the world and second in the EU. In France, the NoV risk is managed and anticipated by a technical instruction from the agri-food Ministry, through the monitoring of four alert factors:

1. Microbiological monitoring
2. High rainfall
3. Malfunction of sewerage systems and/or wastewater treatment plants
4. Suspected or confirmed NoV foodborne outbreaks within shellfish

French shellfish production works to understand and balance these four risk factors to ensure contaminated products don't reach consumers. When an alert level is triggered, warning messages are distributed and/or shellfish production areas are closed for at least 28 days. Measures to withdraw batches or recall products may also be implemented. Adaptions to shellfish industry practices in response to the Covid-19 pandemic have improved traceability from shellfish production areas to dispatch centres. Provision of clean sea water storage allows shellfish to be held safely prior to marketing, and an aquaculture working group has recently been established with the French Ministry by the Comité National De La Conchyliculture to pursue the traceability issue. The European Aquaculture Advisory Council (AAC) has recently published a recommendation for the European Commission and Member States on a Delegated Act to amend Annex III to Regulation 853/2004 of the European Parliament and of the Council on specific hygiene requirements for food of animal origin. This proposed modification details the possibilities of introducing a NoV criterion, stating that as soon as a reliable, rapid and inexpensive NoV test, discriminating between infectious NoV and non-infectious NoV, is made available, the AAC will request the addition of a microbiological criterion relating to NoV, measurable by such a new test.

A counter-proposal put forward by French authorities and shellfish farmers recommended including the NoV risk in the Hazard Analysis and Critical Control Point (HACCP) plan of shellfish operators, implementing a specific health risk management plan by shellfish operators following a risk analysis, improving communication between the shellfish and wastewater sectors, and measures to reduce sources of contamination. Lainé concluded by touching upon the OXYVIR 1 & 2 projects, which seek to further investigate the infectivity of NoV. *(See further information on these projects below).*

### Sarah Horsfall – The Shellfish Association of Great Britain 'The UK Perspective'

Sarah Horsfall provided the UK perspective, outlining that the UK shellfish sector is small, diverse, and primarily aquaculture-based with some wild-capture. Noting Prof. O'Brien's data from earlier in the session, she reasserted that only a very small proportion of NoV cases in the UK originate from shellfish. She stated that measures are in place to maintain watercourse quality, however, some companies continue to pollute them regardless. She highlighted that whilst NoV is a burden on the industry to solve, the wastewater companies and governments are responsible for its prevalence. She added that the industry currently has no sound scientific basis for legislative recommendation because testing techniques aren't able to determine if the NoV identified is actually infectious. However, bringing in a testing regime could lead to a reduction in sales when the product is perfectly safe. These costs would be borne by an industry that is not the polluter, but the victim of pollution. She concluded that the EU is "supposed to operate a polluter-pays principle", adding that the shellfish industry needs to push for cleaner water courses to achieve an environment conducive to rearing shellfish safely.

### Dr Nicolas Boudaud – OXYVIR project

#### 'Development of a method to estimate the infectivity of human noroviruses in oysters'

Dr Nicholas Boudaud detailed his work on the three-year OXYVIR project, which was completed in 2020. Its aim was to develop and define a reliable indicator of NoV contamination in shellfish, through five work packages (WP). He outlined the project's impact and expected benefits, as follows:

- Financial impact – methods for estimating NoV infectivity in shellfish in order to reduce reliance on end-product testing techniques, which are both wasteful and costly
- Social impact – strengthening of food safety; reduction of viral foodborne outbreaks in shellfish
- Environmental impact – reduction of unjustified batch recalls/withdrawals from the EU market (*reduction of food waste by leaving healthy food on the market*)
- Inputs for shellfish producers and regulatory bodies – surveillance plans better adapted to the management of NoV hazard in shellfish

Of the various work packages under the umbrella of the OXYVIR project, WP 3 had yielded the most promising results. It explored the use of F-specific RNA bacteriophages (FRNAPH) to estimate the infectious NoV hazard in oysters. Findings showed there was a correlation between infectious FRNAPH and infectious NoV in retrospective approaches. However, more work is needed to better understand this correlation. This has given rise to the OXYVIR 2 project, which will seek to validate the observed correlation by comparing infectious NoV and infectious bacteriophage, in relation to temperature, salinity and other treatments put in place during shellfish depuration. Boudaud concluded by highlighting that the “infectious FRNAPH criterion” is already being used in practice by two French oyster farm companies to monitor the efficiency of depuration and release batches onto the market, implying its potential.

#### Dr Kata Farkas – Bangor University

##### ‘New Approaches: New and Emerging Technologies for Detecting Viruses’

Dr Kata Farkas opened by summarising methods of virus detection, namely molecular PCR-based detection (qPCR, dPCR, high input sequencing, viromics), integrity assays (DNase/RNase treatment, intercalating dyes, capsid integrity) and infectivity approaches (plaque assay TCID50, ICC-qPCR). She went on to explain these technologies in detail, and drew the following conclusions:

- PCR-based methods are readily available, sensitive, and results are quickly obtainable. However, such methods can only be used as molecular markers as they do not give results on human health risks.
- Viromics is absolutely crucial to identify new and emerging strains.
- Integrity assays are useful for survival and removal assessments and can improve understanding of viral decay in comparison to molecular methods.
- Infectivity approaches provide an alternative validation method and can be implemented in environmental monitoring, but they are complex.

#### Dr Eunice Pinn – Seafish, presented by Professor Lewis LeVay – Bangor University

##### ‘New Approaches: Risk Management and Regulation’

Prof. Lewis LeVay presented on behalf of Dr Eunice Pinn to introduce the concept of integrating risk management and regulation in potential new approaches. He highlighted that we operate in a regulatory environment and that while some regulations point to controls being risk-based, others suggest working towards suitable detection methods to transfer into a regulatory context. Current conditions for the classification and monitoring of classified production and relaying areas for live bivalve molluscs have proven to be effective and ensure a high level of consumer protection. He therefore posed the question “how do we improve on the current situation in terms of regulation?”, adding that “risk management is about problem solving and decision-making” and adapting the way shellfish operators harvest their shellfish, in anticipation of health risks rather than responding to them. In terms of the hazard, NoV causes gastroenteritis in consumers and outbreaks are seasonal with highest risk levels in winter. He reiterated the headlines from Prof. O’Brien’s data earlier in the session, stating that 75% of foodborne cases of NoV can be attributed to food handlers, 20% to fresh salads, 3% to soft fruit and 3% to oysters. Although methods for detecting NoV are well-established, less than 10% of the virus detected are thought to be infectious, thus proposed control measures, which are based on current testing methods are deemed overly restrictive, with high testing frequency also placing significant economic pressure on the industry. In addition, current measures rely on *E. coli* as a proxy for faecal-indicator quality standards but NoV infections associated with shellfish meeting these standards can still occur. He concluded by asserting the need to develop responsive adaptation as knowledge evolves, potentially through identification of viral risk using alternative indicator organisms, and adaptive management utilising environmental indicators of high-risk periods to complement, or be

integrated into, shellfish regulations. This was illustrated by the approach described by Audrey Lainé in her earlier presentation.

### Dr Shelagh Malham – Bangor University

#### ‘New Approaches: Alternative Risk Based Approaches for Shellfish’

Dr Shelagh Malham offered the workshop a detailed insight into her research on risk-based approaches for shellfish management. She described how investigation of environmental predictors of *E. coli* accumulation in shellfish is being applied in the development of predictive catchment-based models. These use environmental data to predict elevated virus risk levels in shellfish and could be used to inform adaptive management responses during periods of predicted high risk. She offered further concepts to support a risk-based approach, namely surveillance-based active management, harvesting controls and wastewater surveillance, the latter of which she noted has grown significantly in response to the Covid-19 pandemic. Combining this information with data for self-reporting of illness, perhaps from an app, could provide a significant step forward in the development of human population health surveillance.

## Open Plenary Discussions

Delegates were invited to enter into open discussion around four key topics, given as follows:

- NoV as a problem for shellfish industry and human health protection.
- Methods for detecting NoV in shellfish – what improvement over current methods is needed to achieve industry trust and public health objectives?
- Is end-product testing a suitable approach, if testing methods can be improved?
- Could NoV regulation be approached differently – for example a risk-based approach with adaptive management?

### NoV as a problem for shellfish industry and human health protection

It was widely agreed that NoV is a problem for the shellfish industry and protection of human health. Representatives from regulatory bodies acknowledged the need for risk-based judgements on regulation and enforcement, adding that work is underway to engage with stakeholder groups to ensure that, wherever possible, management of NoV risk in shellfish can be met with joint solutions. It was noted that a large part of the problem is linked to caterers and food handlers with only a very small number of gastroenteritis cases pertaining to live bivalve molluscs. Sending out clear messaging regarding NoV hygiene controls at both the catering and public levels would be a positive step forward. It was remarked that some production areas vary considerably in terms of risk of shellfish containing NoV at certain times of year, therefore, it is important to try and understand these risks and the methods to control for and mitigate them. It was agreed that a sensible approach would be to treat cooked and raw shellfish independently, as the risks to consumers are substantially lower after cooking, and a majority of participants who commented on this issue agreed that regulatory standards should reflect this. However, it was also pointed out that NoV is not specific to shellfish, indeed, the whole food chain is contaminated including salad, vegetables and fruit. There was strong consensus that a holistic and pragmatic approach, which prioritises improved, long-term wastewater management will be a core part of the solution to safe and healthy shellfish production.

### Methods for detecting NoV in shellfish – what improvement over current methods is needed to achieve industry trust and public health objectives?

The discussion touched upon the fact that proposed EU legislation is targeted at raw shellfish (oyster) consumption. This is set out in the proposed revisions of Regulation (EU) No 853/2004 Annex III, which include testing of 10% of live shellfish destined for raw consumption with a NoV threshold of 500

genome copies per g (DG SANTE working document 10432/2020, March 2020). This level of sampling represents a significant cost burden on producers, while the use of the current testing regime risks over-representation of risk with consequent potential for disproportionate rejection of batches. The group acknowledged that the current NoV control system is inaccurate and unreliable. There is a real need for a new and effective testing methodology to replace the current system, which is fundamentally unable to identify accurate risk of infection. The concept of a simple and practical test for shellfish farmers to use on their products was well-received. Developing an affordable and simple test that can be rolled out to shellfishermen would help inform them as to the level of risk. This would allow farmers to assess NoV in-situ and determine whether to depurate shellfish or transfer them straight to market.

### Is end-product testing a suitable approach, if testing methods can be improved?

There was some debate on this topic, reflecting the concern about the potential impact of proposed introduction of NoV end product testing in regulation (EU) 853/2004 (see above). Views expressed focused on the fact that end-product testing can be expensive and often wasteful, thus the socio-economic cost may be very high. Testing for NoV using the F-specific RNA bacteriophage (FRNAPH) approach could also yield promising results in the future, particularly given that the “infectious FRNAPH criterion” is already being used alongside traditional methods by two French oyster producers to measure depuration efficiency. No formal criteria have been established yet, but for the two years in which both producers have trialled this method, there have been no batch returns.

The majority of delegates agreed with the scientific participants’ position, that the current testing approach was largely irrelevant because quantifying NoV genome copies in shellfish does not indicate the risk to the consumer. Instead, resources should be directed towards researching a technique to discriminate between active and inactive virus and determine the level of infectivity. Understanding the infection threshold of the virus was acknowledged as a critical research gap. A quick and inexpensive test to identify the infectious virus, which can be widely rolled-out and incorporated into legislation would be the gold standard. If the findings of the aforementioned FRNAPH bacteriophage trials are successful, scientists from the OXYVIR research project believe it could become a practical routine testing method within a few years – it’s simple, inexpensive, fast and yields excellent results. In France, the vision would be to incorporate the approach into current ISO controls as an appendix, thus providing an additional management tool within their already adaptive system.

### Could NoV regulation be approached differently – for example a risk-based approach with adaptive management?

A risk-based approach, such as that undertaken in France, which could include wastewater and pollution-source testing combined with weather event and public health alerts, may be a more cost-effective and preventative approach. Equally however, there may be a socio-economic cost associated with a risk-based approach, if a high incidence of NoV exists in the environment. Discussions centred on the issue of wastewater; in order to inform adaptive management plans, water quality information is needed to drive the underpinning of risk models. Wastewater companies and governments do not currently proactively share this information. However, surveillance sampling of wastewater could be an effective basis for risk-based management as it is usually the source of the virus in shellfish and can be easier to work with, as fewer samples would be needed compared to sampling the shellfish themselves. As a consequence, results could proactively inform management. A hope expressed by several presenters and delegates from across the spectrum of stakeholders was that systems adopted in Covid-19 prevalence monitoring efforts focused on wastewater sampling have the potential to improve surveillance techniques both for emerging viruses and for existing risks such as NoV. There was strong consensus that unless governments place greater restrictions on wastewater companies, their current ‘license to pollute’ will continue, and NoV will persist in the environment. In order to enact a risk-based approach, it was felt that the prime responsibility should be placed upon the producer; ultimately

providing them with better business security, and better agency within their own sectoral landscape. However, at the close of the workshop there was also strong agreement that ultimately cleaner water is the best long-term solution to NoV prevalence in shellfish.

## Conclusions

Taking stock of the day's discussions, it was apparent that the full spectrum of stakeholders in attendance – industry representatives, scientists and regulators – engaged deeply on the key issues around NoV contamination in shellfish, including the need to develop effective NoV controls and improve communication of the issues. There was broad acceptance and understanding that the current regulatory system is not fit-for-purpose, using a scientific method that cannot accurately measure and communicate the risk of active infection. Concerns were expressed that proposed changes in EU regulations may create a disproportionate burden on shellfish producers, due to the high cost of the level of sampling required and the uncertainty in measuring actual risk to consumers with the currently available method of testing for NoV. The workshop concluded that developing an effective test, which can determine the infectivity of NoV and the associated risk to consumers, in addition to improving monitoring of environmental water quality should be target priorities. To achieve this, resources should be allocated to targeted scientific research, such as the FRNAPH approach.

The French industry have developed systems for management of NoV in harvested shellfish through improving their understanding of the factors that increase infection risk, and building a system that is both flexible and adaptive, responding to combinations of risks, or increases in individual risk-factors. This approach enables the shellfish industry with agency to participate in regulation of product quality and safety, within the existing regulatory framework. As a result, NoV outbreaks in France are well monitored, and suitable contingency measures are in place to prioritise consumer health should outbreaks occur. It was broadly agreed that this risk-based approach, which doesn't solely rely on tests that have not been proven to accurately capture risk, but instead employ a range of environmental and public health indicators alongside testing, may provide a more accurate picture of outbreak occurrence, as well as greater control and certainty for the industry. Looking ahead, application of improved real-time monitoring of wastewater for levels of viral contamination could further improve the effectiveness of risk-based control on harvesting shellfish. The importance of communication was a common thread in several respects: in terms of accurately communicating where risk exists to producers, in order that they can adapt; in terms of accurately conveying risk to consumers; and in terms of governments and water companies improving communication with industries affected by wastewater pollution. Whilst NoV is a significant burden on the shellfish industry, an overarching perspective from the workshop was that shellfish producers are not the polluter; release of contaminated wastewater into the environment is the source of NoV prevalence in shellfish. In the long term, persuading governments to implement policies for the protection of water quality, and thus ensuring wastewater companies practise transparent reporting and meet their obligation to deliver clean water, will be critical.

## Glossary of Key Terms

Bacteriophage – a virus that grows and divides inside a bacterium

Capsid – protein shell of a virus particle

Capsid integrity assay – a method for detecting the proportion of intact viable virus particles in a sample

Depurate/Depuration – to make or become free from impurities, applied in shellfish to treatment with clean water to reduce bacterial contamination

*E. coli* – an enterobacterium (*Escherichia coli*) that is used in public health as an indicator of faecal pollution (as of water or food) and in medicine and genetics as a research organism

Gastroenteritis – inflammation of the stomach and intestines

High input sequencing – technologies that **sequence** DNA and RNA at high resolution in a rapid and cost-effective manner

Infectivity – the ability to produce infection

Microbe/microbial – a microorganism, especially a pathogenic bacterium

Norovirus – any of various single-stranded RNA viruses of the genus *Norovirus*, of the family Caliciviridae: the most common cause of epidemic viral gastroenteritis in humans

PCR – polymerase chain reaction: An in vitro technique for rapidly synthesizing large quantities of a given DNA segment

qPCR and dPCR – quantitative methods used for measuring amounts of DNA or RNA using PCR

RNA – ribonucleic acid, a nucleic acid present in all living cells. RNA has a range of cellular functions with a principal role in acting a messenger carrying instructions from genetic code in DNA for controlling the synthesis of proteins. In some viruses, including Norovirus, RNA also carries genetic information.

Viral load – the amount or concentration of a virus

Viromics – the study of viruses at genome and population scale



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