Norovirus Detection & Purging Strategies for Oysters:

a Plain English Overview of the State of the Science

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Outline

- What is norovirus?
- Marine water quality standards
  - Illnesses can (& do) still occur
- Challenges measuring norovirus
- Effectiveness of depuration
  - Temperature
  - Salinity
- Relay effectiveness
- Recommendations
- Emerging & Alternative Approaches

Norovirus detection and purging strategies for oysters

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What is norovirus?

- Human norovirus (HuNoV or NoV) is a highly contagious pathogen responsible for gastroenteritis.
- The virus enters marine environments primarily through fecal matter present in wastewater discharge and septic runoff, which increases during periods of frequent rain and storm events.
- Norovirus has a 12-to-48-hour incubation period. People can have, and spread it, before ever experiencing symptoms.
- The increased prevalence of norovirus in winter amplifies risks to public health and the shellfish industry, leading to economic losses and regulatory challenges.

NOTICES

Notice of Illness Outbreaks, Shellfish Closures, Reopenings, & Recalls

Click here to be added to the ISSC Recall Distribution List

In this section

Illnesses

Biotoxins

Norovirus

Vibriosis

What are the symptoms of norovirus?

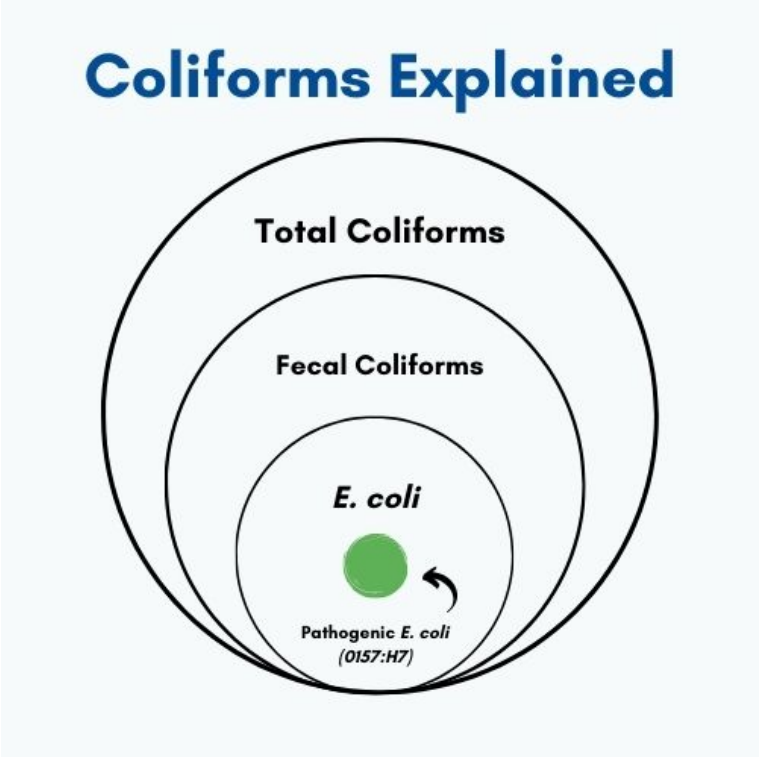
The most common symptoms of norovirus are stomach pain, projectile vomiting, and severe diarrhea. Other symptoms may include fever, headache, and body aches. Some people can be infected with norovirus and have no symptoms. Good hygiene and hand washing, especially after using the bathroom and when handling food, are important to help limit the spread of norovirus.

How soon do symptoms appear?

Symptoms usually appear 24-48 hours after being exposed to the virus. Sometimes symptoms appear as early as 12 hours after exposure. Most people recover in 1 to 3 days.

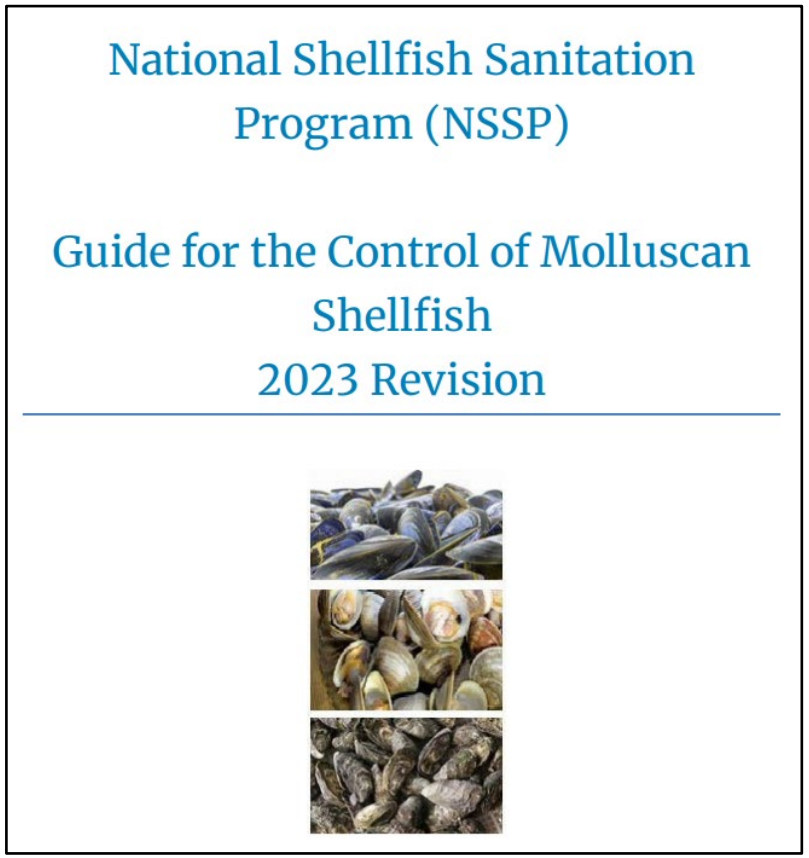
NoV & Marine Water Quality Standards

- NoV testing has been unreliable in the past.
- It's still extremely difficult to obtain NoV testing by an accredited lab.
- Marine water quality monitoring protocols in the U.S., Canada, and Europe instead rely on *fecal coliform levels* as an *indicator of pathogenic contamination*, along with point source contamination assessed during shoreline surveys, to trigger precautionary regulatory classifications and requirements.



Source: Alabama A&M & Auburn Univ. Extension
https://www.aces.edu/blog/topics/fish-water/coliform-bacteria-in-well-water/

NSSP & ISSC



The National Shellfish Sanitation Program (NSSP) is a partnership between states & federal agencies, recognized between the FDA & the Interstate Shellfish Sanitation Conference (ISSC).

Illnesses Can Still Occur

- This approach is problematic since NoV persists longer in oysters and responds differently to commercial purification methods than fecal coliform bacteria.
- As a result, illness outbreaks can occur even when oysters are harvested in approved and open growing areas, including in Europe, where they have also undergone locally prescribed depuration (purification) processes.

Food Standards Agency
food.gov.uk

Shellfish classification

How production areas are classified, the method of treatment and how to apply for classification.

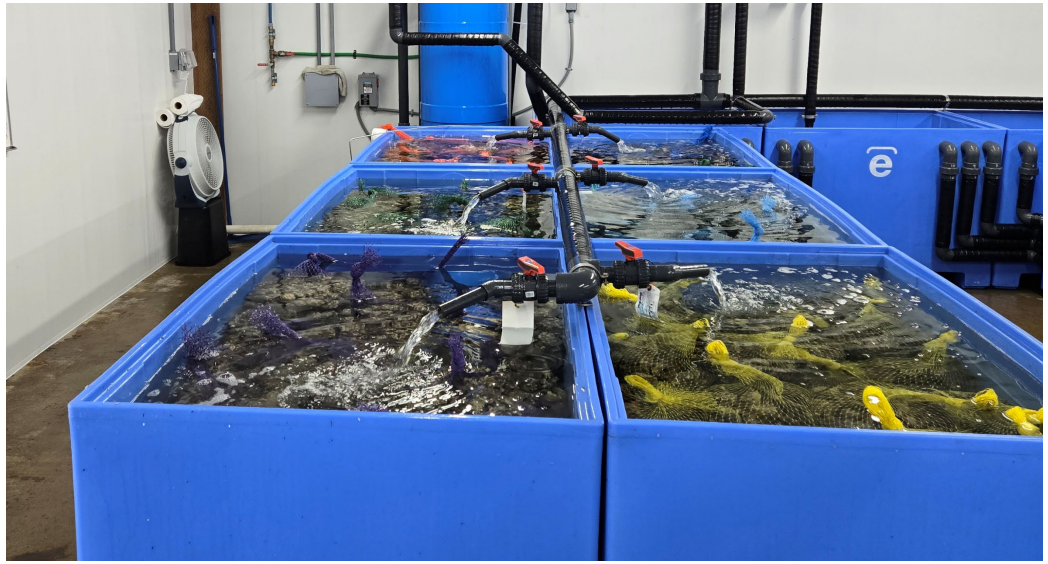
The classification of a production area determines the treatment required before Live Bivalve Molluscs (shellfish) may be marketed for human consumption.

Shellfish production and relay areas are classified according to the levels of E. coli detected in shellfish flesh.

Measuring NoV

- Levels of NoV in seawater and shellfish are currently determined using reverse transcriptase polymerase chain reaction (RT-PCR), which *quantifies total RNA*.
- Total RNA includes *both infectious virus and inactive viral* particles.
- While total RNA below the level of detection (LOD) would suggest that shellfish are safe to consume, total RNA above the LOD does not necessarily indicate infectivity.
- Fecal coliform is not a good measure for determining NoV infectivity, only potential presence.

- NoV in shellfish remains a critical public health concern, necessitating additional research to improve post-harvest treatment methods such as relaying and depuration.



Relay & Depuration: Temperature

- Current relay and depuration parameters are appropriate for removing bacterial pathogens, such as *Vibrio spp.* (e.g. *Vibrio vulnificus, Vibrio parahaemolyticus*) and *Escherichia coli*, but may not be sufficient to remove NoV, especially when contamination levels are high.
- Studies have demonstrated that **temperature** plays a key role in the efficiency of NoV depuration, with elevated temperatures yielding higher rates of reduction.
- Elevated temperatures may increase stress on oysters and increase risk of Vibrio proliferation.
- However, while many recent studies have shown promise in significantly reducing total NoV through depuration, few have achieved removal below the limits of detection (LOD) or quantification (LOQ; 100 genomic copies (gc)/g) within the study timeframe.
- Those that were the most successful, relied on sampling naturally contaminated oysters in which initial NoV concentrations were <1000 gc/g at higher depuration temperatures.

Relay & Depuration: Salinity

- **Salinity** also plays an important role in depuration efficacy.
- Better NoV removal has been observed when salinities closely match those found in estuarine grow-out areas (25-30 ppt).
- Depuration of naturally contaminated Pacific oysters at 25 ppt showed better removal of NoV than at 35 ppt.

Depuration Recommendations

- PSI's literature review concluded that ***depuration to reduce norovirus is likely to require longer durations than are practical***.
- The reviewed literature supports depuration at temperatures between 15-18°C.
- Salinities between 25-30 ppt is most effective.
- Natural, raw (non-UV or chemically treated) seawater is recommended.
- Success is dependent on an initial viral load of <1000 copies/g.
- Some studies show NoV may reach the Limit of Detection within 4-5 weeks.
- There is still uncertainty surrounding the time required to fully inactivate.

Relay Recommendations

- ***We focus our recommendations on relay.*** Relay to reduce NoV in oysters has proven effective, but studies are limited.
- Placing oysters in their natural environment provides optimal growing and flushing conditions and harnesses the bioactive, anti-viral properties of raw seawater.
- Relaying oysters has demonstrated to have the most promising potential for effectively reducing total NoV to background levels within 4-5 weeks.
- There is evidence that a two-week period of relaying at a clean site, followed by six days of depuration at elevated temperatures (15-17°C) is also effective.

Relay Recommendations (cont.)

Battistini et al. 2022 state (emphasis added):

- The application of long-term relaying instead could provide a practical, less expensive, and natural alternative to other methods by enabling the reduction of NoV concentrations to levels that reduce, if not eliminate, the risk to consumers.
- Long-term relaying is especially important for producers that can move the oysters to less contaminated sites close to other more contaminated production areas.
- Considering that the oyster takes 18 to 24 months to become an adult or reach market size (about 3 inches), it would be a matter of moving oysters to the relaying sites one month before being sold, *in the winter season only*, so to minimize the extra costs related to the relocation process.

Battistini, R., Masotti, C., Maurella, C., Costa, E., Orlandi, M., Feletti, M., Ercolini, C., & Serracca, L. (2022). Study on the Effect of Relaying on Norovirus Reduction from *Crassostrea gigas* Oysters. Microorganisms, 10(12), 2389. https://doi.org/10.3390/microorganisms10122389

Does Relay Pose a Risk?

- As filter feeders, oysters can bioaccumulate pathogens from contaminated water, making them potential vectors for foodborne illness when consumed raw.

BUT

- Unlike Vibrios, ***norovirus does not grow in oysters!***
- In marine water, and oysters, norovirus is present or it's not.
  - It doesn't proliferate, it only decays (e.g., dies).
  - It cannot "inoculate" a new marine waterway.
  - If an oyster is contaminated with norovirus, it does not pose a threat to uncontaminated waters.
  - Norovirus is not an invasive species or HAB.

Emerging & Alternative Approaches

- **Plasma-activated seawater (PASW)** has shown promise for total inactivation of NoV within 24 hours of depuration, though its effects on oyster stress and viability need further investigation.
- Addition of **hydrosols** could increase depuration efficiency. C. Limon hydrosol (1%) exposure significantly reduced (.2 log) NoV.
- **Photoinactivation** using Red Bengal encapsulated algal beads is being examined for its efficacy in reducing viral loads. It was shown to reduce other viral pathogens but has not been tested on NoV.
- **High Pressure Processing (HPP)** of 350-600 MPa shows significant reductions of NoV GI and GII at various temperatures.
  - HPP shows promise only at pressures above 600 MPa, but this pressure may reduce the end product shelf life.
  - HPP is not an appropriate for live oysters, but can be applied to fresh, shucked meats, as a non-heat treatment.

Questions?

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