

# Strategies to improve the purification (depuration) of norovirus (NoV) from oysters.



## Introduction

Shellfish originating from moderately polluted areas (e.g. class B areas in Europe) undergo depuration in tanks of clean seawater to allow them to purge themselves of microbiological contaminants. Whilst bacteria may be rapidly removed, it is well documented that purified shellfish shown to be free of *E. coli* have been associated with outbreaks of NoV worldwide.

## The Challenge

- Current norovirus detection methods do not indicate whether post-depuration virus levels are viable.
- F+ bacteriophage testing can potentially help to estimate NoV health risks in some cases.
- These tests are not routinely used as are expensive, to some extent developmental and not part of the legislation as yet.
- In the mean time the industry must produce a safe product.
- Enhanced depuration procedures based on scientific evidence of NoV removal and NoV viability will be useful for the shellfish industry to follow, particularly during the winter months when the NoV risk is typically increased.

## Methods

Environmentally contaminated Pacific oysters were depurated under a range of conditions:

- Increased temperature 18°C vs 8°C
- Feeding - microalgae and shellfish diet
- Light vs dark regimes
- Disturbance vs non-disturbance
- Continuous flow-through vs recirculated natural seawater.

Oysters tested before, during and after purification cycle for *E. coli* MPN, F+ bioassay, F+ PCR and NoV PCR

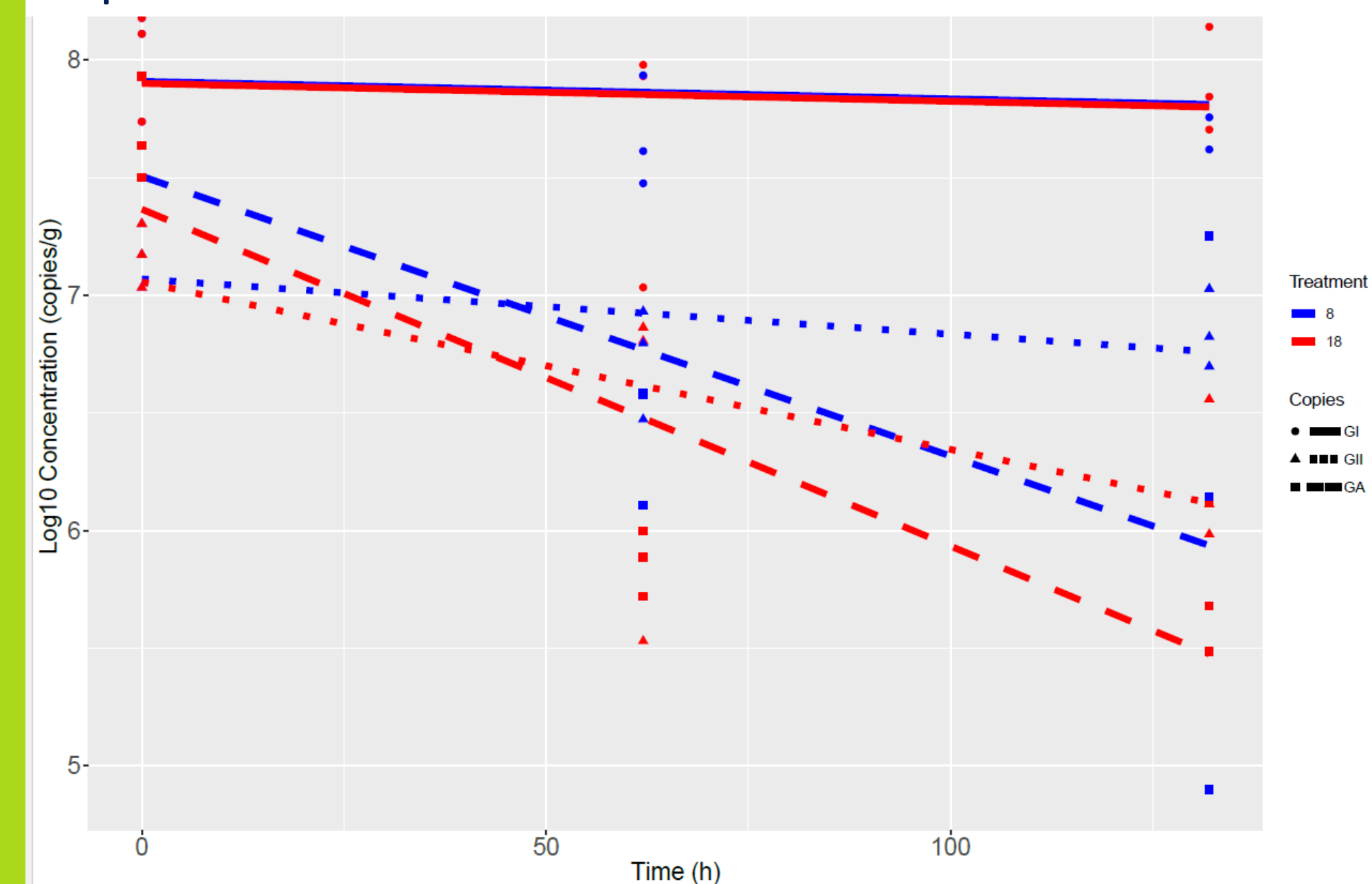


## NoV removal from oysters over 6 days at 2 temperatures.

Graph showing marked differences in NoV GI (continuous lines) and NoV GII (dotted lines) removal.

GA (dashed lines) is a strain of F+ bacteriophage, a surrogate virus indicator also used in these trials for reference purposes.

Reduction of NoV (copies/g) in Pacific oysters during depuration in commercial tanks at 8 and 18°C



## Results and Conclusions

### Key factors

- Temperature: depuration shown to be 2x more effective at 18°C than at 8°C after 5 days ( $p = <0.05$ )
- Salinity: depuration at 25ppt was more effective than at 35ppt ( $p = <0.05$ ) using oysters from a riverine environment with salinity typically in the range 25-30ppt. Suggests salinity in tanks should be aimed to match that of the oyster harvesting area depuration.
- NoV genogroup II was consistently better removed than GI
- Whilst NoV GI removal levels were negligible, NoV GII levels were markedly reduced (up to 60%), indicating that commercial purification can be useful for some strains of NoV.

### Factors with negligible effect

- No beneficial effect of feeding
- No obvious difference between light/dark depuration but warrants further investigation.

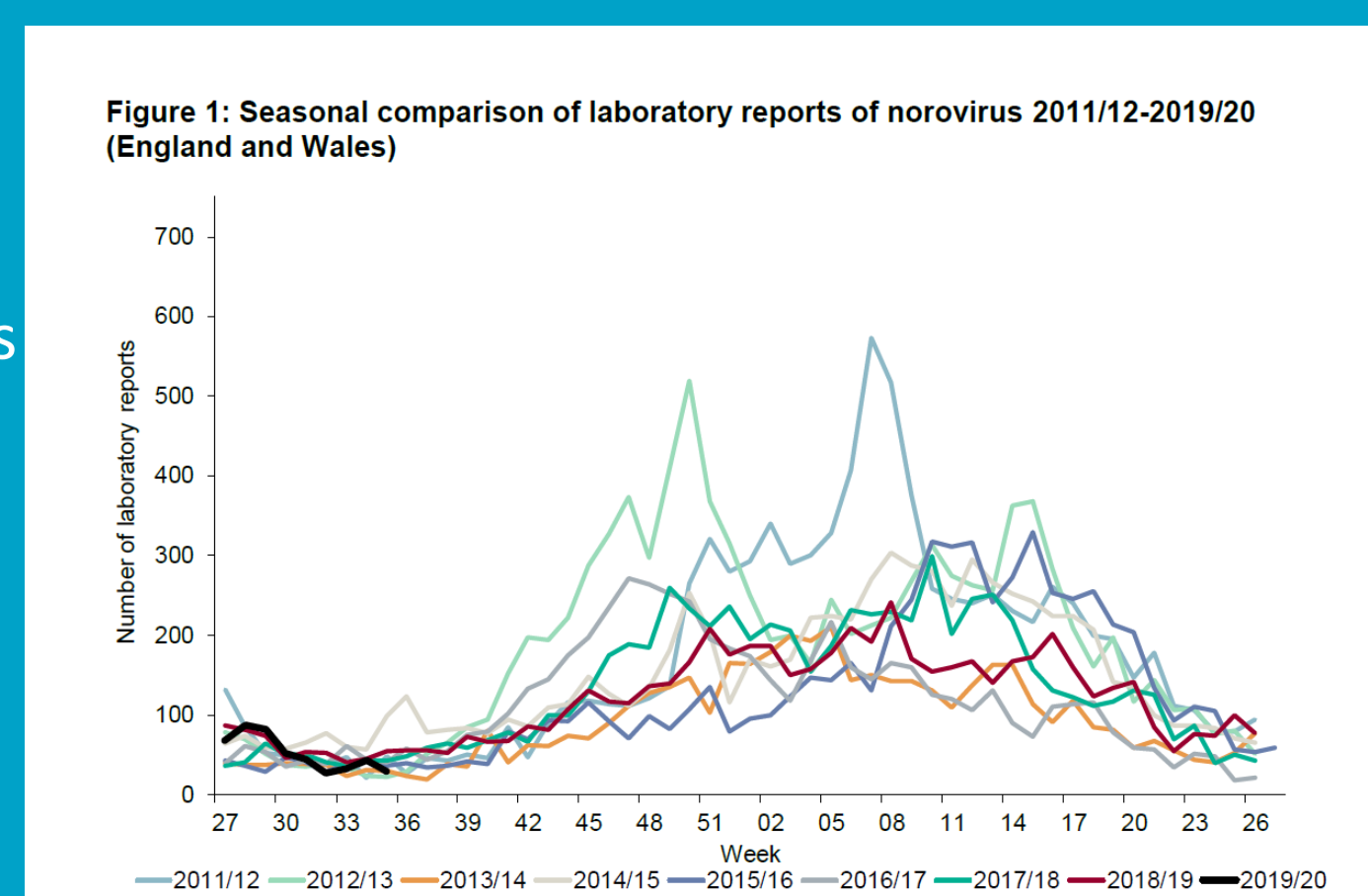
### F+ bacteriophage and *E. coli*

- F+ bacteriophage found to be more readily removed than NoV GI and GII so would not appear to be a good indicator of NoV under depuration conditions based on the results of these trials. *E. coli* consistently removed in all trials (as expected).

## Public Health England NoV Statistics - England and Wales 2011-2019.

### NoV is more prevalent in the winter months.

= Increase in illness in population.  
= Increased NoV in environment.  
= Need for enhanced depuration methods to reduce NoV risk in oysters.



## Further work

- Results of some of the later trials were inconclusive due to low starting NoV levels – need to repeat when NoV levels in environment are higher.
- Further investigation of differences between strains

