Risk management strategy for norovirus contamination in shellfish

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Monitoring Bacteriological and Viral
Contamination of Bivalve Molluscs

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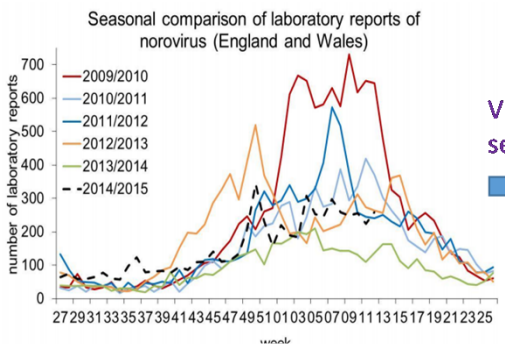
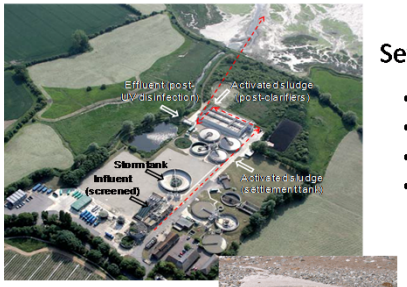
Factors influencing virus risk

Circulation of virus in population

- NoV
  - Season
  - Virus strain
  - Community and institutional outbreaks
- HAV
  - Sporadic (high risk)



Virus loading to sewerage system



Sewage treatment (STW)

- Efficiency of physical removal
- Disinfection
- Reliability (maintenance)
- Location of discharge

Sewer bypasses (CSO)

- Frequency/duration
- Volume
- Location

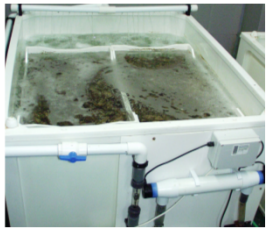
Virus load discharged to marine environment

Contamination of shellfish

- Proximity of discharge to shellfish
- Sewage dilution/dispersion
- Water temperature
- Sunlight
- Meteorological factors
- Environmental reservoirs
- Shellfish species

Contamination levels in harvested products

(FBO monitoring)



Depuration effectiveness

- Water temperature
- Virus loading
- Tank design
- Biology?

Contamination levels in final products

(FBO monitoring)

Illness in consumer

- Virus viability
- Cooking
- Immune status
- Amount consumed
- Epidemiological investigation (meal setting)

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Risk assessment: European Food Safety Authority

Food borne viruses 2011


European Food Safety Authority

EFSA Journal 2011;9(7):2190

SCIENTIFIC OPINION

Scientific Opinion on an update on the present knowledge on the occurrence and control of foodborne viruses[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

Norovirus in oysters 2012


European Food Safety Authority

EFSA Journal 2012;10(1):2500

SCIENTIFIC OPINION

Scientific Opinion on Norovirus (NoV) in oysters: methods, limits and control options[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

Limits: infectivity and dose response

- PCR detects both infectious and non-infectious virus particles
- Growing evidence of a dose response i.e. infectious risk increases with dose (as measured by PCR)
  - In clinical studies (Teunis *et al*., 2008)
  - In restaurant study (Lowther et al., 2010)
  - In outbreak samples (EFSA report, Lowther et al., 2012)
- 'infectious risk associated with low level positive oysters as determined by real-time PCR may be overestimated'
- So ..... although cannot determine safe limit can make risk management decision on a control limit (impact vs public heath gain)
- Since indirect measure of risk sum GI and GII

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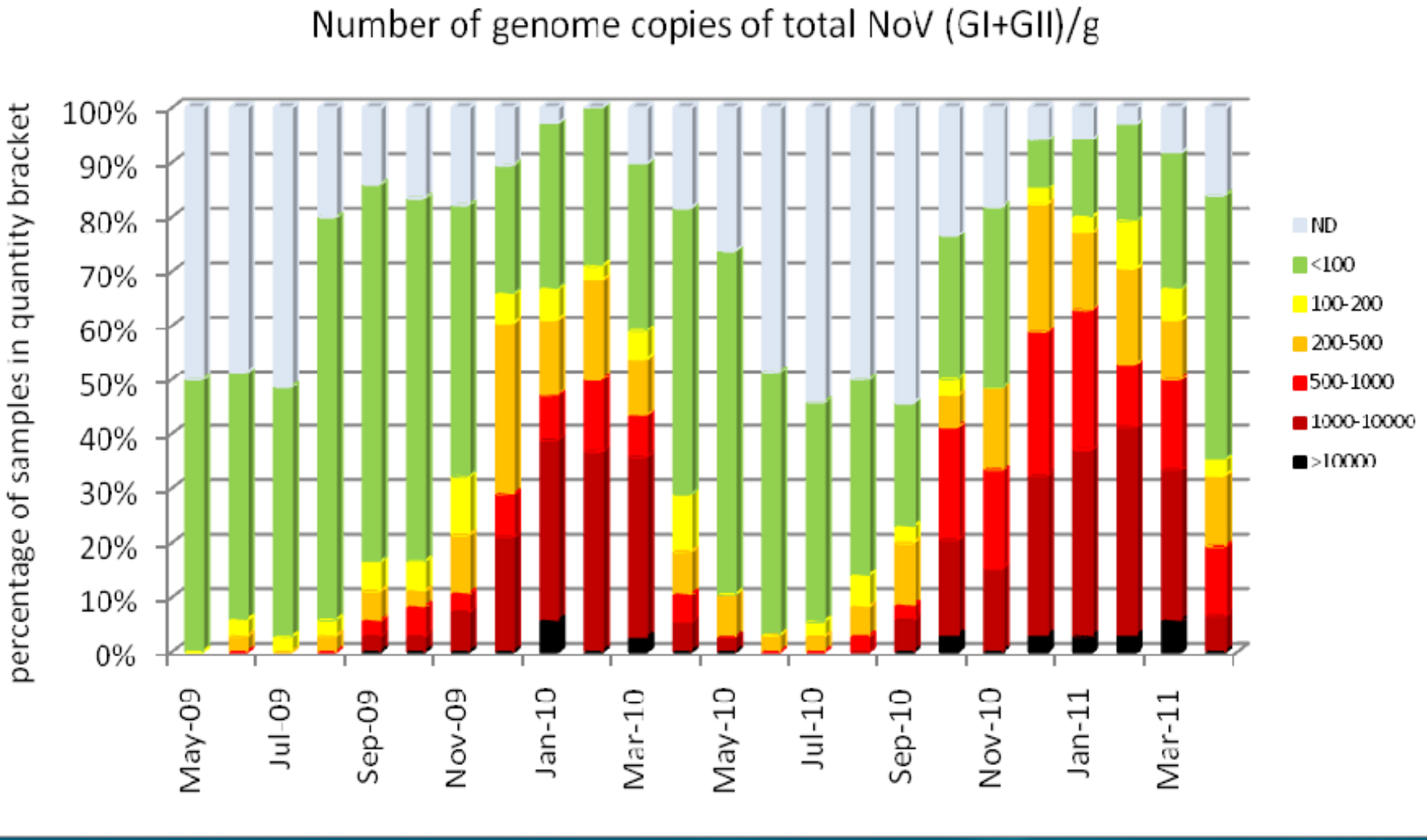
Quantitative data vs possible limits



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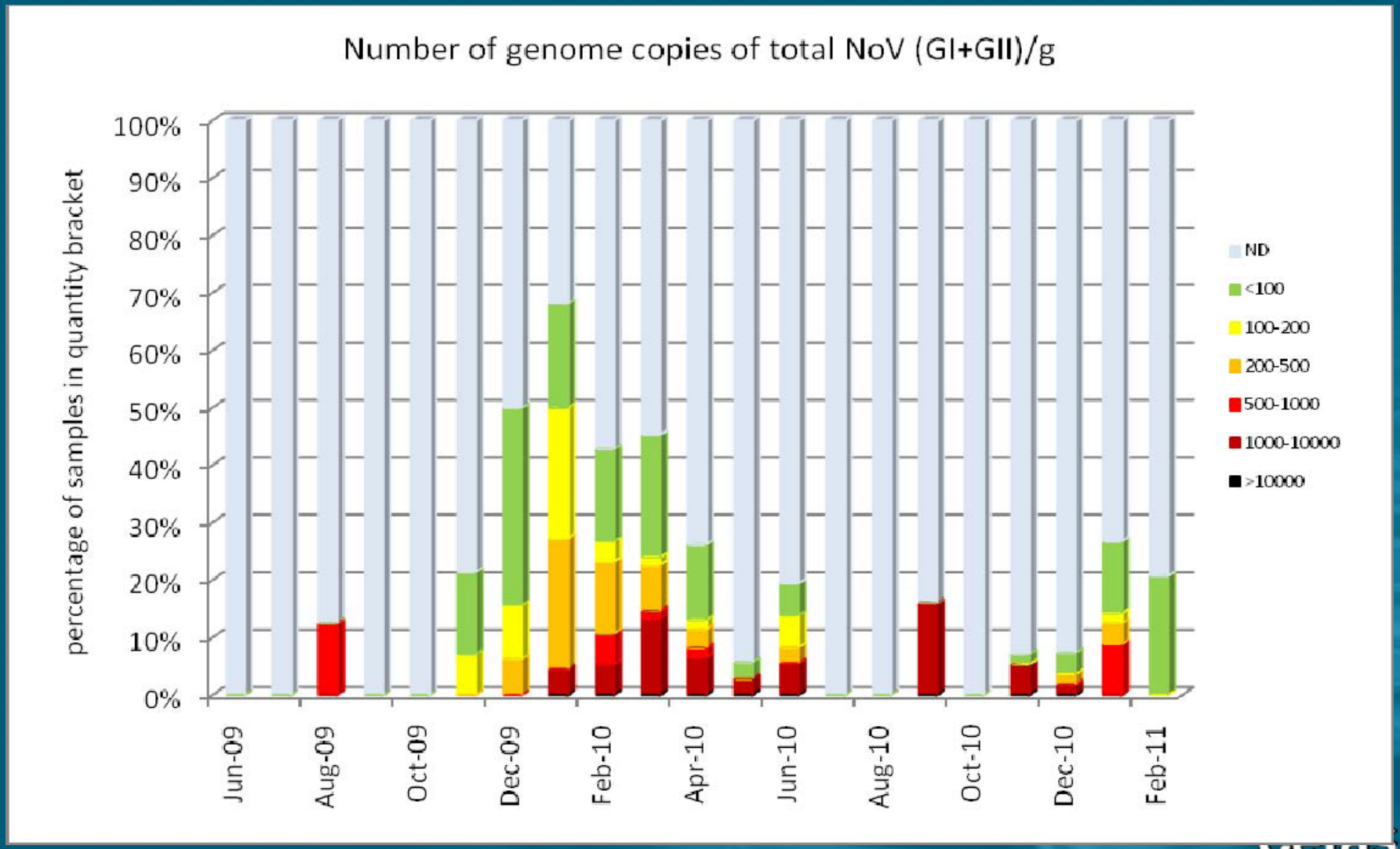
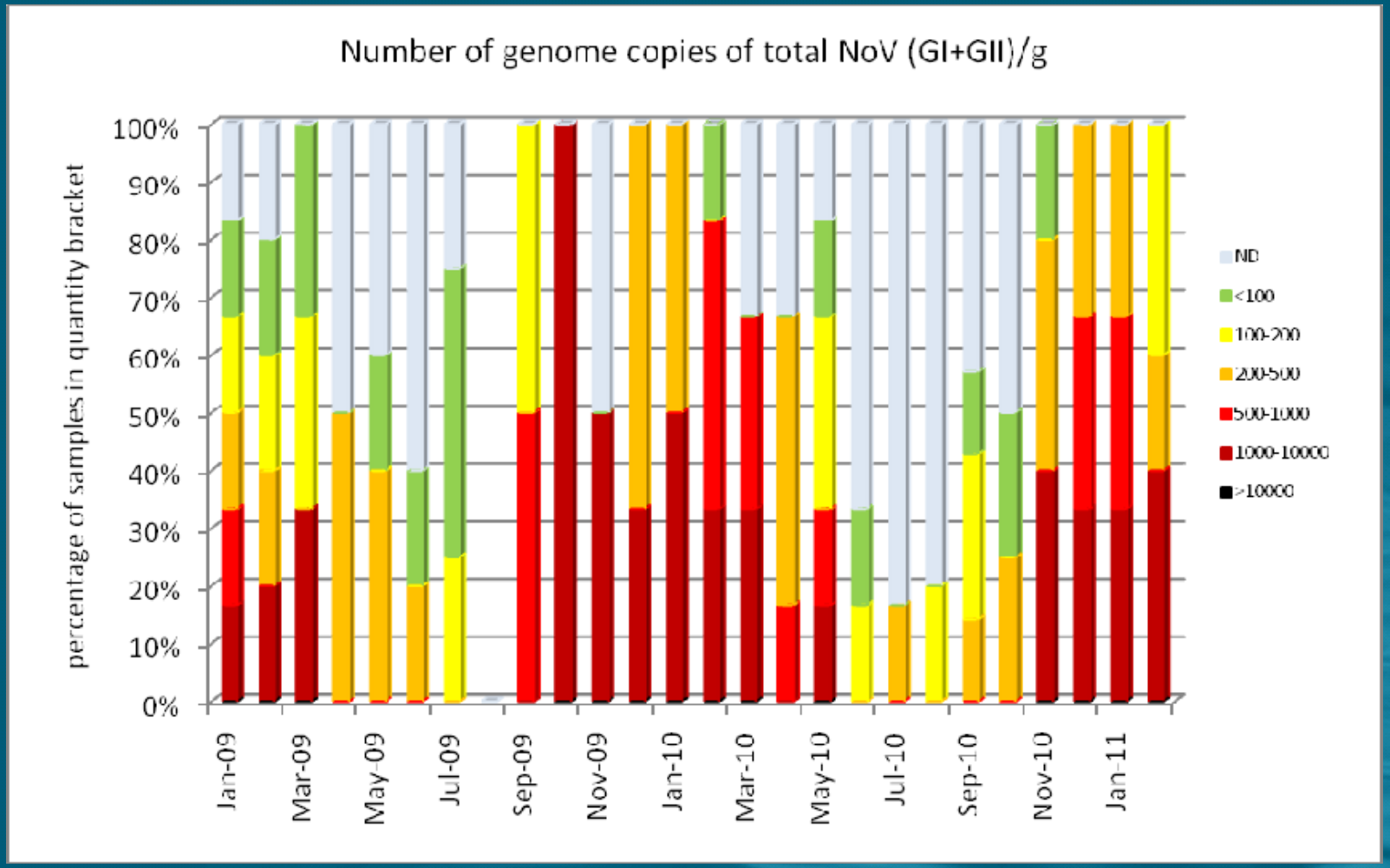
EFSA opinion: Quantitative data vs possible limits France



EFSA opinion: Quantitative data vs possible limits Ireland



Note – worst case scenario (not systematic data)

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EFSA: impact of potential limits for samples from commercial production areas

Table 8: Average percentage of samples that would fail during the high risk season (January to March 2010) if a maximum limit of 100, 200, 500, 1000, or 10,000 genome copies/g were set

	100 c/g	200 c/g	500 c/g	1,000 c/g	10,000 c/g
United Kingdom	65.6%	61.1%	46.9%	37.2%	2.7%
Ireland	83.3%	83.3%	72.2%	44.4%	11.1%
France	33.6%	24.4%	10.0%	7.7%	0%

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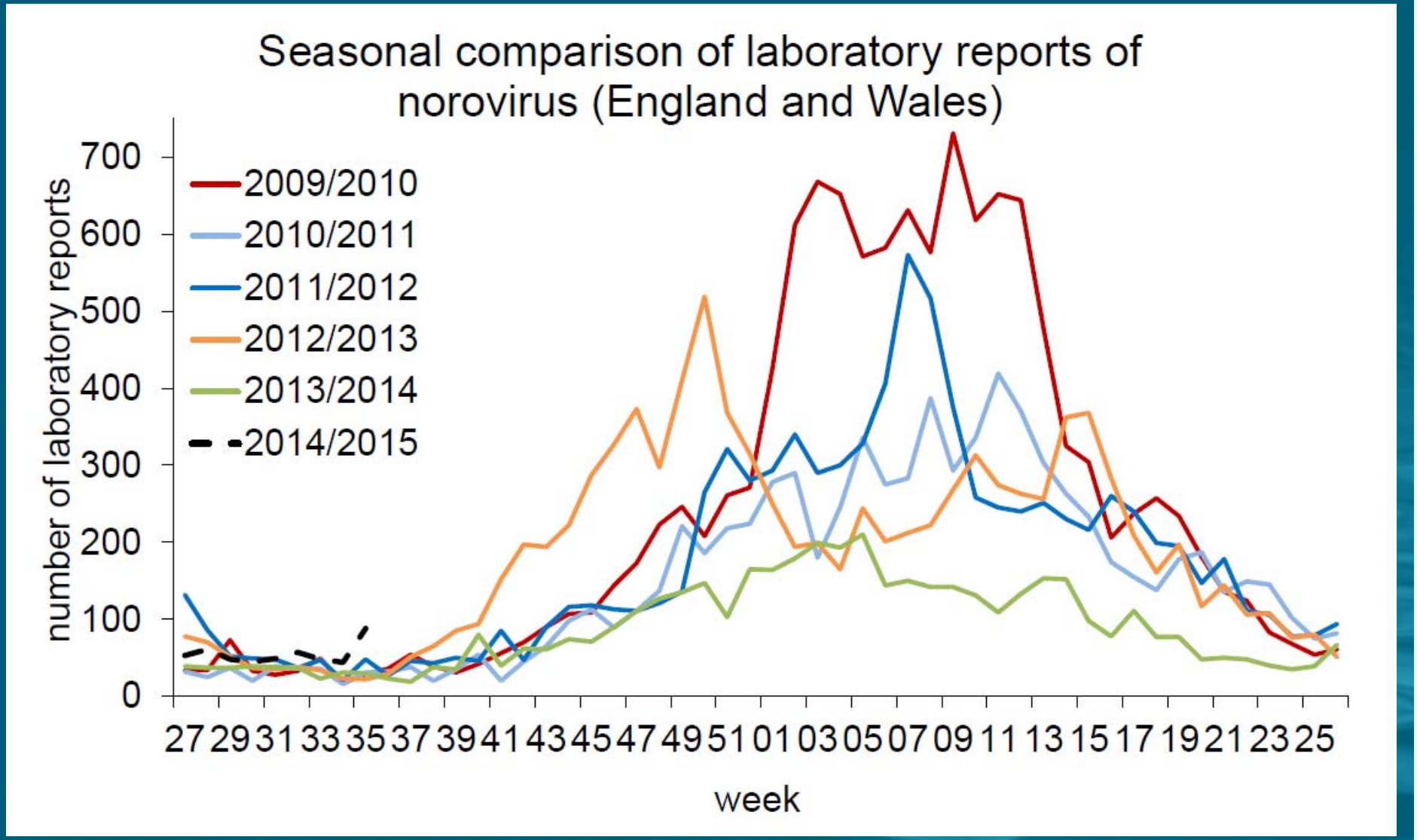
Public Health England – monthly norovirus report



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EFSA conclusions and recommendations

- Virus methods are available and considered suitable for use in legislation
- Dose dependant probability of becoming ill (dose response)
- Risk managers should consider establishing virus limits for high risk LBMs (i.e. those consumed raw)
- Post harvest treatments should be validated for effectiveness against viruses
- Action now rests with the risk managers (European Commission)

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Current state of play

- Virus controls under discussion at EU level
- More data required to underpin any decisions on legislative standards
- EU wide harmonised baseline survey planned (EFSA)

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EU harmonised baseline survey

- Recommendation by European Food Safety Authority (EFSA)
- Supported by EU Member States and producers
- Informed by questionnaire among Member State authorities
  - Target oysters
  - Norovirus and hepatitis A virus analysis using ISO method
  - Include *E.coli* analysis
  - Production areas and end-products but not imports
  - Official sampling (at current RMPs, and in establishments)
  - Sampling design to be established (at least 12 months duration)
  - Collect supporting environmental information
  - Control of quality of analysis (EURL and NRLs)
  - EFSA working group lead/coordinate and report
- Objective
  - Establish levels of Norovirus (and occurrence of HAV) in classified areas, and end-products, representative of production in each MS to understand impact of possible legislative limits

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Interpretation of PCR results vs health risk

- PCR test only detects genetic material of norovirus – no information on infectivity
- Human volunteer studies support concept of dose response
- Correlation between attack rate and norovirus levels in oysters in restaurant study
- Significant difference between levels in outbreak-related and routine +ve samples

- EFSA conclusion: ‘infectious risk associated with low level positive oysters as determined by real-time PCR may be overestimated’

- Research need: methods for differentiation of infectious vs non infectious virus

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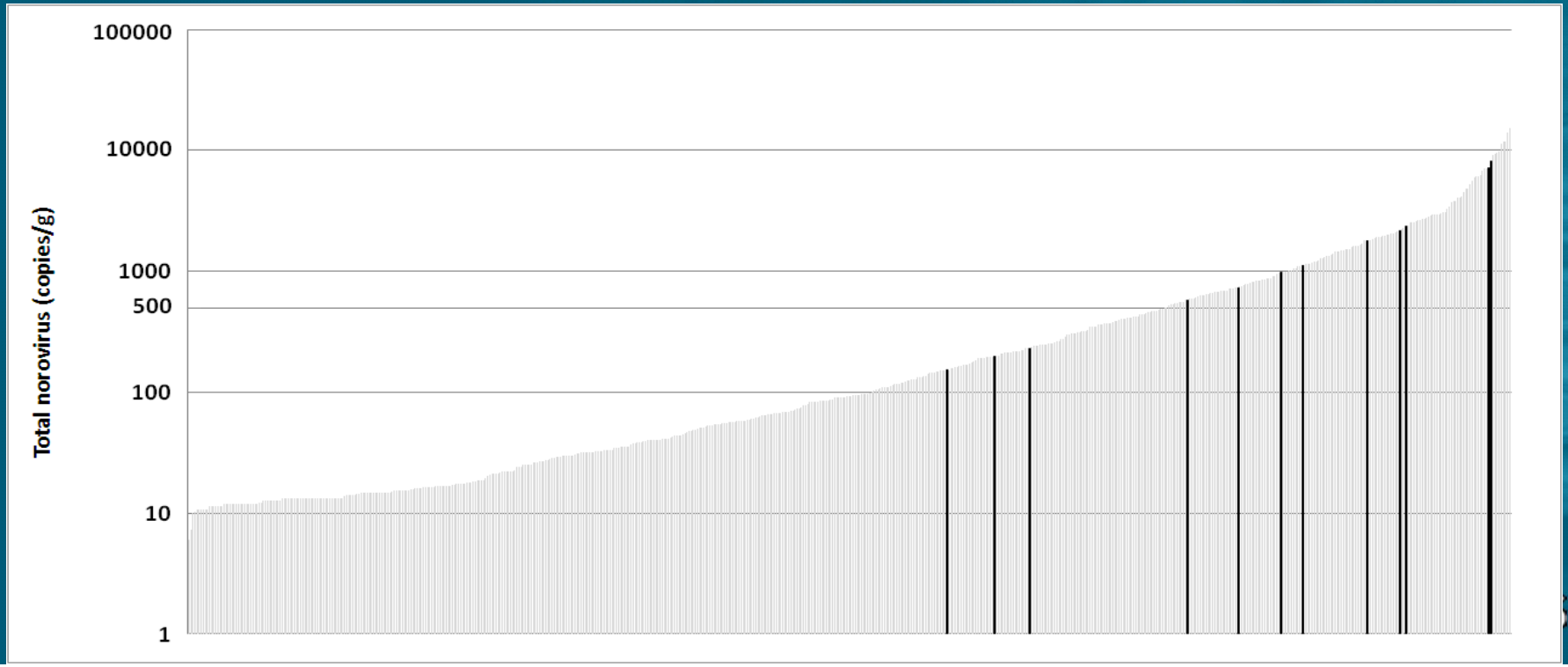
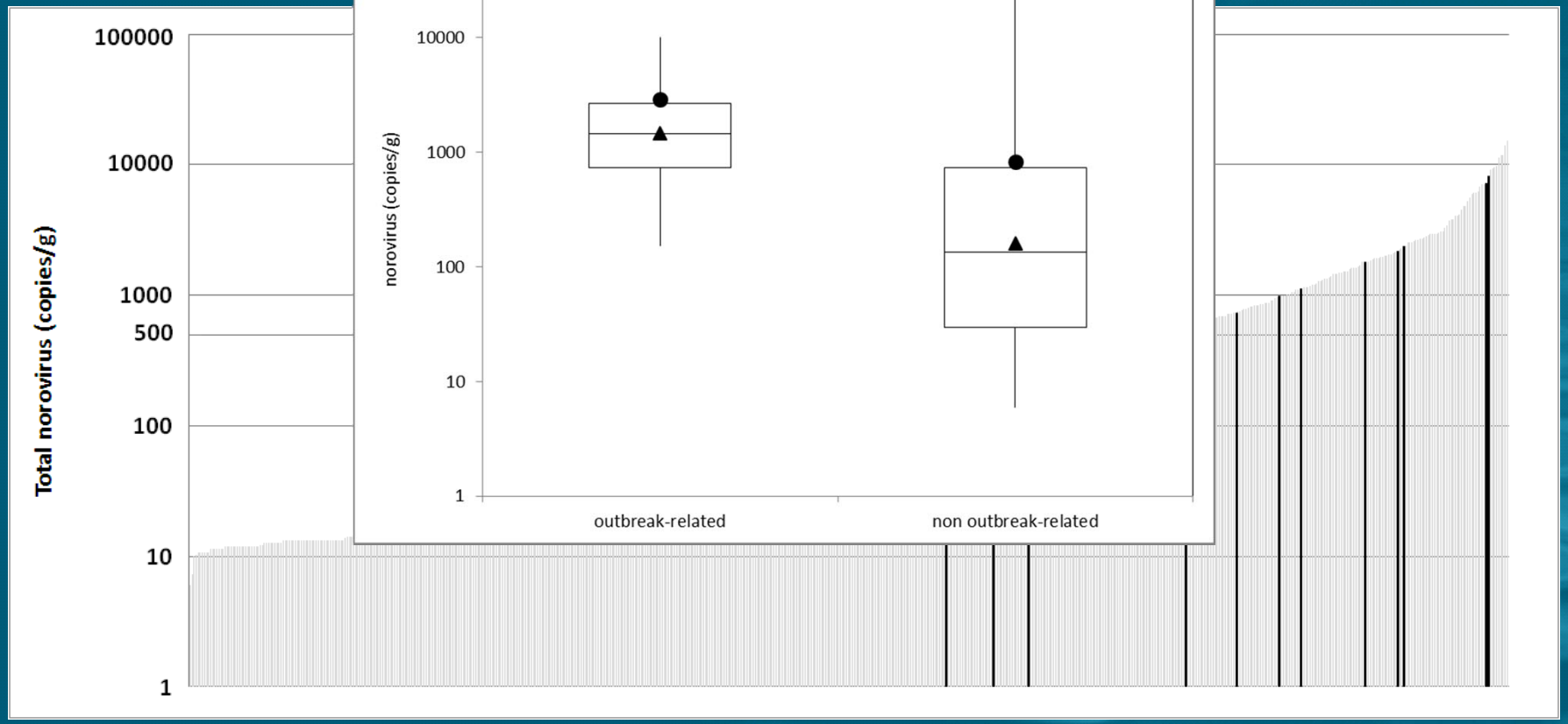
Norovirus levels in outbreak-associated batches of oysters

- All positive samples from 2007-date ranked by norovirus quantity; outbreak samples in black (Lowther et al, J Food Prot. 2012; 75:389)
- Statistically significant difference between levels in outbreak and non-outbreak samples (geomeans 1468 vs 159 copies/g; t test, p<0.0001)
- Lowest total (GI & GII) level observed in outbreak 152 copies/g – levels <100 copies/g frequent (46% of total) in non-outbreak related positives
- Some indication of increased association of outbreak samples with levels >500 copies/g



Norovirus levels in outbreak-associated batches of oysters

- All positive samples from 2007-date ranked by norovirus quantity; outbreak samples in black (Lowther et al, J Food Prot. 2012; 75:389)
- Geomean outbreaks (1,048) vs non-outbreaks (121) – statistically significant difference
- No outbreak



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Health risk vs titre - conclusions

- Oyster samples where norovirus is not detected (using ISO standard methodology) unlikely to present risk of norovirus infection
- Positive samples with levels <100 copies/g unlikely to cause large outbreaks of illness
- Some indication of increased risks as levels increase e.g. >500 copies/g

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Production area pollution control

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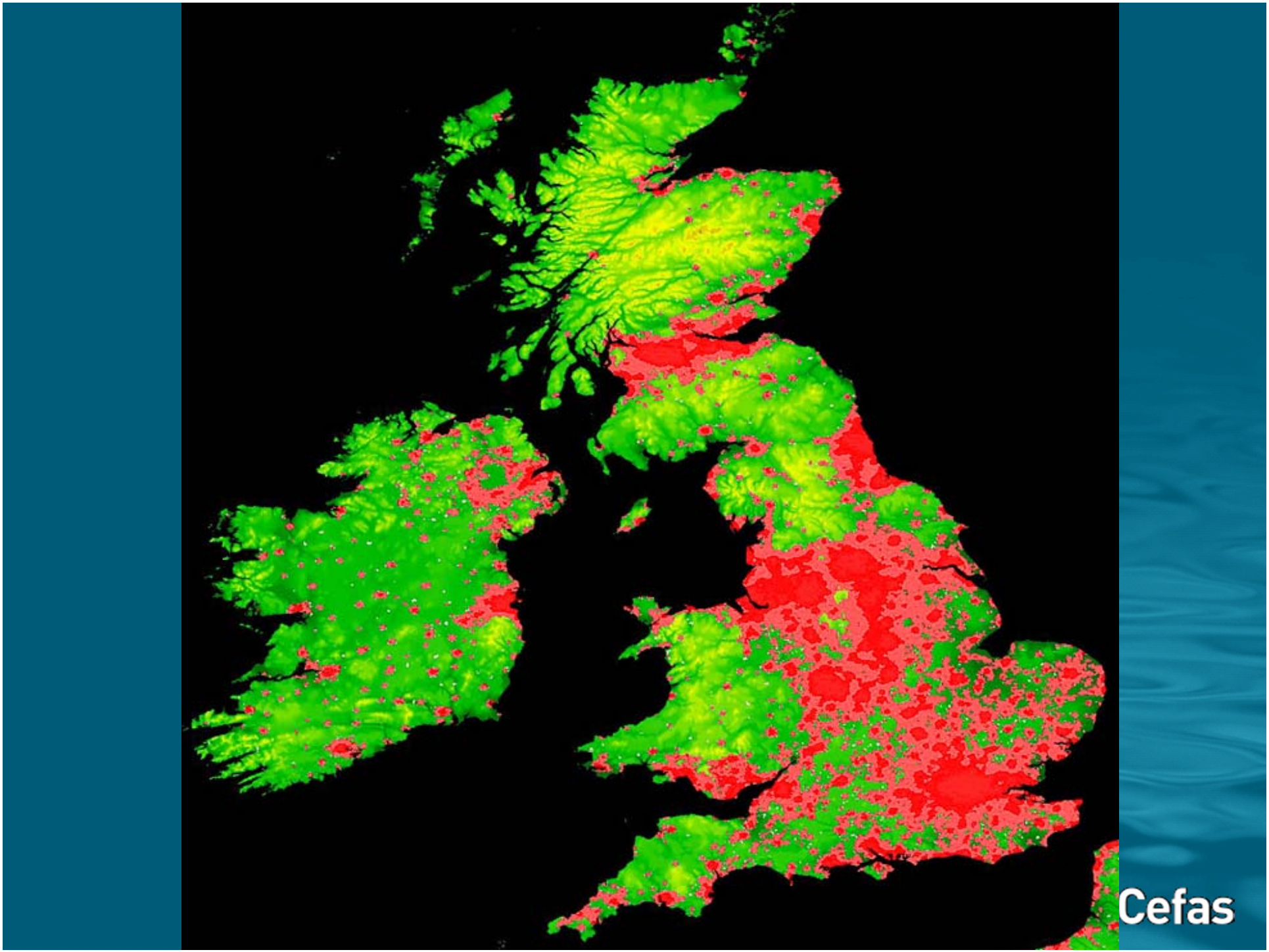
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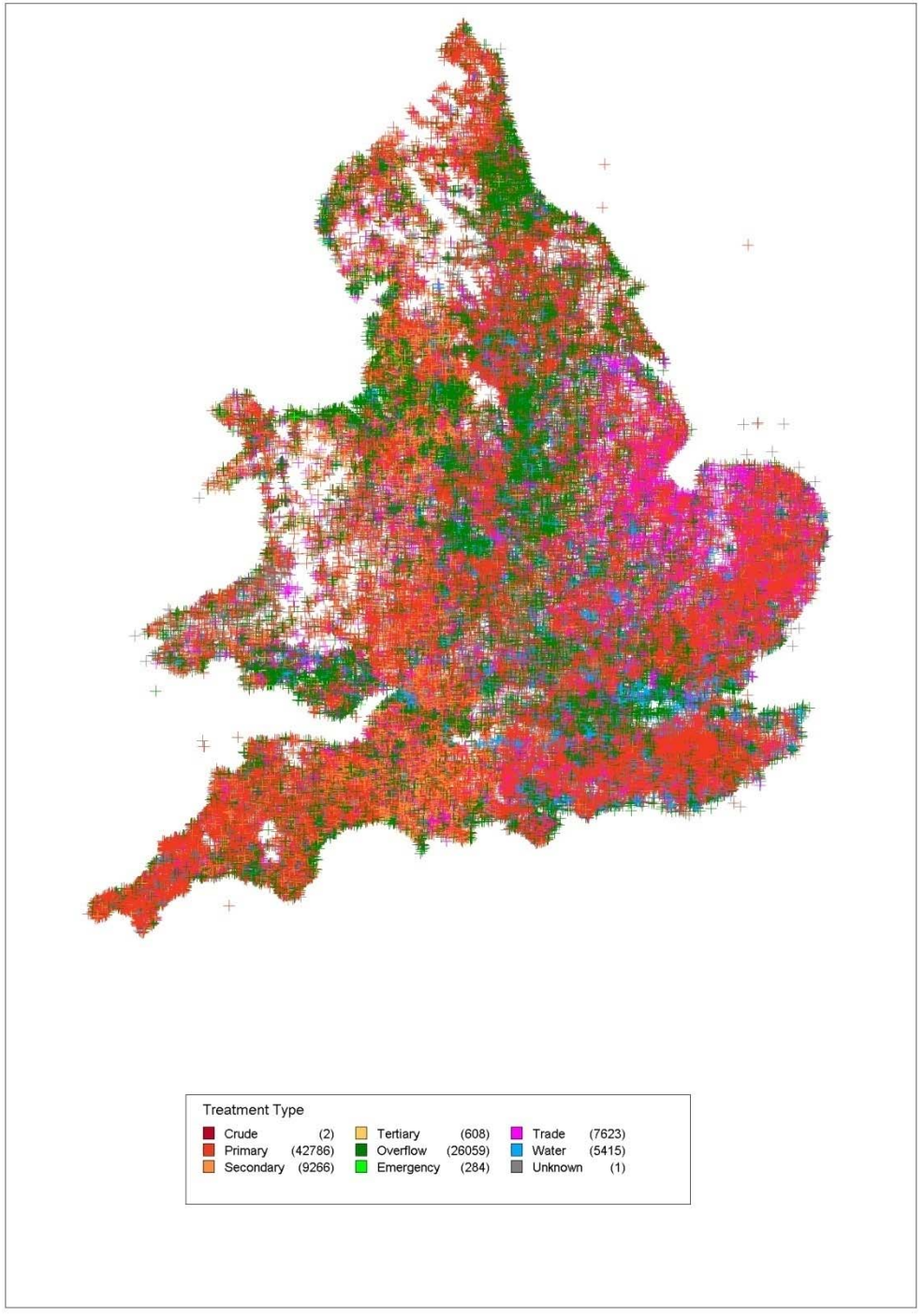
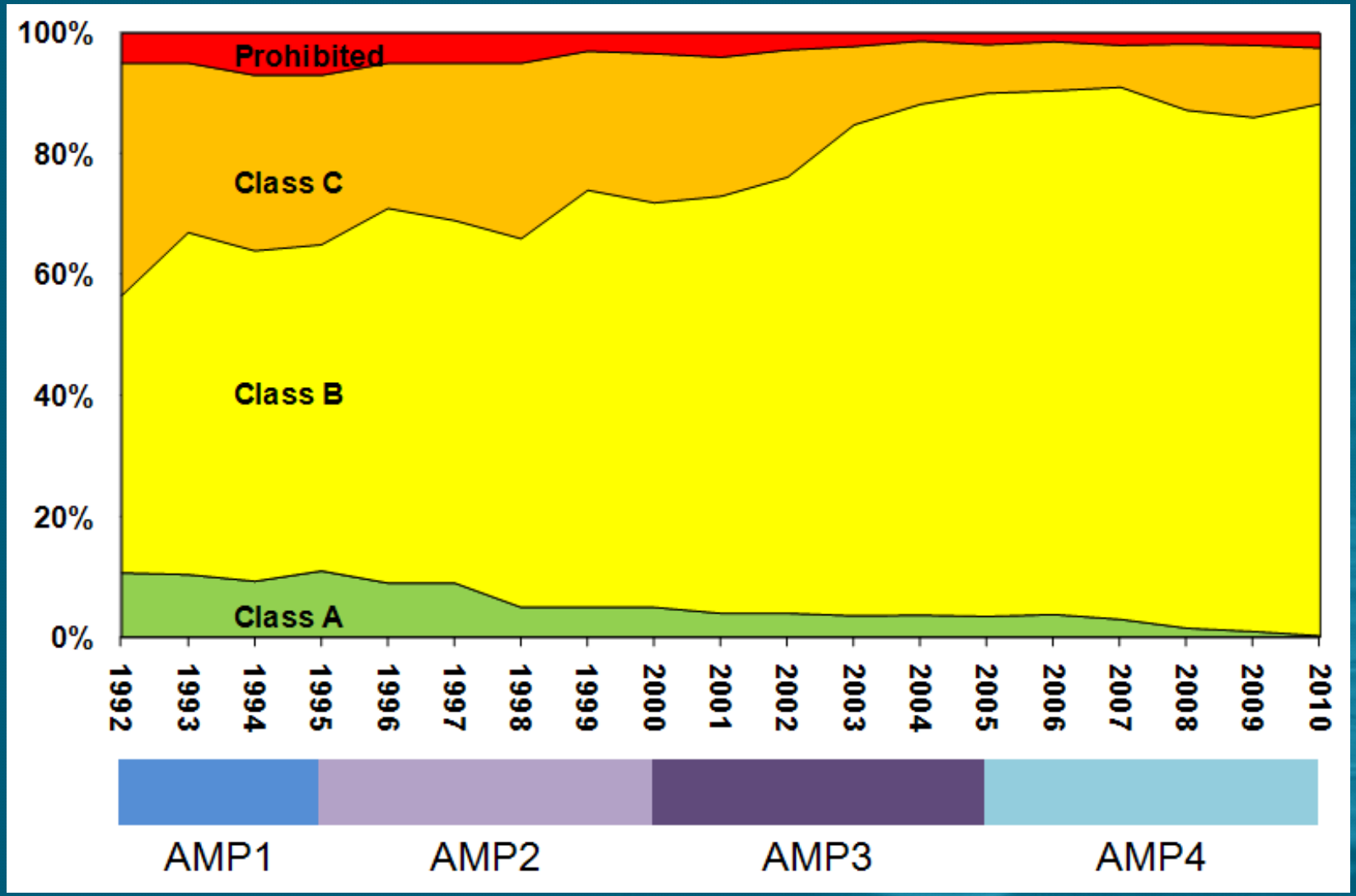
Consented discharges on Environment Agency database

92,044



E.coli classification of shellfisheries in England and Wales



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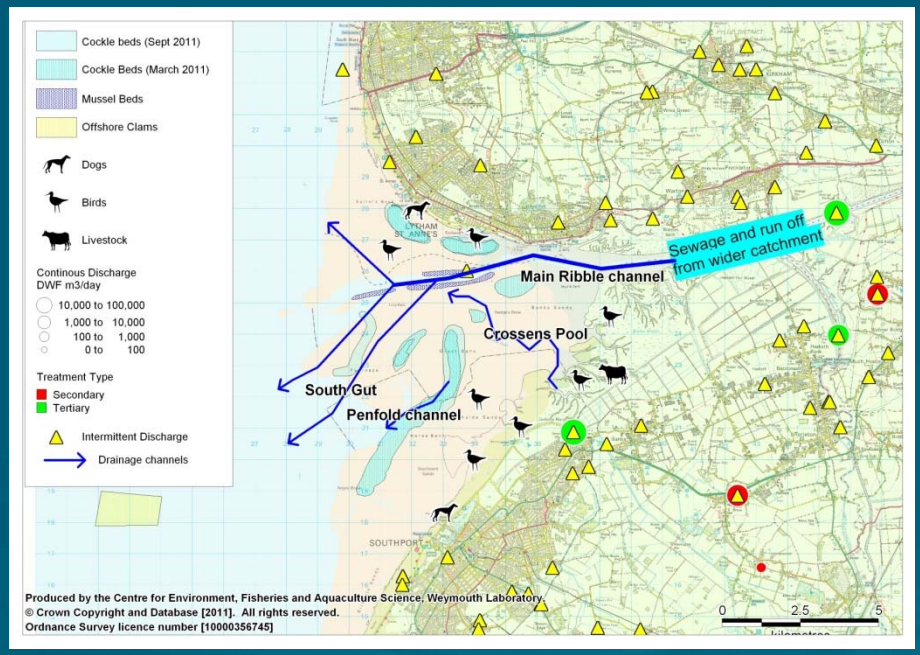
UK Sanitary Survey Programme

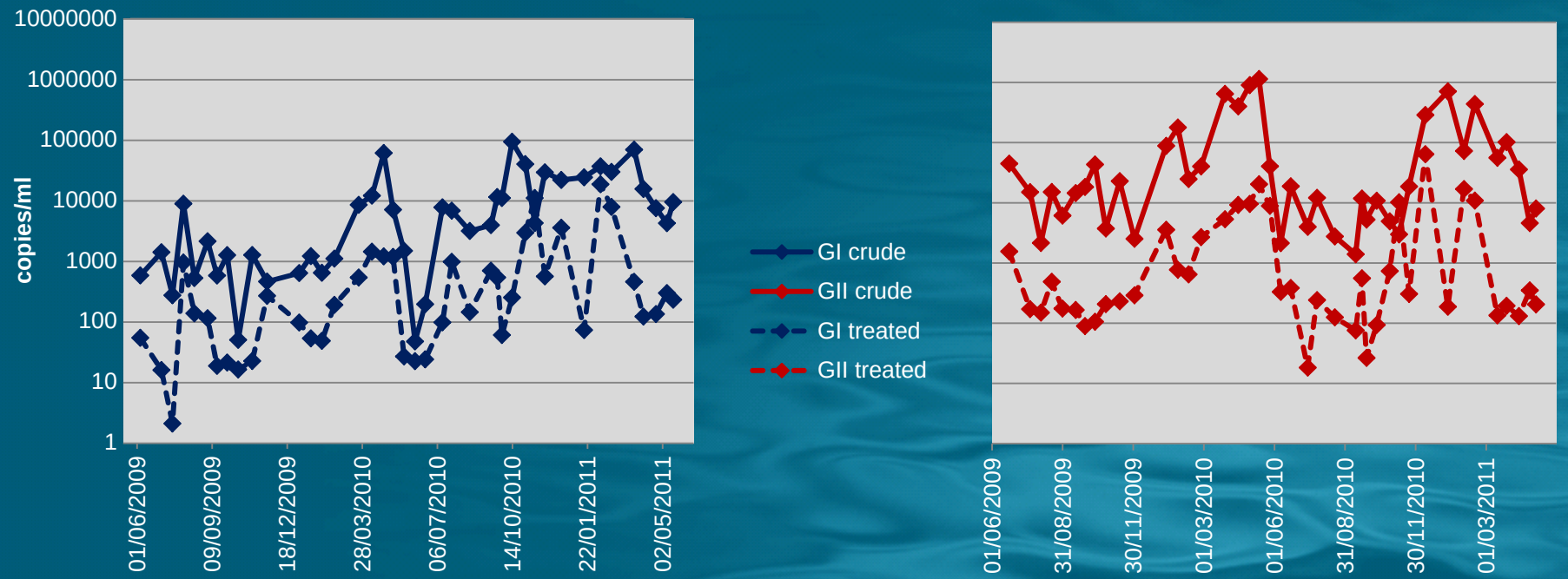
- Sanitary survey: comprehensive assessment of pollution sources
- Legal requirement in EU Food legislation from 2006
- All UK commercial production areas now surveyed (by Cefas and partners)
- >150 surveys
- For survey reports see www.cefas.co.uk



Ribble estuary

Sewage monitoring

- Sewage always contained norovirus
- Peak levels winter-spring
- Treatment causes 1-2 logs reduction



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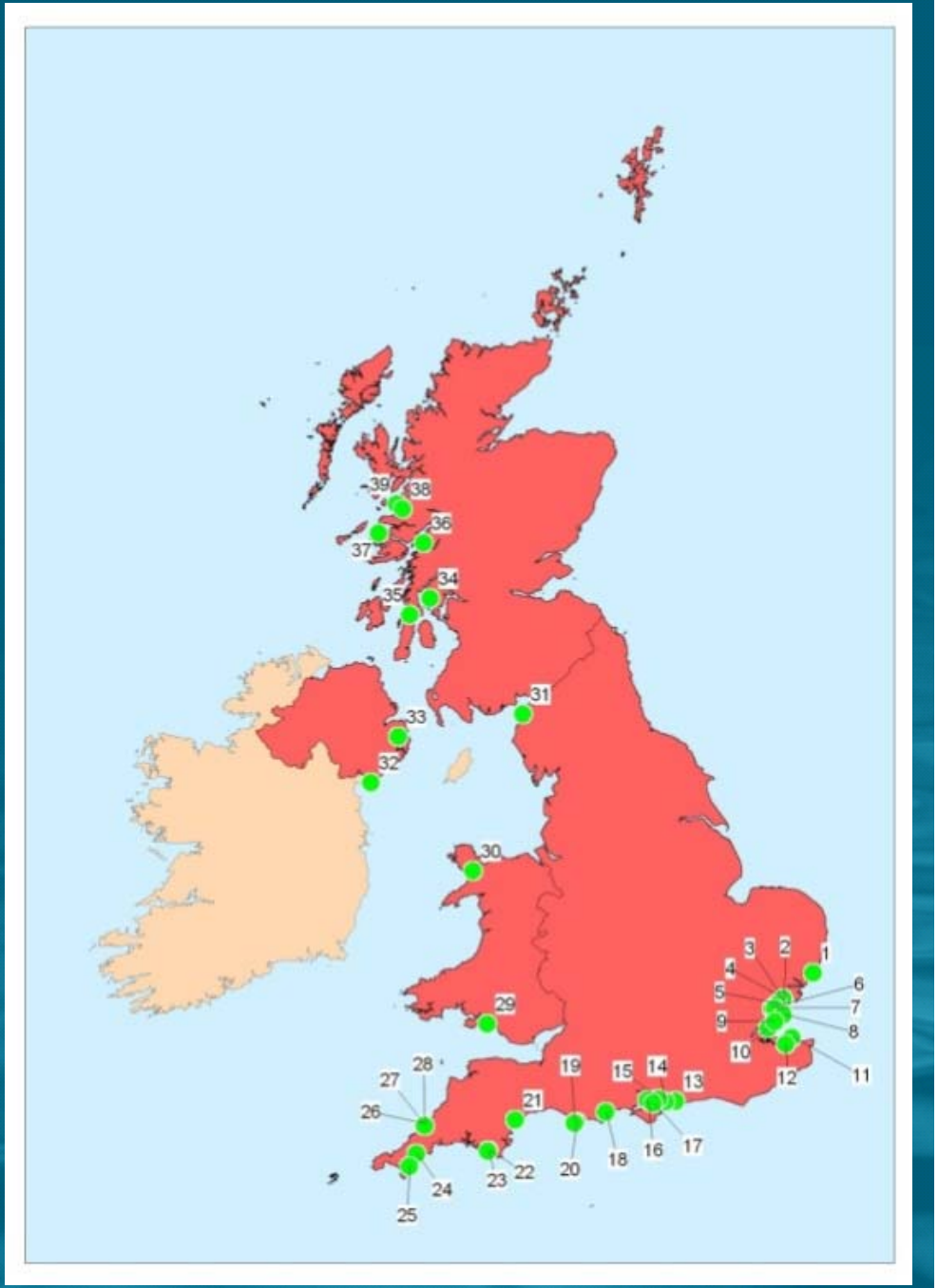
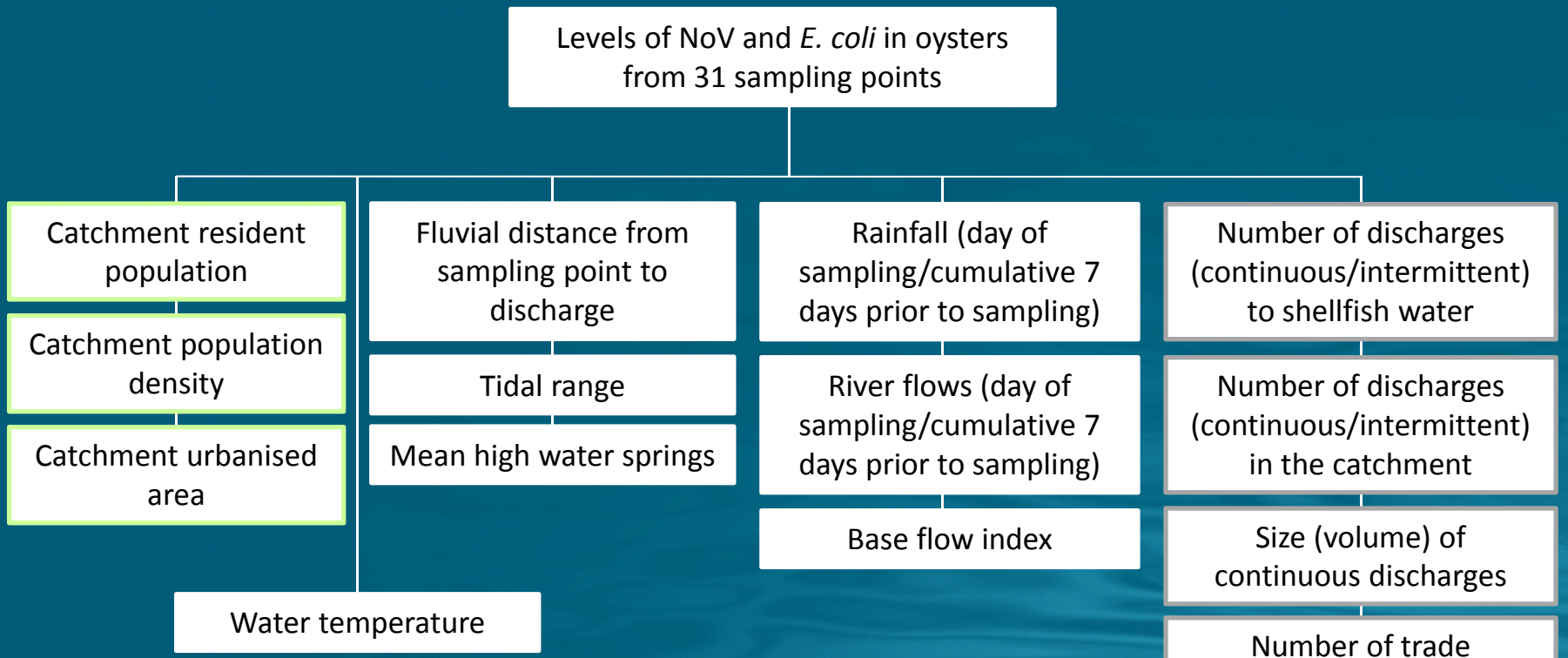
Further analysis of surveillance study data

- Lowther et al, AEM 2012; 78:5812



Desk study of risk factors for NoV in oysters (England and Wales)



- Linear correlation (Pearson's r) analyses
- Linear regression
- Akaike Information Criterion

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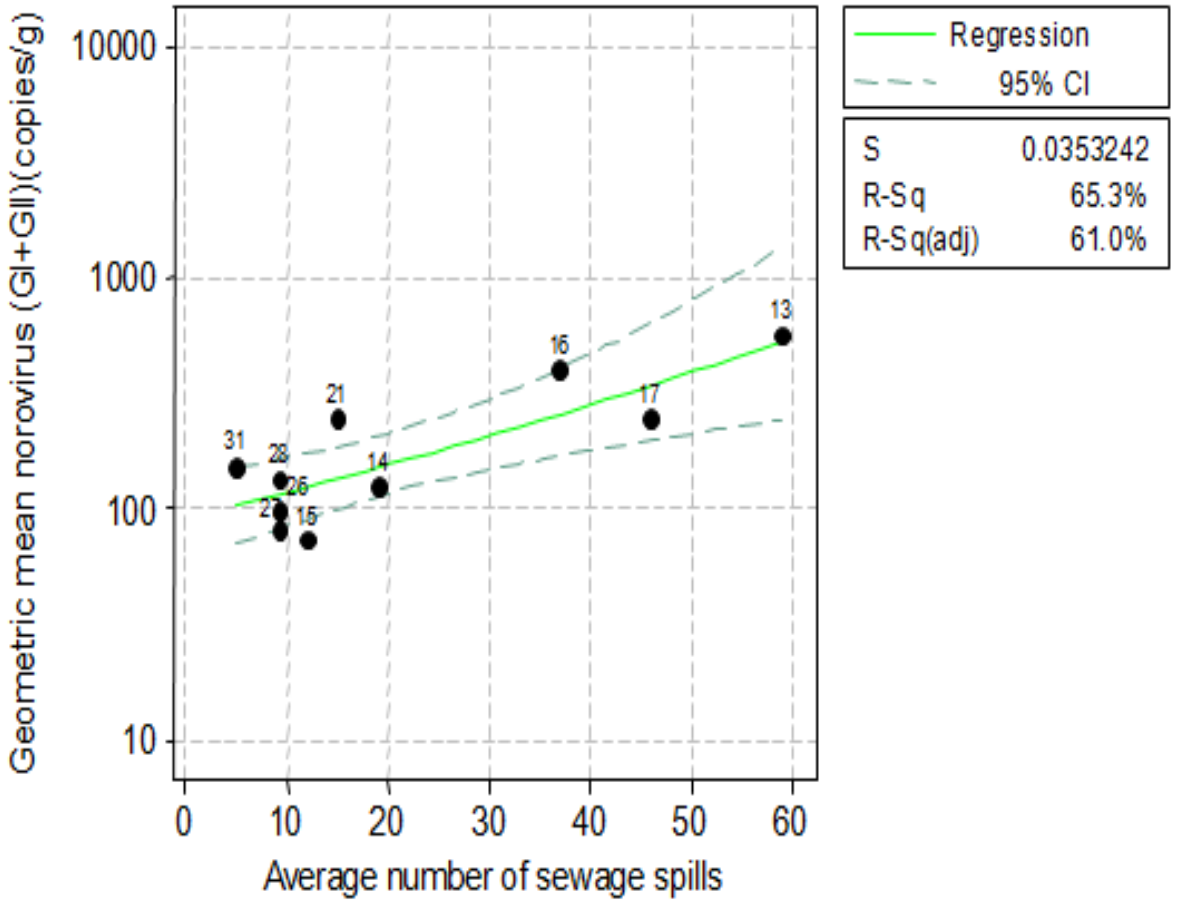
Norovirus levels in oysters vs potential risk factors

Elevated NoV concentrations at a site correlated with:

- catchment area>32,000 hectares
- catchment population>80,000
- >2 continuous discharges (dry weather flows >2,000m^3/s)
- frequency of storm overflows



Number of CSO spills/year

Campos et al., 2015. Risk factors for norovirus contamination of shellfish water catchments in England and Wales. Int. J. Food Microbiol. Submitted.

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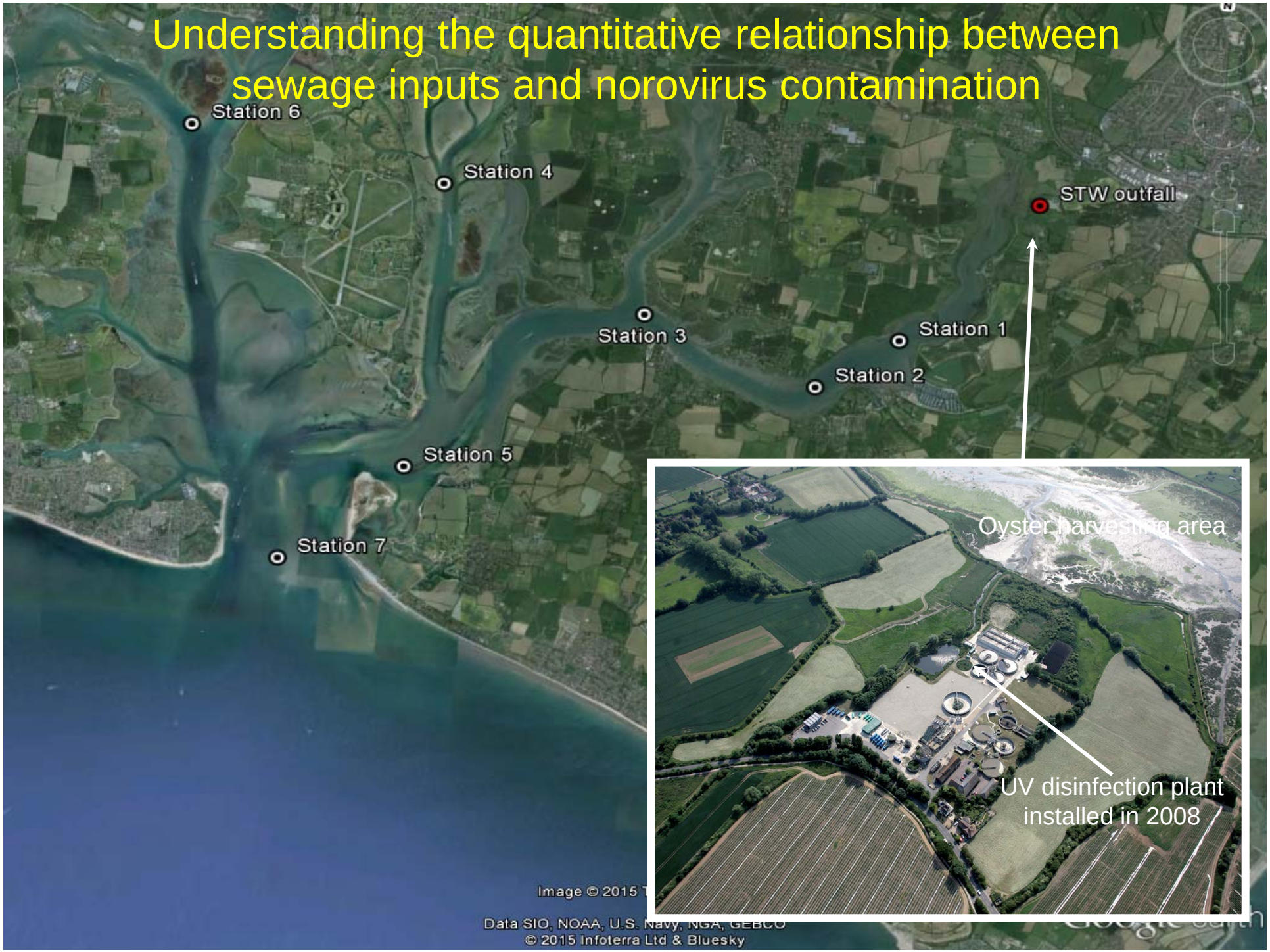
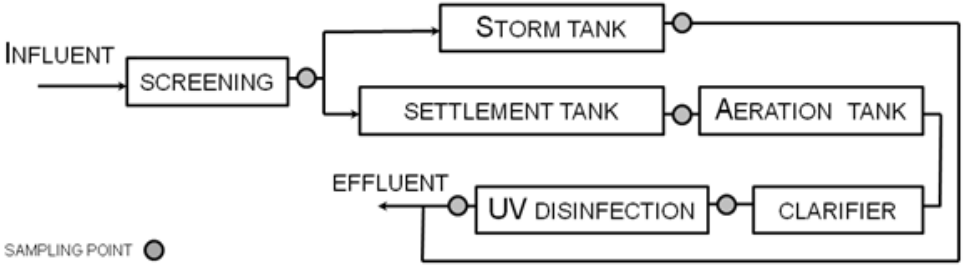
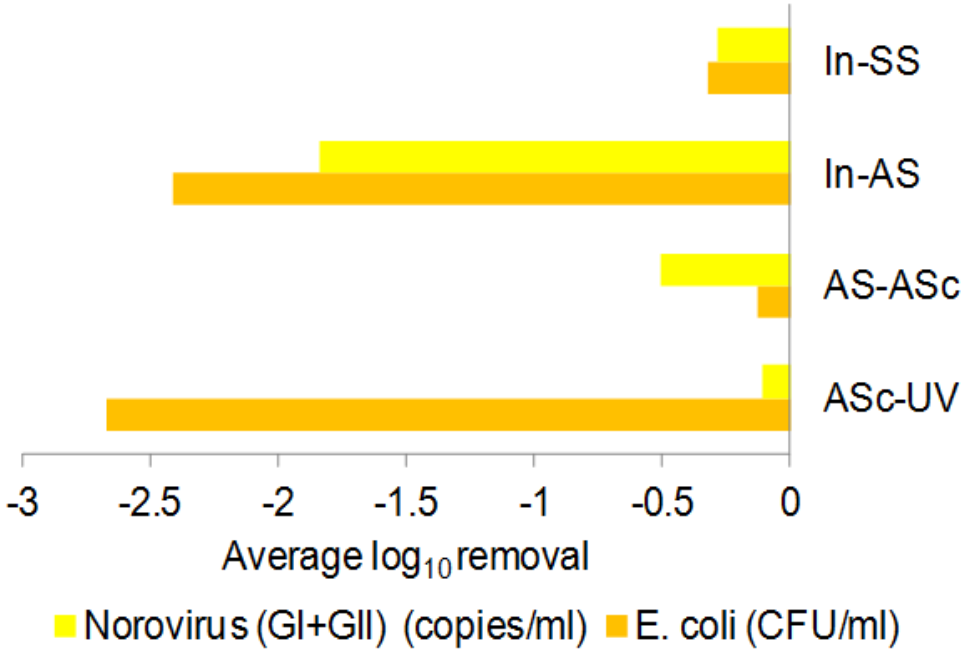
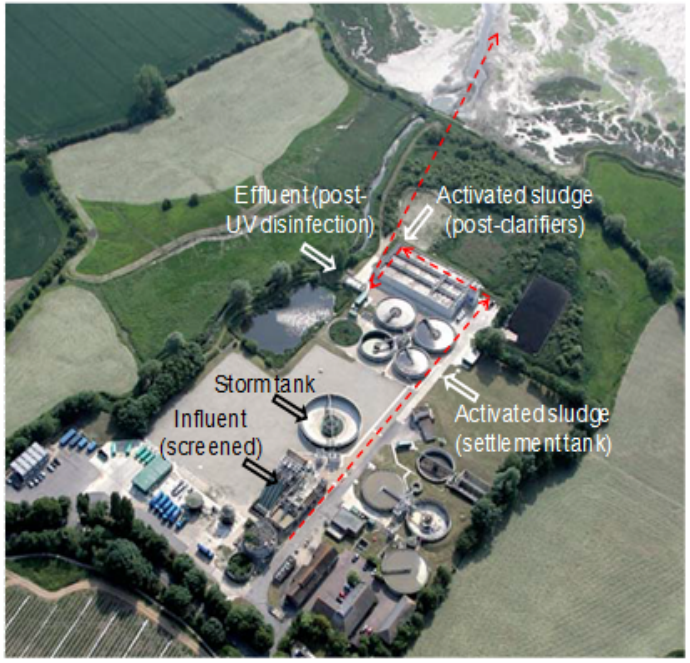
Understanding the quantitative relationship between sewage inputs and norovirus contamination



Comparison of *E. coli* and norovirus removal at single STW







In - influent
SS - settled storm
AS - activated sludge (settlement tank)
ASC - activated sludge (post-clarification)
UV - ultra-violet disinfected effluent

Optimised activated sludge followed by UV disinfection can deliver average total NoV and *E. coli* $\log_{10}$ reductions of 2.9 and 5.2

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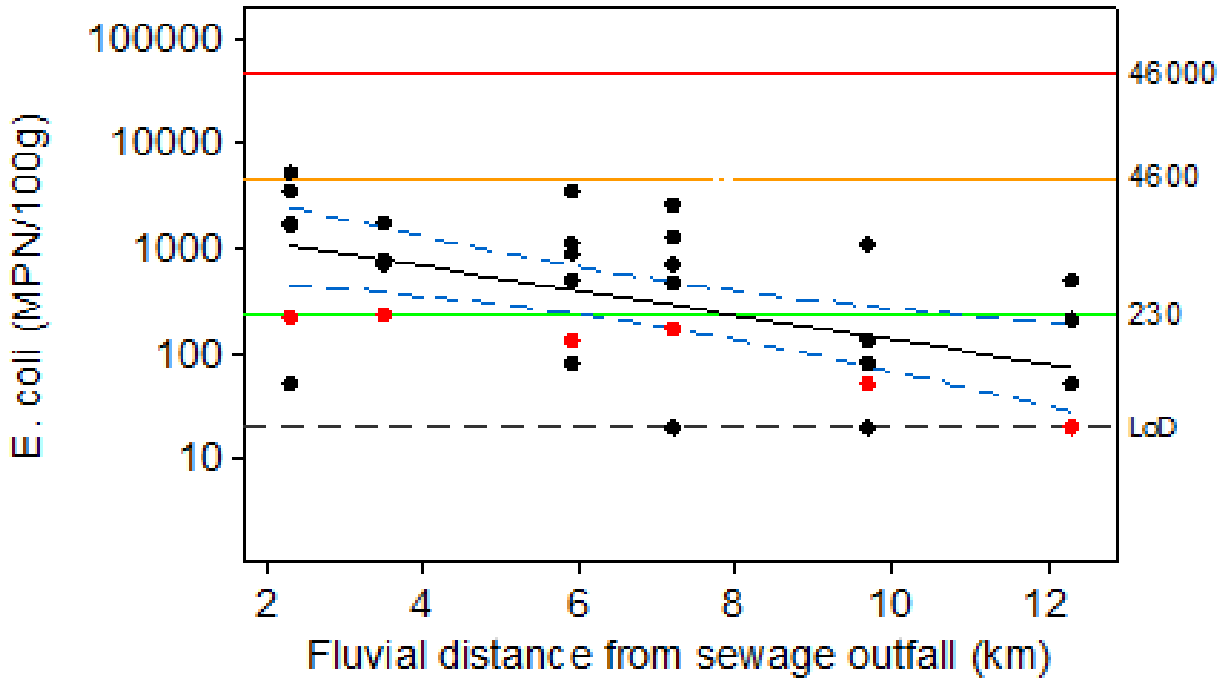
E.coli and norovirus in oysters



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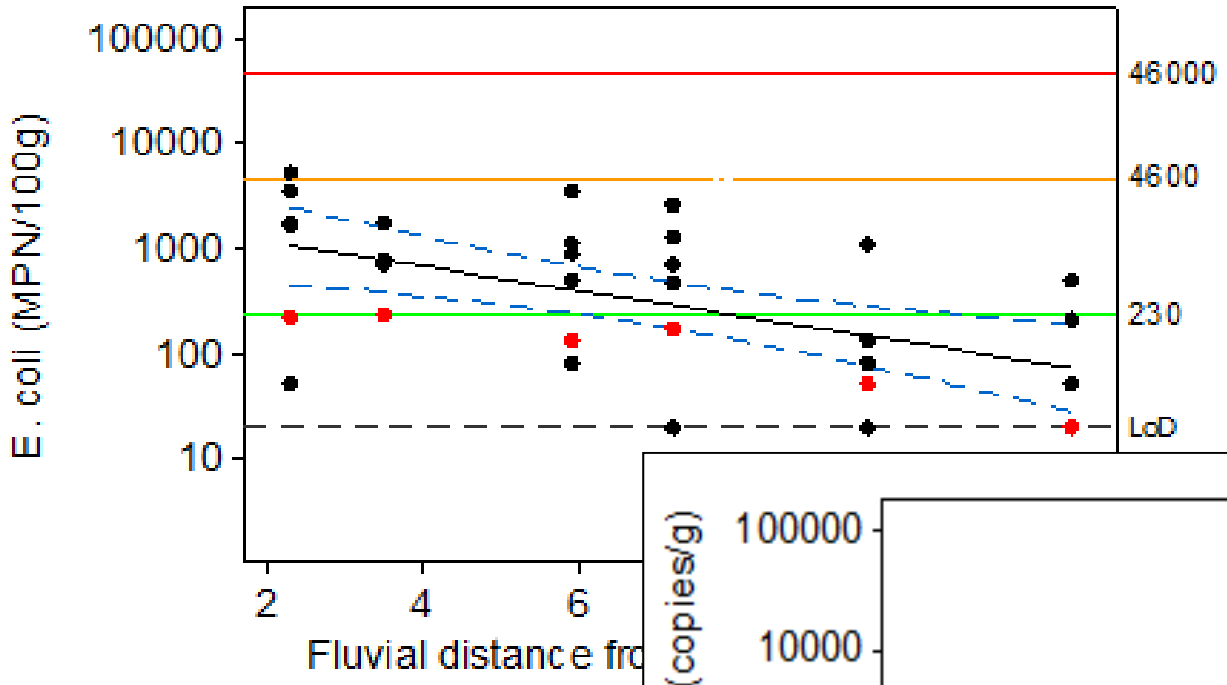
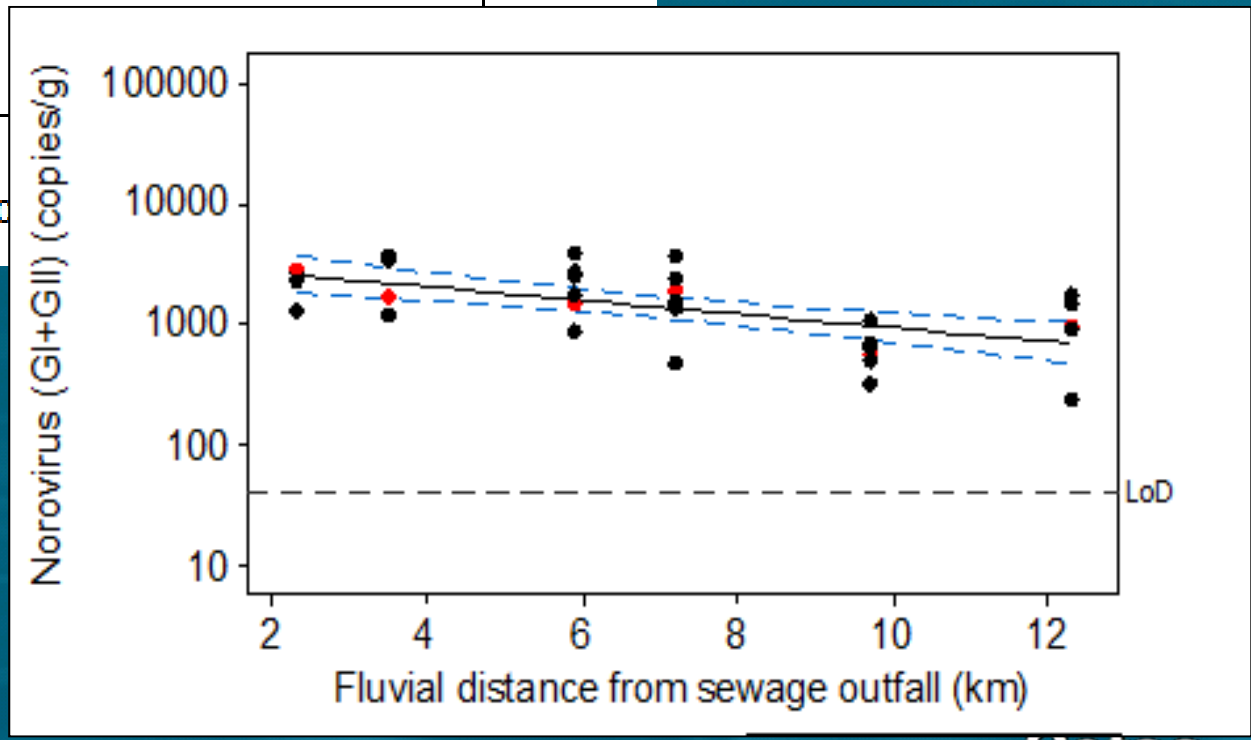
E.coli and norovirus in oysters





Linear models:

E. coli R^2 26.6%

norovirus R^2 32.2%

Campos et al., 2015. Fate of human noroviruses in shellfish impacted by frequent sewage pollution events. *Environ. Sci. Technol*. In press.

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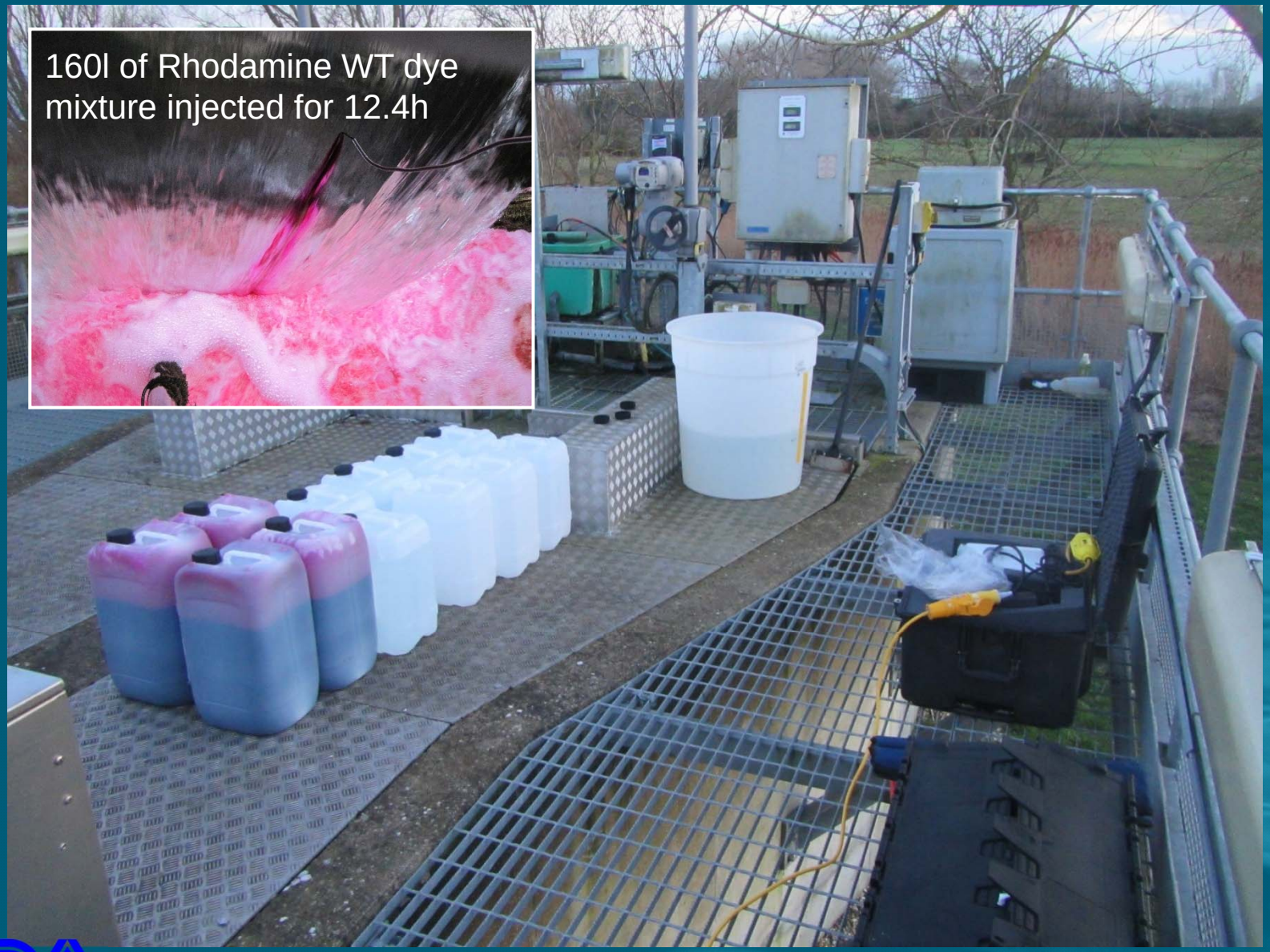
Estimating effluent dilution using dye



Dr Greg Goblick

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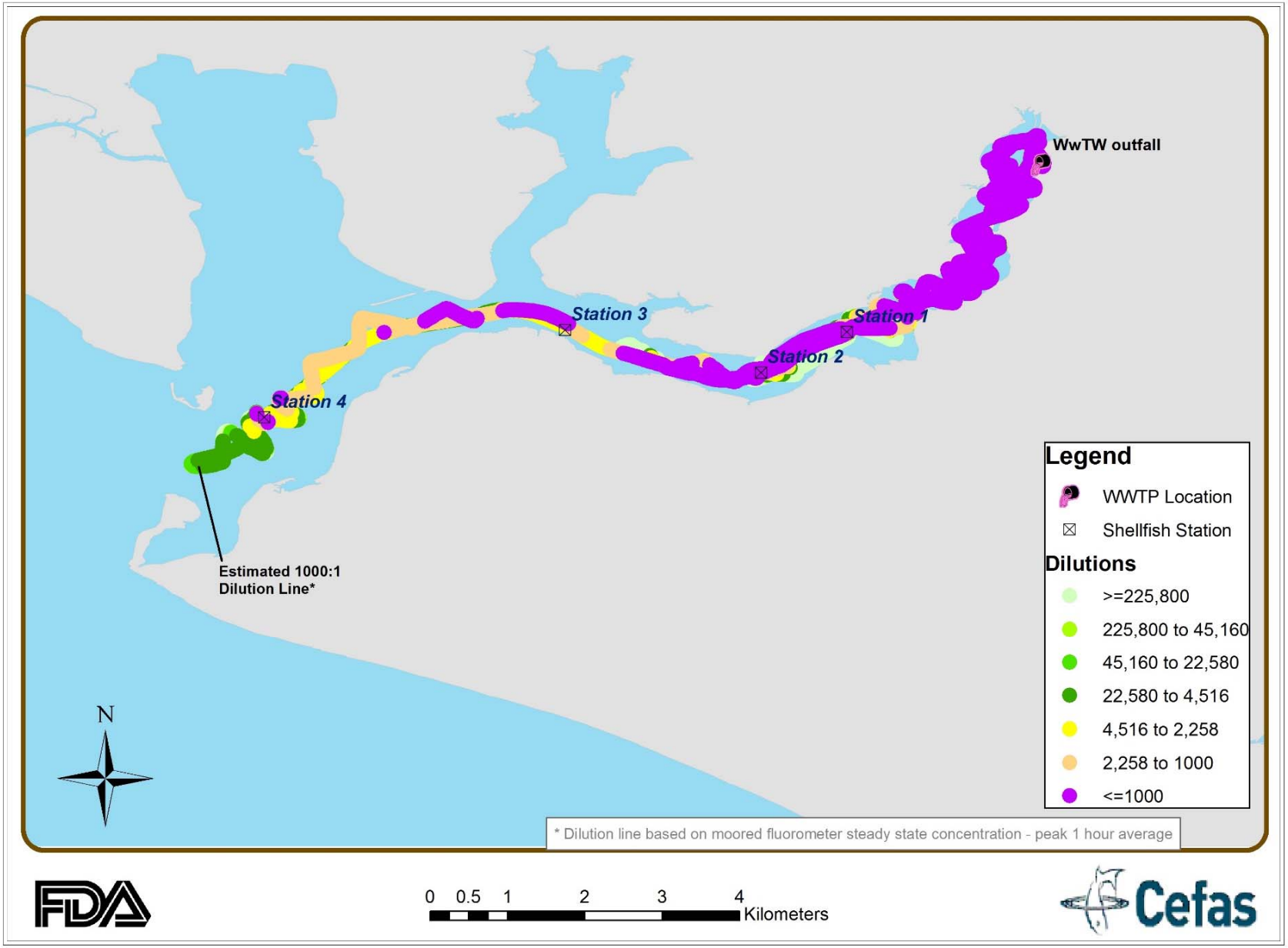
Dilution of dye-tagged effluent evaluated using

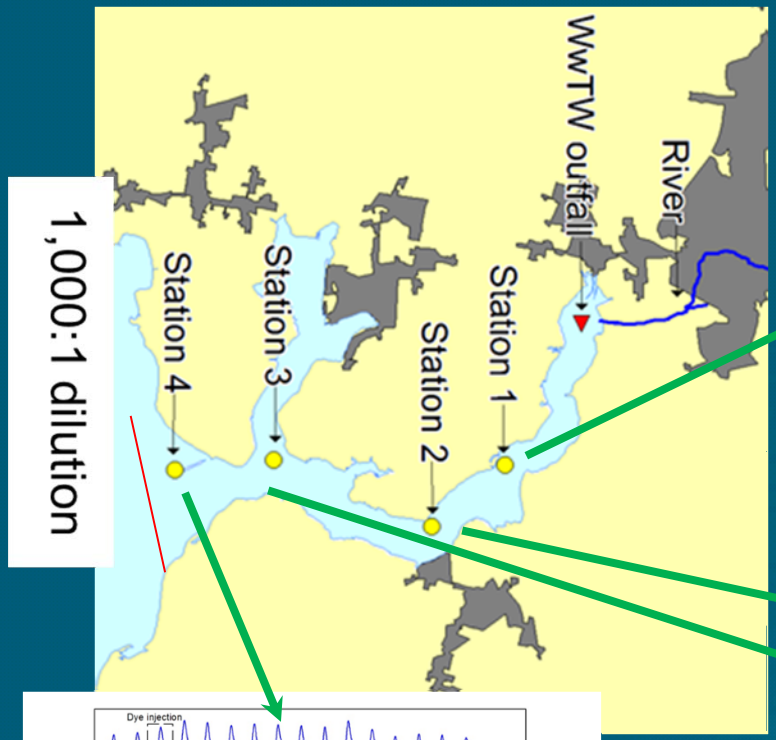
- Fluorometer towed from boat to identify the extent of spatial distribution of dye plume at the surface
- Fluorometers attached to oyster cages placed at four locations downstream from WwTW outfall

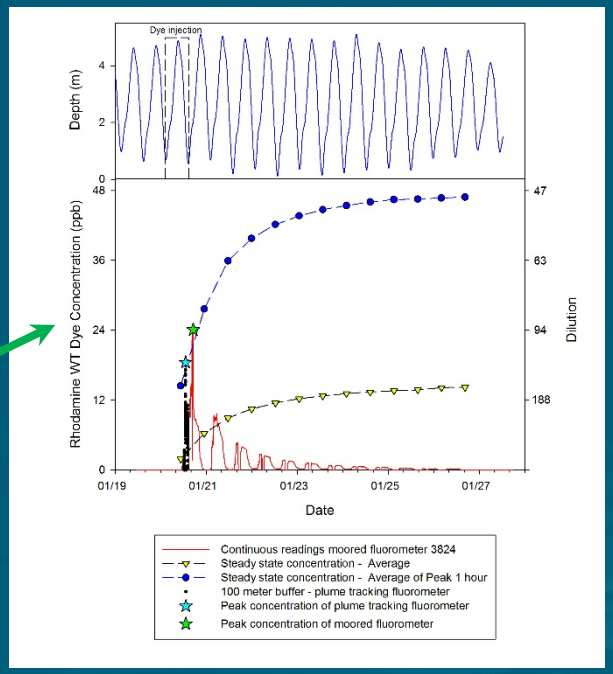
Oyster cage with CTD and fluorometer

Surface tracking

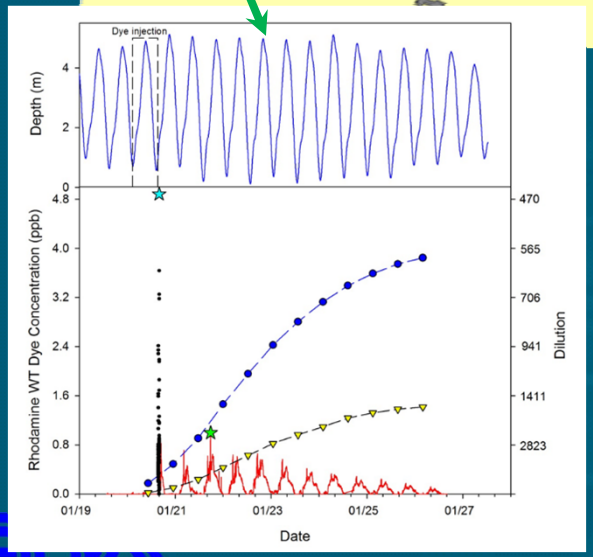


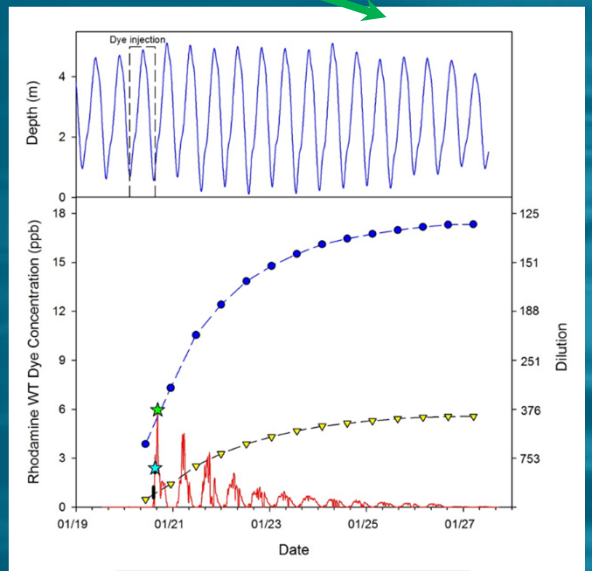
Fixed fluorometers

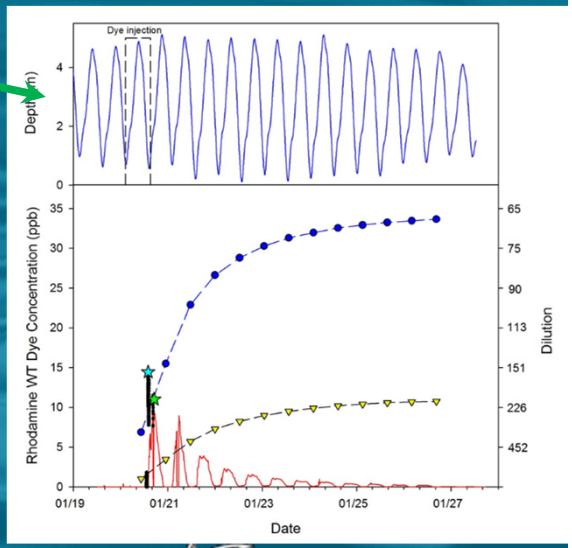




Peak 1h average dilutions represent an hour when, at steady state, the expected exposure of shellfish to NoV is expected to be greatest.







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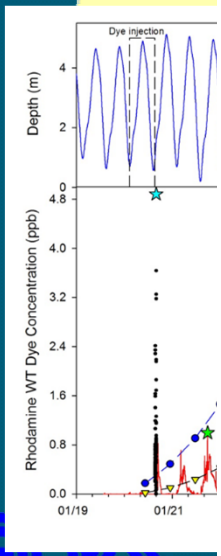
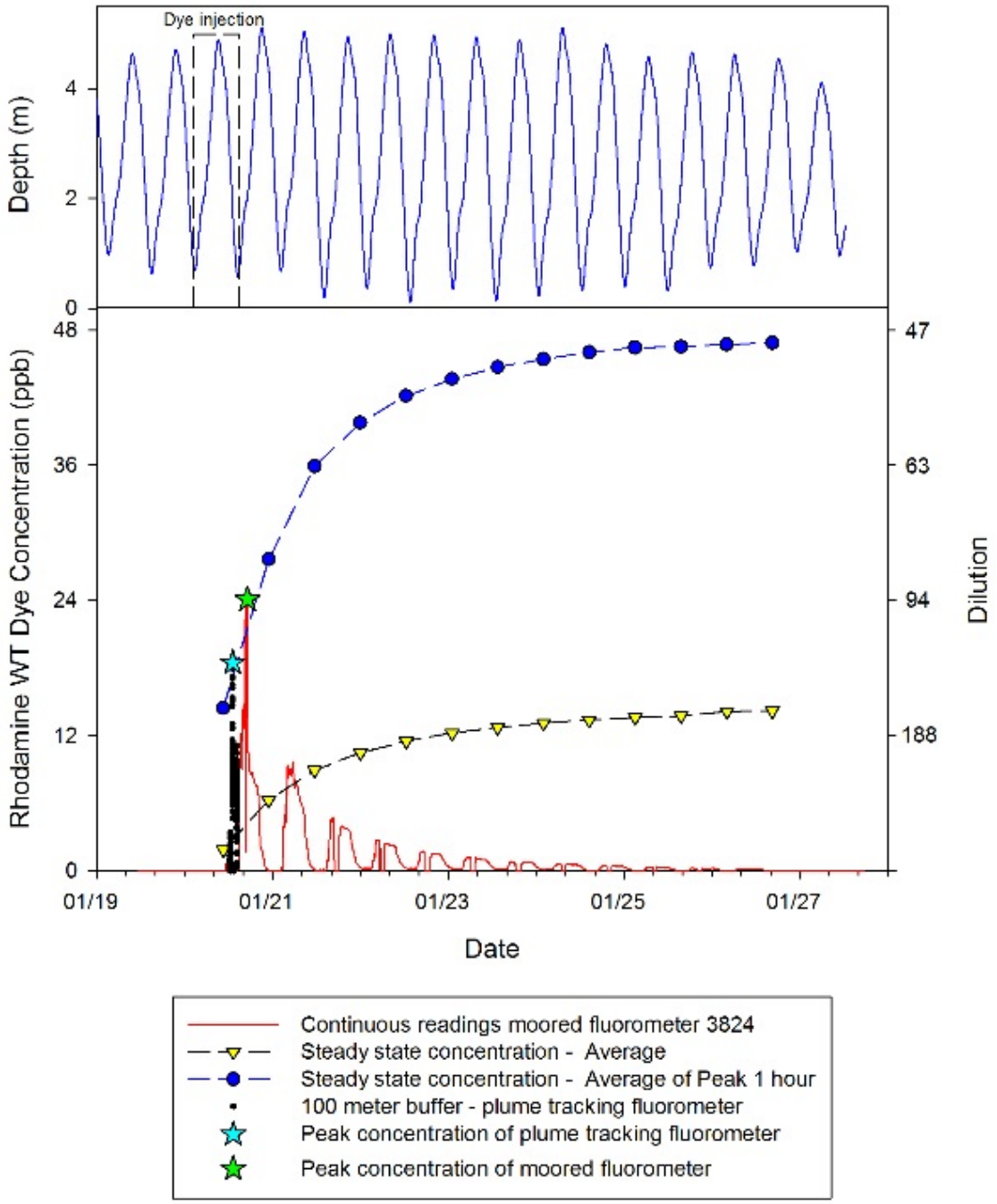
Fixe

1,000:1 dilution





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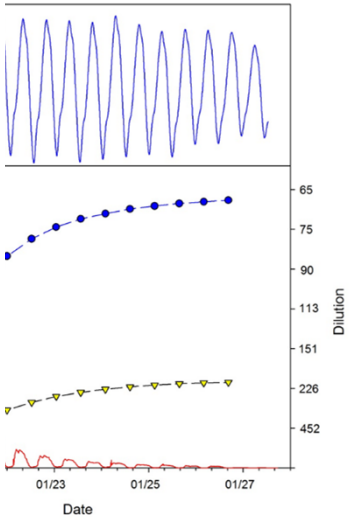

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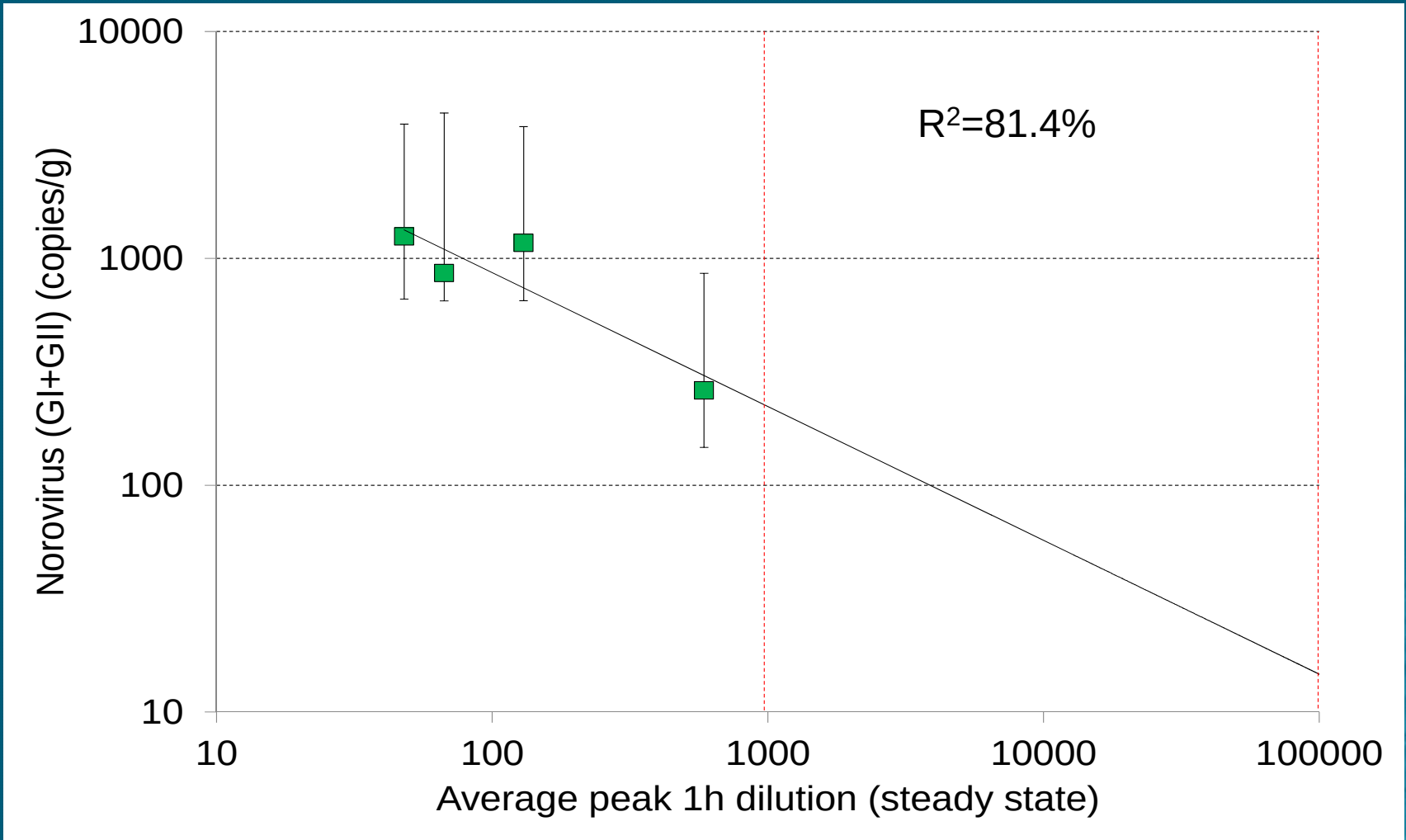
Norovirus in oysters vs effluent dilution



1:1000 - minimum dilution of effluent permitted in USA (WwTW must have management plan)

1:100,000 – dilution required for an approved area in absence of management plan

FDA

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Depuration

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Shellfish depuration

Laboratory based oyster depuration studies

Methodology

- Oysters contaminated with norovirus (GII.4) FRNA Bacteriophage and *E. coli*.
- Depuration as in commercial practices.
- Increased time (14d)
- Temperature 8^{o}C v 16^{o}C
- Norovirus tested using CEN standard quantitative method

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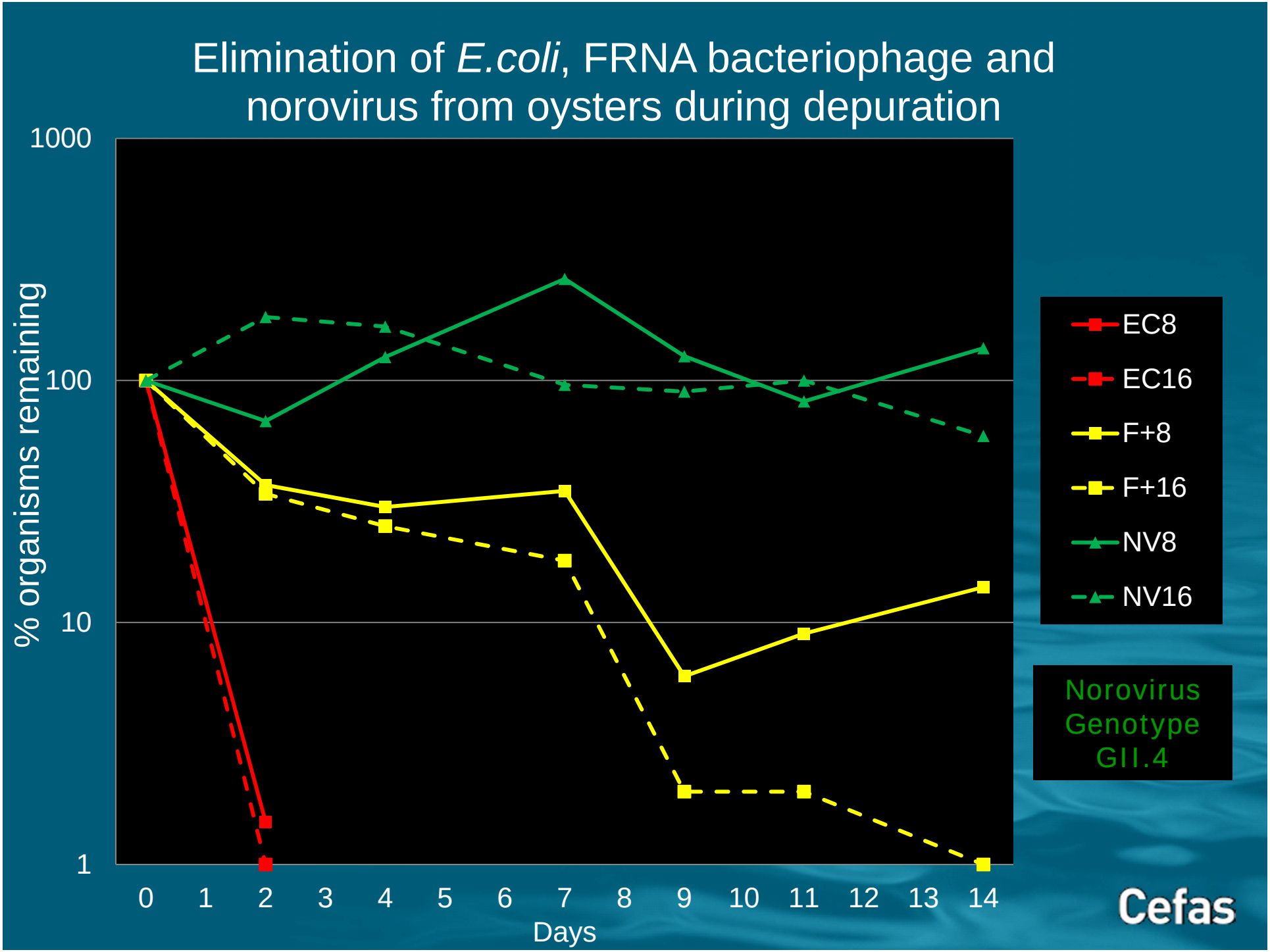
Elimination of *E.coli*, FRNA bacteriophage and norovirus from oysters during depuration



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Summary

- Unexpectedly high levels of norovirus RNA in EU shellfish production areas
- Virus standards for shellfish under consideration – informed by EU baseline survey
- Untreated overflows from sewage works may be a significant contamination mechanism
- Norovirus persists in environment and is not removed by depuration
- Sewage pollution buffer zones – at least 1:1000 effluent dilution
- Determination of virus viability remains a challenge

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