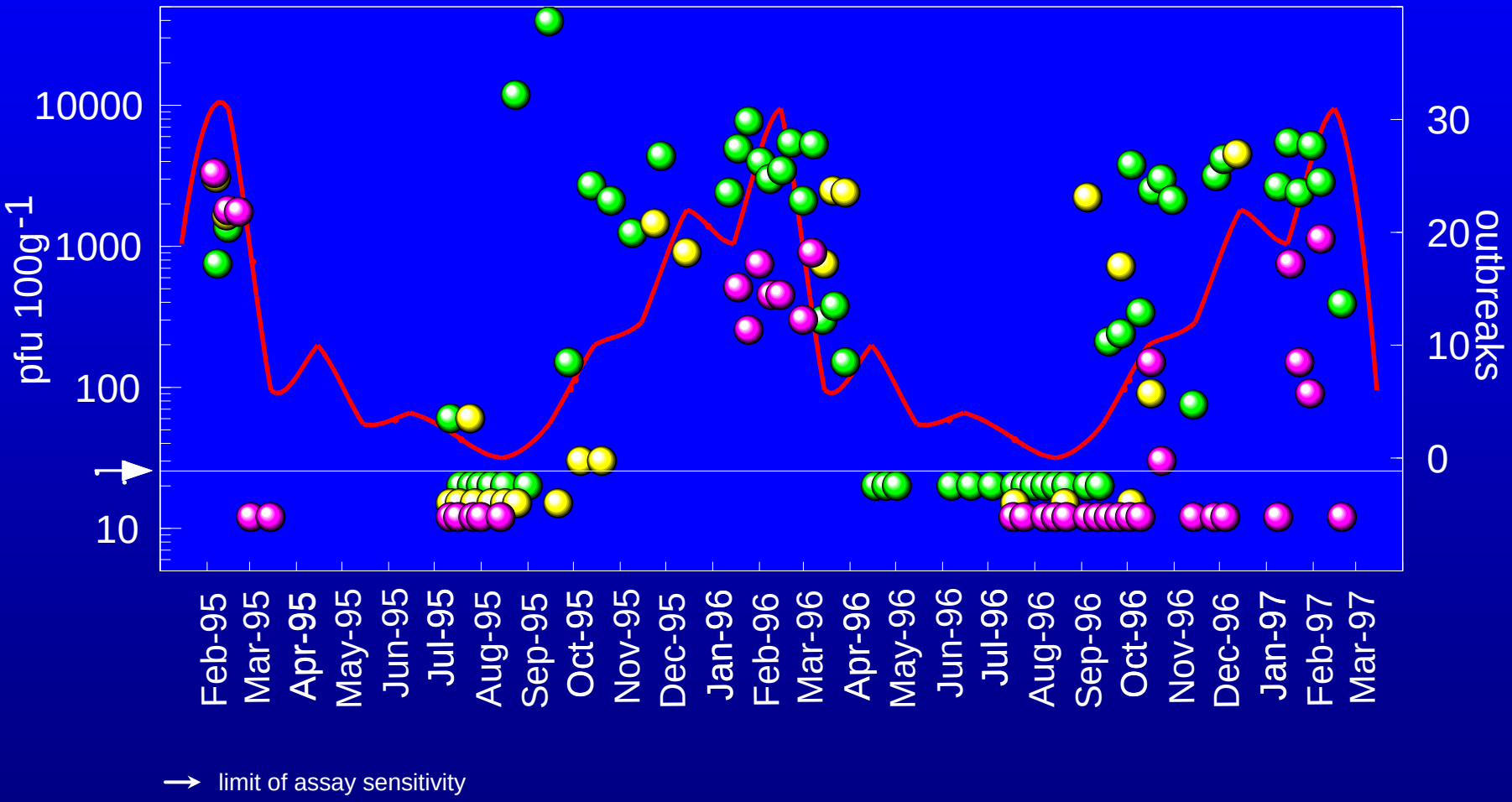
Seasonal or Regional	
3.	What differences in winter vs. summer, if any, were found in the levels of MSC in water in areas of those different classification types around WWTP outfalls?
4.	What differences in winter vs. summer, if any, were found in the background levels of MSC in shellfish (any species) in areas of the different classification types around WWTP outfalls which are continuously exposed to some amount of adequately treated effluent?
5.	Do the accepted levels for regulatory decision making in the US and internationally vary by season or temperature?

Seasonal distribution of F+ bacteriophage at sites 2 (●), 3 (●) and 4 (●)



Dore et al., 2000

Table 1 Number of samples analysed for each shellfish species in each classification category. Categories were ascribed for each harvesting area on the basis of *E. coli* results obtained during this study using the current European Union classification scheme

Classification	*C. gigas*	*O. edulis*	*M. edulis*	All species
Category B	108	127	154	389
Category C	56	24	60	140
Prohibited			79	79
All categories	164	151	293	608

Dore et al., 2003

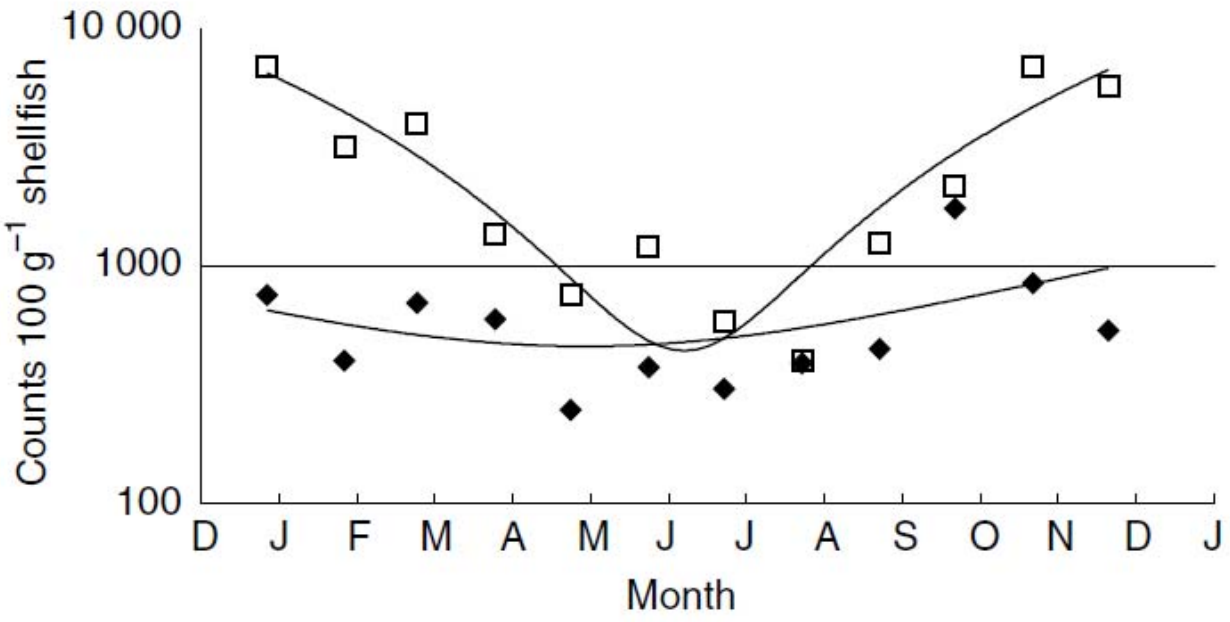
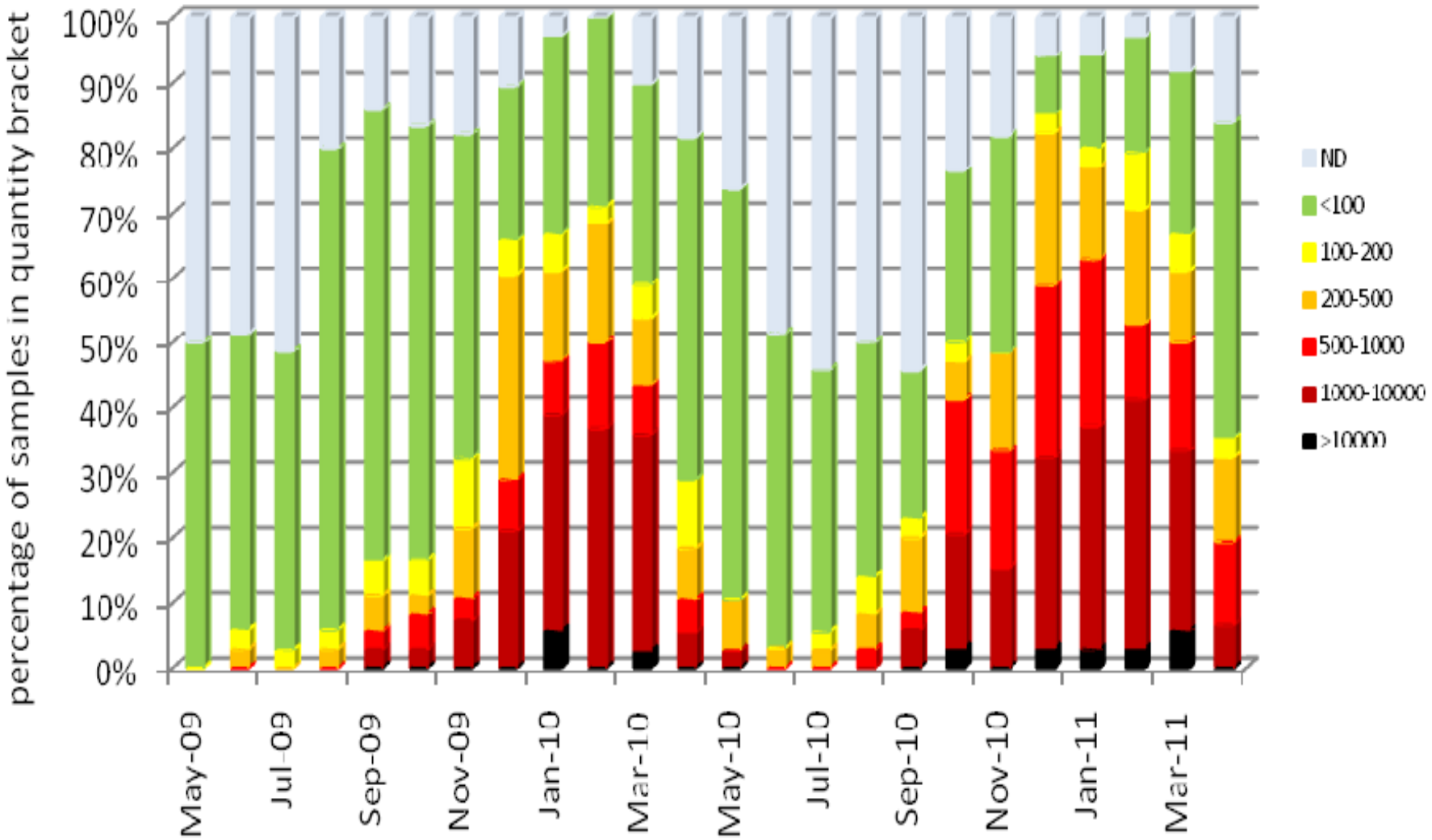


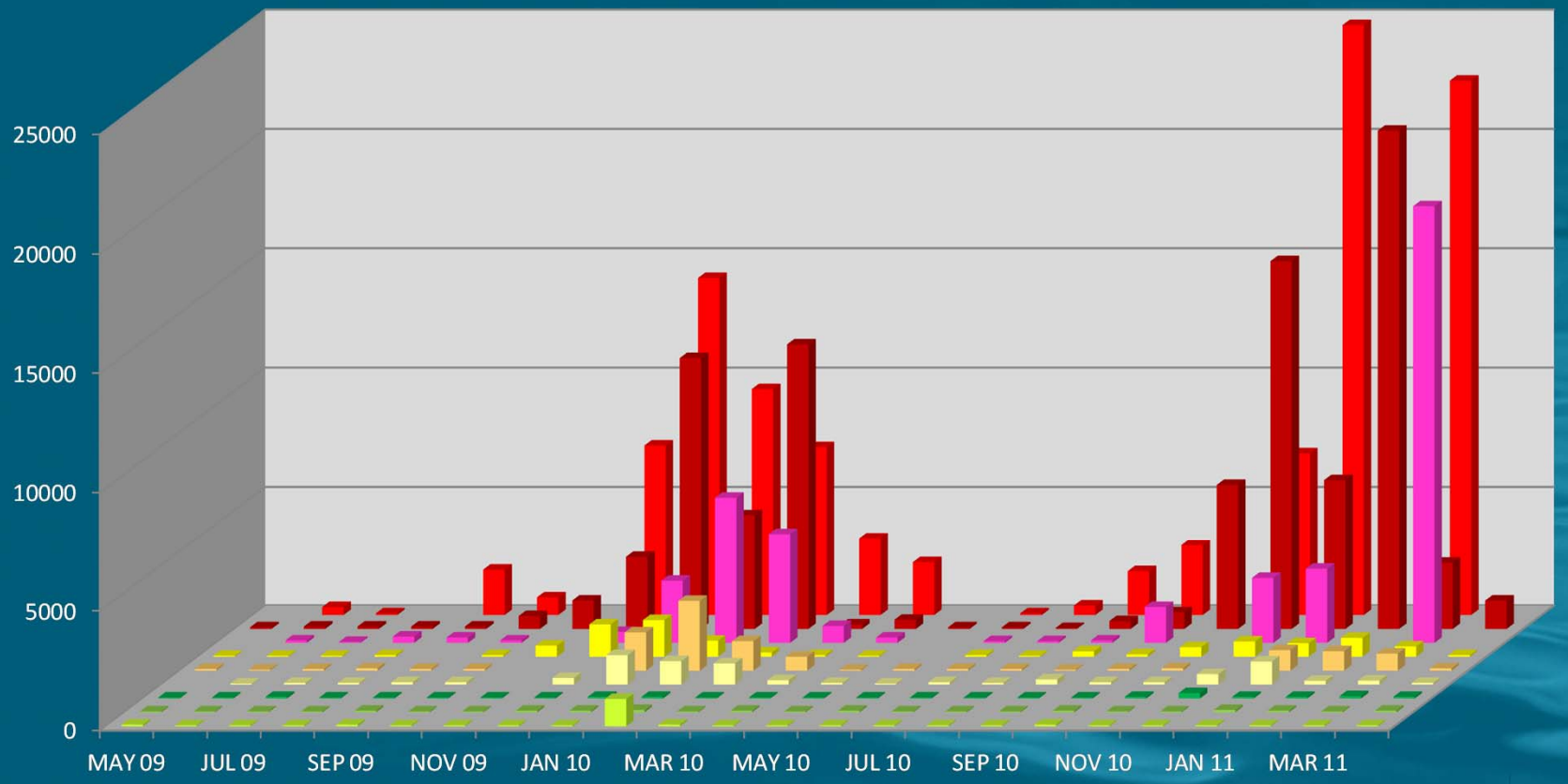
Fig. 2 Geometric mean values of FRNA bacteriophage (□) and *E. coli* (♦) in shellfish by month of sample collection



Oysters from UK production areas 2009 – 20011
39 representative UK sites, 844 monthly samples, 76% NoV positive.
Lowther et al., 2012

Site-by-site variation

- Significant difference in contamination pattern between sites



Cefas

8. What are the options for reducing the lower limits of quantification in existing analysis methods for MSC in water?

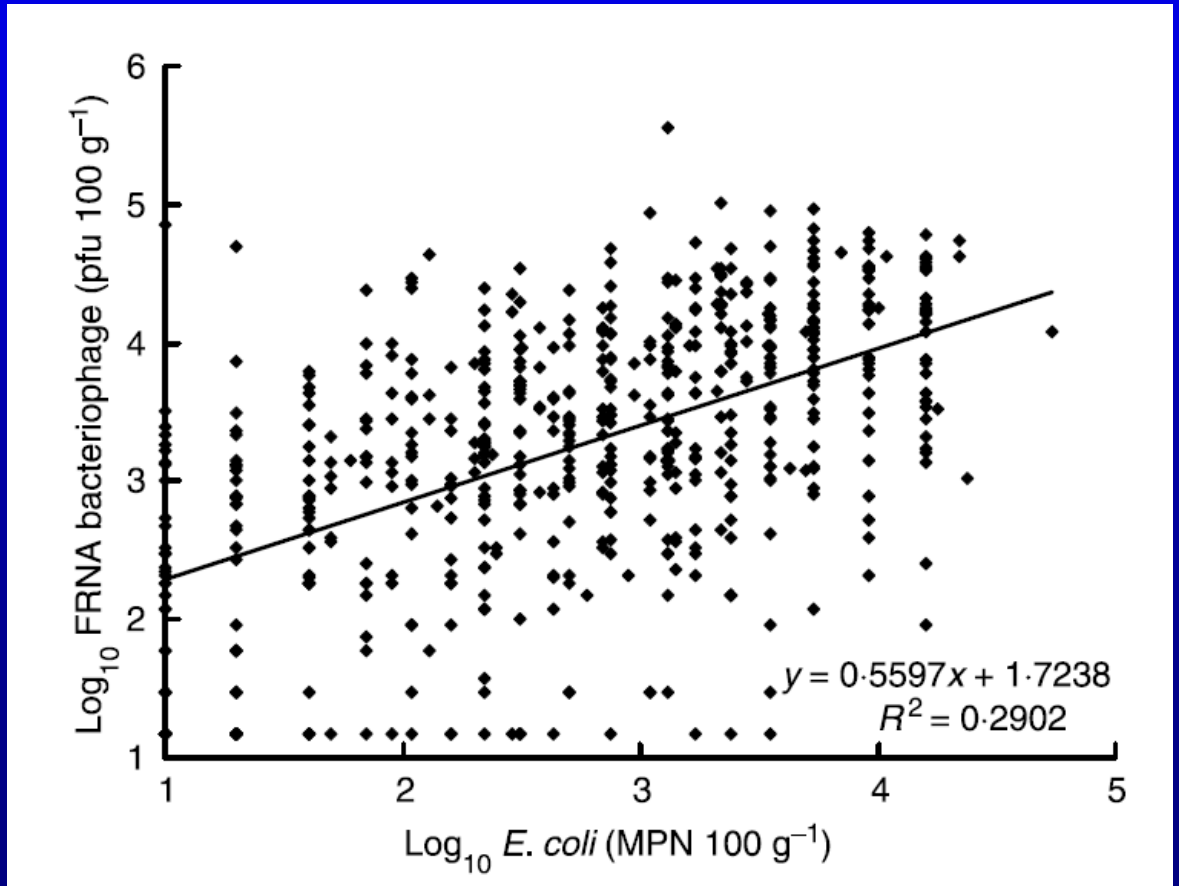
Sensitivity

Shellfish

- increase plate size
- increase no plates (>10)

Water

- concentration (eg Mendez 2004)



Dore et al., 2003

12.	What are the estimated reductions of approved or conditionally approved shellfish growing areas acreage, nationally, anticipated as a result of adopting more stringent growing area standards?

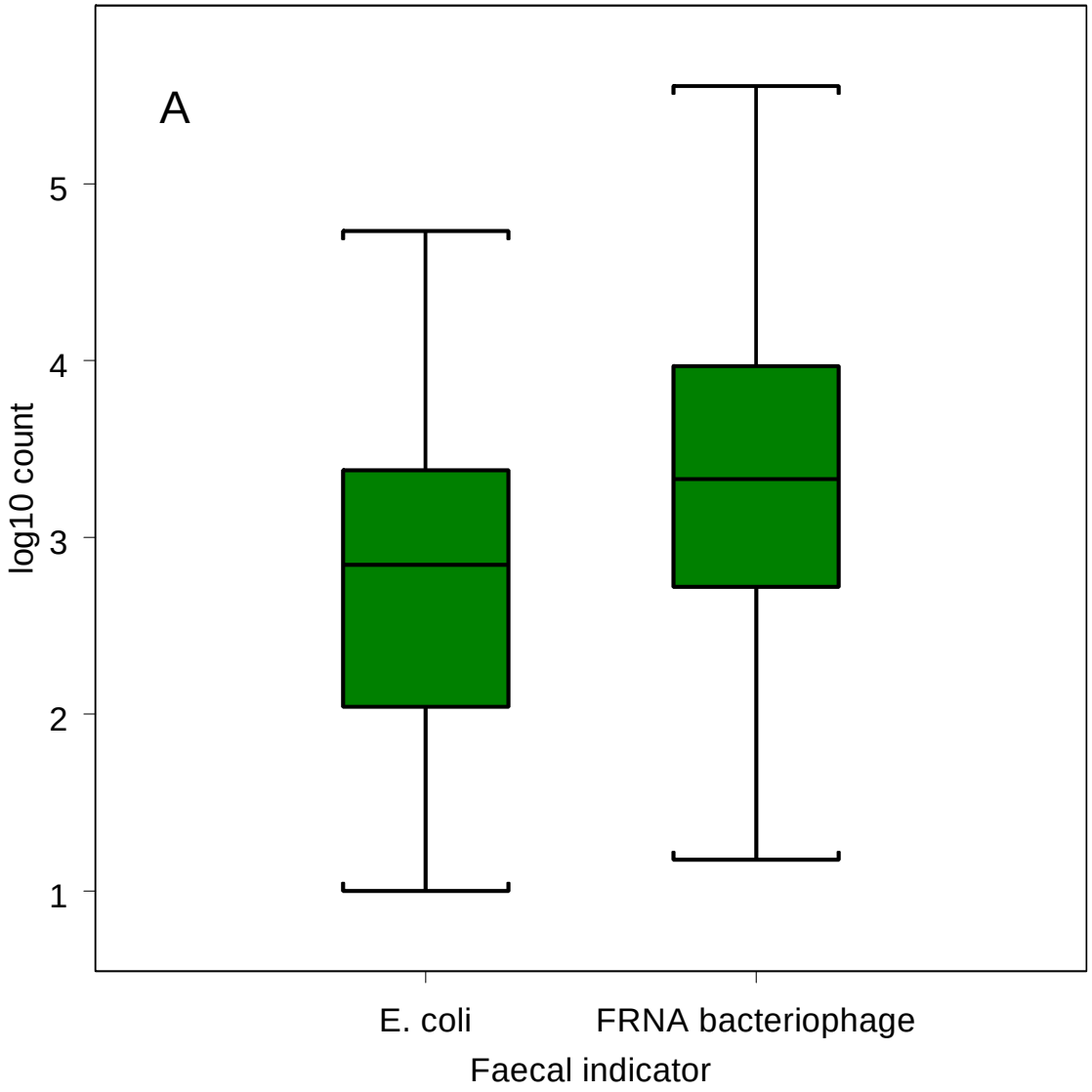

A
log10 count
5
4
3
2
1
E. coli
FRNA bacteriophage
Faecal indicator

Table 1 Number of samples analysed for each shellfish species in each classification category. Categories were ascribed for each harvesting area on the basis of *E. coli* results obtained during this study using the current European Union classification scheme

Classification	*C. gigas*	*O. edulis*	*M. edulis*	All species
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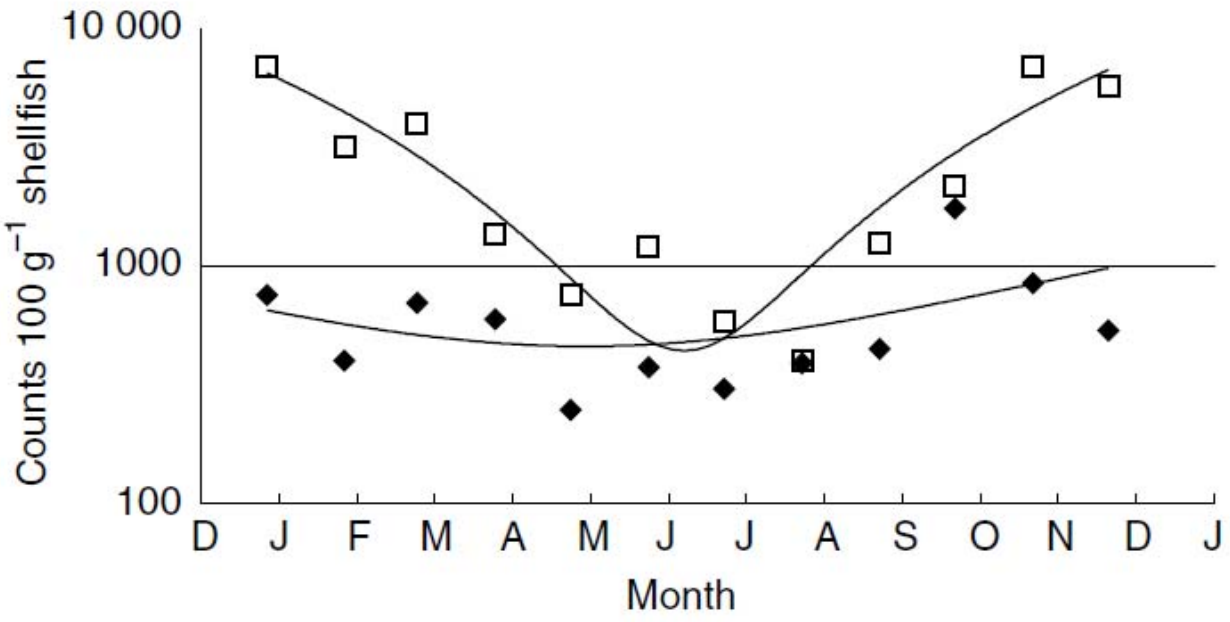


Fig. 2 Geometric mean values of FRNA bacteriophage (□) and *E. coli* (♦) in shellfish by month of sample collection

Industry Impact

458 samples from category B areas

- 0 - 100 pfu 10.2%
- 101 - 1000 pfu 38.4%
- 1001 - 10,000 pfu 42.5%
- >10,000 pfu 8.7%

Correlation to Illness	
27.	27. What is the relationship or correlation between illness and MSC?

Detection of FRNA bacteriophage and NLV's in shellfish associated with outbreaks of gastroenteritis (1996-2001)

Sample	Date	SampleReference	E.coli	FRNA bacteriophage	NLV
Brighton	February 96	96/43	110	750	+
Denmark	January '97	97/419	<20	7,200	+
Sheffield	February '97	97/101	-	7,463	+
Leeds	March '97	97/121	>18,000	34,725	+
Finland	April '97	97/214	<20	2,350	+
Cooked Mussels	February '98	98/86	<20	480	+
Westminster	February '98	98/105	-	540	+
Westminster	February '98	98/286	<20	630	+
Ireland	March '99	99/174	220	240	+
Milton Keynes	March '99	99/245	<20	1275	+
Oxford	March '99	99/256	40	233	-
Danish Batch 1	December '99	99/772	-	570	+
Danish Batch 2	December '99	99/773	-	90	+W
New Zealand	January '00	00/3	9,100	263	+
New Zealand	January '00	00/4	40	<30	+
Belfast Outbreak	January '00	00/18	20	3690	+
Lincoln	January '00	00/23	<20	150	+
Padstow	March '00	00/53	<20	600	+
Denmark (France)	March '00	00/55	<20	<30	-
Denmark (France)	March '00	00/56	<20	300	+
Denmark (France)	January 01	01/31	<20	240	+

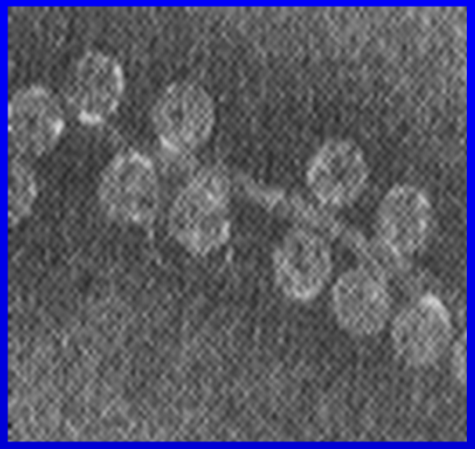
More shellfish associated with outbreaks (2001-2003)

Table 5. FRNA bacteriophage species in outbreak associated samples

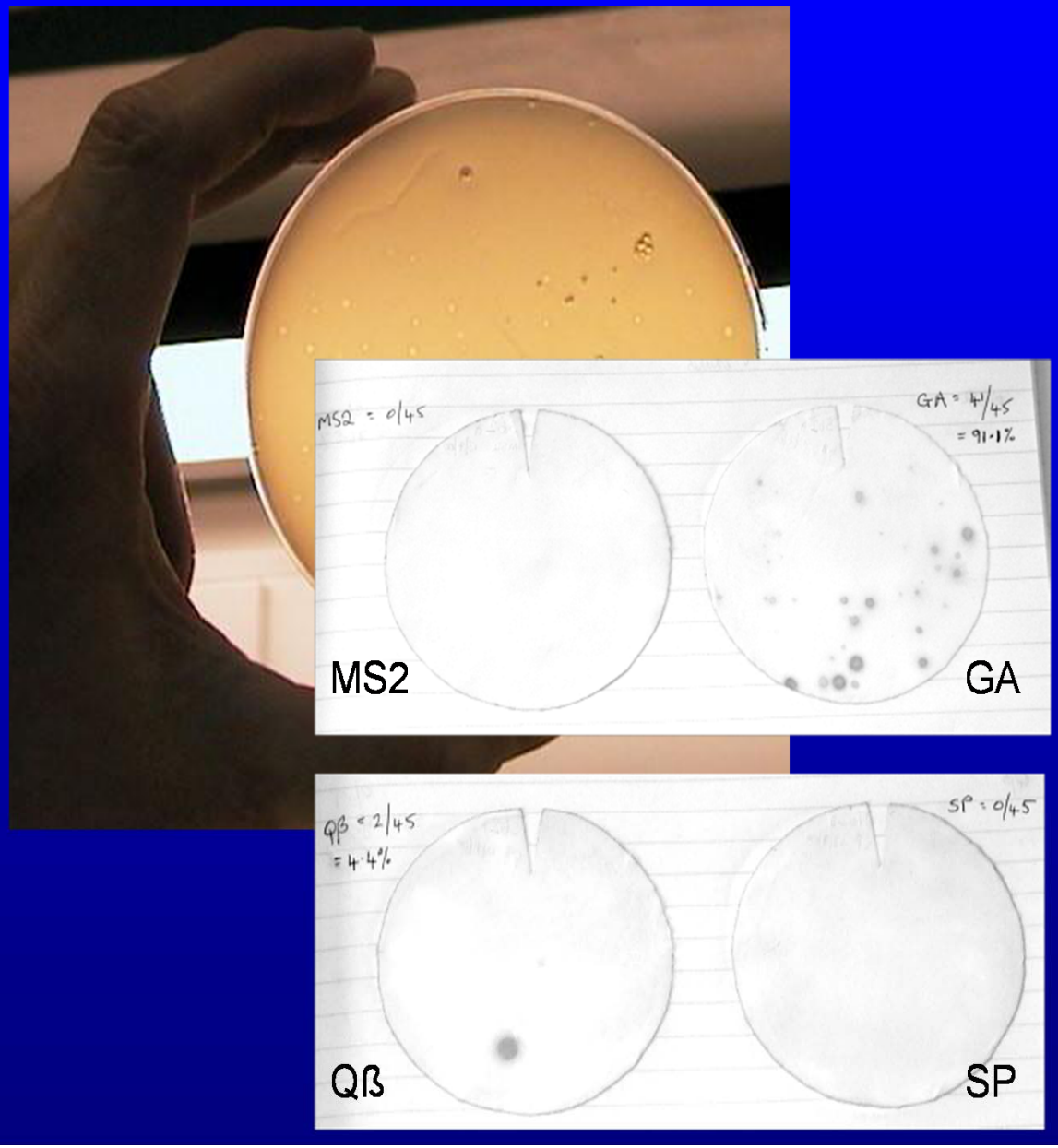
Species	*E. coli*/100 g shellfish flesh	FRNA bacteriophage/100 g with typing results*
C. gigas	40	3930 (84.2, 0, 15.8, 0)
C. gigas	220	14767 (99.4 , 0, 0, 0)
M. edulis	<20	15690 (100, 0, 0, 0)
C. gigas	<20	2850 (83.3,0, 0, 0)
C. gigas	250	5167 (100, 0, 0 ,0)
M.edulis	<20	3000 (56.2, 0, 43.8, 0)

*bracketed figures indicate % occurrence of GA, *Qβ*, *MS2* and SP respectively
nt- not tested

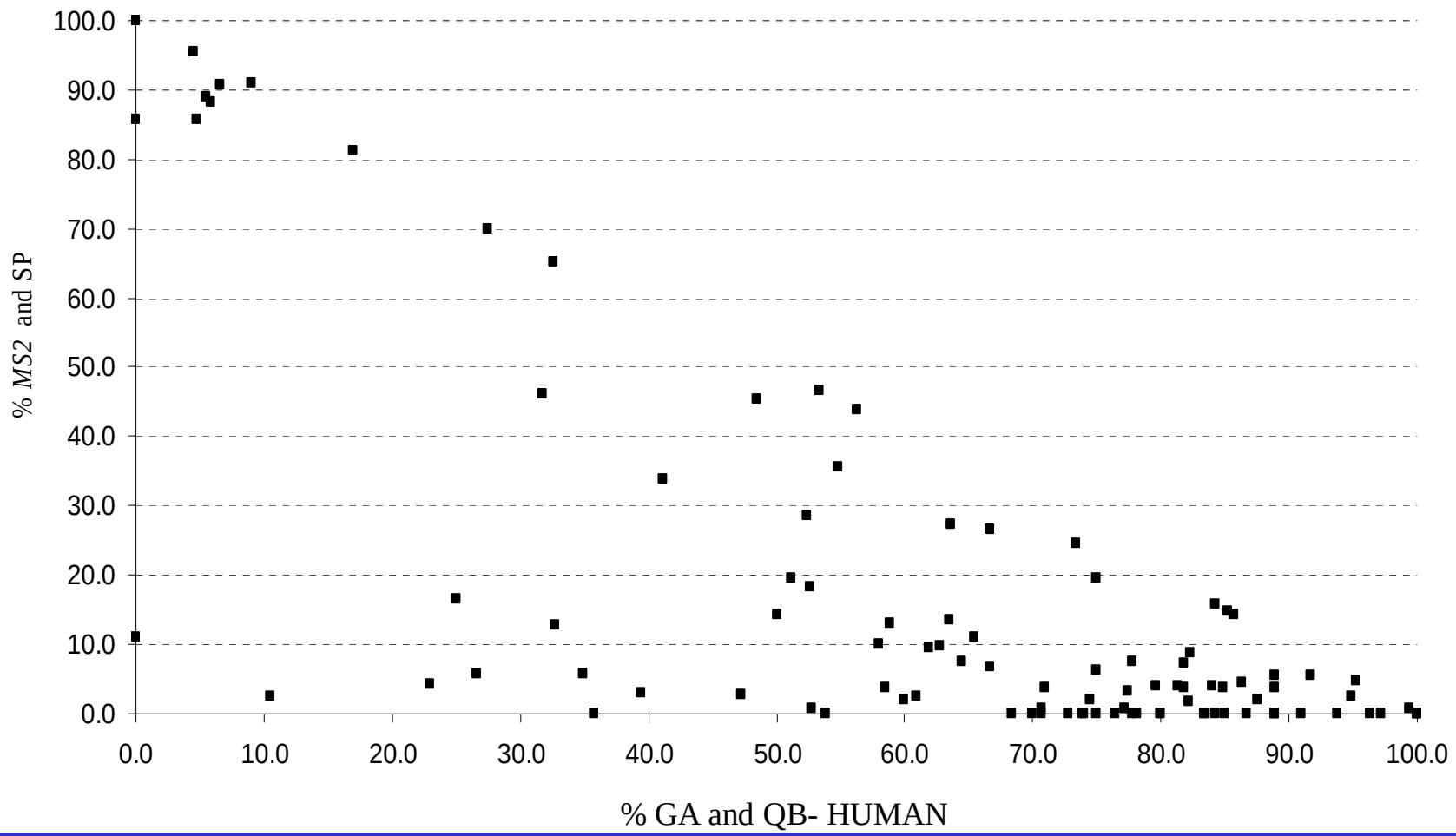
FRNA bacteriophage ‘viral’ indicator



- ssRNA
- simple cubic capsid
- 25-30nm
- hardy in environment
- easy to assay



Animal type FRNA bacteriophage against human type FRNA bacteriophage, isolated from shellfish harvested from site representative of all FSA classification areas



Investigation into the prevalence, distribution and levels of norovirus titre in oyster harvesting areas in the UK

- Testing of monthly oyster samples (May 2009 – April 2011) from representative selection of commercial sites from around UK for norovirus GI and GII
- 39 representative sites
- 844 results for norovirus
- 643 positive for norovirus (76.2%), 402 positive for both genogroups, 176 for GI only, 65 for GII only

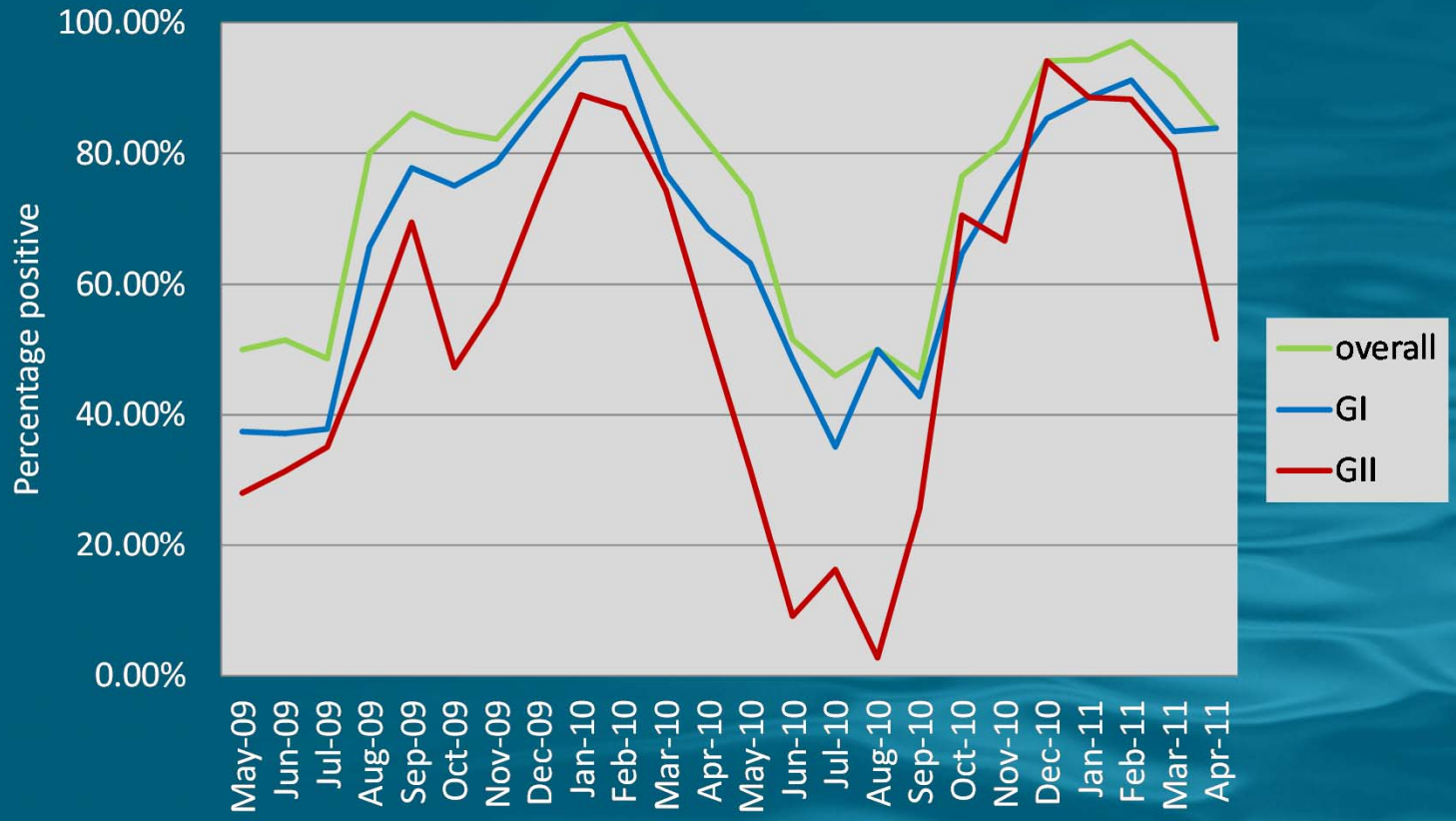
(Lowther et al 2012 AEM)

Cefas

Prevalence

- Prevalence of both GI and GII increased during winter, but overall prevalence >40% even in summer

- GI more commonly recorded than GII

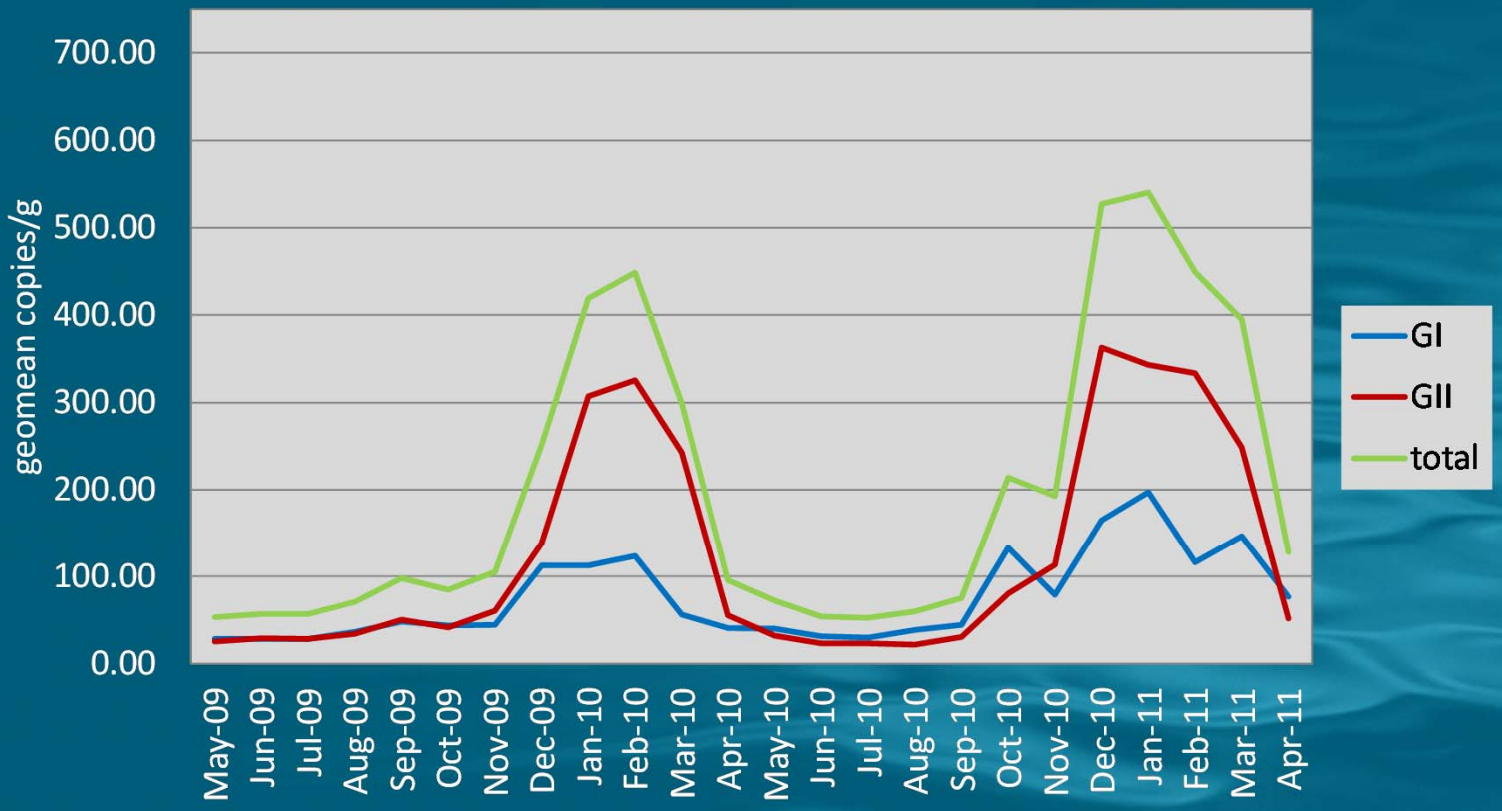


Cefas

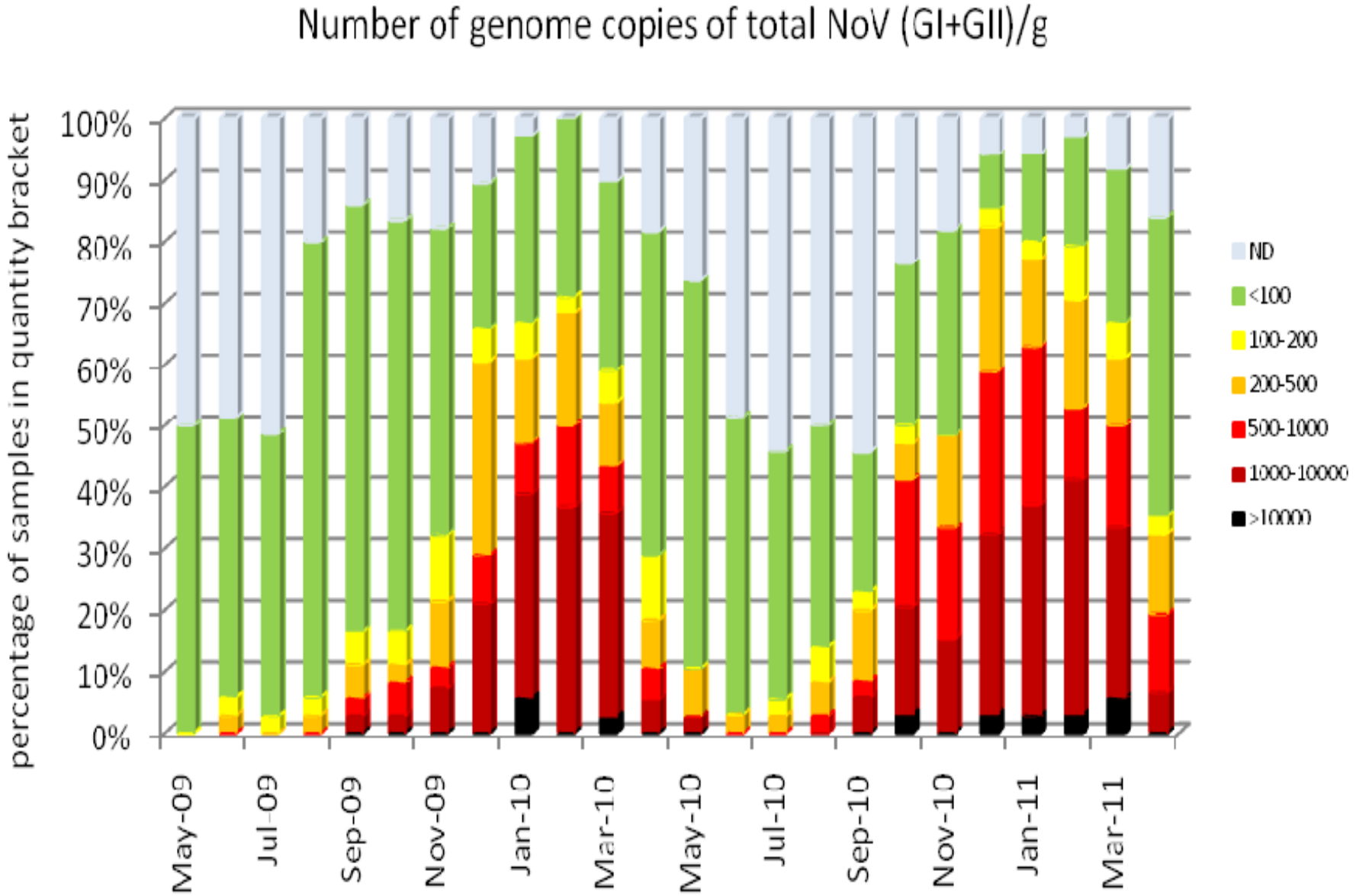
Average norovirus levels

- Clear increase in average norovirus levels (both genogroups) in autumn and decrease in spring

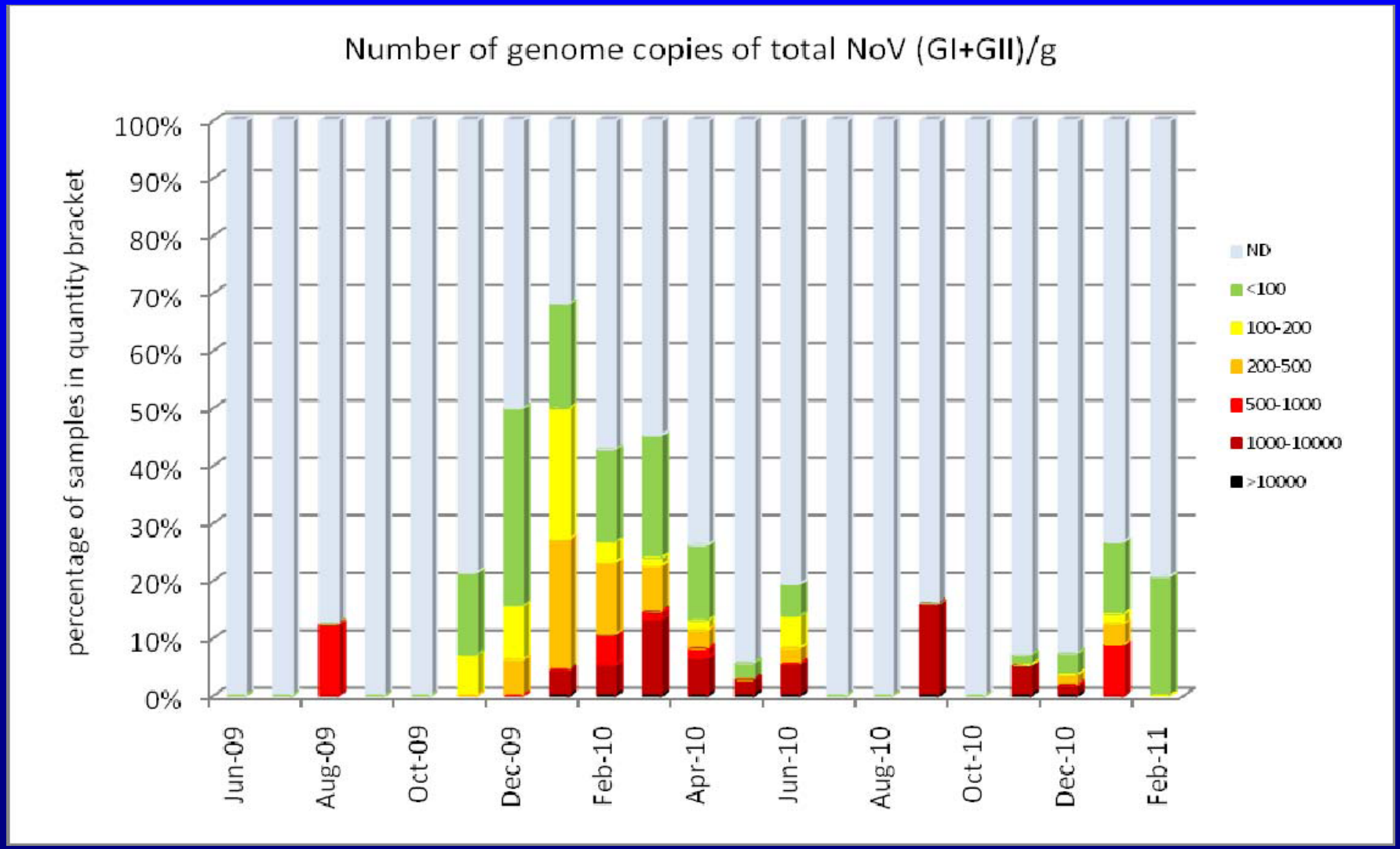
- Recorded GI levels normally lower than GII especially during winter



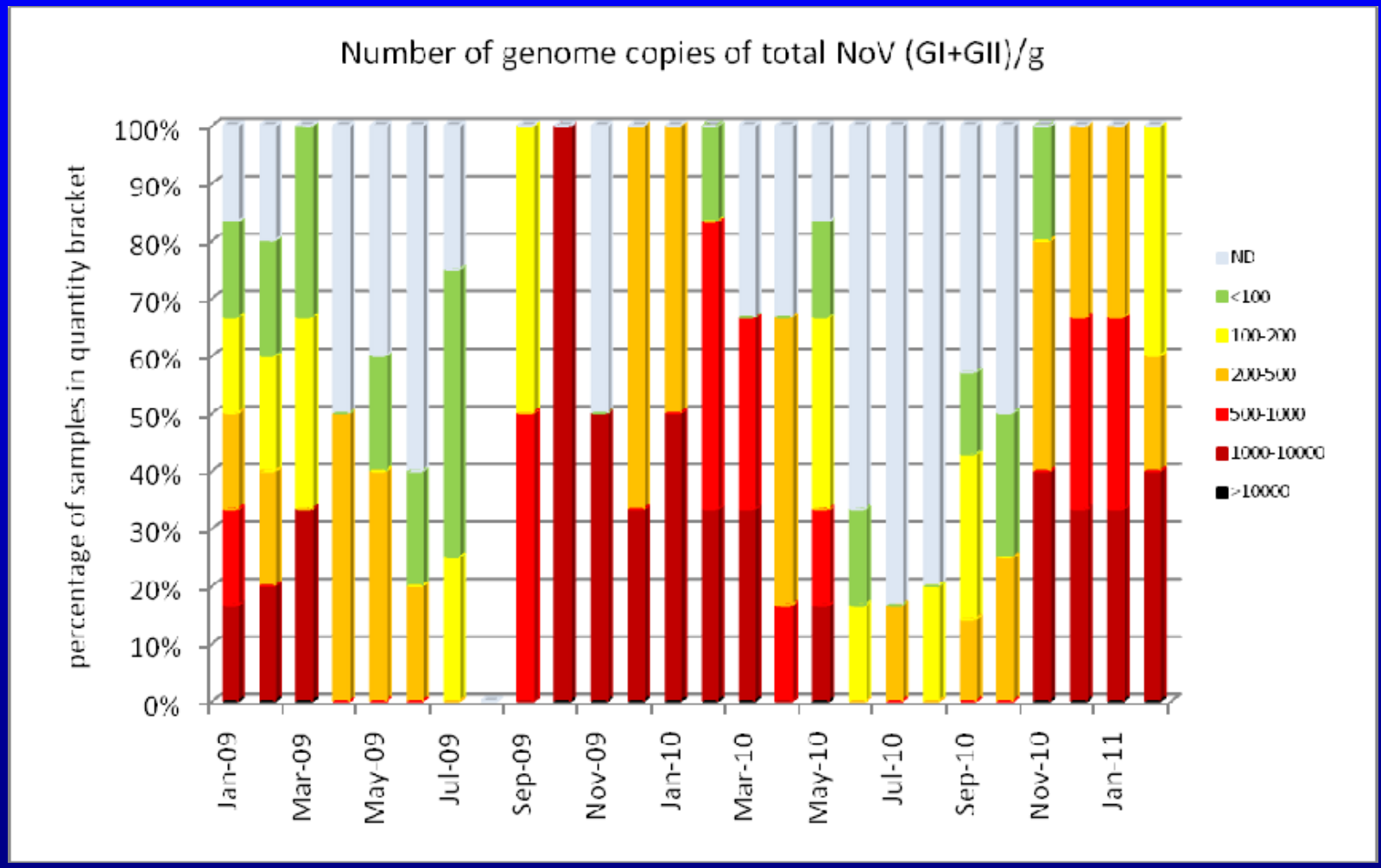
Cefas


Number of genome copies of total NoV (GI+GII)/g
percentage of samples in quantity bracket
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
May-09
Jul-09
Sep-09
Nov-09
Jan-10
Mar-10
May-10
Jul-10
Sep-10
Nov-10
Jan-11
Mar-11
ND
<100
100-200
200-500
500-1000
1000-10000
>10000

EFSA opinion: Quantitative data vs possible limits France



EFSA opinion: Quantitative data vs possible limits Ireland



Note – worst case scenario (not systematic data)

Investigation into the use of F+phage to indicate the viral risk associated with end-product oysters

- End-product oysters obtained from 4 commercial producers.
- Sampled from Feb 95 to Mar 97
- Assayed for
  - E. coli
  - FRNA bacteriophage
  - NLVs

Degree of faecal contamination in oyster harvesting areas during the study period determined by *E.coli* analysis of oysters taken directly from each harvesting area and the reported incidence of gastrointestinal illness associated with products from each site.

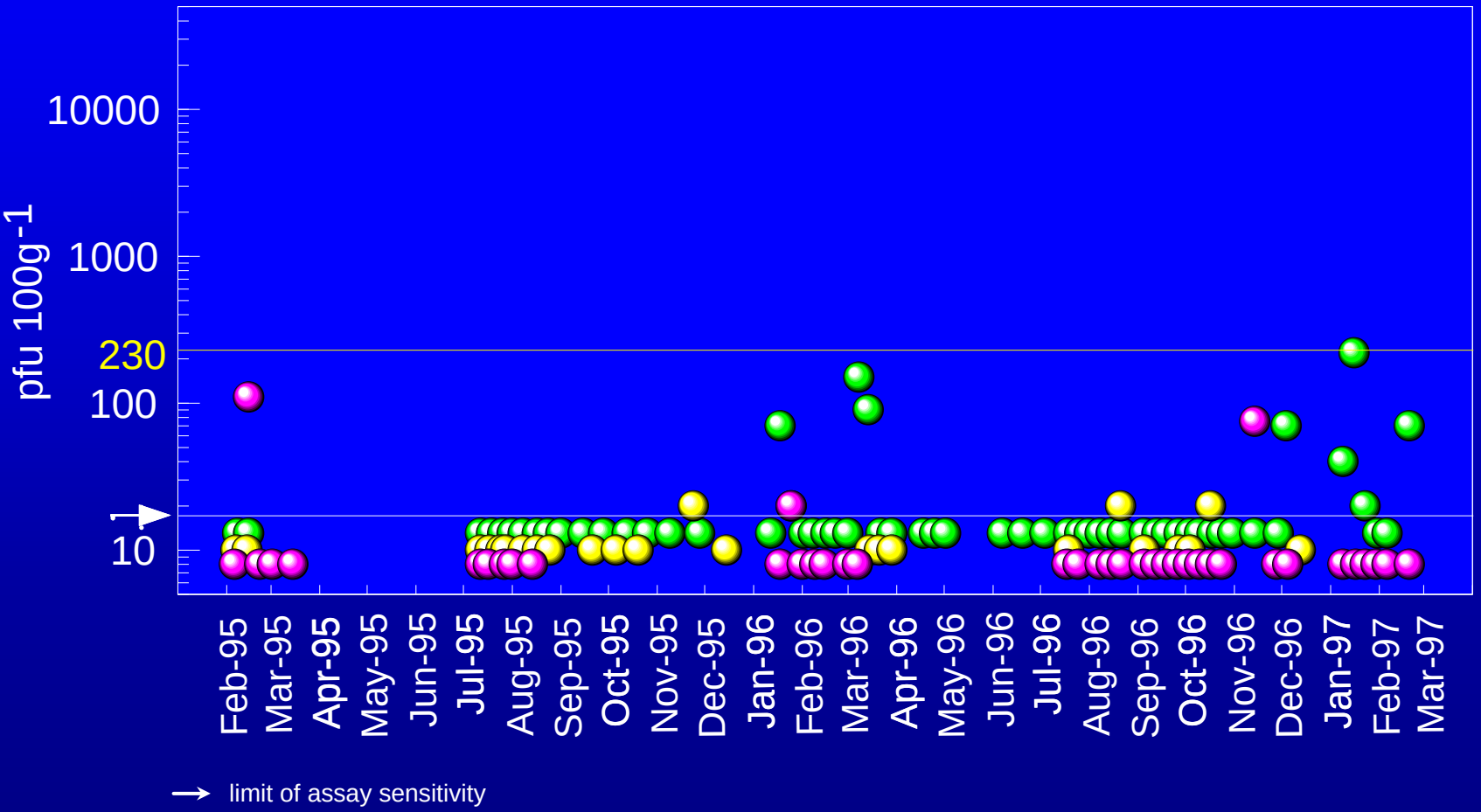
		E.coli (per 100g) from oyster harvesting area			Incidence of Illness[1]	
Site	Classification[2]	Minimum	Maximum	Geometric mean (n)	Outbreaks	Cases
1	A	<20	40	6 (20)	0	0
2	A/B[3]	<20	1300	15 (30)	0	0
3	B	<20	5000	48 (22)	1	10
4	B	<20	22000	363 (59)	6	97

[1] Officially reported incidents of gastrointestinal disease associated with oysters from each site during the study period. Disease statistics kindly provided by Public Health Laboratory Service, Communicable Disease Surveillance Centre, Colindale, UK

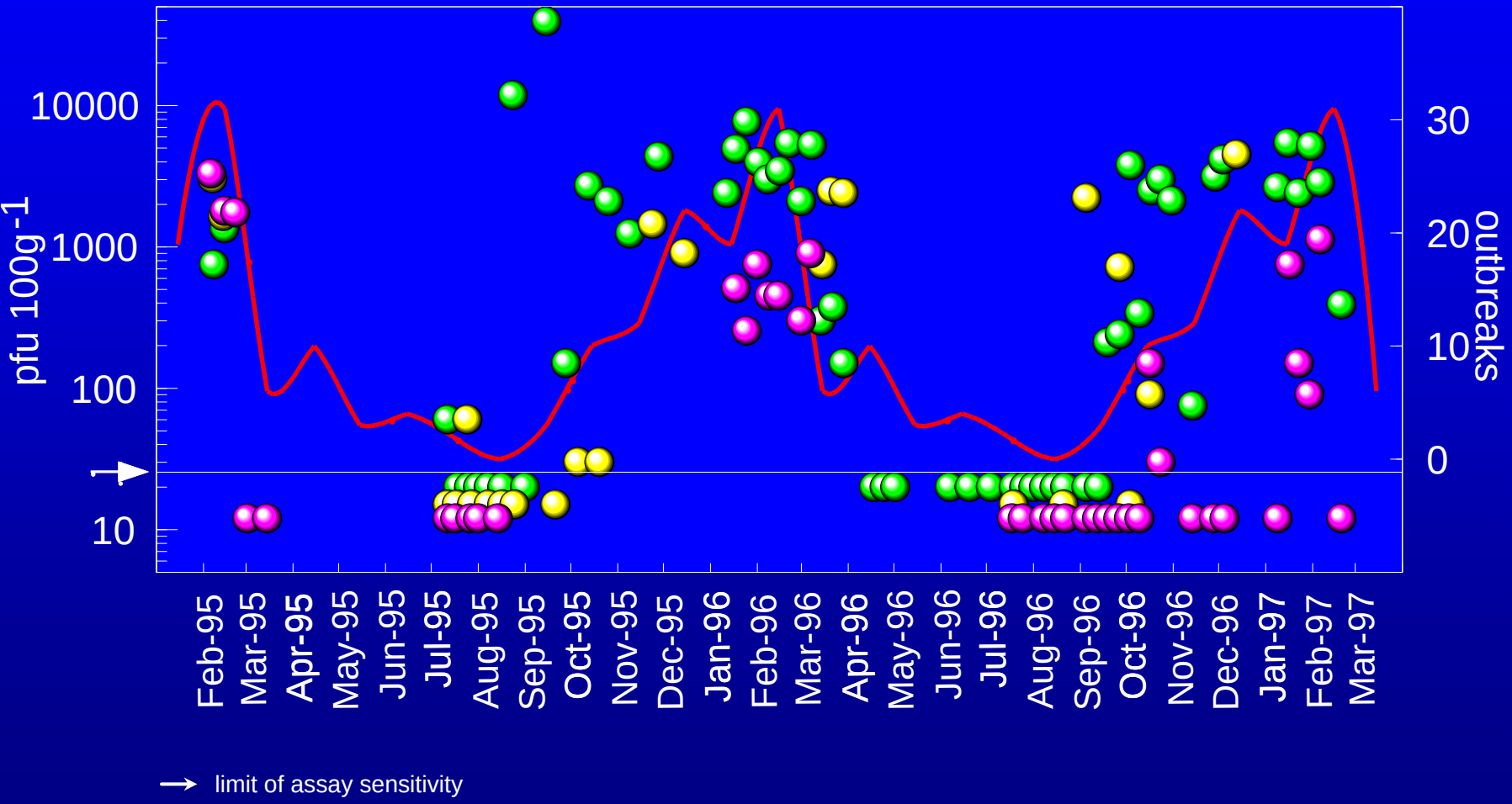
[2] Formal classification of shellfish harvesting area by UK Authorities (Ministry of Agriculture) according to EU Directive 91/492

[3] Both A and B classifications awarded during study period

Seasonal distribution of E. coli at sites 2 (●), 3 (●) and 4 (●)



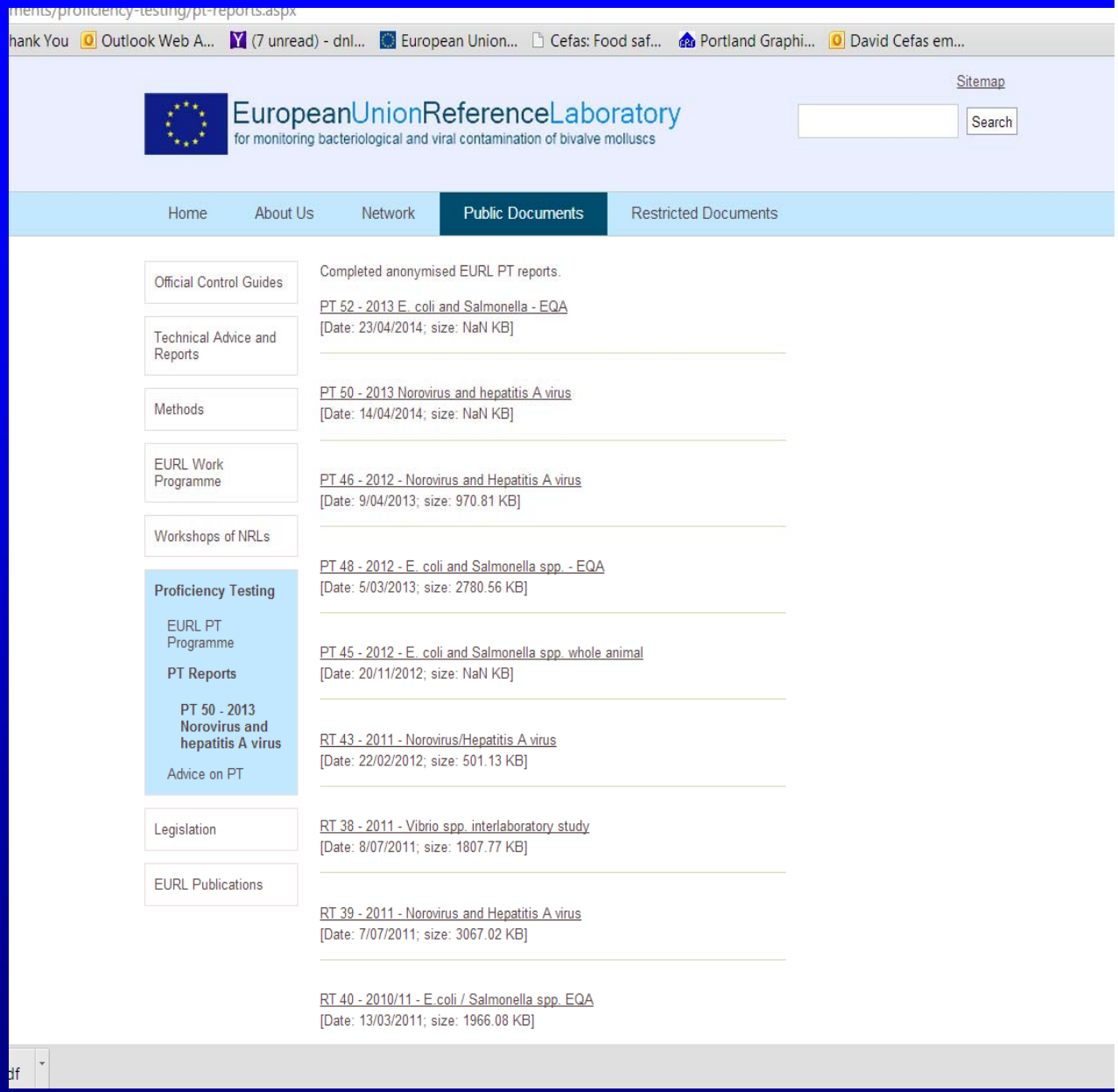
Seasonal distribution of F+ bacteriophage at sites 2 (●), 3 (●) and 4 (●)



Summary of results for faecal pollution indicators and enteric pathogens (NLVs) in market ready oysters from each site during the study period

	E.coli (per 100g)			F+ bacteriophage (per 100g)			NLV (per 7g)
Site	Minimum	Maximum	Geometric mean (n)	Minimum	Maximum	Geometric mean	% positive (n)
1	<20	<20	0.0 (13)	<30	<30	0.0 (13)	0 (7)
2	<20	110	0.4 (39)	<30	3300	11.3 (39)	0 (15)
3	<20	50	0.5 (24)	<30	4500	41.4 (24)	0 (13)
4	<20	220	0.9 (58)	<30	39300	125.9 (58)	37 (35)

- FRNA phage PT until 2007
- all reports on www.eurlcefas.org



CEFAS

The Centre for Environment, Fisheries & Aquaculture Science

European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs

Report on 2nd FRNA bacteriophage ring trial, 2003

Author : Louise Stockley, 22nd March 2004

CRL ring trial reference : RT6 (FRNA bacteriophage, 2003)

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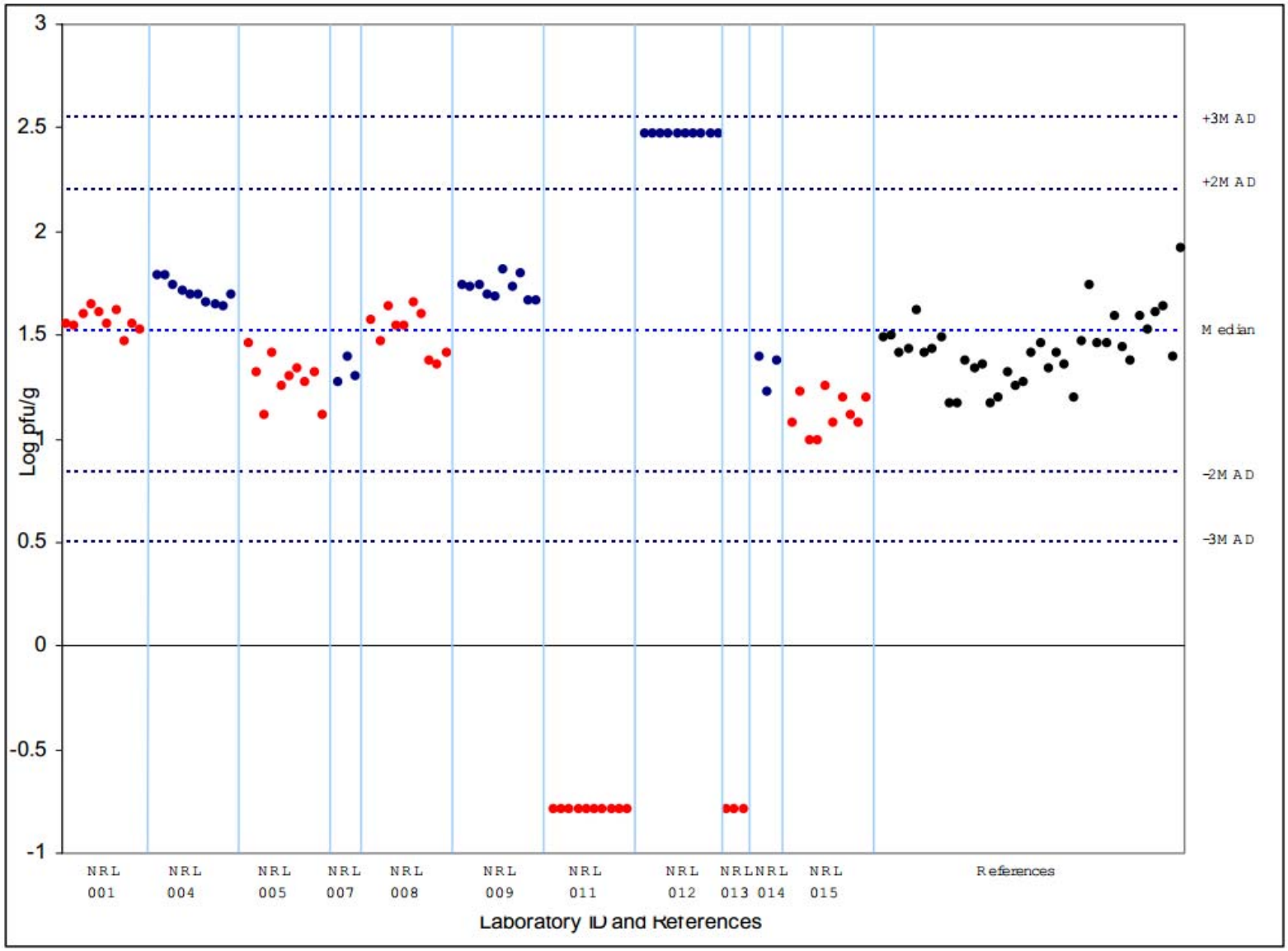

Fig 1. Results for FRNA ring trial distribution vial 1 (September 2003)