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Executive summary

In this two year study, monthly samples of Pacific (*Crassostrea gigas*) or native oysters (*Ostrea edulis*) from 39 production areas around the UK were tested for norovirus genogroups I (GI) and II (GII) using a quantitative real-time RT-PCR method. All samples were also tested for *E. coli* using the regulatory reference method. All sites sampled were designated and classified under EU Regulation 854/2004 and thus available for commercial production. Classifications ranged from class A to class C. Sites included in the study were selected using a risk matrix score to ensure a representative selection of likely faecal contamination levels.

Norovirus was detected in 76.2% (643/844 of samples), with similar prevalences in the two species of oysters tested (76.1% (468/615) for *C. gigas* and 76.4% (175/229) for *O. edulis*). There was a marked seasonality with a positivity rate of 90.0% (379/421) for samples taken between October and March compared with 62.4% (264/423) for samples taken between April and September.

Quantification of positive samples revealed that the majority (52.1%; 335/643) were below the limit of quantification (100 detectable genome copies/g digestive tissues) for both genogroups. However a number of samples (1.4% of all positives; 9/463) contained levels in excess of 10,000 copies/g. As with prevalence, average quantities varied markedly between seasons, with highest levels detected between December and March. The scores for norovirus GI and GII in individual samples were significantly correlated with each other, however results for norovirus GII tended on average to be higher than those for GI.

All 39 sites tested provided at least one norovirus positive result, although prevalence varied from 21% (5/24 samples) to 100% (20/20 samples). Norovirus levels varied markedly between sites with some sites scoring consistently over 1,000 copies/g during the winter while others rarely or never exceeded 100 copies/g.

This study also examined relationships between norovirus contamination and potential risk indicators. A statistically significant correlation between norovirus levels and harvesting area classification was observed. Furthermore a statistically significant and predictive correlation was found between *E. coli* and norovirus levels when data was analysed by site rather than by sample. This finding provides support for the use of *E. coli* as an indicator organism for classification purposes. Strong correlations between norovirus contamination and environmental temperatures were also found on both a nationwide average and in the majority of cases a site-by-site basis.

A statistically significant correlation was also observed between the site-specific risk scores initially assigned and the norovirus contamination levels observed in the study. We were able to further improve the predictive power of the risk matrix based on a combination of the *E. coli* contamination profile for the site and the seawater temperature at the time of sampling. This approach could assist risk management in relation to, for example, highlighting the need for virus monitoring or enhanced operator control measures.

In summary the data generated in this study provides a systematic analysis of norovirus contamination in classified oyster production areas in the UK. The data will help to inform FSA policy on the reduction of viral contamination of bivalve molluscs and to instruct a coherent EU negotiating position.

Acknowledgements

This project was funded by the Food Standards Agency, UK. We would like to thank all participating oyster producers and local authority sampling officers for their co-operation and contributions, without which this project would not have been possible. In addition the Shellfish Association of Great Britain provided valuable assistance in recruiting participants.

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1.0 Introduction

Contamination of bivalve shellfish with norovirus from human faecal sources is recognised as a major human health risk (Lees, 2000). Risk assessment and management currently relies on the use of *Escherichia coli* as an indicator of faecal (sewage) contamination in shellfish (Anon, 2004). However, this approach has been repeatedly demonstrated to inadequately contain the risk from human enteric viruses (Ang, 1998; Chalmers & McMillan, 1995; Gill *et al*., 1983). Over the last decade considerable progress has been made towards development of sensitive detection methods for norovirus in molluscan shellfish and there are numerous publications describing various approaches to virus testing (Höhne and Schreier, 2004; Jothikumar *et al*., 2005; Le Guyader *et al*., 2009; Loisy *et al*., 2005). All available methods are based on detection of virus genome using molecular techniques (PCR), as successful cultivation of norovirus by conventional virological techniques has been reported only rarely and is not feasible for application to foodstuffs. At present no internationally accepted reference method exists, however the European Committee on Normalisation (CEN) has an active working group, chaired by Cefas, addressing the development of a standard method for detection of norovirus and HAV in foodstuffs (including bivalve shellfish). A draft method is nearing completion (Lees and CEN WG6 TAG4, 2010) and this will be succeeded by an international method validation exercise. The timetable for publication of the CEN Technical Specification is 2012. EU legislative text foreshadows the adoption of virus controls when the methods are sufficiently developed and available for use (Anon, 2004; Anon, 2005). Thus it is possible that following publication of a CEN standard viral controls may be adopted into Community feed and food hygiene legislation. In preparation for possible legislative viral standards across the EU it has become essential to gain information about the practical application of the methods and potential impact on UK shellfisheries.

Previously published and informal studies examining norovirus in UK oysters have revealed the presence of viral RNA in a significant proportion of samples, as well as indicating a clear seasonal pattern of contamination with higher prevalence and levels detected during the winter months from October to March (Lowther *et al*., 2008; Lowther *et al*., 2010). These studies have however all targeted specific production areas due to for example historical links to outbreaks of gastroenteritis, and the sampling plans were developed on an *ad hoc* basis according to the particular needs of the project. This project aimed to address these issues through application of a systematic sampling plan to oysters collected from a representative selection of UK oyster production sites and by testing these oysters for norovirus genogroups I and II using the most up-to-date quantitative methods based on the developing CEN standard. In this way the study seeks to generate systematic quantitative data on the occurrence, prevalence and levels of norovirus in UK production areas. The outputs of this project may help to inform FSA discussions with respect to reducing viral contamination of bivalve molluscs.

2.0 Aims and objectives

The aim of this project was to determine the rates of prevalence and typical levels of norovirus in oysters from a representative selection of sites from around the UK. The project also aimed to assess any seasonal variations and any relationships of norovirus levels with *E. coli* and other risk factors (such as population, environmental temperature, etc.).

3.0 Methods

3.1 Sample collection

Samples were collected by the relevant local authority sampling officers in parallel to the collection of samples for the statutory classification monitoring programme. Sampling officers were asked to collect one sample per month of thirty oysters from each selected site from May 2009 to April 2011 inclusive. These were dispatched to the laboratory under temperature controlled conditions. Where practicable, water temperatures at the sampling site were taken using an approved method (http://www.cefas.defra.gov.uk/media/436986/samplingprotocolforlocalauthorities%5b1%5d.pdf) at the time of sampling.

3.2 Detection and quantification of GI and GII norovirus

3.2.1 Preparation of oyster homogenate

Ten oysters from each sample under test were cleaned, opened and removed from their shells. The peripheral flesh and organs of the animals were then cut away from the digestive gland and discarded. The glands of the oysters were pooled together then chopped finely with a razor-blade. A 2g portion of the chopped glands was added to a 50ml centrifuge tube for immediate testing and the remainder was retained frozen at -20ºC. A 10µl volume of Mengo virus strain vMC_0 (to act as a process control) and 2ml of 100 µg/ml proteinase K solution (30U/mg; Promega) were then added to the chopped glands in the 50ml tube. The tube was then incubated at 37°C with shaking at 320 rpm for a duration of 1 hour, and subsequently incubated at 60°C for a duration of 15 min. The tube was centrifuged at 3000 x g for 5 min, then the soluble portion (homogenate) poured into a clean tube. Finally the volume of the homogenate was measured using a pipette, then it was retained at 4ºC for RNA purification and RT-PCR.

3.2.2 Purification of viral RNA

Viral RNA extraction was carried out using NucliSens magnetic extraction technology (BioMerieux). For each sample a 500µl aliquot of sample homogenate was added to 2ml NucliSens lysis buffer in a 15ml centrifuge tube. In addition for each batch of samples tested negative control and positive extraction control samples consisting of 500µl water only and norovirus positive oyster homogenate (derived from bioaccumulated animals) respectively were also prepared and tested in parallel. Samples and controls were vortexed briefly then incubated at room temperature for 10 min before 50µl magnetic silica was added to each tube and the samples incubated at room temperature for a further 10 min. The tubes were centrifuged at 1500 x g for 2 min and the supernatants removed by aspiration. The pelleted silica beads were resuspended in 400µl wash buffer 1 then transferred to individually labelled 1.5ml tubes on the MiniMag extraction station. The magnet of the MiniMag was raised to collect the silica beads on the walls of the tubes, then the beads washed for 30 secs using the wash function of the MiniMag. The supernatants were removed by aspiration, then the magnet lowered and the silica beads resuspended with 400µl of wash buffer 1, washed and the supernatant aspirated as before. The resuspension/wash/aspiration cycle was then repeated using 500µl wash buffer 2 then 500µl wash buffer 3 (wash for 15 secs only). The pelleted silica beads were then resuspended with 100µl of elution buffer. The tubes were capped and transferred to the thermoshaker at 60°C and shaken at 1400 rpm for 5 min, to allow elution of DNA and RNA from the silica beads. After elution the tubes were transferred to a portable magnet to collect the silica beads on the walls of the tubes, then the supernatant (sample nucleic acid (NA) extract) was transferred to a clean 0.5ml tube and stored at -20°C until required for reverse transcription.

3.2.3 One-step RT-PCR

For GI QNIF4 (da Silva *et al.,* 2007), and NV1LCR primers (Svraka *et al.,* 2007), and TM9 probe (Höhne and Schreier, 2006) were used. For GII, QNIF2 (Loisy *et al.,* 2005), and COG2R primers (Kageyama *et al.,* 2003), and QNIFS probe (Loisy *et al.,* 2005) were used. For each sample or extraction control and both norovirus genogroups three aliquots of 5µl NA extract were added to adjacent wells of a 96-well optical reaction plate and made up to 25µl with (GI or GII) TaqMan reaction mix (final concentration of 1x each Ultrasense reaction mix, Rox reference dye and RNA Ultrasense enzyme mix (Invitrogen), 500 nM forward primer, 900 nM reverse primer, and 250 nM probe). Positive (dilution series prepared from a known concentration of plasmid carrying a copy of the target sequence) and negative (three aliquots of 5µl water only) PCR control materials were also tested. The plate was placed in a Stratagene Mx3005P real-time PCR machine with the following amplification program; 55°C for 60 min, then 95°C for 5 min, followed by 45 cycles of 95°C for 15 secs, 60°C for 1 min and 65°C for 1 min. For analysis, threshold values were set at 0.20 fluorescence units, then threshold cycle (Ct) values were determined using the Mx3005P system software. Unexpected results in any positive or negative extraction or RT-PCR control triggered retesting of any affected samples.

3.2.4 Quantification of norovirus using dsDNA standard curve analysis

Norovirus titres were calculated based upon the principles of the forthcoming CEN standard method for quantification of viruses from CEN/TC275/WG6/TAG4. On each TaqMan run a log dilution series of dsDNA control corresponding to a range of approx 1 to 10,000 template copies/µl (quantified using spectrophotometry at 260nm) was included. The Ct values from this dilution series were then used to produce a standard curve. For each TaqMan replicate for the samples under test a quantity in copies/µl was determined using the corresponding standard curve. Not detected replicates were ascribed a quantity of zero. The average quantities from the three replicates in each norovirus genogroup-specific TaqMan assay were calculated to give an overall quantity in detectable copies/µl NA extract for that sample and genogroup. This was multiplied by 100 to give detectable copies/test portion of homogenate (500µl), further multiplied by a factor based on the proportion of the complete homogenate tested, and finally divided by 2 to give a quantity in detectable genome copies/g digestive gland. This value does not take account of any losses during processing or RT-PCR inhibition and should be regarded as a minimum. Uncertainty of measurement of this method has been estimated at ±0.17 $\log_{10}$ copies/g.

3.2.5 Calculation of RT-PCR efficiency

For each sample and each norovirus genogroup one aliquot of 5µl NA extract was added to a 96-well optical reaction plate and made up to 25µl with (GI or GII) TaqMan reaction mix (final concentrations as described above). A 1µl volume containing a high concentration of GI or GII RNA sequences (produced by *in vitro* transcription from the dsDNA control) was then added to each aliquot of sample NA extract in addition to a 5µl aliquot of water in a separate well. The plate was placed in a Stratagene Mx3005P real-time PCR machine and amplified using the program described above. The percentage RT-PCR efficiency for each sample and each gengroup was determined by comparing the Ct values for the sample NA extract plus control NA extract with that for the water plus control NA extract. Any sample with an RT-PCR efficiency of <25% was subjected to retesting, in the first instance by re-extracting the viral RNA from stored homogenate, then by testing stored digestive glands. Results for any sample providing three RT-PCR efficiency results of <25% were considered invalid and are recorded as “no result” in Appendix 3.

3.2.6 Calculation of extraction efficiency

For each sample two aliquots of 5µl NA extract were added to adjacent wells of a 96-well optical reaction plate and made up to 25µl with Mengo virus-specific TaqMan reaction mix (final concentrations as described above). A dilution series prepared from the Mengo virus process control material was also tested. The plate was placed in a Stratagene Mx3005P real-time PCR machine and amplified using the program described above. The percentage extraction efficiency for each sample was determined by comparing the Ct values for the sample NA extract with those for the Mengo virus dilution series. Any sample with an extraction efficiency of <1% was subjected to retesting, in the first instance by re-extracting the viral RNA from stored homogenate, then by testing stored digestive glands. Results for any sample providing three extraction efficiency results of <1% were considered invalid and are recorded in Appendix 3 as “no result”.

3.2.7 Expression of results

Samples where all three RT-PCR reactions for a given genogroup were negative are described as “not detected; <40 copies/g” in Appendix 3 (40 copies/g is the practical limit of detection of the method). Samples where the calculated quantity (as described above) was >500 copies/g are described as “positive; x copies/g” where x is the calculated quantity. Samples where the calculated quantity was between 100 and 500 copies/g are described as “positive; x copies/g” unless there were significant differences between results in individual RT-PCR reactions, in which case they are described as “positive; 100-500 copies/g”. Samples where the calculated quantity was <100 copies/g (100 copies/g is the practical limit of quantification of the method) are described as “positive; <100 copies/g).

3.3 Detection and quantification of *Escherichia coli*

3.3.1 Preparation of oyster homogenate

Ten oysters from each sample under test were cleaned, opened and removed from their shells. The flesh was added to a blender with 2 volumes (per weight) of a 0.1% (w/v) solution of peptone (Oxoid) then homogenized using 4 bursts of 15 seconds at high speed. The homogenate was then retained at 4ºC for up to 24 hrs prior to enumeration.

3.3.2 Enumeration of *E. coli*

Oyster homogenates were assayed for *E. coli* using a standard most probable number method derived from ISO TS 16649-3. The homogenate was initially diluted using 0.1% (w/v) peptone to a concentration of 10% (v/v) ("neat"). A serial $\log_{10}$ dilution series from neat to 10^{-2} was then prepared. Diluted homogenate was inoculated into five tubes containing mineral-modified glutamate broth (Oxoid) for each of the three dilutions and the tubes were then incubated at 37°C for 18-24 hrs.

Confirmation of acid producing tubes was carried out by subculture onto a chromogenic agar containing 5-bromo-4-chloro-3-indolyl-β-D glucoronide (LabM) and subsequent incubation at 44°C for at further 18-24 hrs. Blue-green colonies indicative of the action of β-glucoronidase activity were considered to be *E. coli* positive. The most probable number (MPN) was derived by reference to MPN tables contained within ISO 7218. The theoretical limit of detection of the assay was 20 MPN/100g shellfish and intravalvular fluid.

3.4 Statistical analysis

All statistical analysis was carried out using Minitab 16 software. For the purposes of analysis samples giving not detected results for a particular norovirus genogroup were scored 20 copies/g for that genogroup (half the limit of detection (LOD) of 40 copies/g). Samples giving positive results below the limit of quantification (LOQ; 100 copies/g) were scored 50 copies/g. For the purposes of figures and analyses where total counts of norovirus (GI and GII summed) were used, samples giving positive results <LOQ for both genogroup scored 100 copies/g; such results were included in the <100 copies/g bracket where such brackets were used. For *E. coli* samples giving results <20 MPN/100g were scored 10 MPN/100g.

4.0 Comparison of native and Pacific oysters as a test matrix

4.1 Introduction

Prior to this project the laboratory had previous experience of testing large numbers of samples of Pacific oysters (*Crassostrea gigas*) for norovirus using the detection methods described but had tested comparatively small numbers of samples of native oysters (*Ostrea edulis*). For this reason, prior to selection of sites for surveillance it was necessary to determine the suitability of the test method for use with both species of oysters.

4.2 Experimental approach

For both oyster species, a number of animals were opened and the digestive glands excised as described in section 3.1.1. Separately for each species, glands were pooled together until >10g was present, chopped finely with a razor-blade, and apportioned in 2g aliquots into five tubes. Each aliquot was artificially contaminated with an identical volume of norovirus GII positive faecal suspension, then subjected to Proteinase K treatment, RNA purification in duplicate and quantitative RT-PCR for GII as described in section 3.1.

This procedure was repeated a total of three times using different contamination levels designed to result in approximate concentrations of 500,000, 50,000 and 5,000 copies/g respectively ('high', 'medium' and 'low').

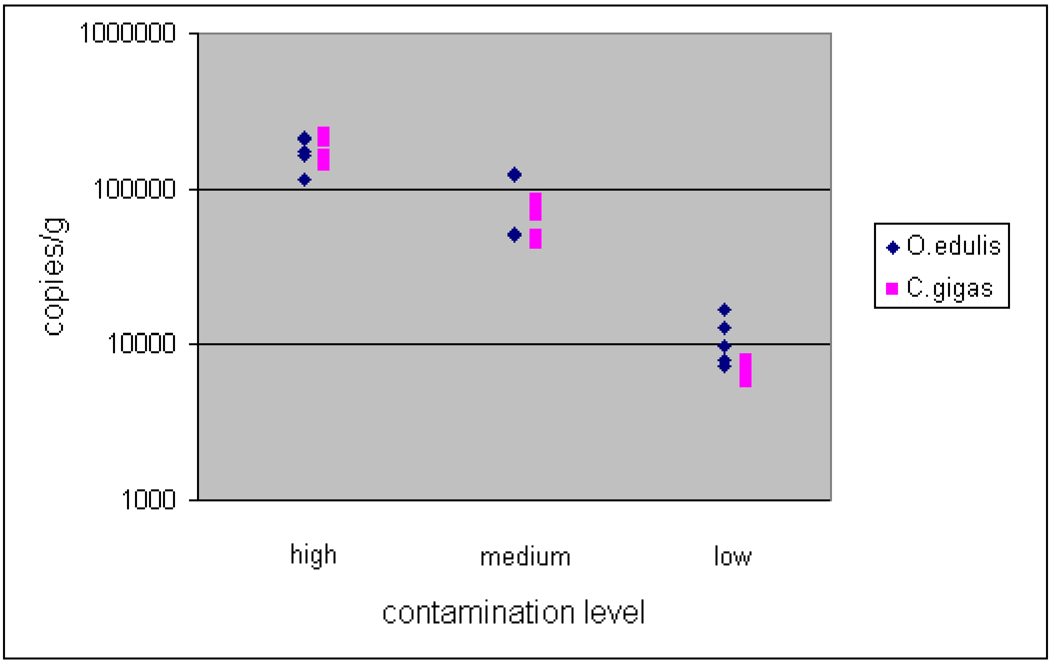

Figure 1: Recovery of norovirus genogroup II from aliquots of native (*O. edulis*) and Pacific (*C. gigas*) oysters contaminated with faecal suspension.

4.3 Results

Quantities in copies/g were calculated for all extractions (see Appendix 1). Results for duplicate extractions from the same homogenate were then averaged (Figure 1). Two-way analysis of variance of the log_{10} transformed averaged data revealed that as expected contamination level had a significant impact on calculated quantities ($p<0.001$), however there was no significant difference between species ($p=0.232$), and there was also no significant interaction between species and contamination level ($p=0.233$; see Appendix 3 for statistical analysis).

4.4 Conclusion

Based on the data generated there was no evidence that the performance of the selected detection method varies significantly between the two oyster species of commercial significance in the UK. Both species were therefore included in the surveillance part of this project.

5.0 Selection of oyster sites for surveillance

5.1 Risk Assessment

5.1.1 Introduction

In this study risk scores were used to rank sites in order to provide a more scientific basis for sampling site selection. They should not be over-interpreted since the actual scores are susceptible to the weightings applied for each factor and for many risks the correct public health weighting cannot readily be established. In addition risk scores derived for England and Wales, and for Scotland, cannot be directly compared since it was necessary to use different population geographic factors (see below). Risk assessment was not carried out for sites in Northern Ireland due to the small number of potential candidate sites.

5.1.2 England and Wales

All active representative monitoring points (RMPs) with 75% or more sampling compliance in official control monitoring in the previous sampling year were selected for risk assessment (total 121 sites from 38 production areas). Each site was assigned a risk score based on a risk matrix as detailed in Table 1.

Table 1: Risk based scoring system (risk matrix)

	Factor	Low (score 1)	Medium (score 3)	High (score 5)
1	Classification status 2006	A or unclassified	A/B, B, B-LT or provisional classification	A/B/C, B/C or C
2	Classification status 2007	As above	As above	As above
3	Classification status 2008	As above	As above	As above
4	Changes in classification status	None (or change from not classified or provisional)	One in three years	Two in three years
5	Unusual *E. coli* results (per 100g) 2006[1]	All results normal relative to annual classification scores 1 Class A $\leq$230 Class B $\leq$4,600 Class C $\leq$10,000	Each "high" result relative to annual classification scores 3[2] Class A 230-1,000 Class B 4,600-18,000 Class C 10,000-18,000	Each "very high" result relative to annual classification scores 5 Class A >1,000 Class B >18,000[3] Class C >18,000
6	Unusual *E. coli* results (per 100g) 2007	As above	As above	As above
7	Unusual *E. coli* results (per 100g) 2008	As above	As above	As above
8	Population in adjacent area[4]	<200,000	200,000-399,999	$\geq$400,000
9	Confirmed outbreaks[5]	None	-	One or more in three years
10	Unconfirmed outbreaks[6]	None	Between one and three in three years	More than three in three years

[1] Split classifications considered at an equivalent annual classification, e.g. results for an A/B area judged against class B criteria.
[2] Where no results were taken site scored 3 for the year in question.
[3] Working range TS ISO16649-3 is generally up to 18,000 *E. coli* MPN/100g so all results above this are reported as >18,000.
[4] Including all census areas of which at least part lies within 10km of the sampling point.
[5] Reported by Health Protection Agency or Health Protection Scotland for the years 2005, 2006 and 2007.
[6] Notified to FSA or the National Reference Laboratory for the years 2005, 2006 and 2007.

5.1.3 Scotland

Generation of risk data was carried out as part of FSAS SPIN 069 as previously described (Price-Hayward, 2010). All active production areas were selected for risk assessment (total 41 areas). Each area was assigned a risk score based on a risk matrix as detailed in Table 1, with the exception of different population cut-offs (Table 2) to take account of differences in population pressures.

Table 2: Population cut-off for risk assessment of oyster sites in Scotland

	Factor	Low (score 1)	Medium (score 3)	High (score 5)
8	Population in adjacent area[1]	<600	600-1,200	≥1,200

[1]Including all census areas of which at least part lies within 2km of the sampling point.

5.2 Site selection

5.2.1 England and Wales

A total of 31 sites (20 Pacific and 11 native oysters, Table 3) were selected using the following criteria.

1) sites with a broad range of risk scores
2) sites from a broad range of geographical locations
3) sites from a wide range of production areas; oyster production in England and Wales is concentrated on the South and East coasts so these regions are well represented

5.2.2 Northern Ireland

Two sites (both Pacific oysters) were selected in direct consultation with FSA Northern Ireland.

5.2.3 Scotland

A total of 6 sites (all Pacific oysters) were selected using the same criteria as outlined above.

Table 3: Sites included in surveillance

Site number	Region	Species	Risk score	Classification[1]
1	England - East Coast[2]	*C. gigas*	24	B
2	England - East Coast	*C. gigas*	23	B
3	England - East Coast	*O. edulis*	16	B
4	England - East Coast	*C. gigas*	18	B
5	England - East Coast	*C. gigas*	25	B
6	England - East Coast	*O. edulis*	20	B
7	England - East Coast	*C. gigas*	22	B
8	England - East Coast	*C. gigas*	18	B
9	England - East Coast	*O. edulis*	22	B
10	England - East Coast	*O. edulis*	12	A
11	England - East Coast	*C. gigas*	30	C
12	England - East Coast	*C. gigas*	20	B
13	England - South Coast[3]	*C. gigas*	38	B
14	England - South Coast	*O. edulis*	25	C
15	England - South Coast	*C. gigas*	22	B
16	England - South Coast	*O. edulis*	20	B
17	England - South Coast	*O. edulis*	27	B
18	England - South Coast	*C. gigas*	17	B
19	England - South Coast	*C. gigas*	14	B
20	England - South Coast	*C. gigas*	22	B
21	England - South Coast	*C. gigas*	14	B
22	England - South Coast	*C. gigas*	20	B
23	England - South Coast	*O. edulis*	22	B
24	England - South Coast	*O. edulis*	32	B
25	England - South Coast	*O. edulis*	54	B
26	England - West Coast, Wales & NI[4,5]	*C. gigas*	12	B
27	England - West Coast, Wales & NI	*C. gigas*	14	B
28	England - West Coast, Wales & NI	*C. gigas*	20	B
29	England - West Coast, Wales & NI	*C. gigas*	n/a	B
30	England - West Coast, Wales & NI	*C. gigas*	21	B
31	England - West Coast, Wales & NI	*C. gigas*	n/a	A
32	England - West Coast, Wales & NI	*O. edulis*	20	B
33	England - West Coast, Wales & NI	*C. gigas*	18	B
34	Scotland	*C. gigas*	32	A
35	Scotland	*C. gigas*	36	B
36	Scotland	*C. gigas*	16	A
37	Scotland	*C. gigas*	50	B
38	Scotland	*C. gigas*	20	A
39	Scotland	*C. gigas*	26	A

[1] For sites with seasonal classification, or where classification changed during the sampling period, the classification covering the majority of samples taken is shown.
[2] Coast of England from Eastern border with Scotland to North Foreland.
[3] Coast of England from North Foreland to Land's End.
[4] Coast of England and Wales from Land's End to Western border with Scotland, plus coast of Northern Ireland.
[5] Two sites without risk scores included to provide appropriate geographical coverage.

6.0 Surveillance results

6.1 Prevalence of norovirus in tested samples

Full results for each site are included in Appendix 2. Of 857 samples tested 844 (98.5%) provided valid results (i.e. with acceptable extraction and amplification efficiencies) for norovirus GI and GII. Of these 844, 643 samples (76.2% [95% CI; 73.1-79.0%]) tested positive for norovirus; 402 (47.6% [95% CI; 44.2-51.1%]) were positive for both genogroups, 176 (20.9% [95% CI; 18.2-23.8%]) were positive for GI only and 65 (7.7% [95% CI; 6.0-9.8%]) were positive for GII only. Analysis of the two-by-two contingency table of positive and not detected results for GI and GII (Table 4) using Fisher's exact test showed a highly significant difference between prevalence for the two genogroups (13.2% more GI positives; $p<0.0001$). Norovirus prevalence varied from month-to-month (Figure 2) ranging from a maximum of 100% positive (38/38 samples; 95% CI; 88.6-100%) in February 2010 to a minimum of 45.7% samples positive (16/35; 95% CI; 29.2-63.1%) in September 2010. Analysis of the two-by-two contingency table of positive and not detected samples from the summer months (April-September) and the winter months (October-March; Table 5) using Fisher's exact test showed a highly significant difference between prevalence in the two seasons (27.6% higher in the winter; $p<0.0001$).

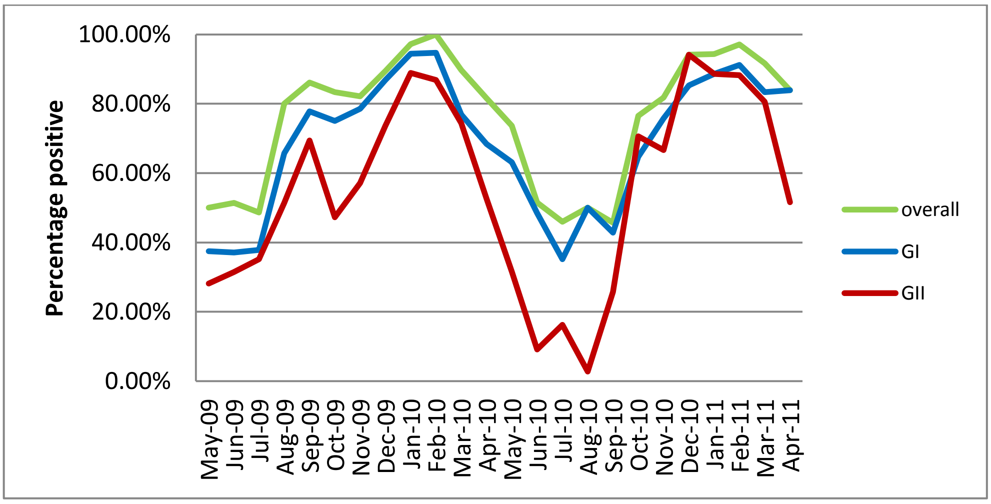

Figure 2: Month-by-month percentages of samples positive for norovirus.

Table 4: Two-by-two contingency table of samples testing positive and not detected for norovirus GI and GII

	Result[1]	
	Positive	Not detected
Norovirus GI	578 (68.5%) [95% CI; 65.2-71.6%]	266 (31.5%) [95% CI; 28.4-34.8%]
Norovirus GII	467 (55.3%) [95% CI; 51.9-58.7%])	377 (44.7%) [95% CI; 41.3-48.1%]

[1] Percentage of samples testing positive or not detected in period shown in brackets

Table 5: Two-by-two contingency table of positive and not detected samples taken during summer and winter

	Norovirus result[1]	
	Positive	Not detected
Summer (April-September)	264 (62.4%) [95% CI; 57.6-67.0%]	159 (37.6%) [95% CI; 33.0-42.4%]
Winter (October-March)	379 (90.0%) [95% CI; 86.7-92.6%]	42 (10.0%) [95% CI; 7.4-13.3%]

[1] Percentage of samples testing positive or not detected in period shown in brackets

6.2 Levels of norovirus in tested samples

Levels of norovirus in tested samples varied widely. For norovirus GI the maximum recorded quantity was 16,507 copies/g and for GII it was 18,024 copies/g. However a large proportion of positive results (65.1% for GI [376/578 samples; 95% CI; 61-68.9%] and 42.8% for GII [200/467; 95% CI; 38.3-47.5%]) were below the limit of quantification of the method (100 copies/g). The numbers and percentages of samples giving GI, GII and total norovirus results in different quantity brackets is shown in Table 6.

Table 6: Number of samples giving norovirus results in different quantity brackets

	Number of samples in norovirus quantity brackets (copies/g)[1]				
	Not detected	<100 copies/g	100-999 copies/g	1,000-10,000 copies/g	>10,000 copies/g
GI	266 (31.5%) [95% CI; 28.4-34.8%]	376 (44.5%) [95% CI; 41.2-48.0%]	168 (19.9%) [95% CI; 17.3-22.8%]	31 (3.7%) [95% CI; 2.6-5.2%]	3 (0.4%) [95% CI; 0.1-1.1%]
GII	377 (44.7%) [95% CI; 41.3-48.1%]	200 (23.7%) [95% CI; 20.9-26.8%]	172 (20.4%) [95% CI; 17.7-23.3%]	89 (10.5%) [95% CI; 8.6-12.9%]	6 (0.7%) [95% CI; 0.3-1.6%]
Sum	201 (23.8%) [95% CI; 21.0-26.9%]	335 (39.7%) [95% CI; 36.4-43.1%]	185 (21.9%) [95% CI; 19.2-24.9%]	114 (13.5%) [95% CI; 11.3-16.1%]	9 (1.1%) [95% CI; 0.5-2.1%]

[1] Percentage of samples in given bracket shown in parentheses

As with prevalence (see section 6.1), norovirus levels varied seasonally (Figure 3) with annual peaks occurring between December and March and lowest levels recorded between May and August. Levels of norovirus GII were on average higher than those for GI. Analysis of GI and GII levels for the same samples (samples testing positive for one or both genogroups only) using the Wilcoxon signed ranks test indicated that within samples GII levels were significantly higher than GI (p=0.000). In order to confirm that this result was not unduly affected by the arbitrary scores given to <LOD and <LOQ results, sensitivity analysis was carried out by repeating the Wilcoxon test after rescoring <LOD and <LOQ results as 40 and 100 copies/g respectively as well as after rescoring <LOD and <LOQ results as 0 and 1 copy/g respectively. In both cases differences between the two genogroups were still found to be significant (p=0.000). The Spearman's rank correlation coefficient between norovirus GI and GII scores for individual samples (r=0.622) indicated that these two variables were significantly correlated (p=0.000).

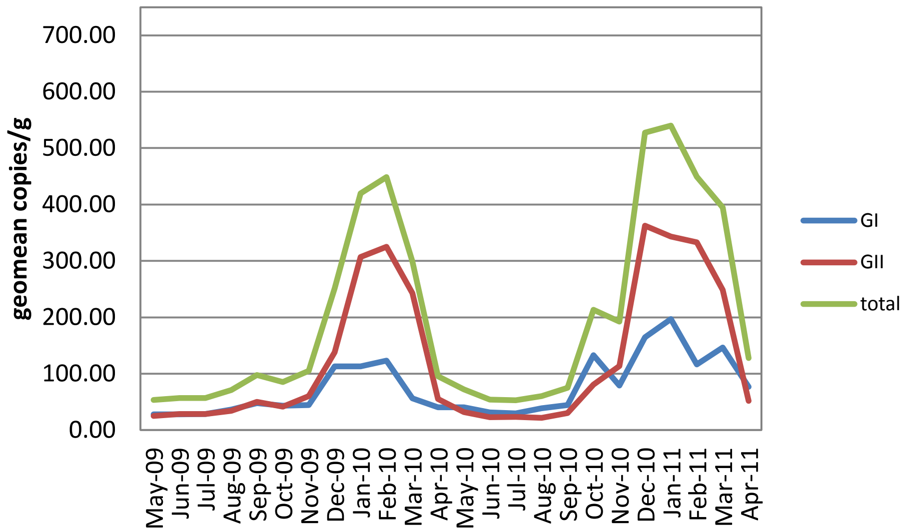

Figure 3: Monthly geometric mean norovirus levels.

For each month of the project, the percentage of samples with total norovirus (GI plus GII summed) in different quantity brackets is shown in Figure 4. This figure illustrates the potential burden of different theoretical safety cut-offs for total norovirus on UK production areas at different times of year.

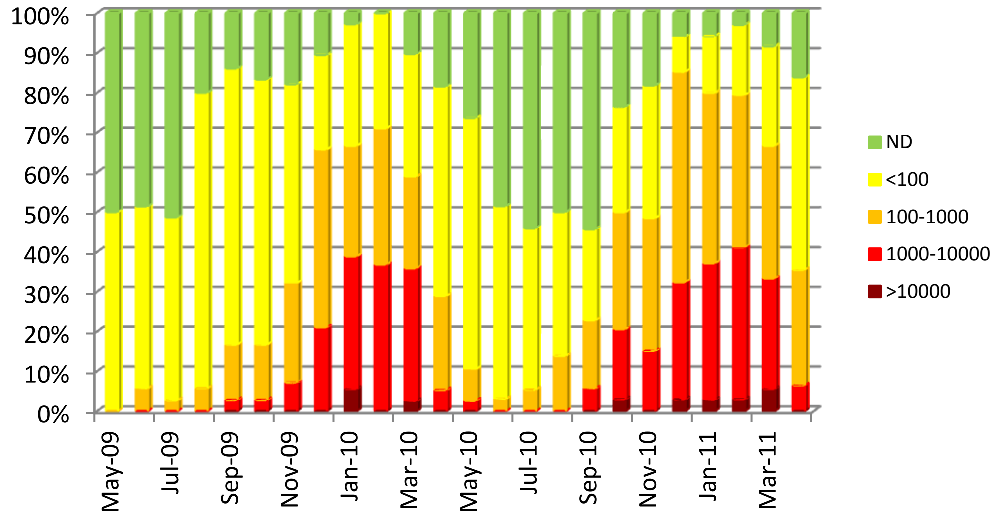

Figure 4: Monthly proportion of samples giving total norovirus results in different quantity brackets (copies/g).

6.3 Comparison of project year 1 and year 2

Overall prevalence, average levels and seasonal trends were broadly similar between the two years of the project (May 2009-April 2010 and May 2010-April 2011; Figures 2, 3 and 4). A greater proportion of samples tested positive in year 1 (Table 7), however this difference was not significant when analysed using Fisher's exact test (p=0.090). On the other hand average levels were higher in year 2 (geomean 175 copies/g (95% CI; 151-203 copies/g) for year 2 compared with 144 copies/g (95% CI; 126-164 copies/g) for year 1. Analysis of levels from year 1 and 2 using the Kruskal-Wallis test showed no significant difference between the genogroups however (p=0.513).

Table 7: Two-by-two contingency table of positive and not detected samples taken during project years 1 and 2

	Norovirus result[1]	
	Positive	Not detected
Year 1 (May 2009-April 2010)	337 (78.7%) [95% CI; 74.5-82.5%]	91 (21.3%) [95% CI; 17.5-25.5%]
Year 2 (May 2010-April 2011)	306 (73.6%) [95% CI; 69-77.7%]	110 (26.4%) [95% CI; 22.3-31%]

[1] Percentage of samples testing positive or not detected in period shown in brackets

6.4 Comparison of different sites

All 39 sites sampled during the project returned at least one positive result. Site-by-site prevalence varied from a minimum of 20.8% norovirus positive (5/24 samples [95% CI; 7.9-42.7%]) up to 100% positive (20/20 samples [95% CI; 80-100%]). Average norovirus levels varied very widely between sites, with the within-site geometric mean norovirus scores (GI and GII summed, samples not detected for both genogroups score 40) ranging from 50 (95% CI; 40-68 copies/g) – 2243 copies/g (95% CI; 883-5701 copies/g). To illustrate typical contamination patterns monthly results for three sites with comparatively low norovirus, three with medium norovirus and three with high norovirus are shown in Figure 5.

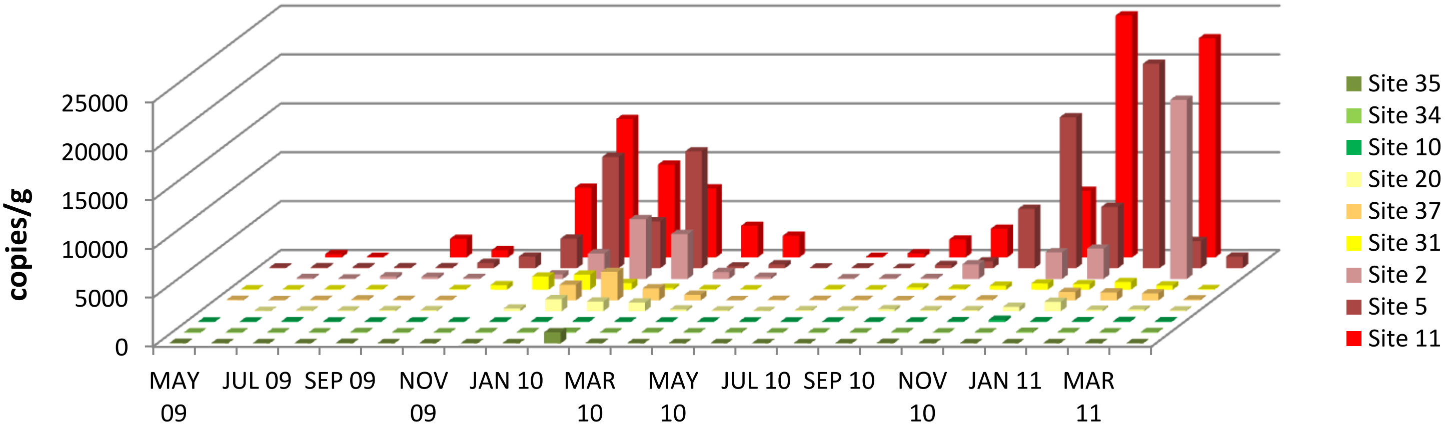

Figure 5; Month-by-month results for representative low, medium and high norovirus sites. Low, medium and high norovirus sites shown in green, yellow and red tones respectively.

6.5 Comparison of different regions

The winter seasonality in the data set as a whole was also apparent when the different regions of the UK were examined separately, although average contamination levels varied (Figure 6). Average levels for sites in Scotland in particular seemed lower than for other regions. Within-site geometric mean norovirus scores grouped by region are shown in Figure 7. Median values for these sets ranged from 82 copies/g (Scotland [95% CI; 50-169 copies/g]) to 226 copies/g (England – East Coast [95% CI; 73-537 copies/g]) and comparison of the sets using the Kruskal-Wallis test revealed a significant difference in average norovirus contamination for tested sites for the four regions of the UK (p=0.029). Post hoc analysis using pairwise comparisons indicated that there were significant differences in average norovirus contamination between tested sites in Scotland and both England – East Coast and England – South Coast, but no differences between any of the other pairs.

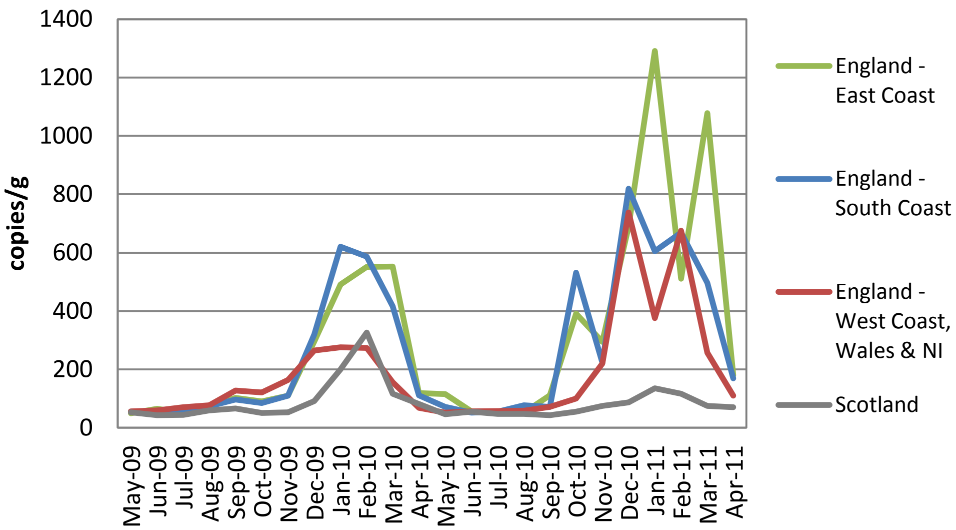

Figure 6: Monthly geometric mean norovirus levels for different UK regions.

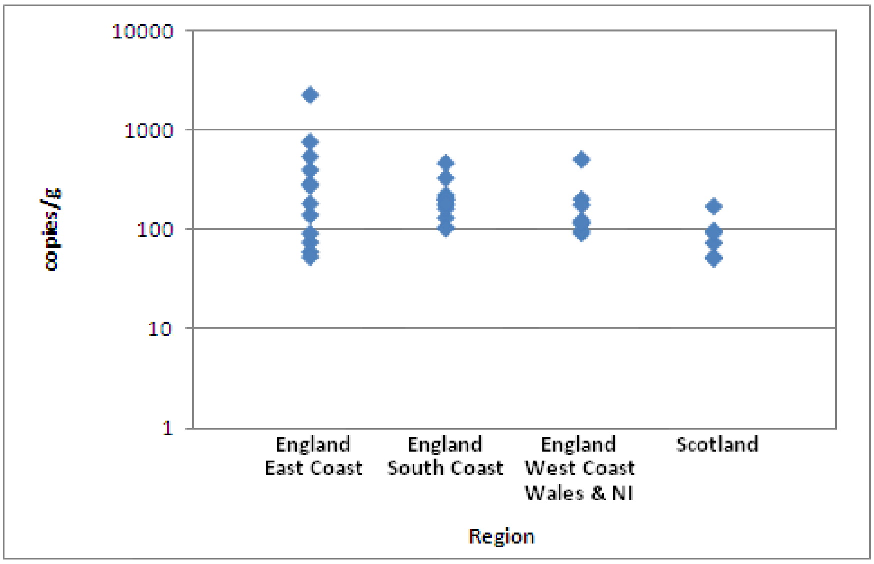

Figure 7: Within-site geometric means of individual sample norovirus scores grouped by UK region.

6.6 Comparison of different oyster species

The 39 sites analysed included 28 for Pacific oysters (*C. gigas*) and 11 for native oysters (*O. edulis*). The prevalence of norovirus in the two species was highly similar with 468 out of 615 *C. gigas* and 175 out of 229 *O. edulis* samples testing positive (76.1% [95% CI; 72.5-79.4%] and 76.4% [95% CI; 70.3-81.7%] respectively). Within-site geometric mean norovirus scores grouped by species are shown in Figure 8. Comparison of the two sets using the Kruskal-Wallis test revealed no significant difference in median norovirus contamination for tested sites for the two species (p=0.901; *C. gigas* median = 165 copies/g [95% CI; 102-197 copies/g], *O. edulis* median = 174 copies/g [95% CI; 59-328 copies/g]).

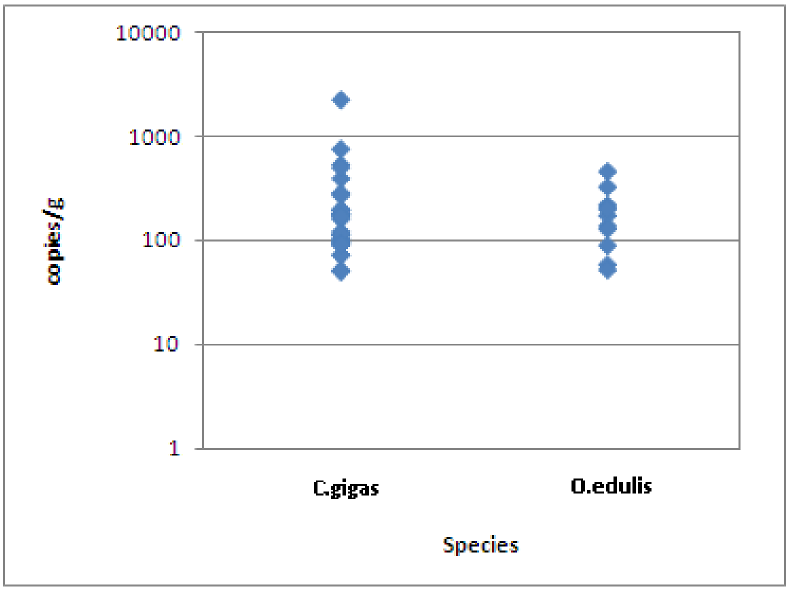

Figure 8: Within-site geometric means of individual sample norovirus scores grouped by species.

6.7 Comparison of shellfish harvesting area classifications

The 39 sites analysed included 6 sites classified A for the majority of the sampling period, 31 sites classified B and 2 sites classified C. Within-site geometric mean norovirus scores grouped by classification are shown in Figure 9. Median values for these three sets increased with classification (82 copies/g [95% CI; 52-175 copies/g], 180 copies/g [95% CI; 116-209 copies/g] and 1,208 copies/g [range 174-2243 copies/g; too few measurements to calculate CI] for class A, B and C respectively) and comparison of the three sets using the Kruskal-Wallis test revealed a significant difference in average norovirus contamination for tested sites for the three classifications (p=0.018). Post hoc analysis using pairwise comparisons indicated that there were significant differences in average norovirus contamination between A and B and A and C classification sites, but not between B and C classification sites (although the analysis was confounded by the small number of C classification sites in the study).

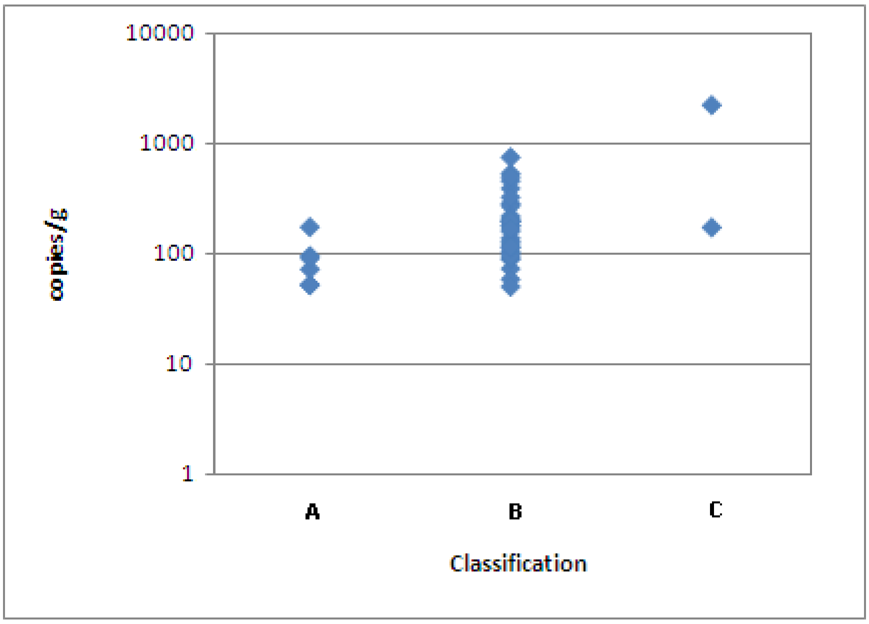

Figure 9; Within-site geometric means of individual sample norovirus scores grouped by classification.

6.8 Correlation of norovirus with *E. coli*

All 857 samples received were tested for *E. coli* (see section 3.3.2). One sample produced an invalid result (non-interpretable MPN tube combination on successive tests) however valid results were obtained for all other samples (n=856). In total 514 out of 856 samples (60.0% [95% CI; 56.7-63.3%]) contained levels which would be compliant with the end product standard (≤230 MPN/100g); the highest level recorded was 16,000 MPN/100g (found in 3 samples). Norovirus scores for individual samples plotted against the corresponding *E. coli* result are shown in Figure 10.

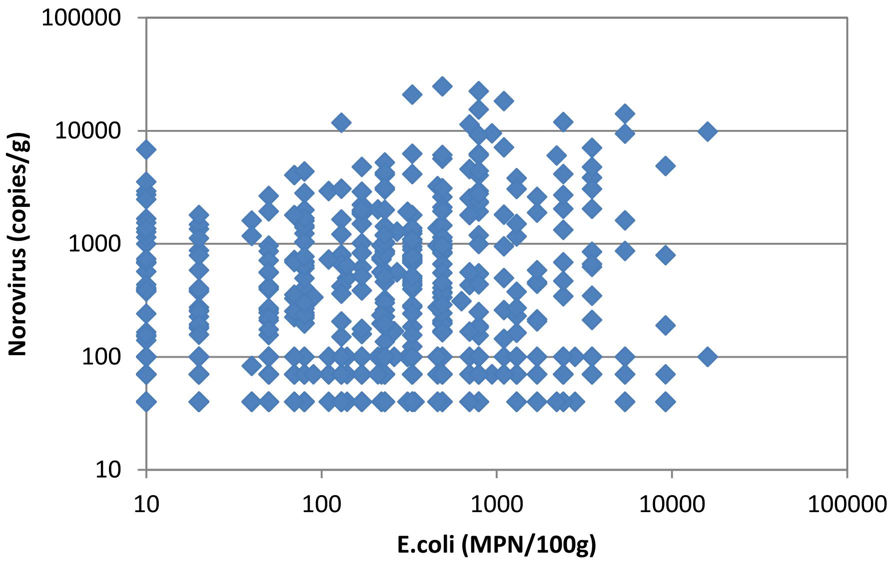

Figure 10: Norovirus vs. *E. coli* for individual samples.

As can be seen from Figure 10 a large number of samples returned low levels for norovirus and high levels for *E. coli*, or vice versa with little visual evidence of any correlation. Supporting this, the Spearman's rank correlation coefficient between norovirus and *E. coli* scores on an individual sample basis was relatively low (r=0.195). However the existence of a significant interdependence of the two variables was strongly supported statistically (p=0.000). The rate of norovirus positivity for samples ≤230 MPN/100g was 73.9% (376/509 samples giving valid norovirus results; 95% CI; 69.8-77.6%); above this level it was 79.6% (266/334; 95% CI; 74.8-83.7%).

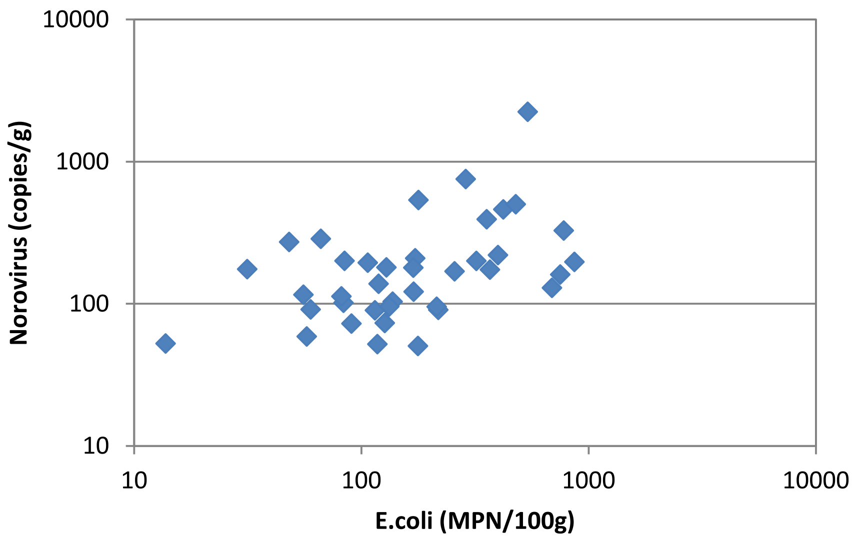

Figure 11: Norovirus vs. *E. coli*; comparison of within-site geometric means of individual sample scores (all months).

Within-site geometric mean values for norovirus and *E. coli* are shown in Figure 11. The Spearman's rank correlation coefficient was higher than for individual sample results (r=0.453), and indicated that these two variables were significantly correlated (p=0.004). When results from the winter months (October-March) were considered the strength of the relationship between norovirus and *E. coli* geometric mean values increased markedly (r = 0.676, p=0.000; Figure 12).

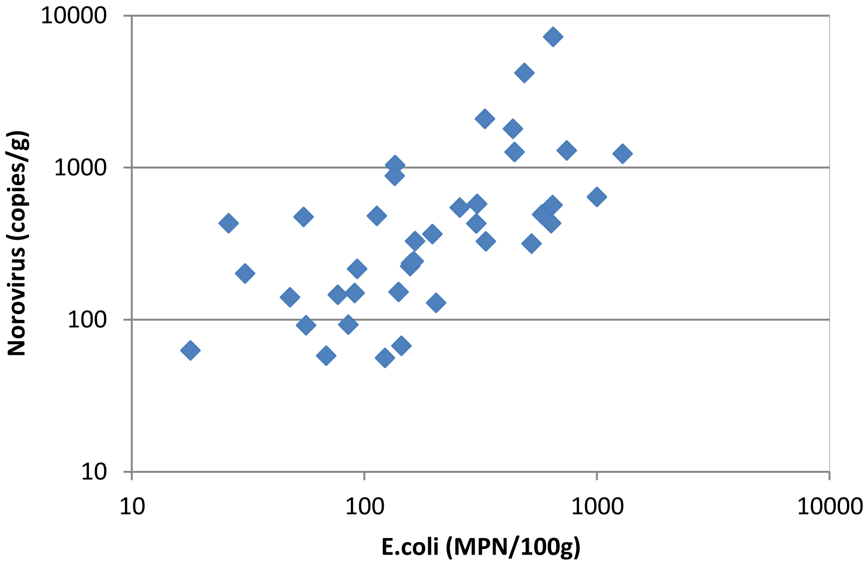

Figure 12: Norovirus vs. *E. coli*; comparison of within-site geometric means of individual sample scores (October-March).

6.9 Correlation of norovirus in oysters with clinical laboratory reports

Monthly geometric mean norovirus levels (both genogroups summed) for the samples tested in this study are shown in Figure 13 alongside monthly total numbers of laboratory reports of norovirus infections received by the Health Protection Agency from England and Wales and reported on their website, see e.g. http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Norovirus

For the period of the study the two variables shared a common winter seasonality and the Spearman's rank correlation coefficient between them (r=0.761) indicated that they were significantly correlated (p=0.000). However in contrast to the peak for norovirus in oyster samples, more lab reports were received by the HPA in the winter of 2009/10 than in the following winter (2010/11) although lab reports tend to be related to outbreaks associated with healthcare institutions and may not reflect the overall levels of norovirus in the community.

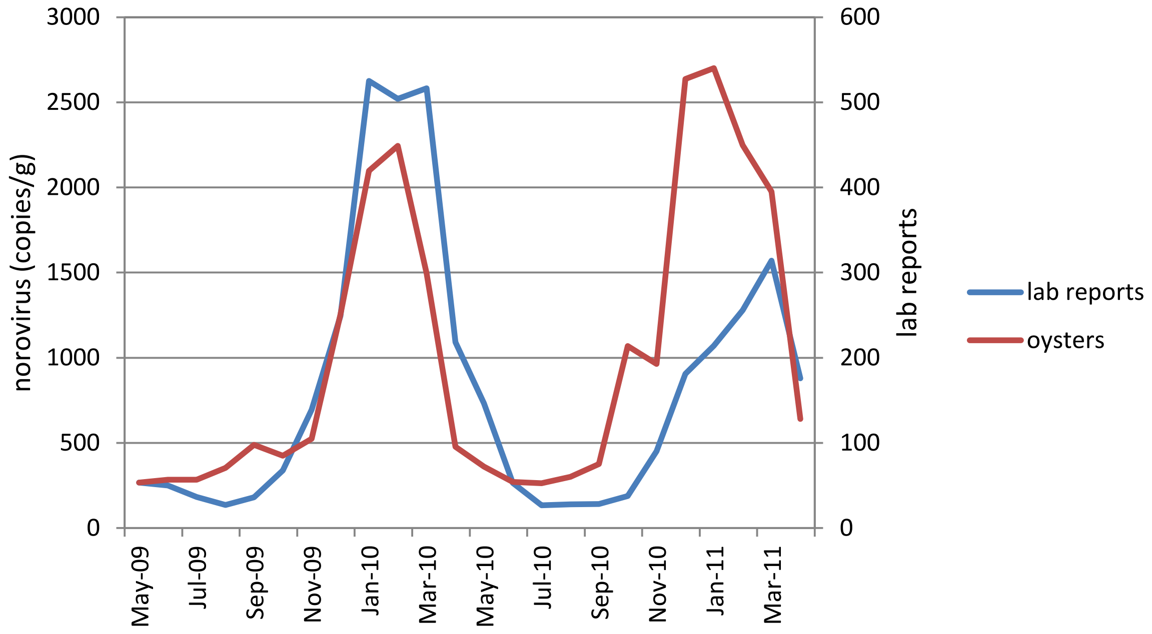

Figure 13: Comparison of monthly geometric mean norovirus levels in oysters with lab reports received by the HPA.

6.10 Correlation of norovirus in oysters with environmental temperature

Monthly geometric mean norovirus levels (both genogroups summed) for the samples tested in this study are shown in Figure 14 alongside monthly UK national average air temperatures (plotted with a reversed y axis to aid visualisation) as reported by the Met Office e.g.
http://www.metoffice.gov.uk/climate/uk/actualmonthly

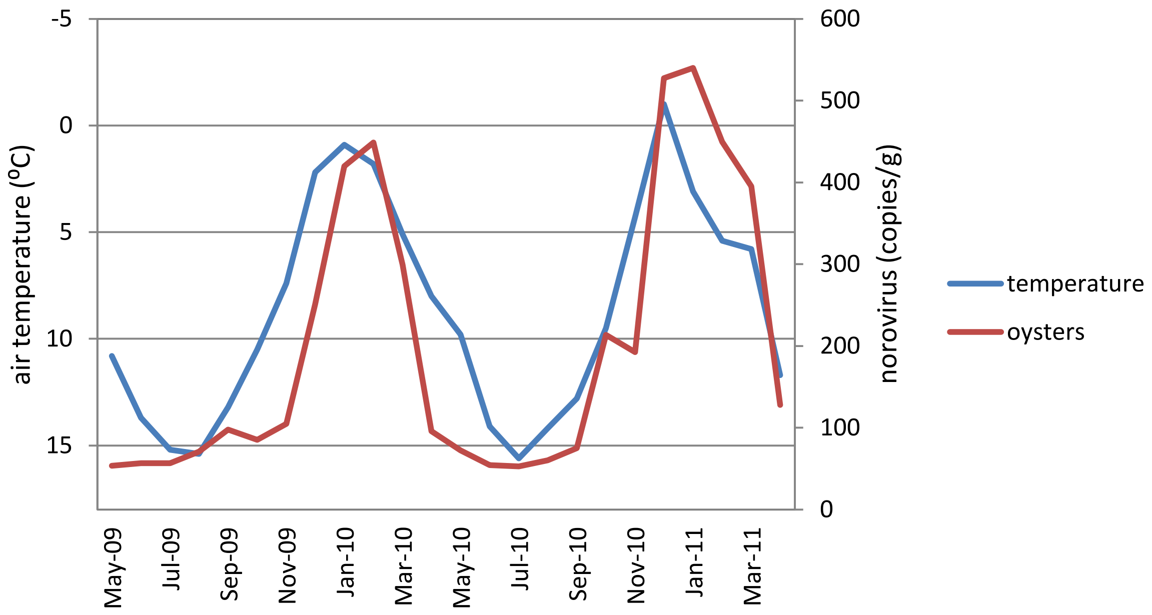

Figure 14: Comparison of monthly geometric mean norovirus levels in oysters with national average air temperatures.

For the period of the study the two variables shared a very strong, inverse, common seasonality (highest norovirus during periods of lowest air temperature) and the Spearman's rank correlation coefficient between them (r=-0.877) indicated that they were significantly dependent (p=0.000). The maximum monthly geometric mean for norovirus in oysters (January 2011) followed shortly after the lowest monthly average air temperature of -1^{o}C in December 2010.

Seawater temperatures at the time of sampling were available for all or most samples for 36 out of 39 sites (for some sites seawater temperatures for a proportion of samples were missing due to particular issues, tides, problems with equipment etc. at the time of sampling). In order to investigate whether there was a common (although possibly variable) within-site correlation between temperature and norovirus Spearman's rank analysis was carried out on data from each site. In 27 out of 36 sites there was a significant negative correlation between water temperature and norovirus levels (correlation coefficients of r=-0.460 to r=-0.879, see Appendix 3). For illustrative purposes Figure 15 shows norovirus scores plotted against seawater temperatures for samples from two sites where there was a particularly strong relationship. Of those sites where no statistically significant correlation was apparent the majority had low overall norovirus contamination levels (5 out of 9 with a within-site geometric mean norovirus score less than 100 copies/g).

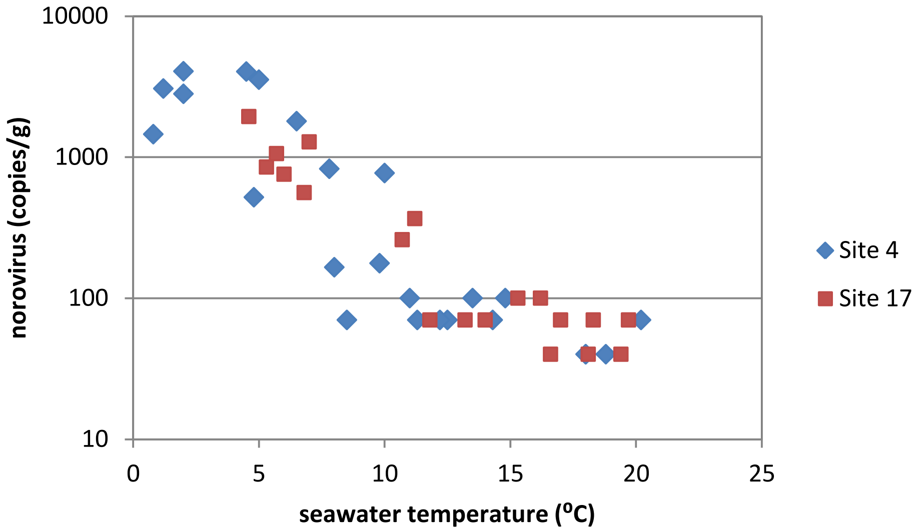

Figure 15: Norovirus vs. seawater temperature; comparison of individual sample norovirus scores with seawater temperatures at the time of sampling.

6.11 Correlation of norovirus in oysters with risk scores

Figure 16 shows within-site geometric mean norovirus scores for sites in England and Wales plotted against the risk scores for these sites (as calculated in section 5.1.1 and detailed in Table 3; risk scores for sites in Scotland used a different matrix and are therefore not included in this analysis). The Spearman's rank correlation coefficient between the two variables (r=0.546) indicated that they were significantly correlated (p=0.001), illustrating the value of the risk-based site selection method used.

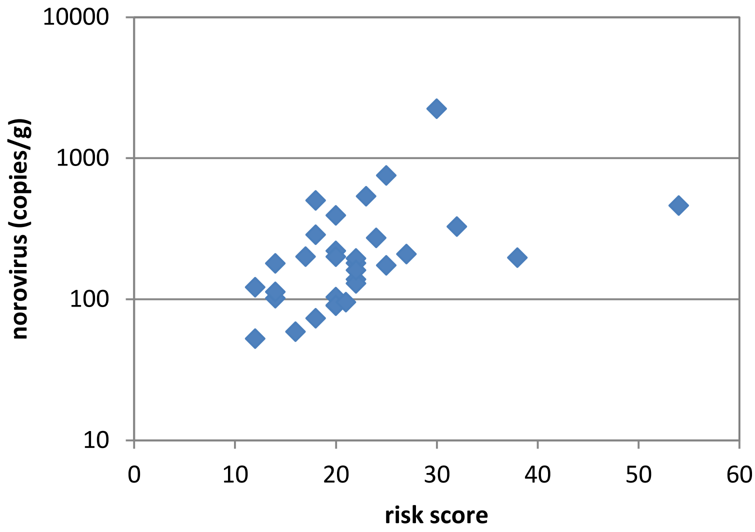

Figure 16: Norovirus vs. risk scores; comparison of within-site geometric means of individual sample norovirus scores with risk scores (England and Wales).

6.12 Further development of a risk scoring matrix

The elaboration of a predictive matrix for norovirus risk could be a useful risk management tool. A correlation was found between the risk scoring matrix developed for site selection and norovirus geometric mean scores (see Figure 16). Further analysis attempted to improve the predictive potential of this approach using the data obtained in this study to develop the model. The existence of a significant within-site relationship between *E. coli* and norovirus scores, particularly during the winter, has been shown in section 6.8 (see Figure 12). In addition a correlation between seawater temperatures and norovirus scores for the majority of sites was found (section 6.10), while the seasonal variation in norovirus risk was clearly apparent (sections 6.1 and 6.2). We therefore elaborated an improved risk scoring system based on these factors as follows:-

Factor *a* = within site geometric mean of *E. coli* scores from samples taken October-March (proxy for the overall norovirus risk of the site).

Factor *b* = 20 – (seawater temperature at the time of sampling); a minimum baseline value of 1 was used for this factor such that seawater temperatures ≥19ºC all score 1.

Factor *c* = seasonal multiplier dependent on month of sampling as follows:-

May-August	0.75
March, April, September, October	1.00
November-February	1.25

Risk score = ($\log_{10}$(product of factors *a* and *b*)) multiplied by factor *c*.

Following this approach risk scores were calculated for all samples where seawater temperature data existed. These are plotted against norovirus scores in Figure 17. The Spearman's rank correlation coefficient between the two variables (r=0.645) indicated that they were significantly correlated (p=0.000).

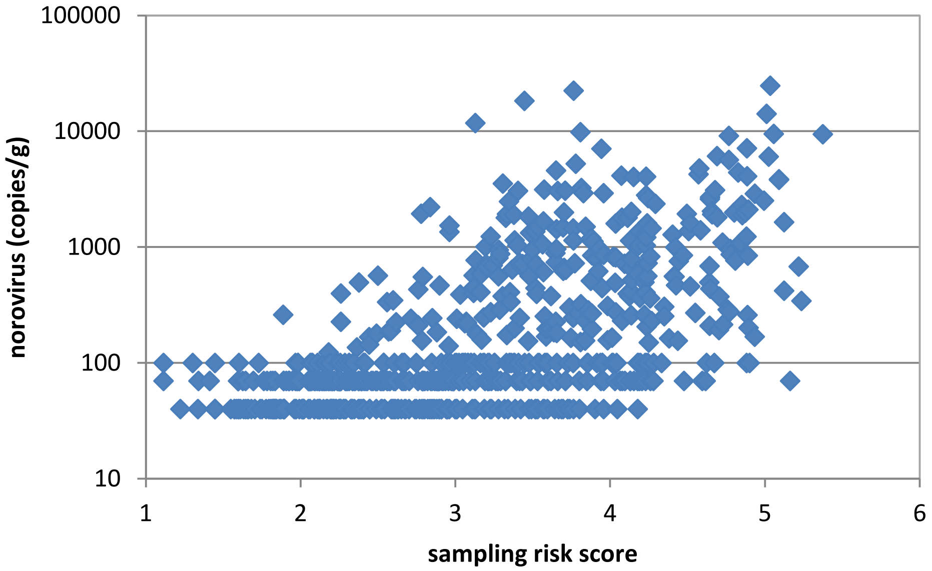

Figure 17: Norovirus vs. sampling risk scores.

The distribution of norovirus results in different quantity brackets according to sampling risk score is shown in Figure 18. Defining a norovirus score of >100 copies/g as a gold standard positive and a sampling risk score of >3.0 as a test outcome positive, this risk scoring system applied to the data in this study would have had an overall accuracy of 76.1% (95% CI; 72.7-79.2%), a sensitivity of 88.8% (95% CI; 84.1-92.2%), a specificity of 68.7% (95% CI; 64.1-72.9%), a positive predictive value of 62.2% (95% CI; 57.0-67.2%) and a negative predictive value of 91.3% (95% CI; 87.6-94.0%) (see Table 8).

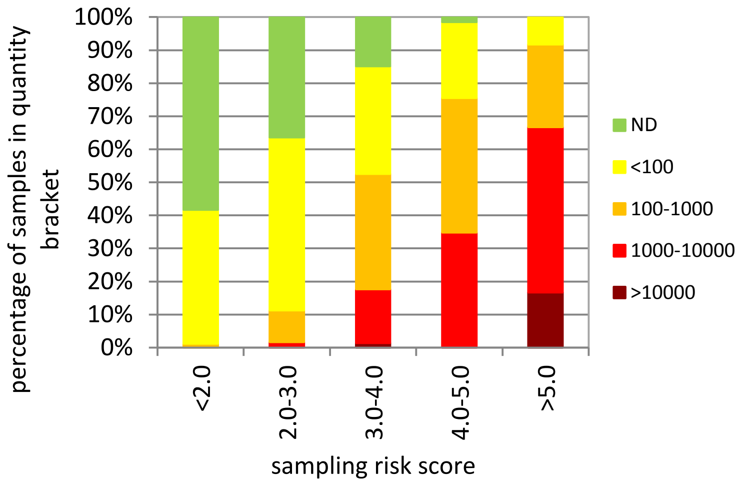

Figure 18: Proportion of samples giving total norovirus results in different quantity brackets (copies/g) for different sampling risk scores.

Table 8: Two-by-two contingency table of norovirus scores <100 or >100 copies/g from samples with sampling risk scores <3.0 or >3.0

		Norovirus result	
		<100 copies/g	>100 copies/g
Sampling risk score	<3.0	305	29
	>3.0	139	229

7.0 Discussion

This study comprises the first systematic survey of the occurrence of norovirus in oyster production areas in the UK. The overall prevalence of samples testing positive for one or both norovirus genogroups was 76.2% (643/844). This was very similar for both oyster species tested in the study. GI norovirus was detected more frequently than GII (in 68.5% (578/844) and 55.3% (467/844) of samples respectively). This is in contrast to clinical samples where the overwhelming majority of norovirus outbreaks are caused by GII strains (Kroneman *et al*., 2008). However a greater frequency of norovirus GI in foodborne infections has previously been reported (Verhoef *et al*., 2010). Although relatively high prevalences (20-59%) have been recorded in several previous studies targeting oysters and other bivalve spp. (Costantini *et al*., 2006, Elamri *et al.*, 2006, Hansman *et al*., 2008, Loisy *et al*., 2005, Lowther *et al.*, 2008, Lowther *et al*., 2010) to the best of our knowledge the overall prevalence reported here is higher than that recorded in any other similar study. This may be due, at least in part, to progressive improvements in the sensitivity of the test method over recent years. It is also possible that the UK coastal environment is under greater population pressure and hence subject to more severe faecal contamination than other comparable countries. It is notable that within the different UK regions, norovirus levels appeared lowest in Scotland, where population pressures would be expected to be lowest.

The significant proportion of positive samples highlights the importance of quantification for assessing the burden of norovirus contamination. Of samples testing positive, in the majority of cases (52.1%; 335/643) neither genogroup contained levels in excess of the limit of quantitation (LOQ) of the assay (100 copies/g). On the other hand for a small number of samples results of >10,000 copies/g were recorded. However, it should be noted that it is not currently possible to determine the absolute human health risks posed by different levels of norovirus. There is growing evidence of a dose response for norovirus in human volunteer studies (Teunis *et al*., 2008) and also evidence from a restaurant oyster consumption study of a correlation between norovirus levels in the batch consumed and likelihood of illness (Lowther *et al*., 2010). Several agencies (e.g. the European Food Safety Authority) are currently considering possible norovirus threshold levels for risk management. It was noted that different possible threshold levels have widely different impacts. For example a threshold level (GI and GII combined) of 100 copies/g would have resulted in a sample failure rate of >60% during the winter (October to March) for both years of the study. In contrast a threshold level of 1,000 copies/g would have resulted in a sample failure rate of <30%.

The data presented in this study should prove valuable in determining the impacts of the use of such threshold levels for the UK. It should however be noted that oysters placed on the market in the UK generally receive additional commercial processing through depuration (self-purification) in tanks. Further processing is a legal requirement for class B and C oyster production areas and is also frequently applied to class A oysters. Although depuration is generally considered to be relatively ineffective for safeguarding against viral contamination (Doré *et al.*, 1995, Schwab *et al*., 1998, Muniain-Mujika *et al*., 2002) its contribution in reducing norovirus levels has not been systematically assessed, and this area may therefore warrant further investigation. Likewise a study of norovirus levels in oysters placed on the market following commercial processing would help establish the levels of exposure of the UK consumer.

In common with previous studies in Northern Europe (Formiga-Cruz *et al*., 2002, LeGuyader *et al*., 2000, Lowther *et al.*, 2008) a very marked seasonality was observed, with highest prevalences and levels during the winter periods. Previously possible contributors to this seasonal pattern of occurrence have been suggested to include; the greater persistence of viral particles under typical Northern European winter environmental conditions of low solar irradiation, low temperature and higher turbidity (Allwood *et al*., 2005, Cannon *et al*., 2006); more effective viral clearance in oysters during summer months due to the higher rate of shellfish metabolism in warmer seawaters (Doré *et al*., 1998, Hernroth *et al*., 2007); and the higher prevalence of noroviruses in the human population, and thus seeded into the marine environment, during the winter (Blanton *et al*., 2006, Lopman *et al*., 2003, Mounts *et al*., 2000). Possibly all are contributory factors, however in this large systematic study the relationship of norovirus to environmental temperature data was found to be stronger than that with numbers of infections reported to national surveillance. Specifically, higher levels of norovirus were found in oysters in the second winter of the study period corresponding to the record low winter temperature but contrasting with a relatively low rate (compared with the first winter period) of lab reports of infections. However this may be due to limits in the surveillance systems currently in place to monitor human norovirus infections; lab reports tend to be related to outbreaks associated with healthcare institutions and may not reflect the overall levels of norovirus in the community.

The findings related to seasonality are consistent with the winter seasonality of norovirus illness incidents following shellfish consumption in epidemiological data both for the UK and for other countries (Doré *et al*., 2000). During the early months of 2010 an exceptionally large number of incidents of gastroenteritis linked to oysters were reported from restaurants, hotels and similar establishments in the UK. In total for the period July 2009-June 2010, details of 53 clusters of norovirus-type illness associated with consumption of oysters were received by the Cefas Liaison for Outbreaks Associated with Bivalve Molluscs, compared with an average of 10.9 clusters per season (July-June) in 1999-2009 and a previous maximum of 26 clusters in 2005-2006 (personal communication). There were also high numbers of clusters reported in the same period from several other European countries (Westrell *et al*., 2010); this was linked to unusually severe weather conditions in northern Europe, for example the winter period of 2009-2010 (December to February) was officially the coldest in the United Kingdom since 1978-1979 (Met Office press release 1st March, 2010;

http://www.metoffice.gov.uk/news/releases/archive/2010/coldest-uk-winter).

On the other hand despite more extreme low temperatures in the following winter (December 2010 was the coldest on record in the UK; Met Office press release 5th January, 2011; http://www.metoffice.gov.uk/news/releases/archive/2011/cold-dec), and the corresponding high levels of norovirus in oysters found in this study, a relatively small number of illness clusters linked to oyster consumption were recorded (11 clusters from July 2010-June 2011; Cefas Liaison for Outbreaks Associated with Bivalve Molluscs, personal communication). It is possible that, following the unusually high number of clusters in the winter of 2009/10, some producers in the UK took steps to lower the risk of illness outbreaks, including voluntary restrictions on harvesting during the winter, and the use of extended depuration times, resulting in a lower number of illness incidents linked to oysters.

In the past many outbreaks of norovirus have been linked to consumption of shellfish that were compliant with bacteriological faecal pollution criteria (Ang, 1998; Chalmers & McMillan, 1995; Gill *et al*., 1983). However analysis of the relationships between norovirus contamination, and either *E. coli* levels or classification status in this study offers support for the use of *E. coli* as an indicator organism for faecal pollution monitoring and risk management purposes. Although on an individual sample basis *E. coli* was poorly predictive of norovirus risk, on a site-specific basis average *E. coli* levels were found to correlate with average norovirus scores, particularly when data from the winter only was used. Significant differences were also found in norovirus levels in class A, B and C sites supporting the site specific classification approach. It should however be noted that class B is a very wide category accommodating *E. coli* levels of between 230 and 46,000 (in 10% of samples) MPN/100g shellfish. Based on the data presented in this study there is clearly scope to further refine the classification categories to improve the correlation with norovirus risk, for example by combining seasonal or temperature-based criteria with *E. coli*-based classification.

A risk matrix incorporating various weighted factors (population density, *E. coli* scores, outbreak history etc.) was developed to aid site selection and ensure representative sampling in this study. It is informative that a positive correlation was found between the assigned risk score for each site and the average norovirus levels subsequently determined. Further analysis attempted to refine this risk ranking approach to improve the predictive value for norovirus contamination modelled using the data generated in this study. We have elaborated a risk scoring system based on a combination of the *E. coli* contamination profile of the site, the site seawater temperature, and the month of sampling. This system performed relatively well for the data generated in this study with, for example, a negative predictive value of 91.3%, although the positive predictive value of 62.2% indicates that it could be further improved. It would be interesting to validate this approach in a selection of areas not monitored during this study. A simple risk scoring matrix based on the principal factors identified here has the potential to focus enhanced risk management measures, such as virus monitoring or enhanced depuration practices, where they are most needed.

In summary this is the first systematic study of norovirus contamination levels in UK oyster production areas and should help inform both the FSA and the producer sector with regard to this risk. It should also assist the FSA with consideration of the impact of possible norovirus threshold risk management levels.

8.0 References

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Appendix 1: Oyster species comparison results

		GII quantity (copies/g)			
		O. edulis		*C. gigas*	
	aliquot	extraction 1	extraction 2	extraction 1	extraction 2
High	1	93454	333641	141626	140236
	2	181164	240309	252699	147574
	3	142665	185666	159136	291543
	4	138860	92330	104058	225560
	5	135668	207534	225154	211363
Medium	1	26099	74961	26250	74309
	2	72738	168943	43008	91931
	3	32311	69099	51439	117865
	4	30796	72071	44471	104847
	5	68940	181471	26764	61494
Low	1	8508	6101	7227	6982
	2	16282	9318	8433	3545
	3	24900	8082	10705	5396
	4	11994	7732	9213	5809
	5	10967	4913	7605	3779

Appendix 2: Surveillance sample results

Site 1

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	12.5	<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 09	15.8	50	not detected	<40	copies/g	positive	<100	copies/g
JUL 09	19	<20	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	20	50	not detected	<40	copies/g	positive	<100	copies/g
SEP 09	17	<20	positive	<100	copies/g	positive	<100	copies/g
OCT 09		<20	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	7.8	490	positive	<100	copies/g	positive	<100	copies/g
DEC 09	7.5	330	positive	209	copies/g	positive	100-500	copies/g
JAN 10	1.6	220	positive	119	copies/g	positive	609	copies/g
FEB 10	1.5	50	positive	739	copies/g	positive	1914	copies/g
MAR 10	4	790	positive	<100	copies/g	positive	1882	copies/g
APR 10	9.2	<20	not detected	<40	copies/g	positive	100-500	copies/g
MAY 10	9.8	130	not detected	<40	copies/g	positive	<100	copies/g
JUN 10	13.5	20	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	19.3	<20	not detected	<40	copies/g	positive	<100	copies/g
AUG 10	18	<20	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	13.5	<20	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	10	130	positive	11741	copies/g	positive	<100	copies/g
NOV 10	5.5	50	positive	<100	copies/g	positive	346	copies/g
DEC 10	1	170	positive	100-500	copies/g	positive	658	copies/g
JAN 11	2	330	positive	260	copies/g	positive	774	copies/g
FEB 11	5.5	700	positive	<100	copies/g	positive	1745	copies/g
MAR 11	3.5	<20	positive	683	copies/g	positive	1794	copies/g
APR 11	12	<20	positive	<100	copies/g	positive	340	copies/g

Site 2

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
JUN 09	16	20	positive	<100	copies/g	positive	<100	copies/g
JUL 09	20.9	20	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	21.5	50	positive	<100	copies/g	positive	211	copies/g
SEP 09	16.5	80	positive	<100	copies/g	positive	170	copies/g
OCT 09	13.5	50	positive	<100	copies/g	positive	<100	copies/g
DEC 09	7.5	1700	positive	334	copies/g	positive	100-500	copies/g
JAN 10	4.2	1700	positive	<100	copies/g	positive	2554	copies/g
FEB 10	2.8	490	positive	371	copies/g	positive	5743	copies/g
MAR 10	6.4	700	positive	<100	copies/g	positive	4536	copies/g
APR 10	9.9	330	positive	<100	copies/g	positive	656	copies/g
MAY 10	10.6	70	positive	<100	copies/g	positive	100-500	copies/g
JUL 10	16.5	230	positive	<100	copies/g	not detected	<40	copies/g
AUG 10	18.8	330	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	15.4	260	positive	<100	copies/g	positive	<100	copies/g
OCT 10	9.6	170	positive	574	copies/g	positive	928	copies/g
DEC 10		<20	positive	1920	copies/g	positive	803	copies/g
JAN 11	3.2	490	positive	1116	copies/g	positive	1990	copies/g
MAR 11	11.5	1100	positive	13193	copies/g	positive	5138	copies/g

Site 3

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	14.5	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	18	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	18.5	20	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	16.5	20	positive	<100	copies/g	not detected	<40	copies/g
OCT 09		3500	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	10.8	50	not detected	<40	copies/g	not detected	<40	copies/g
DEC 09	8	130	not detected	<40	copies/g	not detected	<40	copies/g
JAN 10	3.2	20	positive	<100	copies/g	not detected	<40	copies/g
FEB 10	2.2	1700	positive	<100	copies/g	not detected	<40	copies/g
MAR 10	4.8	50	not detected	<40	copies/g	not detected	<40	copies/g
APR 10	9.3	20	not detected	<40	copies/g	not detected	<40	copies/g
MAY 10	4	<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	18	50	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	17	80	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	10.8	<20	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	6.8	<20	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	6	80	positive	<100	copies/g	not detected	<40	copies/g
DEC 10	1.5	460	positive	<100	copies/g	positive	<100	copies/g
JAN 11	3	1700	positive	186	copies/g	not detected	<40	copies/g
FEB 11	6	<20	positive	<100	copies/g	not detected	<40	copies/g
MAR 11	8.5	330	positive	<100	copies/g	positive	<100	copies/g
APR 11	14	20	positive	<100	copies/g	not detected	<40	copies/g

Site 4

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	11.3	20	positive	<100	copies/g	positive	<100	copies/g
JUN 09	14.3	<20	not detected	<40	copies/g	positive	<100	copies/g
JUL 09	18.8	110	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	20.2	50	not detected	<40	copies/g	positive	<100	copies/g
SEP 09	14.8	<20	positive	<100	copies/g	positive	<100	copies/g
OCT 09	13.5	50	positive	<100	copies/g	positive	<100	copies/g
NOV 09	8	<20	positive	<100	copies/g	positive	100-500	copies/g
DEC 09	7.8	490	positive	203	copies/g	positive	623	copies/g
JAN 10	0.8	490	positive	213	copies/g	positive	1240	copies/g
FEB 10	2	70	positive	782	copies/g	positive	3277	copies/g
MAR 10	1.2	3500	positive	1168	copies/g	positive	1894	copies/g
APR 10	8.5	<20	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	11	<20	positive	<100	copies/g	positive	<100	copies/g
JUN 10	12.5	20	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	18	490	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	18	80	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	12.2	20	not detected	<40	copies/g	positive	<100	copies/g
OCT 10	9.8	170	positive	<100	copies/g	positive	100-500	copies/g
NOV 10	4.8	170	positive	100-500	copies/g	positive	396	copies/g
DEC 10	2	80	positive	867	copies/g	positive	1942	copies/g
JAN 11	4.5	790	positive	767	copies/g	positive	3274	copies/g
FEB 11	6.5	70	positive	133	copies/g	positive	1664	copies/g
MAR 11	5	<20	positive	1166	copies/g	positive	2372	copies/g
APR 11	10	80	positive	288	copies/g	positive	483	copies/g

Site 5

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		80	positive	<100	copies/g	not detected	<40	copies/g
JUN 09		310	positive	<100	copies/g	positive	<100	copies/g
JUL 09		460	positive	<100	copies/g	positive	<100	copies/g
AUG 09		80	positive	<100	copies/g	positive	<100	copies/g
SEP 09		170	positive	<100	copies/g	positive	<100	copies/g
OCT 09		790	positive	150	copies/g	positive	389	copies/g
NOV 09		790	positive	180	copies/g	positive	1012	copies/g
DEC 09		230	positive	1042	copies/g	positive	1981	copies/g
JAN 10		700	positive	501	copies/g	positive	10883	copies/g
FEB 10		170	positive	953	copies/g	positive	3841	copies/g
MAR 10		2400	positive	1063	copies/g	positive	10898	copies/g
APR 10		230	positive	<100	copies/g	positive	136	copies/g
MAY 10		170	positive	172	copies/g	positive	100-500	copies/g
JUN 10		230	positive	<100	copies/g	not detected	<40	copies/g
JUL 10		330	positive	<100	copies/g	not detected	<40	copies/g
AUG 10		80	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10		70	positive	278	copies/g	positive	<100	copies/g
OCT 10		80	positive	648	copies/g	positive	<100	copies/g
NOV 10		790	positive	1153	copies/g	positive	4919	copies/g
DEC 10		790	positive	4625	copies/g	positive	10820	copies/g
JAN 11		330	positive	2615	copies/g	positive	3657	copies/g
FEB 11		330	positive	2890	copies/g	positive	18025	copies/g
MAR 11		790	positive	791	copies/g	positive	1983	copies/g
APR 11		230	positive	350	copies/g	positive	838	copies/g

Site 6

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09		1300	positive	<100	copies/g	not detected	<40	copies/g
JUL 09		330	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09		460	not detected	<40	copies/g	positive	<100	copies/g
SEP 09		230	positive	<100	copies/g	positive	<100	copies/g
NOV 09		700	positive	<100	copies/g	not detected	<40	copies/g
DEC 09		230	positive	<100	copies/g	not detected	<40	copies/g
JAN 10		490	positive	<100	copies/g	not detected	<40	copies/g
FEB 10		80	positive	<100	copies/g	not detected	<40	copies/g
MAR 10		<20	positive	<100	copies/g	not detected	<40	copies/g
APR 10		20	positive	<100	copies/g	not detected	<40	copies/g
MAY 10		140	positive	<100	copies/g	not detected	<40	copies/g
JUN 10		230	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10		5400	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10		40	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10		20	positive	369	copies/g	not detected	<40	copies/g
DEC 10		50	positive	100-500	copies/g	positive	<100	copies/g
JAN 11		20	positive	100-500	copies/g	positive	<100	copies/g
FEB 11		80	positive	<100	copies/g	positive	185	copies/g
MAR 11		70	positive	172	copies/g	positive	182	copies/g
APR 11		20	positive	<100	copies/g	positive	<100	copies/g

Site 7

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	10.4	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	10.8	80	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09		50	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	20.4	80	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	17	80	not detected	<40	copies/g	not detected	<40	copies/g
OCT 09	16.2	170	positive	<100	copies/g	positive	<100	copies/g
NOV 09	9.5	1300	positive	<100	copies/g	positive	113	copies/g
DEC 09	8.7	790	positive	592	copies/g	positive	405	copies/g
JAN 10		230	positive	113	copies/g	positive	348	copies/g
FEB 10	3	490	positive	<100	copies/g	positive	100-500	copies/g
MAR 10		490	positive	<100	copies/g	positive	2548	copies/g
APR 10	10	20	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	9.5	330	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10		170	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	17.6	80	not detected	<40	copies/g	not detected	<40	copies/g
DEC 10	3.6	170	positive	<100	copies/g	positive	<100	copies/g
JAN 11	5.1	230	positive	315	copies/g	positive	3944	copies/g
FEB 11		80	positive	220	copies/g	positive	1465	copies/g
MAR 11	5.2	230	positive	247	copies/g	positive	1180	copies/g
APR 11	9	<20	positive	100-500	copies/g	positive	279	copies/g

Site 8

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	12	80	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	15	140	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	19	2200	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	20	3500	positive	<100	copies/g	positive	<100	copies/g
SEP 09	17	230	positive	<100	copies/g	positive	<100	copies/g
OCT 09	15	40	not detected	<40	copies/g	not detected	<40	copies/g
NOV 09	10	110	positive	<100	copies/g	not detected	<40	copies/g
DEC 09		130	positive	<100	copies/g	positive	<100	copies/g
JAN 10	3	50	positive	<100	copies/g	positive	<100	copies/g
FEB 10	3	140	positive	<100	copies/g	positive	<100	copies/g
MAR 10	7	50	positive	<100	copies/g	positive	<100	copies/g
APR 10	8	310	positive	<100	copies/g	positive	<100	copies/g
MAY 10	11	<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 10		130	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	19	230	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	19	790	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	17	70	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	15.9	790	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	8	40	positive	<100	copies/g	positive	<100	copies/g
DEC 10	5	220	not detected	<40	copies/g	positive	100-500	copies/g
JAN 11	5	80	positive	115	copies/g	positive	152	copies/g
FEB 11	5.8	130	positive	<100	copies/g	positive	<100	copies/g
MAR 11	7.5	<20	not detected	<40	copies/g	positive	<100	copies/g
APR 11	11	20	positive	<100	copies/g	not detected	<40	copies/g

Site 9

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	12.1	<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 09	17	20	positive	<100	copies/g	not detected	<40	copies/g
JUL 09	21	220	positive	<100	copies/g	not detected	<40	copies/g
AUG 09	18	330	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	19	50	not detected	<40	copies/g	not detected	<40	copies/g
OCT 09	10	330	not detected	<40	copies/g	not detected	<40	copies/g
NOV 09	12	490	positive	<100	copies/g	not detected	<40	copies/g
DEC 09	8.5	490	positive	<100	copies/g	not detected	<40	copies/g
JAN 10	2.9	1100	positive	1597	copies/g	positive	100-500	copies/g
FEB 10	3.5	1300	positive	<100	copies/g	positive	<100	copies/g
MAR 10	6	50	not detected	<40	copies/g	not detected	<40	copies/g
APR 10	12.5	<20	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	17	50	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	17.1	80	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	20.9	70	positive	<100	copies/g	not detected	<40	copies/g
AUG 10	19	50	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	15	50	positive	<100	copies/g	not detected	<40	copies/g
OCT 10	12	80	positive	635	copies/g	positive	<100	copies/g
NOV 10	6	80	positive	575	copies/g	positive	824	copies/g
JAN 11	6.2	3500	positive	2043	copies/g	positive	2739	copies/g
FEB 11	1.8	330	positive	571	copies/g	positive	523	copies/g
MAR 11	4.8	130	positive	528	copies/g	positive	134	copies/g
APR 11	13.2	20	positive	<100	copies/g	positive	<100	copies/g

Site 10

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	17.6	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	19	<20	not detected	<40	copies/g	positive	<100	copies/g
AUG 09	20.5	<20	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	16.5	<20	not detected	<40	copies/g	not detected	<40	copies/g
OCT 09	14.1	20	not detected	<40	copies/g	not detected	<40	copies/g
NOV 09	10.8	20	not detected	<40	copies/g	not detected	<40	copies/g
DEC 09	8.7	<20	not detected	<40	copies/g	not detected	<40	copies/g
JAN 10	3.2	<20	positive	<100	copies/g	not detected	<40	copies/g
FEB 10		20	positive	<100	copies/g	not detected	<40	copies/g
MAR 10	5.8	<20	not detected	<40	copies/g	not detected	<40	copies/g
APR 10	9.8	<20	not detected	<40	copies/g	not detected	<40	copies/g
MAY 10	12.1	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	16.6	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	20	20	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	18.9	<20	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	17.9	<20	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	10.5	<20	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	8.5	20	not detected	<40	copies/g	positive	<100	copies/g
DEC 10	3.8	50	positive	171	copies/g	positive	<100	copies/g
JAN 11	6.2	130	positive	<100	copies/g	not detected	<40	copies/g
FEB 11	6.4	<20	positive	<100	copies/g	not detected	<40	copies/g
MAR 11	5.6	<20	positive	<100	copies/g	positive	<100	copies/g
APR 11	9.8	<20	positive	<100	copies/g	not detected	<40	copies/g

Site 11

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
JUN 09	16	490	positive	<100	copies/g	positive	286	copies/g
JUL 09	20.9	3500	not detected	<40	copies/g	positive	<100	copies/g
SEP 09	16.3	170	positive	741	copies/g	positive	1163	copies/g
OCT 09	13.1	330	positive	<100	copies/g	positive	690	copies/g
DEC 09	7.5	1100	positive	2190	copies/g	positive	4949	copies/g
JAN 10	4.2	5400	positive	887	copies/g	positive	13272	copies/g
FEB 10	2.8	940	positive	1124	copies/g	positive	8380	copies/g
MAR 10	6.4	3500	positive	149	copies/g	positive	6923	copies/g
APR 10	9.9	460	positive	<100	copies/g	positive	3190	copies/g
MAY 10	10.6	170	positive	558	copies/g	positive	1660	copies/g
JUL 10	16.5	1100	positive	<100	copies/g	not detected	<40	copies/g
AUG 10	18.4	330	positive	378	copies/g	not detected	<40	copies/g
SEP 10	15.4	170	positive	1379	copies/g	positive	459	copies/g
OCT 10	9.6	790	positive	1649	copies/g	positive	1287	copies/g
DEC 10		<20	positive	4319	copies/g	positive	2496	copies/g
JAN 11	3.5	490	positive	9385	copies/g	positive	15369	copies/g
MAR 11	11	790	positive	16507	copies/g	positive	5920	copies/g

Site 12

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
JUN 09	15.2	1700	positive	<100	copies/g	not detected	<40	copies/g
JUL 09	20.9	330	positive	<100	copies/g	positive	<100	copies/g
AUG 09	19	170	positive	<100	copies/g	positive	<100	copies/g
SEP 09	17	230	positive	<100	copies/g	positive	<100	copies/g
OCT 09	18.5	490	not detected	<40	copies/g	positive	<100	copies/g
NOV 09	8.3	140	positive	<100	copies/g	positive	100-500	copies/g
DEC 09	6.8	490	positive	324	copies/g	positive	<100	copies/g
FEB 10	2.8	790	positive	255	copies/g	positive	2055	copies/g
MAR 10	6.4	230	positive	115	copies/g	positive	5135	copies/g
APR 10	9.9	230	positive	<100	copies/g	positive	177	copies/g
MAY 10	10.6	330	positive	131	copies/g	positive	100-500	copies/g
JUL 10	16.5	130	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	18.4	230	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	15.2	330	positive	998	copies/g	positive	798	copies/g
OCT 10	9.6	1300	positive	2268	copies/g	positive	789	copies/g
JAN 11	3.5	790	positive	1301	copies/g	positive	3114	copies/g
MAR 11	11.5	230	positive	1743	copies/g	positive	1395	copies/g

Site 13

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		2800	positive	<100	copies/g	positive	<100	copies/g
JUN 09		1100	not detected	<40	copies/g	positive	<100	copies/g
JUL 09		2400	not detected	<40	copies/g	positive	<100	copies/g
AUG 09		1700	positive	<100	copies/g	positive	<100	copies/g
SEP 09		3500	no result			no result		
OCT 09		1300	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	8.6	2200	no result			no result		
DEC 09	7.4	230	positive	212	copies/g	positive	3881	copies/g
JAN 10	4.6	700	positive	668	copies/g	positive	1852	copies/g
FEB 10		790	positive	123	copies/g	positive	6127	copies/g
MAR 10	11.5	230	positive	<100	copies/g	positive	100-500	copies/g
APR 10	11.7	220	positive	<100	copies/g	positive	<100	copies/g
MAY 10	14.2	220	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	17.9	790	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	18.4	330	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10		9200	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	18.9	5400	positive	<100	copies/g	not detected	<40	copies/g
OCT 10	15.1	1100	positive	901	copies/g	positive	<100	copies/g
NOV 10		790	positive	<100	copies/g	positive	<100	copies/g
DEC 10	10	490	positive	<100	copies/g	positive	239	copies/g
JAN 11	7.3	490	positive	479	copies/g	positive	366	copies/g
FEB 11	13.2	1300	positive	168	copies/g	positive	100-500	copies/g
MAR 11	9.3	220	positive	108	copies/g	positive	125	copies/g
APR 11		230	positive	<100	copies/g	positive	<100	copies/g

Site 14

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	16	20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09		790	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09		1300	not detected	<40	copies/g	positive	<100	copies/g
SEP 09	17	490	positive	<100	copies/g	positive	119	copies/g
OCT 09	16	130	positive	<100	copies/g	not detected	<40	copies/g
DEC 09	9.5	1100	positive	<100	copies/g	not detected	<40	copies/g
JAN 10	4.5	5400	positive	3190	copies/g	positive	6249	copies/g
FEB 10		9200	positive	926	copies/g	positive	3957	copies/g
MAR 10	6.5	40	positive	115	copies/g	positive	1490	copies/g
APR 10	7.5	220	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	10.5	50	positive	<100	copies/g	positive	<100	copies/g
JUN 10	15.5	80	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	19.5	130	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10		330	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	17	2400	positive	<100	copies/g	positive	<100	copies/g
OCT 10	15	16000	positive	4794	copies/g	positive	5042	copies/g

Site 15

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13.8	<20	positive	<100	copies/g	positive	<100	copies/g
JUN 09	17.9	110	no result			no result		
JUL 09	18.4	80	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	18.8	50	no result			no result		
SEP 09	17.7	<20	positive	<100	copies/g	not detected	<40	copies/g
OCT 09	16.3	50	positive	<100	copies/g	positive	106	copies/g
NOV 09	9.7	20	positive	<100	copies/g	positive	224	copies/g
DEC 09	7.9	1300	positive	<100	copies/g	positive	326	copies/g
JAN 10	5.3	330	positive	179	copies/g	positive	1123	copies/g
FEB 10		230	positive	230	copies/g	positive	637	copies/g
MAR 10		170	positive	<100	copies/g	positive	964	copies/g
APR 10	10.5	230	positive	155	copies/g	positive	854	copies/g
MAY 10	11.9	130	positive	<100	copies/g	positive	<100	copies/g
JUN 10	15	230	positive	<100	copies/g	positive	<100	copies/g
JUL 10	19.8	<20	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	18.3	<20	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	17.4	790	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	16.5	700	positive	381	copies/g	positive	<100	copies/g
NOV 10		20	positive	<100	copies/g	positive	<100	copies/g
DEC 10	5	220	positive	<100	copies/g	positive	513	copies/g
JAN 11	5.4	460	positive	<100	copies/g	positive	<100	copies/g
FEB 11	4.5	130	positive	<100	copies/g	positive	312	copies/g
MAR 11		130	positive	<100	copies/g	positive	<100	copies/g
APR 11	12	490	positive	192	copies/g	positive	100-500	copies/g

Site 16

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13.1	490	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	16.4	460	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	18.5	490	positive	<100	copies/g	not detected	<40	copies/g
AUG 09	19.6	460	positive	<100	copies/g	positive	<100	copies/g
SEP 09	16.9	220	positive	<100	copies/g	positive	<100	copies/g
OCT 09	14.9	790	positive	<100	copies/g	positive	100-500	copies/g
NOV 09	11.8	5400	positive	<100	copies/g	not detected	<40	copies/g
DEC 09	10	230	positive	<100	copies/g	positive	143	copies/g
JAN 10	4.7	260	positive	<100	copies/g	positive	100-500	copies/g
FEB 10	5.6	80	positive	<100	copies/g	positive	<100	copies/g
MAR 10	5.5	170	positive	<100	copies/g	positive	569	copies/g
APR 10		230	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	14	50	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	17.3	230	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	19.4	2400	positive	<100	copies/g	not detected	<40	copies/g
AUG 10	18.6	330	positive	100-500	copies/g	not detected	<40	copies/g
OCT 10	15.3	330	positive	664	copies/g	positive	<100	copies/g
NOV 10	10.8	3500	positive	401	copies/g	positive	1640	copies/g
DEC 10	5.8	490	positive	884	copies/g	positive	1225	copies/g
JAN 11	6.8	1700	positive	488	copies/g	positive	1401	copies/g
FEB 11	7.1	330	positive	531	copies/g	positive	558	copies/g
MAR 11	8	1300	positive	383	copies/g	positive	1118	copies/g
APR 11	6.5	20	positive	865	copies/g	positive	100-500	copies/g

Site 17

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13.2	80	not detected	<40	copies/g	positive	<100	copies/g
JUN 09	16.2	170	positive	<100	copies/g	positive	<100	copies/g
JUL 09	18.1	40	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	19.7	220	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	17	130	not detected	<40	copies/g	positive	<100	copies/g
OCT 09	15.3	70	positive	<100	copies/g	positive	<100	copies/g
NOV 09	11.8	330	positive	<100	copies/g	not detected	<40	copies/g
DEC 09	10.7	230	positive	240	copies/g	not detected	<40	copies/g
JAN 10	5.3	3500	positive	102	copies/g	positive	748	copies/g
FEB 10	4.6	50	positive	489	copies/g	positive	1449	copies/g
MAR 10	5.7	490	positive	<100	copies/g	positive	1009	copies/g
APR 10		230	positive	<100	copies/g	positive	<100	copies/g
MAY 10	14	80	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	16.6	50	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	19.4	130	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	18.3	230	positive	<100	copies/g	not detected	<40	copies/g
OCT 10		40	positive	340	copies/g	positive	835	copies/g
NOV 10	11.2	490	positive	<100	copies/g	positive	316	copies/g
DEC 10	6	220	positive	100-500	copies/g	positive	611	copies/g
JAN 11	6.8	700	positive	146	copies/g	positive	414	copies/g
FEB 11	7	270	positive	161	copies/g	positive	1120	copies/g
MAR 11		220	positive	208	copies/g	positive	759	copies/g
APR 11		80	positive	251	copies/g	positive	<100	copies/g

Site 18

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13.6	170	positive	<100	copies/g	not detected	<40	copies/g
JUN 09	19.1	<20	positive	<100	copies/g	positive	<100	copies/g
JUL 09	16.3	700	positive	<100	copies/g	positive	<100	copies/g
AUG 09	17.5	1300	positive	<100	copies/g	positive	<100	copies/g
SEP 09	16.7	110	positive	<100	copies/g	not detected	<40	copies/g
OCT 09		130	positive	<100	copies/g	not detected	<40	copies/g
NOV 09		<20	positive	<100	copies/g	positive	<100	copies/g
DEC 09	10.1	50	positive	195	copies/g	positive	<100	copies/g
JAN 10		<20	positive	487	copies/g	positive	512	copies/g
FEB 10		<20	positive	<100	copies/g	positive	1223	copies/g
MAR 10	6.1	20	not detected	<40	copies/g	positive	164	copies/g
APR 10	11.5	20	positive	<100	copies/g	positive	<100	copies/g
MAY 10	13.2	170	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	16.8	790	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	15.1	230	positive	<100	copies/g	not detected	<40	copies/g
AUG 10	17.3	20	positive	<100	copies/g	not detected	<40	copies/g
SEP 10		130	positive	<100	copies/g	positive	<100	copies/g
OCT 10		3500	positive	322	copies/g	positive	340	copies/g
NOV 10		70	positive	<100	copies/g	positive	<100	copies/g
DEC 10		20	positive	331	copies/g	positive	1468	copies/g
JAN 11	8.1	<20	positive	<100	copies/g	positive	1312	copies/g
FEB 11	9	490	positive	786	copies/g	positive	1158	copies/g
MAR 11		230	positive	202	copies/g	positive	835	copies/g

Site 19

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		50	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09		<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09		50	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09		790	positive	<100	copies/g	not detected	<40	copies/g
SEP 09		80	not detected	<40	copies/g	positive	<100	copies/g
OCT 09		80	positive	<100	copies/g	not detected	<40	copies/g
DEC 09		2400	positive	1137	copies/g	positive	905	copies/g
JAN 10		130	positive	<100	copies/g	positive	<100	copies/g
FEB 10		170	not detected	<40	copies/g	positive	<100	copies/g
MAR 10		<20	not detected	<40	copies/g	positive	<100	copies/g
APR 10		<20	positive	<100	copies/g	not detected	<40	copies/g
MAY 10		<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 10		<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10		<20	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10		<20	positive	<100	copies/g	not detected	<40	copies/g
SEP 10		1700	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10		50	not detected	<40	copies/g	not detected	<40	copies/g
DEC 10		330	positive	<100	copies/g	positive	100-500	copies/g
JAN 11		330	positive	<100	copies/g	positive	465	copies/g
FEB 11		130	not detected	<40	copies/g	positive	185	copies/g
MAR 11		330	positive	<100	copies/g	positive	4089	copies/g
APR 11		700	not detected	<40	copies/g	not detected	<40	copies/g

Site 20

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
JUN 09		790	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09		2400	positive	<100	copies/g	not detected	<40	copies/g
AUG 09		2400	positive	<100	copies/g	not detected	<40	copies/g
SEP 09		3500	positive	<100	copies/g	positive	<100	copies/g
OCT 09		1300	positive	<100	copies/g	positive	<100	copies/g
NOV 09	8.6	490	no result			no result		
DEC 09	7.4	330	positive	<100	copies/g	positive	226	copies/g
JAN 10	4.6	330	positive	191	copies/g	positive	1043	copies/g
FEB 10		490	positive	<100	copies/g	positive	933	copies/g
MAR 10	11.5	330	positive	<100	copies/g	positive	838	copies/g
APR 10	11.7	330	positive	<100	copies/g	positive	100-500	copies/g
MAY 10	14.2	70	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	17.9	790	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	18.4	1700	positive	<100	copies/g	positive	<100	copies/g
AUG 10		2400	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	18.9	3500	positive	192	copies/g	not detected	<40	copies/g
OCT 10	15.1	790	positive	<100	copies/g	positive	<100	copies/g
NOV 10		790	positive	<100	copies/g	positive	<100	copies/g
DEC 10	10	790	positive	118	copies/g	positive	320	copies/g
JAN 11	7.3	460	positive	445	copies/g	positive	517	copies/g
FEB 11	13.2	330	positive	<100	copies/g	positive	100-500	copies/g
MAR 11	9.3	490	positive	115	copies/g	positive	<100	copies/g
APR 11		790	positive	<100	copies/g	not detected	<40	copies/g

Site 21

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
AUG 09	14.3	460	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	14.6	790	positive	<100	copies/g	positive	<100	copies/g
OCT 09	15.4	330	no result			no result		
DEC 09	8.5	110	positive	<100	copies/g	not detected	<40	copies/g
JAN 10	7.8	1100	positive	278	copies/g	positive	100-500	copies/g
FEB 10	7.3	<20	positive	<100	copies/g	positive	<100	copies/g
MAR 10	6.7	<20	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	11.3	20	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	13.5	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	15.6	330	positive	<100	copies/g	not detected	<40	copies/g
AUG 10	15.9	230	positive	<100	copies/g	not detected	<40	copies/g
NOV 10	13.1	9200	positive	<100	copies/g	positive	743	copies/g
DEC 10	8.7	1700	positive	<100	copies/g	positive	534	copies/g
JAN 11	8.5	330	positive	720	copies/g	positive	655	copies/g
FEB 11		80	positive	296	copies/g	positive	4075	copies/g
MAR 11	9	50	positive	<100	copies/g	positive	124	copies/g

Site 22

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09		330	no result			no result		
JUL 09		330	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09		310	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	11.8	80	not detected	<40	copies/g	not detected	<40	copies/g
OCT 09	14	130	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	11	170	positive	<100	copies/g	positive	<100	copies/g
DEC 09	8	70	positive	114	copies/g	positive	605	copies/g
JAN 10	4	130	positive	<100	copies/g	positive	100-500	copies/g
FEB 10	7	20	positive	<100	copies/g	positive	100-500	copies/g
MAR 10	8	130	positive	<100	copies/g	not detected	<40	copies/g
APR 10	8	230	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	10	70	not detected	<40	copies/g	positive	<100	copies/g
JUN 10	14	490	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	18	80	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	18	130	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	17	50	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	15	9200	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	13	490	positive	<100	copies/g	not detected	<40	copies/g
DEC 10	7	210	positive	<100	copies/g	positive	1967	copies/g
JAN 11	7	130	not detected	<40	copies/g	positive	769	copies/g
FEB 11	7	50	not detected	<40	copies/g	positive	392	copies/g
MAR 11		70	not detected	<40	copies/g	positive	669	copies/g
APR 11		40	not detected	<40	copies/g	not detected	<40	copies/g

Site 23

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		310	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	17	2800	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	18.5	790	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09		790	positive	<100	copies/g	positive	<100	copies/g
SEP 09	17	330	positive	<100	copies/g	positive	<100	copies/g
OCT 09	16	330	positive	<100	copies/g	not detected	<40	copies/g
DEC 09	9.5	2200	positive	1311	copies/g	positive	4736	copies/g
JAN 10	4.5	2400	positive	<100	copies/g	positive	294	copies/g
FEB 10	5	330	positive	<100	copies/g	positive	630	copies/g
MAR 10	6.5	490	positive	<100	copies/g	positive	1088	copies/g
APR 10	7.5	460	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	10	16000	positive	<100	copies/g	positive	<100	copies/g
JUN 10	15.5	230	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	19.5	80	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	19	230	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	17	330	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	15	3500	positive	100-500	copies/g	positive	100-500	copies/g

Site 24

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
JUL 09	15.6	9200	positive	<100	copies/g	positive	140	copies/g
AUG 09	15.8	790	positive	<100	copies/g	positive	137	copies/g
SEP 09	14.8	1700	positive	237	copies/g	positive	206	copies/g
OCT 09	14.5	230	positive	<100	copies/g	positive	<100	copies/g
NOV 09	12.2	940	positive	<100	copies/g	not detected	<40	copies/g
DEC 09	10.4	1700	positive	165	copies/g	positive	<100	copies/g
JAN 10	7.3	170	positive	<100	copies/g	positive	<100	copies/g
FEB 10	7	490	positive	151	copies/g	positive	<100	copies/g
MAR 10	7.9	230	positive	<100	copies/g	positive	798	copies/g
APR 10	8.8	790	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	10.3	940	not detected	<40	copies/g	positive	<100	copies/g
JUN 10	15.4	3500	positive	<100	copies/g	positive	297	copies/g
JUL 10	10	490	positive	100-500	copies/g	not detected	<40	copies/g
AUG 10	16.6	230	positive	548	copies/g	not detected	<40	copies/g
SEP 10	17	270	positive	510	copies/g	positive	<100	copies/g
OCT 10	15.2	2400	positive	757	copies/g	positive	571	copies/g
NOV 10	13.5	5400	positive	504	copies/g	positive	1108	copies/g
DEC 10	7.5	170	positive	423	copies/g	positive	1561	copies/g
FEB 11	8.9	330	positive	109	copies/g	positive	657	copies/g
MAR 11	8.3	1300	positive	<100	copies/g	positive	1112	copies/g

Site 25

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13	490	positive	<100	copies/g	not detected	<40	copies/g
JUN 09	18	20	positive	<100	copies/g	not detected	<40	copies/g
JUL 09	19	210	positive	<100	copies/g	positive	<100	copies/g
AUG 09	20	3500	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	18	170	positive	<100	copies/g	positive	109	copies/g
NOV 09	13	2400	positive	<100	copies/g	positive	637	copies/g
DEC 09	9	1100	positive	209	copies/g	positive	<100	copies/g
JAN 10	4	3500	positive	218	copies/g	positive	3638	copies/g
FEB 10	4	1300	positive	361	copies/g	positive	3452	copies/g
MAR 10	4	2400	positive	639	copies/g	positive	3505	copies/g
APR 10	8	330	positive	286	copies/g	positive	157	copies/g
AUG 10	18	80	positive	139	copies/g	positive	357	copies/g
SEP 10	15	330	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	11	5400	positive	100-500	copies/g	positive	739	copies/g
DEC 10	3	130	positive	1178	copies/g	positive	466	copies/g
JAN 11	3	130	positive	186	copies/g	positive	235	copies/g
FEB 11	8	170	positive	286	copies/g	positive	2606	copies/g
MAR 11	12	110	positive	151	copies/g	positive	575	copies/g
APR 11	13	140	positive	519	copies/g	positive	118	copies/g

Site 26

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13	50	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	8	230	positive	<100	copies/g	not detected	<40	copies/g
JUL 09	16	330	positive	<100	copies/g	positive	<100	copies/g
AUG 09	12	1300	not detected	<40	copies/g	positive	<100	copies/g
SEP 09	10	330	not detected	<40	copies/g	positive	<100	copies/g
OCT 09	10	2400	not detected	<40	copies/g	positive	<100	copies/g
DEC 09	9	330	positive	<100	copies/g	not detected	<40	copies/g
JAN 10	2	50	positive	<100	copies/g	positive	<100	copies/g
FEB 10	4	1300	positive	<100	copies/g	positive	100-500	copies/g
MAR 10	6	90	positive	<100	copies/g	positive	286	copies/g
APR 10	7	490	not detected	<40	copies/g	positive	<100	copies/g
MAY 10	10	130	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	11	70	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	11	70	positive	<100	copies/g	not detected	<40	copies/g
AUG 10	12	230	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	10	790	positive	<100	copies/g	not detected	<40	copies/g
OCT 10	9	130	positive	<100	copies/g	positive	<100	copies/g
NOV 10		490	positive	247	copies/g	positive	669	copies/g
DEC 10	-1	50	positive	506	copies/g	positive	<100	copies/g
JAN 11	6	110	positive	<100	copies/g	positive	<100	copies/g
FEB 11	5	130	positive	344	copies/g	positive	872	copies/g
MAR 11	4	<20	positive	402	copies/g	positive	332	copies/g
APR 11	10	<20	positive	<100	copies/g	positive	<100	copies/g

Site 27

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	11	<20	not detected	<40	copies/g	positive	<100	copies/g
JUN 09	13	20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	12	330	not detected	<40	copies/g	positive	<100	copies/g
AUG 09		1100	positive	<100	copies/g	positive	<100	copies/g
SEP 09	10	230	positive	<100	copies/g	positive	<100	copies/g
OCT 09	11	330	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	14	330	positive	<100	copies/g	not detected	<40	copies/g
DEC 09	12	80	positive	147	copies/g	positive	<100	copies/g
JAN 10	9	80	positive	120	copies/g	positive	100-500	copies/g
FEB 10	8	<20	positive	102	copies/g	positive	<100	copies/g
MAR 10	7	110	positive	<100	copies/g	positive	<100	copies/g
APR 10	8	<20	not detected	<40	copies/g	not detected	<40	copies/g
MAY 10	14	<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	14	80	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	17	50	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	17	170	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10		330	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	15	80	positive	<100	copies/g	not detected	<40	copies/g
NOV 10	13	1300	not detected	<40	copies/g	not detected	<40	copies/g
DEC 10	11	50	positive	<100	copies/g	positive	914	copies/g
JAN 11	10	130	positive	317	copies/g	positive	2756	copies/g
FEB 11	9	80	positive	197	copies/g	positive	1254	copies/g
MAR 11	9	<20	positive	<100	copies/g	positive	191	copies/g
APR 11	9.6	50	positive	<100	copies/g	not detected	<40	copies/g

Site 28

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	11	50	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	13	230	not detected	<40	copies/g	positive	116	copies/g
JUL 09	12	5400	positive	<100	copies/g	positive	<100	copies/g
AUG 09	17	330	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	16	1100	positive	<100	copies/g	positive	<100	copies/g
OCT 09	11	<20	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	14	5400	positive	<100	copies/g	positive	<100	copies/g
DEC 09	12	130	positive	<100	copies/g	positive	<100	copies/g
JAN 10	9	460	positive	<100	copies/g	positive	<100	copies/g
FEB 10	8	50	positive	<100	copies/g	positive	<100	copies/g
MAR 10		130	positive	<100	copies/g	not detected	<40	copies/g
APR 10	8	130	not detected	<40	copies/g	not detected	<40	copies/g
MAY 10	14	220	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	17	170	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	17	490	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	16	230	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	15	2200	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	13	1700	positive	<100	copies/g	not detected	<40	copies/g
DEC 10	11	80	positive	<100	copies/g	positive	100-500	copies/g
JAN 11	10	490	positive	130	copies/g	positive	532	copies/g
FEB 11	9	80	positive	<100	copies/g	positive	985	copies/g
MAR 11	9	20	positive	<100	copies/g	positive	<100	copies/g
APR 11	9.6	<20	positive	<100	copies/g	not detected	<40	copies/g

Site 29

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		20	positive	<100	copies/g	not detected	<40	copies/g
JUN 09	15.1	130	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	19.9	170	positive	<100	copies/g	not detected	<40	copies/g
AUG 09		230	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	16.1	5400	positive	<100	copies/g	positive	<100	copies/g
OCT 09	15.3	20	positive	<100	copies/g	positive	<100	copies/g
NOV 09	10.1	330	positive	<100	copies/g	positive	<100	copies/g
DEC 09	8.5	50	positive	109	copies/g	positive	138	copies/g
JAN 10	4	<20	positive	<100	copies/g	positive	<100	copies/g
FEB 10	5.6	20	positive	<100	copies/g	positive	327	copies/g
MAR 10	8.9	<20	not detected	<40	copies/g	positive	<100	copies/g
APR 10	11.5	330	positive	<100	copies/g	positive	<100	copies/g
MAY 10	16.2	20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	18	20	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	21	20	positive	<100	copies/g	positive	<100	copies/g
AUG 10	17.5	<20	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	15.2	230	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10		50	not detected	<40	copies/g	positive	<100	copies/g
NOV 10	6	20	positive	265	copies/g	positive	609	copies/g
DEC 10	6.3	130	positive	505	copies/g	positive	100-500	copies/g
JAN 11	6.1	80	positive	238	copies/g	positive	<100	copies/g
FEB 11	7.2	<20	positive	408	copies/g	positive	287	copies/g
MAR 11	10	<20	positive	<100	copies/g	not detected	<40	copies/g
APR 11	10	330	positive	<100	copies/g	not detected	<40	copies/g

Site 30

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	11	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	13	460	not detected	<40	copies/g	positive	<100	copies/g
JUL 09	12	3500	positive	<100	copies/g	positive	<100	copies/g
AUG 09	17	790	positive	<100	copies/g	positive	<100	copies/g
SEP 09	16	330	positive	<100	copies/g	positive	<100	copies/g
OCT 09	11	80	positive	<100	copies/g	positive	<100	copies/g
NOV 09	14	790	positive	132	copies/g	positive	<100	copies/g
DEC 09	12	230	positive	184	copies/g	positive	100-500	copies/g
JAN 10	9	630	positive	<100	copies/g	positive	100-500	copies/g
FEB 10	8	130	positive	<100	copies/g	not detected	<40	copies/g
MAR 10		20	positive	<100	copies/g	positive	<100	copies/g
APR 10	8	340	not detected	<40	copies/g	not detected	<40	copies/g
MAY 10	14	330	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	14	130	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	17	490	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	17	1100	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	16	2400	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	15	460	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	13	230	not detected	<40	copies/g	not detected	<40	copies/g
DEC 10	11	230	positive	<100	copies/g	positive	462	copies/g
JAN 11	10	490	positive	138	copies/g	positive	824	copies/g
FEB 11	9	20	positive	<100	copies/g	positive	100-500	copies/g
MAR 11	9	<20	positive	<100	copies/g	positive	<100	copies/g
APR 11	9.6	20	positive	<100	copies/g	positive	<100	copies/g

Site 31

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	12	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	12.8	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	14	130	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	14	3500	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	14	490	no result			no result		
OCT 09	13	230	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	12	330	positive	<100	copies/g	positive	417	copies/g
DEC 09	11	20	positive	100-500	copies/g	positive	1240	copies/g
JAN 10	11	<20	positive	396	copies/g	positive	1140	copies/g
FEB 10	6	<20	positive	<100	copies/g	positive	626	copies/g
MAR 10	8.1	20	positive	<100	copies/g	positive	100-500	copies/g
APR 10	10.1	<20	not detected	<40	copies/g	positive	<100	copies/g
MAY 10	11	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	12	50	no result			no result		
JUL 10	13	<20	not detected	<40	copies/g	positive	<100	copies/g
AUG 10	15	40	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	13	20	positive	100-500	copies/g	not detected	<40	copies/g
OCT 10	10	<20	positive	<100	copies/g	positive	<100	copies/g
NOV 10	8	20	positive	277	copies/g	positive	126	copies/g
DEC 10	1	80	positive	167	copies/g	positive	474	copies/g
JAN 11	8	<20	positive	519	copies/g	positive	<100	copies/g
FEB 11		20	positive	204	copies/g	positive	586	copies/g
MAR 11		<20	positive	157	copies/g	positive	100-500	copies/g
APR 11		<20	positive	<100	copies/g	not detected	<40	copies/g

Site 32

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	12	2400	positive	<100	copies/g	positive	<100	copies/g
JUN 09	12	50	positive	<100	copies/g	not detected	<40	copies/g
JUL 09	12	490	positive	<100	copies/g	positive	<100	copies/g
AUG 09	12	460	positive	<100	copies/g	positive	<100	copies/g
SEP 09	12	16000	positive	<100	copies/g	positive	<100	copies/g
OCT 09	12	490	positive	393	copies/g	positive	751	copies/g
DEC 09	12	80	positive	218	copies/g	positive	100-500	copies/g
MAR 10	7	170	positive	<100	copies/g	positive	<100	copies/g
APR 10		790	positive	<100	copies/g	positive	<100	copies/g
MAY 10	11	<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	13	170	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	14	700	positive	147	copies/g	not detected	<40	copies/g
AUG 10	14	1100	positive	124	copies/g	not detected	<40	copies/g
SEP 10	12	80	positive	100-500	copies/g	not detected	<40	copies/g
OCT 10		790	positive	199	copies/g	positive	<100	copies/g
NOV 10	12	790	positive	278	copies/g	positive	270	copies/g
DEC 10	11	790	positive	1625	copies/g	positive	743	copies/g
JAN 11	10	void	positive	256	copies/g	positive	<100	copies/g
FEB 11	10	70	positive	<100	copies/g	positive	203	copies/g
APR 11	10	50	positive	256	copies/g	positive	306	copies/g

Site 33

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	11.6	130	positive	<100	copies/g	positive	<100	copies/g
JUN 09	17.2	2400	positive	<100	copies/g	positive	<100	copies/g
JUL 09	17.5	700	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	17	130	no result			no result		
SEP 09	15.6	330	positive	224	copies/g	positive	722	copies/g
OCT 09	14.8	490	positive	<100	copies/g	positive	358	copies/g
NOV 09	10.7	460	positive	<100	copies/g	positive	1328	copies/g
DEC 09	8	2400	positive	<100	copies/g	positive	2651	copies/g
JAN 10	4	80	positive	126	copies/g	positive	1869	copies/g
FEB 10	5	490	positive	2952	copies/g	positive	2714	copies/g
MAR 10	4.5	110	positive	214	copies/g	positive	2718	copies/g
APR 10	11	490	positive	<100	copies/g	positive	146	copies/g
MAY 10	16.9	790	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	13.2	1300	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	17	1700	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	16.1	460	positive	<100	copies/g	positive	224	copies/g
OCT 10	12.9	330	positive	359	copies/g	positive	433	copies/g
NOV 10	12	2400	positive	354	copies/g	positive	100-500	copies/g
DEC 10	5	790	positive	2356	copies/g	positive	6779	copies/g
FEB 11	7.7	310	positive	746	copies/g	positive	1175	copies/g
MAR 11	8.4	230	positive	1133	copies/g	positive	863	copies/g
APR 11	10.5	230	positive	146	copies/g	positive	<100	copies/g

Site 34

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	13	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	16	80	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	15	9200	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	15	490	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	14	230	positive	<100	copies/g	not detected	<40	copies/g
OCT 09	9	230	not detected	<40	copies/g	not detected	<40	copies/g
NOV 09	11	1300	not detected	<40	copies/g	not detected	<40	copies/g
DEC 09	8	230	not detected	<40	copies/g	positive	<100	copies/g
JAN 10	2	20	not detected	<40	copies/g	positive	<100	copies/g
FEB 10	2	<20	positive	<100	copies/g	positive	<100	copies/g
MAR 10	3	<20	not detected	<40	copies/g	not detected	<40	copies/g
APR 10	9	20	positive	<100	copies/g	not detected	<40	copies/g
MAY 10	13	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	17	50	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	16	1700	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	15	20	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	14	170	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	6	130	not detected	<40	copies/g	positive	<100	copies/g
NOV 10	8	1300	not detected	<40	copies/g	not detected	<40	copies/g
DEC 10	2	700	not detected	<40	copies/g	not detected	<40	copies/g
JAN 11	6	330	positive	<100	copies/g	positive	<100	copies/g
FEB 11	5	210	positive	<100	copies/g	not detected	<40	copies/g
MAR 11	6	<20	not detected	<40	copies/g	not detected	<40	copies/g
APR 11	12	90	positive	<100	copies/g	not detected	<40	copies/g

Site 35

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09		80	not detected	<40	copies/g	positive	<100	copies/g
JUN 09	13	2400	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	18	2400	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	14	5400	not detected	<40	copies/g	not detected	<40	copies/g
SEP 09	14	2400	positive	<100	copies/g	not detected	<40	copies/g
OCT 09	14	330	not detected	<40	copies/g	not detected	<40	copies/g
NOV 09	12	1300	not detected	<40	copies/g	not detected	<40	copies/g
DEC 09	9	170	not detected	<40	copies/g	not detected	<40	copies/g
JAN 10	5	70	not detected	<40	copies/g	not detected	<40	copies/g
FEB 10	5	<20	positive	1123	copies/g	not detected	<40	copies/g
MAR 10	8	<20	positive	<100	copies/g	not detected	<40	copies/g
APR 10	5	220	not detected	<40	copies/g	not detected	<40	copies/g
MAY 10	5	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	7	130	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	7	1300	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	10	9200	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	10	220	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	9	790	not detected	<40	copies/g	positive	<100	copies/g
NOV 10	6	130	not detected	<40	copies/g	not detected	<40	copies/g
DEC 10	6	20	not detected	<40	copies/g	not detected	<40	copies/g
JAN 11	7	20	not detected	<40	copies/g	not detected	<40	copies/g
FEB 11	8	<20	not detected	<40	copies/g	not detected	<40	copies/g
MAR 11	7	50	not detected	<40	copies/g	not detected	<40	copies/g
APR 11	7	20	not detected	<40	copies/g	not detected	<40	copies/g

Site 36

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
JUN 09	13	700	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	13	70	no result			no result		
AUG 09	15	<20	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	14	490	no result			no result		
OCT 09	14	<20	positive	<100	copies/g	not detected	<40	copies/g
NOV 09	11	1300	not detected	<40	copies/g	positive	<100	copies/g
DEC 09	9	80	positive	<100	copies/g	positive	987	copies/g
JAN 10	5	<20	positive	<100	copies/g	positive	1619	copies/g
FEB 10	5	80	not detected	<40	copies/g	positive	589	copies/g
MAR 10	8	50	not detected	<40	copies/g	positive	100-500	copies/g
APR 10	5	<20	not detected	<40	copies/g	not detected	<40	copies/g
MAY 10	5	130	not detected	<40	copies/g	positive	<100	copies/g
JUN 10	7	80	not detected	<40	copies/g	positive	<100	copies/g
JUL 10	6	790	not detected	<40	copies/g	positive	<100	copies/g
AUG 10	10	130	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	10	20	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	8	<20	positive	<100	copies/g	not detected	<40	copies/g
NOV 10	7	20	not detected	<40	copies/g	not detected	<40	copies/g
DEC 10	6	330	not detected	<40	copies/g	positive	<100	copies/g
JAN 11	6	130	not detected	<40	copies/g	not detected	<40	copies/g
FEB 11	8	20	positive	<100	copies/g	positive	<100	copies/g
MAR 11	7	20	not detected	<40	copies/g	positive	<100	copies/g
APR 11	7	<20	not detected	<40	copies/g	not detected	<40	copies/g

Site 37

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	10	490	positive	<100	copies/g	not detected	<40	copies/g
JUN 09	14	2400	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	16	5400	positive	<100	copies/g	not detected	<40	copies/g
AUG 09	15	1300	positive	<100	copies/g	positive	<100	copies/g
SEP 09	13	9200	positive	<100	copies/g	not detected	<40	copies/g
OCT 09	12	790	positive	<100	copies/g	not detected	<40	copies/g
JAN 10	5	80	positive	<100	copies/g	positive	1549	copies/g
FEB 10	7	<20	positive	563	copies/g	positive	2360	copies/g
MAR 10	5	80	positive	<100	copies/g	positive	1186	copies/g
APR 10	7	20	positive	<100	copies/g	positive	534	copies/g
MAY 10	11	20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	12	50	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	14	1300	not detected	<40	copies/g	positive	<100	copies/g
AUG 10	14	2400	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	13	170	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	10	130	not detected	<40	copies/g	positive	<100	copies/g
NOV 10	8	790	positive	<100	copies/g	positive	<100	copies/g
JAN 11	7	50	positive	297	copies/g	positive	564	copies/g
FEB 11	5	230	positive	160	copies/g	positive	657	copies/g
MAR 11	6	50	positive	252	copies/g	positive	462	copies/g
APR 11	10	170	positive	<100	copies/g	positive	<100	copies/g

Site 38

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	10	5400	not detected	<40	copies/g	not detected	<40	copies/g
JUN 09	14	330	positive	<100	copies/g	not detected	<40	copies/g
JUL 09	15	490	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	14	50	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	15	230	positive	<100	copies/g	positive	<100	copies/g
OCT 09	13	330	not detected	<40	copies/g	positive	<100	copies/g
NOV 09	12	330	not detected	<40	copies/g	not detected	<40	copies/g
DEC 09	10	<20	not detected	<40	copies/g	not detected	<40	copies/g
JAN 10	7	490	no result			no result		
FEB 10	6.5	330	positive	<100	copies/g	positive	235	copies/g
MAR 10	7	230	not detected	<40	copies/g	positive	100-500	copies/g
APR 10	10	<20	not detected	<40	copies/g	positive	100-500	copies/g
MAY 10	10	<20	positive	<100	copies/g	not detected	<40	copies/g
JUN 10	11	20	positive	<100	copies/g	not detected	<40	copies/g
JUL 10	14	9200	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	15	170	positive	<100	copies/g	not detected	<40	copies/g
SEP 10	16	700	positive	<100	copies/g	not detected	<40	copies/g
OCT 10	13	330	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	10	20	not detected	<40	copies/g	positive	1462	copies/g
DEC 10	8	50	not detected	<40	copies/g	positive	249	copies/g
JAN 11	6	230	positive	<100	copies/g	positive	1148	copies/g
FEB 11	7	<20	positive	<100	copies/g	positive	106	copies/g
MAR 11	9	<20	not detected	<40	copies/g	not detected	<40	copies/g
APR 11	11	130	not detected	<40	copies/g	not detected	<40	copies/g

Site 39

Month	Water temperature	*E. coli*	Norovirus GI			Norovirus GII		
MAY 09	10	80	not detected	<40	copies/g	positive	<100	copies/g
JUN 09	14	790	not detected	<40	copies/g	not detected	<40	copies/g
JUL 09	15	330	not detected	<40	copies/g	not detected	<40	copies/g
AUG 09	16	1300	positive	<100	copies/g	not detected	<40	copies/g
SEP 09	14	50	not detected	<40	copies/g	not detected	<40	copies/g
OCT 09	12	790	not detected	<40	copies/g	not detected	<40	copies/g
NOV 09	11	790	positive	<100	copies/g	positive	<100	copies/g
DEC 09	8	50	positive	<100	copies/g	positive	<100	copies/g
JAN 10	7	50	positive	<100	copies/g	positive	<100	copies/g
FEB 10	6	<20	positive	<100	copies/g	positive	329	copies/g
MAR 10	6	20	positive	<100	copies/g	not detected	<40	copies/g
APR 10	9	80	not detected	<40	copies/g	positive	<100	copies/g
MAY 10	10	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUN 10	14	<20	not detected	<40	copies/g	not detected	<40	copies/g
JUL 10	14	2200	not detected	<40	copies/g	not detected	<40	copies/g
AUG 10	15	790	not detected	<40	copies/g	not detected	<40	copies/g
SEP 10	15	<20	not detected	<40	copies/g	not detected	<40	copies/g
OCT 10	13	220	not detected	<40	copies/g	not detected	<40	copies/g
NOV 10	9	70	positive	<100	copies/g	not detected	<40	copies/g
DEC 10	7	20	positive	<100	copies/g	positive	203	copies/g
JAN 11	6	50	not detected	<40	copies/g	positive	<100	copies/g
FEB 11	5	20	positive	<100	copies/g	positive	<100	copies/g
MAR 11	9	<20	positive	<100	copies/g	not detected	<40	copies/g
APR 11	9	490	positive	533	copies/g	not detected	<40	copies/g

Appendix 3: Statistical analysis

Page 6: Oyster species comparison
Two-way ANOVA: norovirus versus species, contamination

Source	DF	SS	MS	F	P
species	1	0.0614	0.06138	1.46	0.232
contamination	2	18.4199	9.20993	219.40	0.000
Interaction	2	0.1257	0.06287	1.50	0.233
Error	54	2.2668	0.04198		
Total	59	20.8738			

S = 0.2049   R-Sq = 89.14%   R-Sq(adj) = 88.13%

```
                   Individual 95% CIs For Mean Based on
                   Pooled StDev
species      Mean  ----+---------+---------+---------+-----
C.gigas   4.60887  (-----------*------------)
O.edulis  4.67284            (------------*-----------)
                   ----+---------+---------+---------+-----
                     4.560     4.620     4.680     4.740
```

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
contamination     Mean  -----+---------+---------+---------+----
high           5.23487                                    (-*-)
medium         4.78641                        (--*-)
low            3.90129  (--*-)
                        -----+---------+---------+---------+----
                           4.00      4.40      4.80      5.20
```

Page 10: GI vs GII
Tabulated statistics: genogroup, result

Using frequencies in C3

Rows: genogroup   Columns: result

	-	+	All
GI	266	578	844
GII	377	467	844
All	643	1045	1688

Cell Contents: Count

Fisher's exact test: P-Value = 0.0000000

Page 10: Summer vs winter prevalence
Tabulated statistics: season, norovirus

```
Using frequencies in C3


Rows: season   Columns: norovirus

            -    +  All

summer    159  264  423
winter     42  379  421
All       201  643  844

Cell Contents:      Count


Fisher's exact test: P-Value =  0.0000000
```

Page 11: GI vs GII
Wilcoxon Signed Rank Test: Diff

```
Test of median = 0.000000 versus median not = 0.000000

          N for   Wilcoxon         Estimated
       N   Test  Statistic      P     Median
Diff  643    521    44434.0  0.000     -39.50
```

Wilcoxon Signed Rank Test: Diff; <LOD = 0, <LOQ = 1

```
Test of median = 0.000000 versus median not = 0.000000

          N for   Wilcoxon         Estimated
       N   Test  Statistic      P     Median
Diff  643    521    43352.0  0.000     -60.55
```

Wilcoxon Signed Rank Test: Diff; <LOD = 40, <LOQ = 100

```
Test of median = 0.000000 versus median not = 0.000000

          N for   Wilcoxon         Estimated
       N   Test  Statistic      P     Median
Diff  643    521    48094.0  0.000     -30.00
```

Page 11: Spearman’s rank of GI vs GII
Correlations: GIrank, GIIrank

```
Pearson correlation of GIrank and GIIrank = 0.622
P-Value = 0.000
```

Page 13: Yr1 vs Yr2 prevalence
Tabulated statistics: year, norovirus

Using frequencies in C3

Rows: year   Columns: norovirus

	-	+	All
yr1	91	337	428
yr2	110	306	416
All	201	643	844

Cell Contents: Count

Fisher's exact test: P-Value = 0.0895321

Page 13: Yr1 vs Yr2
Kruskal-Wallis Test: norovirus versus year

Kruskal-Wallis Test on norovirus

year	N	Median	Ave Rank	Z
yr1	428	100.0	417.1	-0.65
yr2	416	100.0	428.1	0.65
Overall	844		422.5	

H = 0.43 DF = 1 P = 0.513
H = 0.44 DF = 1 P = 0.506 (adjusted for ties)

Page14: Between regions comparison
Kruskal-Wallis Test: norovirus versus region

Kruskal-Wallis Test on norovirus

region	N	Median	Ave Rank	Z
England - East Coast	12	226.30	22.8	1.00
England - South Coast	13	194.60	23.9	1.52
England - West Coast, Wales and NI	8	118.74	18.5	-0.42
Scotland	6	81.92	8.0	-2.80
Overall	39		20.0	

H = 9.02 DF = 3 P = 0.029

Page14: Between regions comparison – post hoc analysis
Kruskal-Wallis: All Pairwise Comparisons

Comparisons: 6
Family Alpha: 0.2
Bonferroni Individual Alpha: 0.033
Bonferroni Z-value (2-sided): 2.128

Standardized Absolute Mean Rank Differences
|Rbar(i)-Rbar(j)| / Stdev

Rows: Group i = 1,...,n
Columns: Group j = 1,...,n

1. Table of Z-values

England - East Coast	0.00000	*	*	*
England - South Coast	0.25701	0.00000	*	*
England - West Coast, Wales & NI	0.81665	1.05848	0.00000	*
Scotland	2.58732	2.82961	1.70520	0

2. Table of P-values

England - East Coast	1.00000	*	*	*
England - South Coast	0.79717	1.00000	*	*
England - West Coast, Wales & NI	0.41413	0.28984	1.00000	*
Scotland	0.00967	0.00466	0.08816	1

Sign Confidence Intervals controlled at a family error rate of 0.2

Desired Confidence: 86.761

Sign confidence interval for median

				Confidence Interval		
	N	Median	Achieved Confidence	Lower	Upper	Position
norovirus_England - East Coast	12	226.3	0.8540	90.0	393.3	4
			0.8676	89.2	399.9	NLI
			0.9614	73.3	537.3	3
norovirus_England - South Coast	13	194.6	0.7332	173.9	200.5	5
			0.8676	166.0	205.6	NLI
			0.9077	160.6	209.0	4
norovirus_England - West Coast,	8	118.7	0.7109	112.8	175.3	3
			0.8676	104.8	186.7	NLI
			0.9297	95.3	200.3	2
norovirus_Scotland	6	81.9	0.7813	52.0	95.7	2
			0.8676	51.8	106.4	NLI
			0.9688	50.5	169.2	1

Kruskal-Wallis: Conclusions

The following groups showed significant differences:

Groups	Z vs. Critical value	P-value
England - South Coast vs. Scotland	2.82961 >= 2.128	0.0047
England - East Coast vs. Scotland	2.58732 >= 2.128	0.0097

Page15: Between species comparison
Kruskal-Wallis Test: norovirus versus species

```
Kruskal-Wallis Test on norovirus

species    N  Median  Ave Rank      Z
C.gigas   28   164.9      19.9  -0.12
O.edulis  11   173.9      20.4   0.12
Overall   39              20.0

H = 0.02  DF = 1  P = 0.901
```

Page 16: Between classifications comparison
Kruskal-Wallis Test: norovirus versus classification

```
Kruskal-Wallis Test on norovirus

classification   N   Median  Ave Rank      Z
A                6    81.92       8.7  -2.65
B               31   179.55      21.5   1.67
C                2  1208.33      30.0   1.27
Overall         39               20.0

H = 8.04  DF = 2  P = 0.018

* NOTE * One or more small samples
```

Page16: Between classifications comparison – post hoc analysis
Kruskal-Wallis: All Pairwise Comparisons

```
----------------------------------------

Comparisons:                     3
Family Alpha:                    0.2
Bonferroni Individual Alpha:     0.067
Bonferroni Z-value (2-sided):    1.834

----------------------------------------

Standardized Absolute Mean Rank Differences
|Rbar(i)-Rbar(j)| / Stdev

Rows:    Group i = 1,...,n
Columns: Group j = 1,...,n

1. Table of Z-values

A  0.00000        *  *
B  2.53313  0.00000  *
C  2.29157  1.01603  0

2. Table of P-values

A  1.00000        *  *
B  0.01130  1.00000  *
C  0.02193  0.30961  1

----------------------------------------------------------
```

```
Sign Confidence Intervals controlled at a family error rate of 0.2


Desired Confidence:    80.529


Sign confidence interval for median

                                              Confidence
                                   Achieved    Interval
                N  Median  Confidence  Lower  Upper  Position
norovirus_1_A   6    81.9      0.7813   52.5   95.7         2
                               0.8053   52.5   98.0       NLI
                               0.9688   52.0  175.3         1
norovirus_1_B  31   179.5      0.7190  138.3  197.1        13
                               0.8053  133.5  198.9       NLI
                               0.8504  129.5  200.3        12
norovirus_1_C   2    1208      0.5000    174   2243         1

The highest attainable confidence has been achieved.
```

Kruskal-Wallis: Conclusions

```
The following groups showed significant differences:

Groups          Z vs. Critical value       P-value
A vs. B         2.53313 >= 1.834           0.0113
A vs. C         2.29157 >= 1.834           0.0219
```

Page 17: Spearman's rank norovirus vs. *E. coli* sample-by-sample Correlations: ranknorovirus, rankE.coli

```
Pearson correlation of ranknorovirus and rankE.coli = 0.195
P-Value = 0.000
```

Page 18: Spearman's rank norovirus vs. *E. coli* site-by-site Correlations: ranknorovirus, rankE.coli

```
Pearson correlation of ranknorovirus and rankE.coli = 0.453
P-Value = 0.004
```

Page 18: Spearman's rank norovirus vs. *E. coli* site-by-site, winter Correlations: ranknorovirus, rankE.coli

```
Pearson correlation of ranknorovirus and rankE.coli = 0.676
P-Value = 0.000
```

Page 18: Spearman's rank norovirus in oysters vs. HPA lab reports Correlations: rankoyster, rankHPA

```
Pearson correlation of rankoyster and rankHPA = 0.761
P-Value = 0.000
```

Page 20: Spearman's rank norovirus in oysters vs. UK average air temperatures
Correlations: rankoyster, ranktemp

```
Pearson correlation of rankoyster and ranktemp = -0.877
P-Value = 0.000
```

Page 20: Spearman's rank norovirus in oysters vs. Water temperatures (individual sites)
Site 1
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.797
P-Value = 0.000
```

Site 2
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.783
P-Value = 0.000
```

Site 3
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.528
P-Value = 0.014
```

Site 4
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.879
P-Value = 0.000
```

Site 7
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.766
P-Value = 0.001
```

Site 8
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.676
P-Value = 0.001
```

Site 9
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.596
P-Value = 0.003
```

Site 10
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.603
P-Value = 0.002
```

Site 11
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.865
P-Value = 0.000
```

Site 12
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.755
P-Value = 0.000
```

Site 13
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.747
P-Value = 0.003
```

Site 14
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.433
P-Value = 0.160
```

Site 15
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.737
P-Value = 0.000
```

Site 16
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.573
P-Value = 0.005
```

Site 17
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.870
P-Value = 0.000
```

Site 18
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.587
P-Value = 0.027
```

Site 20
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.737
P-Value = 0.004
```

Site 21
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.359
P-Value = 0.208
```

Site 22
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.862
P-Value = 0.000
```

Site 23
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.783
P-Value = 0.001
```

Site 24
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = 0.002
P-Value = 0.992
```

Site 25
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.704
P-Value = 0.001
```

Site 26
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.690
P-Value = 0.000
```

Site 27
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.549
P-Value = 0.008
```

Site 28
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.407
P-Value = 0.060
```

Site 29
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.618
P-Value = 0.003
```

Site 30
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.330
P-Value = 0.124
```

Site 31
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.615
P-Value = 0.005
```

Site 32
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.242
P-Value = 0.333
```

Site 33
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.832
P-Value = 0.000
```

Site 34
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.373
P-Value = 0.080
```

Site 35
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = 0.013
P-Value = 0.954
```

Site 36
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.142
P-Value = 0.539
```

Site 37
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.754
P-Value = 0.000
```

Site 38
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.460
P-Value = 0.027
```

Site 39
Correlations: ranktemp, ranknoro

```
Pearson correlation of ranktemp and ranknoro = -0.746
P-Value = 0.000
```

Page 20: Spearman's rank norovirus vs. risk score
Correlations: rankrisk, ranknorovirus

```
Pearson correlation of rankrisk and ranknorovirus = 0.546
P-Value = 0.001
```

Page 21: Spearman's rank norovirus vs. sampling risk score
Correlations: ranksampling risk score, ranknorovirus

```
Pearson correlation of ranksampling risk score and ranknorovirus = 0.645
P-Value = 0.000
```