Envirolink Project No. 162 NLRC20

Report : NLRC Shellfish safety following sewage spills

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Recommendations

- Following significant rainfall and recorded sewage spills or overflows, shellfish samples are collected from selected sites relevant to the spill site and analysed for presence of enteric viruses (Adenovirus and Norovirus), *E coli* and faecal coliforms within 24-48 hours of the spill.

- Additional samples should be collected and analysed for the presence of viruses at 2, 4 and 6 weeks following the spill. If viruses are still detected after this period, then a further sample should be taken after 8 weeks.

- Sampling sites should be located downstream of the reported pollution source at appropriate distances

- It is recommended that sewage effluent be analysed for presence of infectious viruses during winter periods when heavy rainfall is expected.

- Baseline data for background levels of both bacterial and viral contamination should be collected via ongoing regular monitoring programmes in the area.

- Climate and seawater data should be collected at every sampling.

Project Brief

NRC is undertaking initiatives to minimise the prevalence of viruses in shellfish in the Northland region. Therefore in order to achieve this, NRC wishes to undertake monitoring of shellfish following known sewerage spill events.

After a sewerage spill shellfish are often contaminated with viruses and sampling of the shellfish is needed to determine if they are once again safe to collect for consumption.

This report offers advice which will allow NRC to adequately monitor shellfish for viruses after spills and to provide this information to Health Authorities who are required to make public health decisions on the collection of shellfish. The advice will be used when monitoring the effects of sewage spills into waters from which shellfish are collected.

The report draws on data collected in recent research studies carried out on the prevalence of enteric viruses in shellfish collected from sites impacted by sewage pollution in several areas around New Zealand and on viral persistence in shellfish following uptake. Information is provided on the survival and persistence of enteric viruses in shellfish following pollution events and potential sampling and monitoring strategies are outlined.

The information will allow for development of cost effective programmes to monitor viral contamination of shellfish following sewage spills and overflows across Northland.

Background to project

Contamination of bivalve shellfish by human enteric viruses is an increasing problem for environmental and public health agencies. Extensive urban and residential development is impacting on water quality in traditional shellfish growing areas and therefore on the safety of the product for human consumption. Human viruses, including noroviruses, adenoviruses and enteroviruses, are able to contaminate shellfish and therefore present health risks to both the general public collecting shellfish and the aquaculture industry.

Viral uptake and localisation

Viral contamination of shellfish growing waters can result from a number of sources, including septic tank leakages, boat discharges, overflows and spills from sewage treatment plants (STPs) and seepage from sewage reticulation networks. As they feed, bivalve shellfish filter large quantities of water, selectively concentrating particulate matter, bacteria and viruses at the same time. The actual mechanism is not fully understood but several litres of water can be filtered per hour. This can lead to extensive viral contamination within a short time period if the water is contaminated with raw sewage. In contrast to bacterial contaminants such as *E coli* which usually are cleared from shellfish within a few days of uptake, these viruses are known to persist for several weeks or months in the shellfish gut and the environment, although their infectivity status is generally not known (Lees, 2000). Researchers have shown that viruses can be detected in shellfish several weeks after uptake (Greening *et al.,* 2003; Caballero *et al*., 2004; Loisy *et al*., 2005) Greening et al. unpublished data).

It is now known that human susceptibility to norovirus infection has a genetic basis and that noroviruses bind specifically to the human histocompatability blood group antigens. The presence or absence of certain genetic markers determines whether people are susceptible or resistant to norovirus infection. Recent research has shown that oysters possess similar histocompatability blood group antigens to humans and can bind noroviruses in a similar manner to that in the human gut (Le Guyader et al., 2006) but the longevity of virus in the tissues is unknown at this stage. Enteric viruses localise in the shellfish gut and other tissues, and are also excreted in the pseudofaeces and faeces of oysters. However recent research (Seamer, 2007) has shown that enteric viruses may each behave differently, with poliovirus being inactivated and depurated readily from oysters, in contrast to noroviruses which are not easily depurated and may become sequestered in the oyster gut epithelia and other tissues.

This could explain why depuration methods have not always been successful for viral removal and indicates that the only sure safety precaution is zero tolerance for norovirus in shellfish. Pristine water free from human sewage and faecal contamination is a goal which is difficult to achieve for local authorities because of increasing urban development and environmental impact on the quality of marine waters.

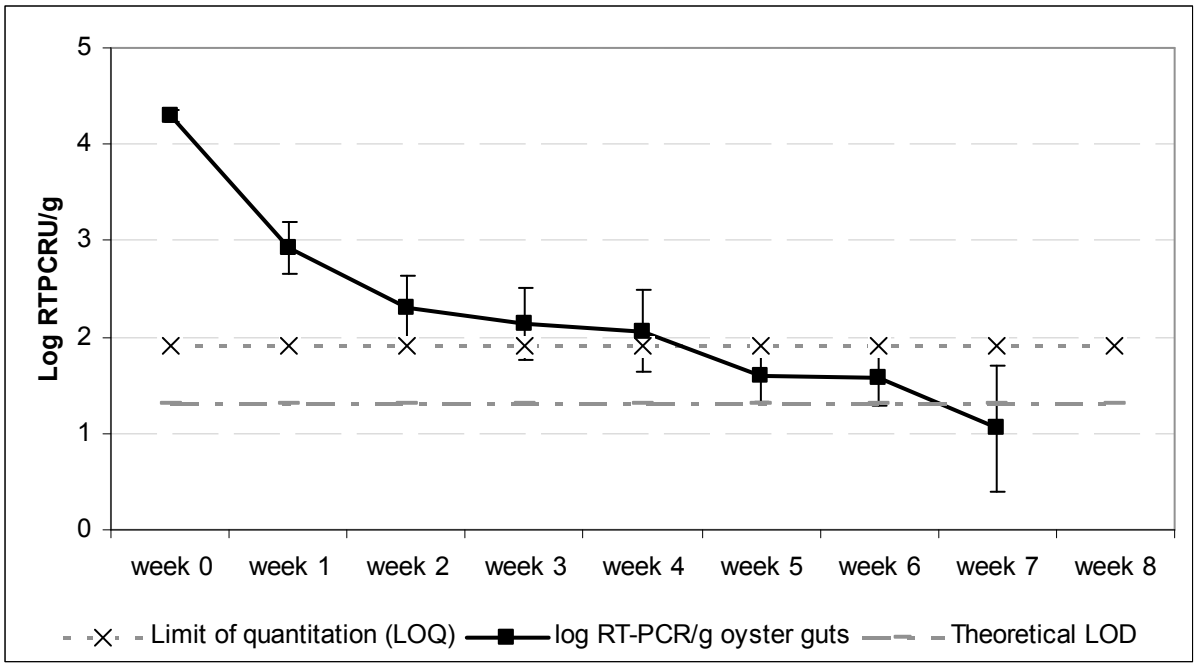
Seasonal effects and viral persistence

Seasonal effects occur due to both virus distribution and to climatic factors, and can affect virus persistence and survival in water. The occurrence of human enteric viruses in wastewater is dependent on the incidence of viral infection in the contributing community and so certain viruses may only occur sporadically. Consequently the level of human virus occurrence in sewage is difficult to predict and will vary during the year. Certain viral infections show seasonal characteristics, with adenovirus infections generally more common in winter and enterovirus infections more common in summer, but it is likely that both of these viruses will be present in raw sewage all of the time in most communities, even if at low levels. Norovirus outbreaks occur throughout the year but do not necessarily occur in a small community all of the time.

During the cooler winter months, viruses are more likely to survive prolonged environmental exposure, and periods of expected high rainfall occur which may reduce the effectiveness of sewage treatment. Thus at this time the risk of virus presence in the effluent is greatest and so, irrespective of the actual prevalence of infection in the community, potentially the greatest risk to shellfish is likely to be when these conditions all occur together. At these times it may be appropriate to test specifically for human viruses in the STP effluent to determine whether the plant is successfully removing or inactivating viruses. Human adenoviruses and enteroviruses are the most appropriate viruses to use for this analysis.

Viruses are known to persist in shellfish for several weeks. Our research has shown that noroviruses can persist for at least 4 weeks in shellfish at 12-18°C (Greening *et al.,* 2003) and even at 20°C (Figure 1) (Greening, unpublished data). Monthly sampling should therefore provide sufficient information on the presence in shellfish at selected sites which could be impacted by sewage discharges.

Figure 1: Persistence of norovirus in shellfish following bioaccumulation and uptake by Pacific oysters. Shellfish were held in netlon bags in the intertidal zone and samples were removed for viral analysis weekly for 8 weeks.



Spills & overflows

Sewage spills and overflows often occur during periods of high rainfall and at these times the shellfish can become heavily contaminated with viral and bacterial pathogens. The data from our shellfish prevalence study shows that the shellfish collected in the Bay of Islands were most contaminated during the winter months and especially following a major spill at the Kawakawa STP in June / July 2004. At this time shellfish collected from most of the main sites were positive for at least one enteric virus (Figure 4).

Role of bacterial indicators

Traditionally bacterial indicators such as faecal coliforms, *E coli* and enterococci are used to assess water and shellfish quality, but these indicators are not representative of viruses. Their role is to show presence of recent heavy faecal contamination and provide subsequent clearance approvals. However, the internationally approved standard microbiological methods cannot discriminate between animal and human pollution sources. Consequently, apart from confirming the occurrence of recent heavy contamination events from unknown sources, these indicators do not provide any accurate information about risks from viruses.

Role of phage as an indicator

FRNA bacteriophage are RNA viruses which infect coliform bacteria, including *E coli*, and are present in the environment. These viruses also possess some similar survival and persistence characteristics to enteric viruses in the environment and so have been suggested as potential viral indicators of pollution (Dore et al., 2000; Lees, 2000). They survive longer than bacteria, are present in large numbers in sewage, and can be quantified in an infectivity assay. However the FRNA phage assay also does not indicate whether pollution is from animal or human sources

and, because the viruses can replicate in the bacterial hosts present in sewage or environmental situations, may provide inaccurate data. In the New Zealand context, no correlation between the occurrence of FRNA phage and enteric viruses was observed overall in our shellfish prevalence study or in any area except for Dunedin where there was a correlation with phage presence in sites heavily impacted by the sewage outfall.

Viral transportation and shellfish

Data on transportation of viruses from a point source of pollution and subsequent uptake by shellfish has been obtained from research studies in New Zealand and overseas. In our recent shellfish study we collected virus data from 6 sites located downstream from a major pollution source - the Dunedin sewage outfall. The Dunedin Tahuna Wastewater Treatment Plant services a population of 80,000 residents and industry, discharging 35 million litres of partially treated sewerage per day into the ocean at a coastal outfall point. A series of sites along the coastline downstream from the outfall were studied as part of our 2 year study of viral contamination in shellfish. We observed that human viruses were always present in shellfish in close proximity to the outfall and at high levels but, as the distance from the point source increased, viral loading was lower and noroviruses were not always detected in shellfish (Table 1, Figure 2).

Table 1. Mean Norovirus levels in shellfish collected from Dunedin sites downstream of the main shoreline sewage outfall

	Distance in km from outfall	GI positive /total	GII positive /total	Mean GI Units/g	Mean GII Units/g
Lawyers Head	0 km	18/25	17/25	226	707
Smaills Beach	2.5 km	13/21	15/21	269	228
Boulder Beach	7.5 km	7/25	10/25	28	41
Sandfly Bay	10 km	6/21	8/21	15	34
Victory Beach	Control	0/13	8/13	<1	61

A gradient of reducing virus occurrence as the distance from the outfall increased was observed with total human enteric virus dropping from approximately 1000 PCR units/g (PCRU) of tissue adjacent to the outfall to 130 PCRU/g at 10km and 100 PCRU/g at 24km (Figure 3). Control samples from a distant coastal site showed little or no virus on any occasion. The occurrence of each virus in shellfish by distance from the outfall in each case was a very good fit to a simple linear model (R^2 >0.95). However, there were differences in the rates of change in occurrence by distance for each virus. F-RNA, noroviruses and enteroviruses had quite similar rates of change (-0.11, -0.12, -0.15 respectively), while adenovirus showed a slower rate of change (-0.02). This data suggests that a differential transport mechanism may act for the adenovirus group, which are large dsDNA viruses compared with the smaller RNA virus types.

Figure 2. Virus presence at major sites in the Dunedin region over the study period. Sample numbers per site are given.

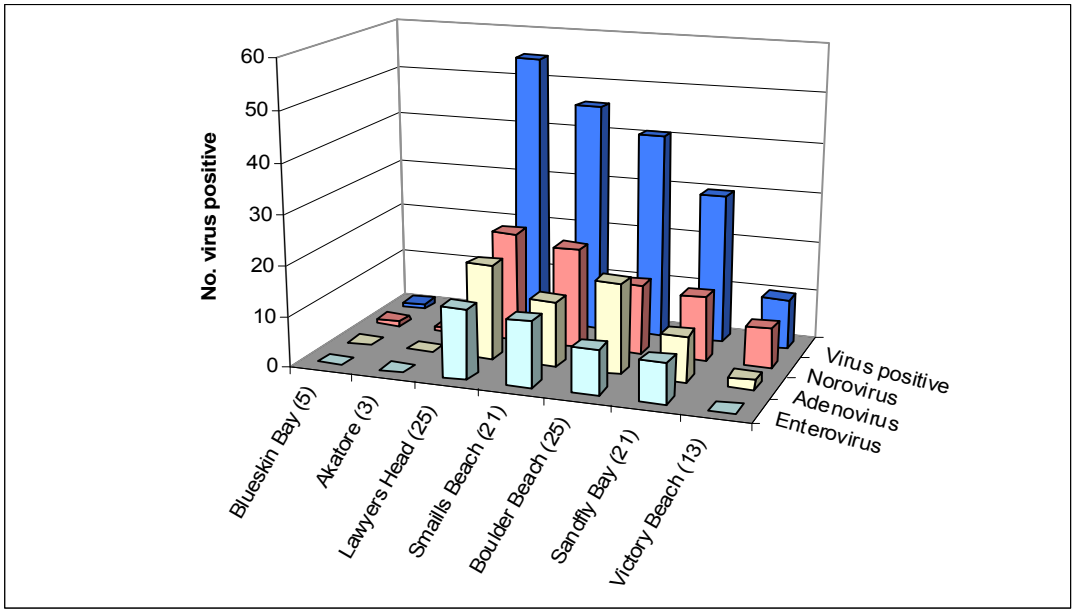
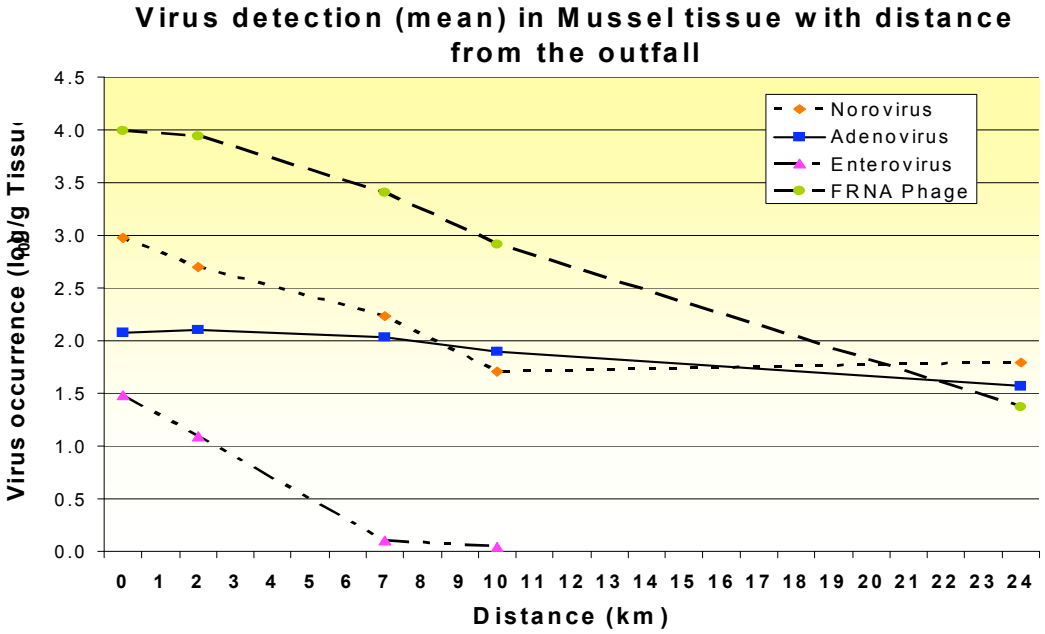


Figure 3. Relationship between mean virus detection and distance shellfish were collected from outfall. Enteric virus units are $\log_{10}$/g of shellfish gut tissue. FRNA units are pfu/g of shellfish flesh.



The Bay of Islands

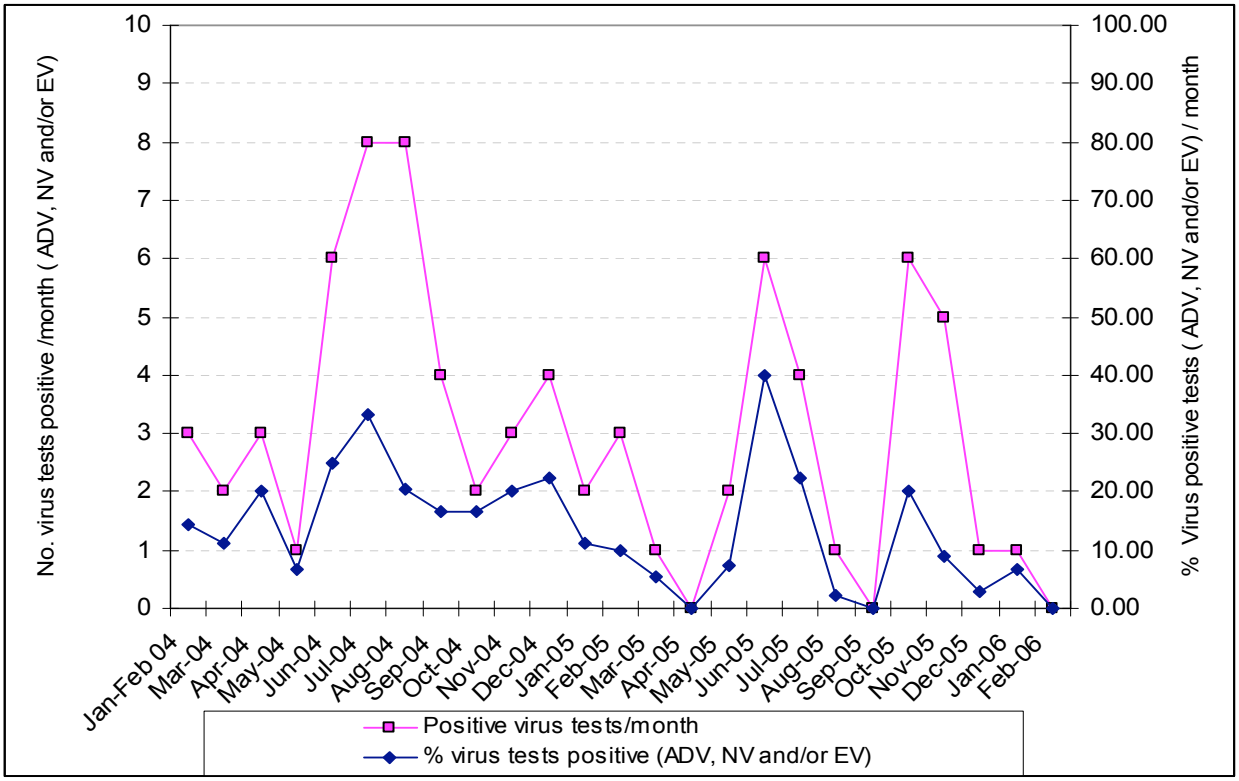
In the virus prevalence in shellfish study, 8 sites were monitored for enteric viruses, FRNA phage and E coli over a 2 year period from January 2004- February 2006. During this time sewage spills and overflows occurred in the area, principally from the Kawakawa Sewage Treatment plant, the Te Haumi pump station and the Haruru Falls pump station on the Waitangi River. Over the period, 61/174 (35.1%) of shellfish samples analysed from the 8 sites were positive for one or more enteric viruses (Table 2).

Table 2. Number and percentage of samples from each main Bay of Islands sampling site positive for any enteric virus over the period January 2004 - February 2006.

Map Code	Site	No. of samples	Virus negative	Virus positive	% Virus positive
F	Kawakawa River (top)	15	8	7	46.7
G	Kawakawa River (mouth)	17	13	4	23.5
H	Opua Marina	18	13	5	27.8
O	Okiato Pt	22	15	7	31.8
P	Waikare Lease 64	25	14	11	44.0
L	Te Haumi Pt	25	16	9	36.0
M	Waitangi Estuary	24	14	10	41.7
N	Matauwhi Bay	28	20	8	28.6
Total	All above sites	174	113	61	35.1

No clear pattern of virus contamination across all sites was observed, even when known sewage spills had occurred. However, there were times during the study when viruses were more prevalent in samples and in some cases these did relate to known spill events. Following major sewage spills at the Kawakawa STP, in June and July 2004 several shellfish samples were positive for one or more of the 3 viruses (Figure 4).

Figure 4. Viruses detected and percentage of total samples tested for 3 viruses per month from all Bay of Islands sites. Adenovirus: ADV, Norovirus: NV, enterovirus: EV



Each sample was analysed separately for the presence of 3 enteric viruses. Figure 4 shows that 25%, 33.3 % and 20.5 % of virus tests carried out in June, July, and August respectively were positive for a virus. The other periods when over 20% of virus tests were positive for a viruses were April 2004 (20% of tests were positive), November and December 2004 (20% and 22%), June and July 2005 (40% and 22.2%) and October 2005 (20%).

No viruses were detected in shellfish collected from Te Haumi Point, Waitangi, Opua Marina and Matauwhi Bay between July 2005 and February 2006 but sites along the Kawakawa River were positive for viruses in October, November and December 2005.

Other anecdotal reports of events following heavy rainfall and sewage leaks were received during the sampling period but it is difficult to directly relate these to specific virus occurrences without detailed spill reports and sampling of shellfish from appropriate sites at the time of the spills.

Figure 5. Adenovirus and norovirus occurrence in shellfish collected from Waitangi Estuary site. Units are PCRU / g of shellfish gut tissue.

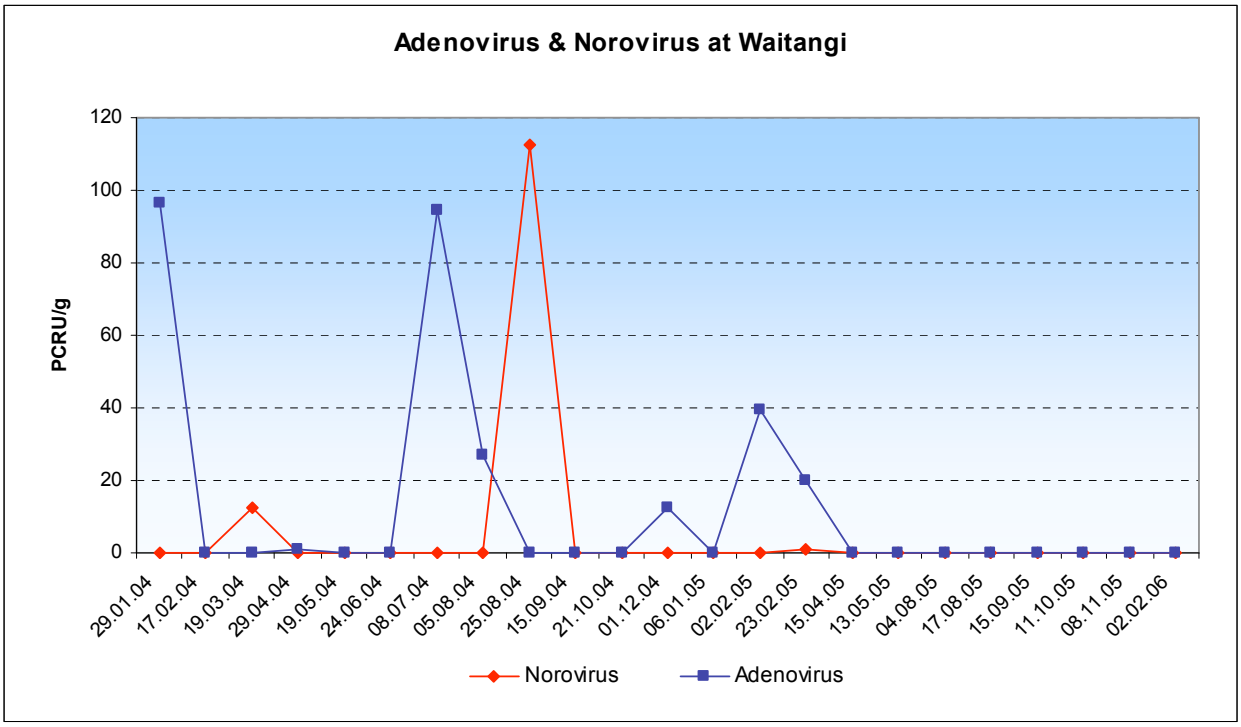
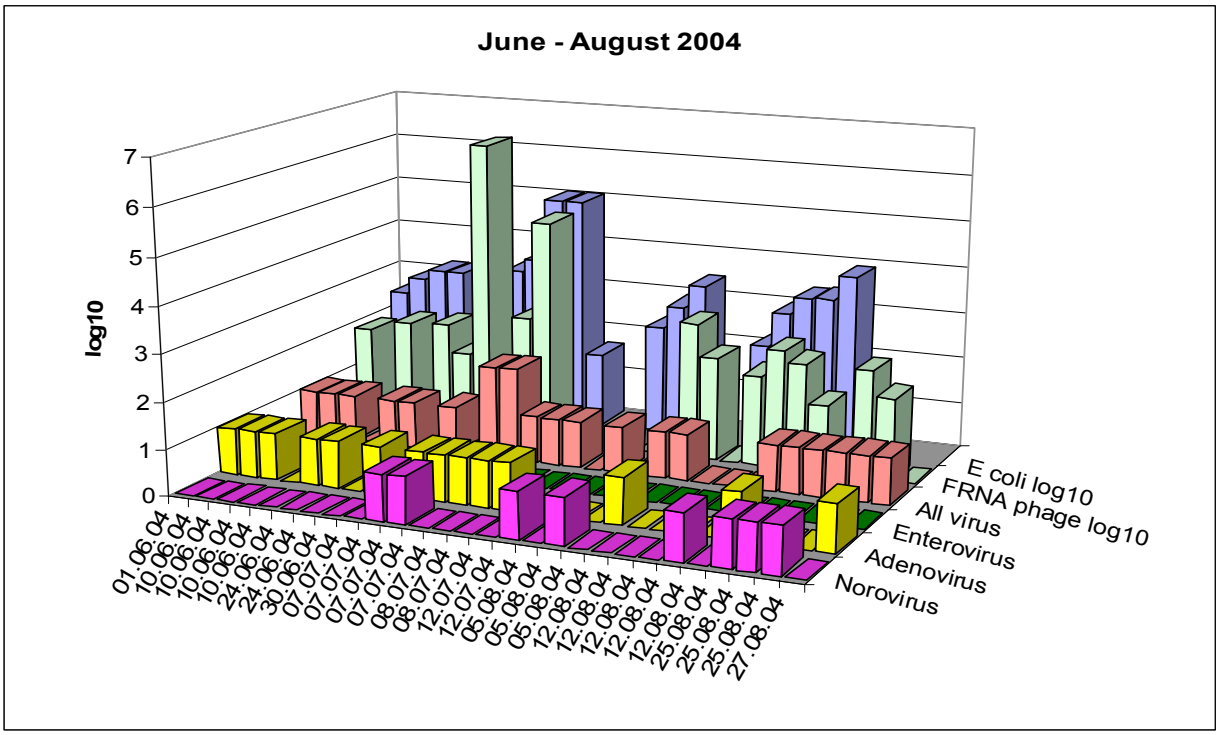
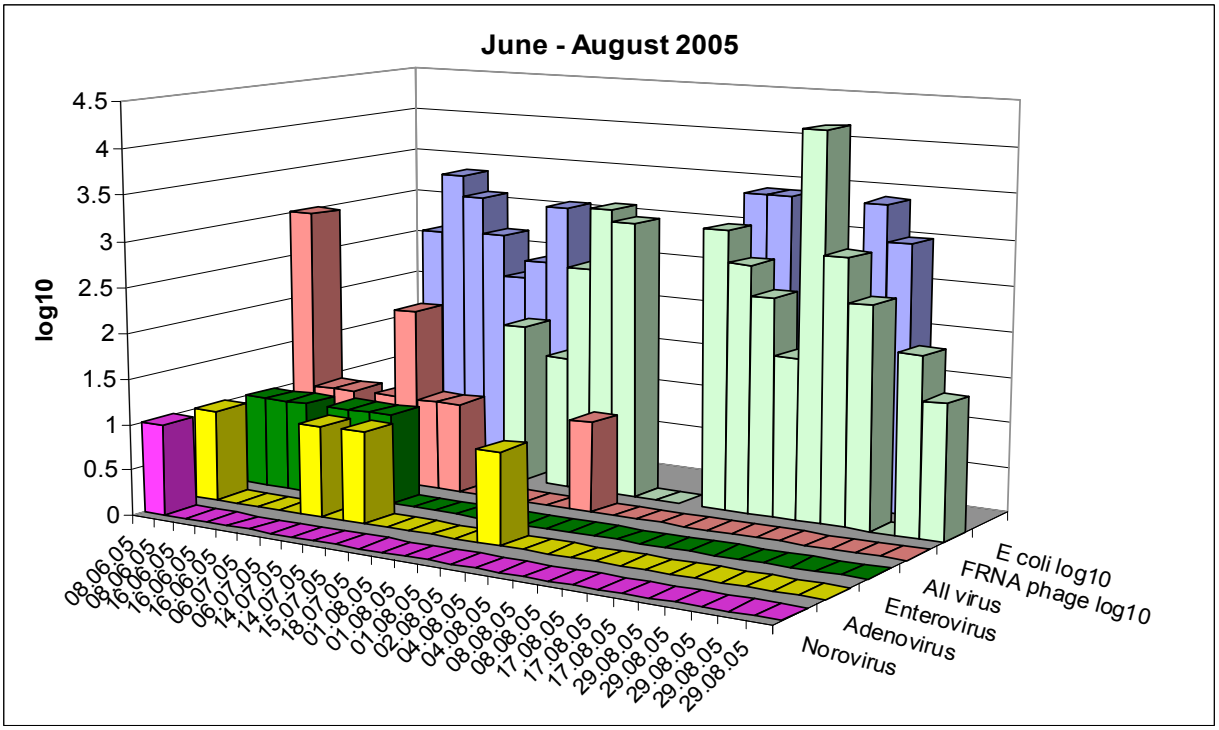


Figure 6. Relationship between virus presence, numbers of FRNA phage and *E coli* in main sampling sites in the Bay of Islands during the winter months of study.

A) June-August 2004.



B) June –August 2005



Rationale for Sampling programme following sewage spill or known discharge

1. **Selection of sampling sites based on distance from pollution source**
   From our Dunedin data and recent Irish studies, where possible a site within 500m downstream of the spill area, and 2 other sites up to 10 km downstream of the source (eg 3-5 km and 10 km) should be monitored. Where there are no shellfish in the downstream vicinity of a spill, then it is recommended to take shellfish samples as close as possible to the spill. Our research studies have shown that viruses can travel several kilometres in favourable current conditions, and so shellfish growing at a distance from a spill site can be expected to show viral contamination. In our recent study, we detected enteric viruses up to 24 km from the outfall (Table 1, Figure 3) on several occasions following ongoing sewage contamination.

   The limit of detection for the current norovirus assay is approximately 20 viral copies / gram of digestive tissue. Climatic conditions, and the species and physiological state of the shellfish will influence viral uptake and retention. In cold weather, shellfish are generally less active and may retain viruses longer. Viruses have been reported to survive longer in cold temperatures although in our virus persistence experiments we detected norovirus presence in oysters for > 6 weeks in both cold (12-14°C) and warm (>18°C) waters (Greening *et al*., 2003; Greening, unpublished). Viral uptake rates vary for both the shellfish species and each individual animal. In order to minimise individual variation, it is recommended that representative samples (> 6 oysters or mussels and ~20-30 cockles or small pipis) are collected. Several replicates from each sample are tested in the viral assay. However even when extensive sampling protocols are used it is not possible to eliminate the individual variation. Although the viral analytical methods have become more sensitive and robust, international agencies have not yet addressed the issue of appropriate sampling protocols for shellfish monitoring programmes.

2. **Frequency of sampling following a spill**
   Initial sampling within 24-48 hr of spill followed by sampling at 2 weeks, 4 weeks and 6 weeks following pollution event. If viruses are still detected after 6 weeks, then a further sample collected at 8 weeks should be analysed.

3. **Limitations of testing**
   For viruses, unless infectivity assays are carried out to establish whether infectious or culturable viruses such as enteroviruses or adenoviruses are present, the risk for human consumption cannot be determined. Norovirus infectivity cannot be determined in shellfish at present but assays may be possible within 1-2 years. The molecular assays provide an estimate of viral load. However this data must be evaluated in conjunction with the knowledge that there is inherent variability in viral uptake by shellfish so there will be limitations on the accuracy of quantitative data. Low levels of virus may be below the LOD for the assay.

4. **Evaluation and comparison of contamination events against background viral levels**.

A monthly monitoring programme for viruses included with the normal monitoring programme for standard bacterial indicators (*E coli* and faecal coliforms) will provide background data. It is recommended to monitor receiving waters for the bacterial indicators as soon as possible after pollution events to determine the extent of immediate pollution and potential public health risk. Further monitoring will provide evidence of clearance for the bacterial indicators but this does not mean that viruses will have also cleared. Monitoring of sites for at least one year will provide useful information on background levels of viral contamination.

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