

Envirolink Project No. 1: NLRC Shellfish Monitoring design and sites

Provider: ESR

Report : NLRC Shellfish Monitoring design and sites

Dr Gail E Greening
Science Leader
Communicable Disease Group,
ESR Kenepuru Science Centre
PO Box 50-348
Porirua

Ph: +64-4-9140765
Fax: +64-4-9140700
Email: gail.greening@esr.cri.nz

Recommendations

- Establishment of a regular monitoring programme for enteric viruses in shellfish
- Ongoing monthly monitoring of shellfish for presence of adenoviruses and noroviruses by quantitative real-time PCR methods
- Samples be collected from 4 sites in the Bay of Islands which frequently had virus contamination over a 2 year period
- Adenovirus positive shellfish samples are further tested to determine the infectivity status of the adenovirus and provide data for a risk assessment
- An estimate of levels of viral contamination present in each sample be provided

Summary

Enteric virus contamination has occurred frequently in shellfish collected from both commercial and non-commercial growing areas in the Bay of Islands. This contamination may have come from a number of sources including the Kawakawa sewage treatment plant, sewage pipe and pump station failures, septic tanks, and boat discharges. The establishment of a monitoring programme to determine the occurrence and frequency of enteric viral contamination of shellfish in the Bay of Islands region is recommended. Four sites which have shown previous viral contamination on a regular basis have been selected for the programme. Monthly sampling for human adenoviruses and noroviruses rather than enteroviruses is recommended because enteroviruses were detected less frequently in shellfish in a recent prevalence study. The infectivity status of adenoviruses detected in shellfish should be determined to provide information on the risk of infection following shellfish consumption.

Background

NRC is undertaking initiatives to minimise the prevalence of viruses in shellfish in the Bay of Islands region. Therefore in order to achieve this, NRC wishes to undertake monitoring of shellfish in the Bay of Islands.

This project provides data collected in a recent research study carried out on prevalence of viruses in shellfish in the Bay of Islands area and offers advice on appropriate sampling sites, sampling frequency, and viral analysis options. Costings for the various analysis options and the type of data output from the viral analyses are provided.

The information will allow for development of a cost effective programme to monitor viral contamination of shellfish.

Introduction

A 2 year programme to determine the extent of viral contamination in shellfish from different regions of New Zealand was carried out from January 2004 to February 2006 as part of the ESR FRST Programme CX0301 Safeguarding Environmental Health and Market Access for New Zealand Foods. The aims of the study were to examine the relationship between the occurrence of FRNA and enteric viruses in shellfish and to determine whether local shellfish were contaminated with human enteric viruses from sewage.

Shellfish collected from 16 sites around New Zealand were sampled monthly or two monthly over the 24 months, including 8 sites in the Bay of Islands. These 8 sites were mostly non-commercial and included two frequently-used customary shellfishing sites at Waitangi and Te Haumi Point (Figure 1). One commercial site was included (SiteP) but this lease has been closed for harvesting for a few years due to ongoing viral contamination problems.

Figure 1. Map of Bay of Islands shellfish sampling sites



Analysis of shellfish was carried out to determine the presences of 3 human enteric viruses, adenoviruses, enteroviruses and noroviruses, FRNA bacteriophage and E coli, the standard bacterial indicator. The results from the 8 Bay of Islands sites are summarised below

1. Virus prevalence over the study period

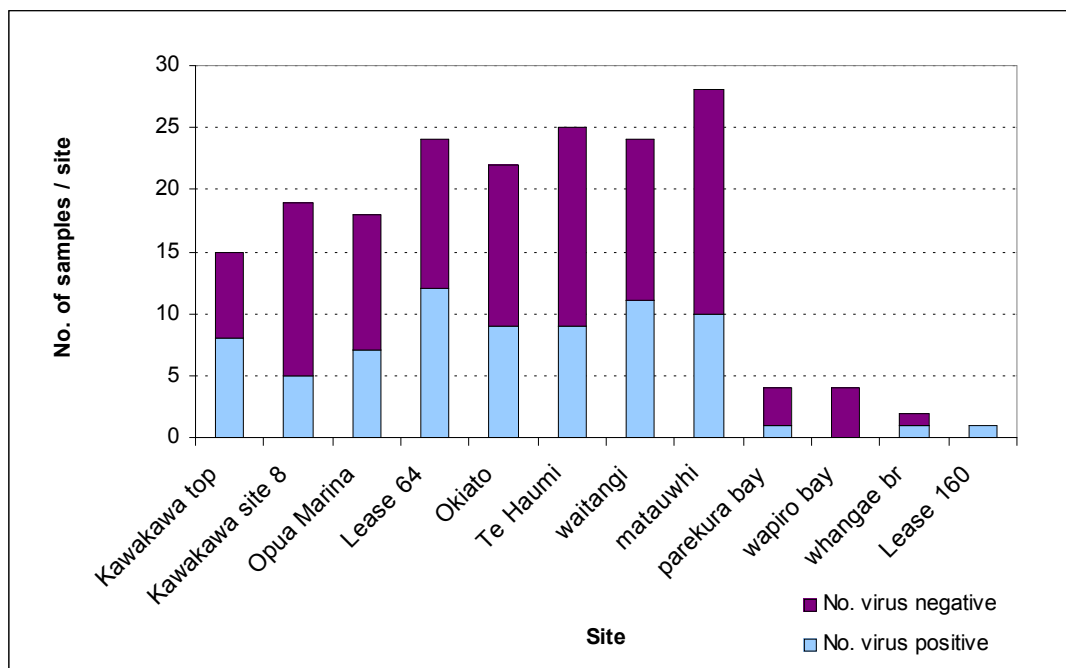
There was no correlation observed between the occurrence of FRNA bacteriophage and any of the 3 enteric viruses studied in the Bay of Islands area, so the use of phage in a monitoring programme would not provide useful information on the presence of human enteric viruses.

Virus contamination of the shellfish in the 8 main Bay of Islands sites occurred frequently during the course of the study (Table 1, Figure 2).

Table 1. 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	Matauwahi Bay	28	20	8	28.6
Total	All sites	174	113	61	35.1

Figure 2. Presence / absence of viruses in all Bay of Islands sites over study period



Over the 2 year prevalence study, the four sites most often contaminated with viruses were Site F, the top end of the Kawakawa River, which was the closest site to the Kawakawa Sewage Treatment Plant (STP) outfall (7/15, 46.7% positive), Site P, Waikare Inlet Lease 64, a commercial growing site which is currently closed for oyster production (11/25, 44.0% positive), and the 2 customary shellfish gathering areas, site M, the Waitangi Estuary (10/24, 41.7% positive) and Site L, Te Haumi Point beach (9/25, 36% positive). Sites at Okiato Point (Site O) and Matauwhi Bay (Site N) were the next most frequently contaminated by viruses. At Okiato Point viruses were detected on 7/22 (31.7%) occasions and at Matauwhi Bay, Russell, on 8/28 (28.7%) occasions. The main site with fewest viruses detected was Site G, near the Kawakawa River mouth (4/17, 23.5% positive).

Samples collected from Te Haumi and Waitangi sites over the last 3 months have shown that viruses are still occurring in shellfish in the area (Table 2).

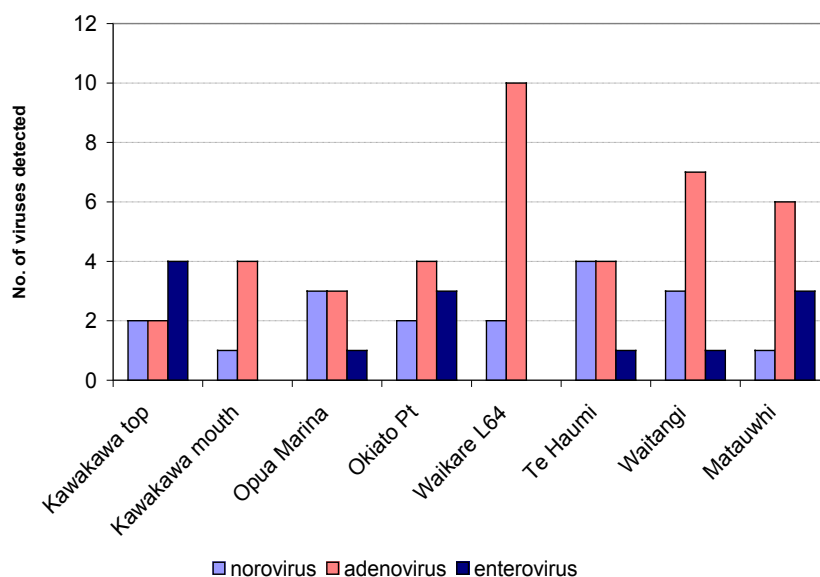
Table 2. Results for the Waitangi and Te Haumi sites for July - October 2006

Site	Sampling date	Adenovirus	Enterovirus	Norovirus
Waitangi Estuary	7 June 2006	Negative	Positive	Negative
	23 August 2006	Negative	Negative	Negative
	3 October 2006	Negative	Negative	Negative
	14 November	In progress	In progress	In progress
Te Haumi Point	7 June 2006	Negative	Negative	Negative
	23 August 2006	Positive	Negative	Negative
	3 October 2006	Negative	Negative	GII positive
	14 November	In progress	In progress	In progress

2. The occurrence of different virus types over study period

Analysis of the virus types found during the study shows that the most commonly detected viruses were adenoviruses (40 / 61, 65.6%) followed by noroviruses (18 / 61, 29.5%) and lastly enteroviruses (13 / 61, 21.3%) (Figure 3).

Figure 3. The occurrence of each virus per site over complete study period



Adenoviruses were detected at all sites at some time during the study, and most frequently at, Waikare Lease 64 (11 occurrences). Adenovirus was also commonly detected at Waitangi (7 occurrences), and Matauwhi (6 occurrences) (Figure 3). The reason for the high detection rate of adenoviruses at Waikare Lease 64a private commercial lease, is unknown. Sequence analysis and genotyping of the adenoviruses from this site has shown that these isolates were human rather than animal strains. Enteroviruses were least frequently detected with the highest number of detections (4) from the Kawakawa River top site (F) closest to the STP outfall. No enteroviruses were detected at either the lower Kawakawa River mouth site or Waikare Lease 64. Noroviruses were detected at every site at some time in the study.

3. Distribution of virus contamination per site over time course of study

The occurrence of viruses at each site over time is shown in Figures 4-11. The Kawakawa River site near the mouth (Site G), and the Opuia Marina (Site H) were added to the study in June 2004 and the Kawakawa River site F (top) was added in September 2004. Consequently no data is available for these sites for the first few months of the study. Despite the lower number of samples for the Kawakawa river (top) site, it was the site that was most frequently positive for viruses. This is also the site nearest to the Kawakawa. The highest number of viruses was detected at Waikare Lease 64 with detection of 12 viruses in total, closely followed by Waitangi (11) and Matauwhi (10) over the course of the study (Figure 3). There is no clear pattern of virus contamination across all sites, even for sampling times when known sewage spills had occurred, although there were a number of samples positive in June and July 2004 following major sewage spills at the Kawakawa STP.

4. Proposed monitoring programme

The results of the prevalence study show that viruses have been regularly occurring in shellfish in the Bay of Islands area. No viruses were detected in samples from Matauwhi Bay, Opuia Marina, Waitangi or Te Haumi in the 6 months from August 2005 to February 2006. This may reflect a number of measures set up by the district council and regional council to improve the general water quality in the area. Changes were made to the marina boat discharge requirements, the operation of the sewage treatment plant, septic tank upgrades and identification of the source of sewage spills and leaking pipes in the district. These changes may have resulted in lower viral levels in the area that were not detectable by the viral analysis methods used in the study. Recent viral analysis using a new, more sensitive analytical method has shown that there are still viruses present in shellfish collected from the Waitangi and Te Haumi customary shellfishing sites. This indicates that there is still a risk of infection for people consuming the shellfish, which comprise a major source of food for the local iwi. Therefore a monitoring programme to determine the extent of ongoing viral contamination is recommended. This programme will provide data on the occurrence and levels of enteric viruses contaminating the local shellfish beds. The data can be used in risk assessments and can link in with communicable disease data for the local community collected by the Northland District Health Board.

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 12-18C (ref) and even at 20 C (Greening, unpublished data). Monthly sampling should therefore provide sufficient information to determine the ongoing prevalence of viruses in shellfish, with additional samples collected from appropriate sites when a spill or sewage discharge event occurs. The additional samples will provide data on the extent of viral contamination and the impact on

the shellfish. It could also provide information on when shellfish can be safely harvested again.

Therefore, based on the viral occurrence data, it is recommended shellfish are analysed on a monthly basis for adenoviruses and noroviruses by a quantitative real-time PCR-based method. Quantitative real-time PCR can provide an estimate of the level of viral contamination which can subsequently be used in risk assessments. It is also recommended that adenoviruses recovered from the shellfish be grown in cell culture to determine their infectivity.

The sampling sites are recommended for the monitoring programme are as follows:

1. **Kawakawa River top site.** This site will monitor viruses discharged in effluent from the Kawakawa STP and will be especially useful following unplanned discharge events and spills. It was the site most frequently positive (7/15, 46.7% occasions) for viruses in the study.

2. **Waitangi estuary.** This site is frequently used by local iwi for food gathering, and is potentially impacted by many sources of pollution. These include a pump station behind the marae, the Waitangi River, the Haruru Falls pump station and boat discharges from the small marina at Waitangi. Shellfish have been contaminated with viruses on over 40% of sampling occasions.

3. **Te Haumi Point beach.** This site is also frequently used by iwi for food gathering. The main source of pollution has been an ongoing seepage of sewage leaking from the pump station behind the beach. Although this leak was repaired on 21 April 2005, viruses are still occurring in shellfish collected from the beach. Therefore there may be other pollution sources in this area – possibly undetected broken pipes, contamination from the Haumi River or boat discharges in the vicinity.

4. **Waikare Lease 64** (if available) or **Okiato Point.** The Waikare Lease 64 site had the second highest frequency of viral positivity in the study and was also implicated in the norovirus outbreaks of 1994 and 1999. The source of these viruses is not clear and opinions differ on whether they have come from the Kawakawa STP plant via the river flows or from other sources such as septic tanks or boats. The high numbers of adenovirus detected are of interest and could represent a possible ongoing source. They may even relate to people using the site who are also carriers of the virus. This is a privately owned commercial site which is closed for harvesting, and so it may not be available or suitable for a regular monitoring programme. An alternative site in the same area is **Okiato Point.** This site is only 300 metres from Lease 64 and was known to be impacted by septic tanks before their remediation and by boat discharges. It is close to the Opuā Marina and borders the narrow Veronica Channel leading into the Waikare Inlet. It is a terminal for the Opuā ferry and several ‘live-aboard’ boats are moored on permanent moorings in the vicinity. The Kawakawa river flow may also impact on this area.

References

Greening GE, Hewitt J, Hay BE, Grant CM. Persistence of Norwalk-like viruses over time in Pacific oysters grown in the natural environment. Proc. 4th Int. Conf Molluscan Shellfish Safety, Santiago de Compostela, Spain, 2003. Ed: A Villalba, B Reguera, JL Romalde, R Beiras. p367-377.

Greening GE. Viruses and Mataitai at Waitangi. Objective 2. Virus prevalence in shellfish.
FRST Programme CX0301 Report FW0630.

Monitoring programme costs

Samples:

12 large or 24 small shellfish collected monthly from each site and couriered overnight on ice to ESR.

Monthly Viral analysis:

Virus recovery from shellfish and quantitative real-time PCR analysis for adenoviruses and GI and GII noroviruses:

Cost per month:	\$1800 (for 4 samples)
Infectivity of adenoviruses by culture:	\$200 per month
Total cost for both molecular and culture options:	\$2000

Ad hoc additional analysis of shellfish samples following spills or other events

Adenovirus and norovirus PCR	\$500 per sample
Adenovirus infectivity:	\$100 per sample

All prices exclude GST.

Individual site graphs

Figures 4-11. Occurrence of all virus types at each of the main Bay of Islands sites for each sample date

Figure 4: Kawakawa river (top)

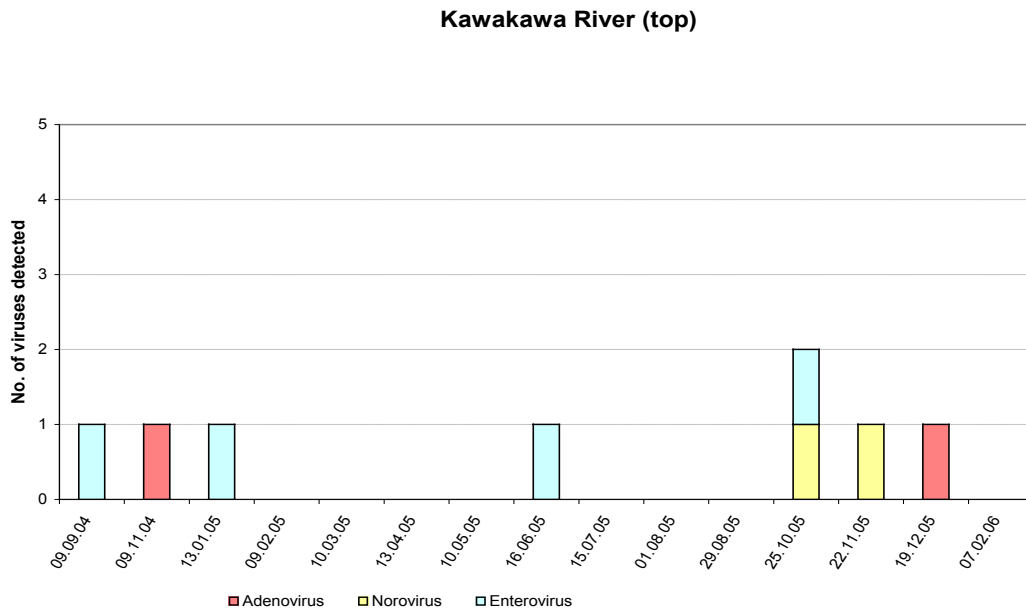


Figure 5. Kawakawa river (mouth)

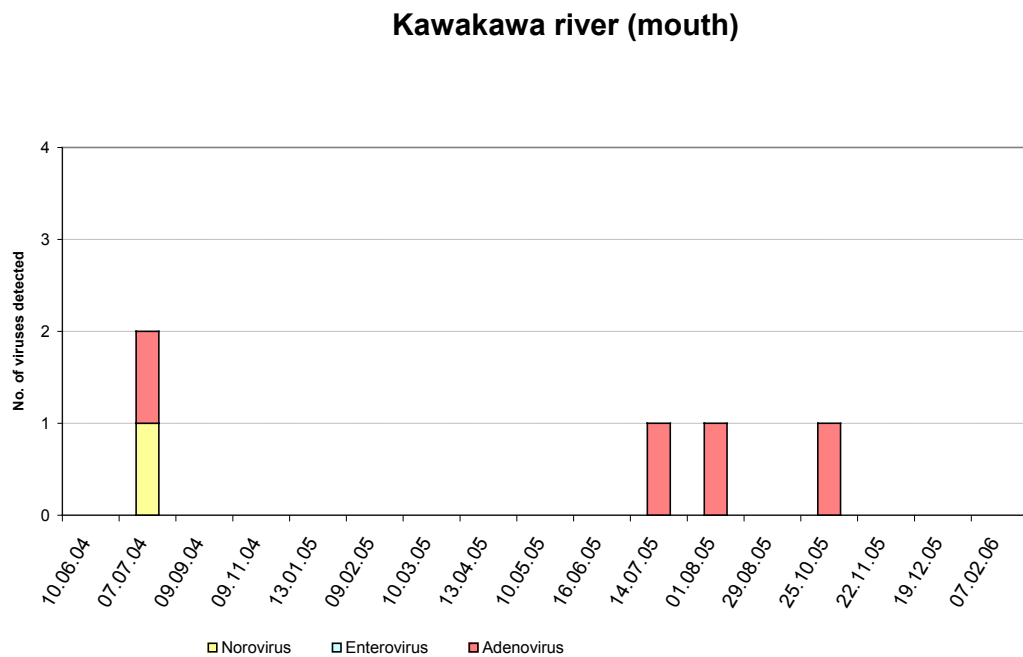


Figure 6. Opuā Marina, Pier D

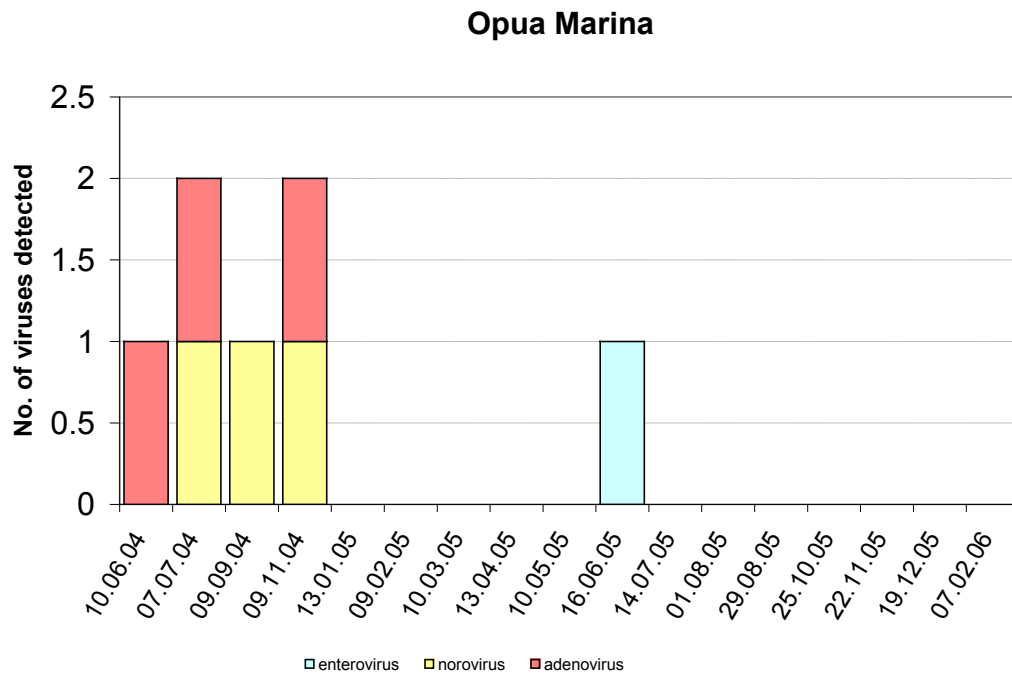


Figure 7. Okiato Point

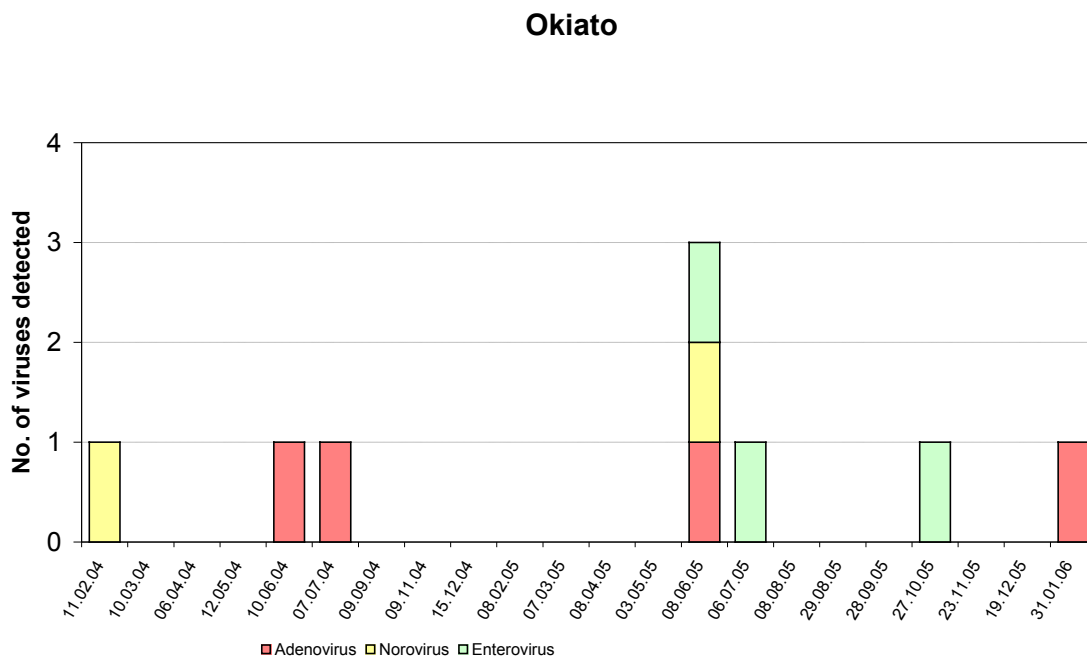


Figure 8. Matauwhi Bay, Russell

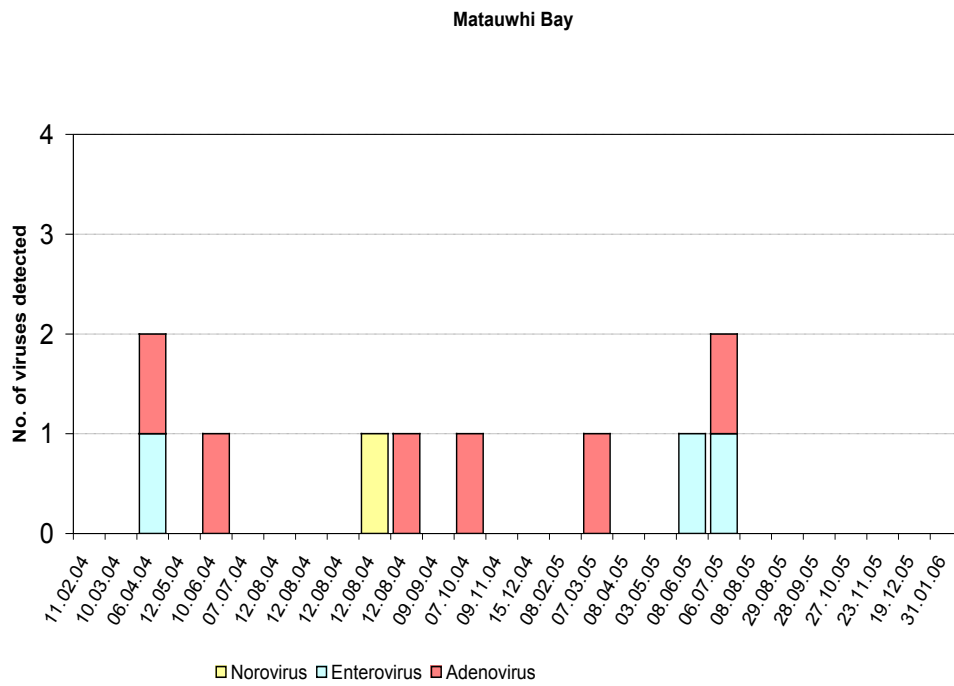


Figure 9. Waikare inlet, Lease 64

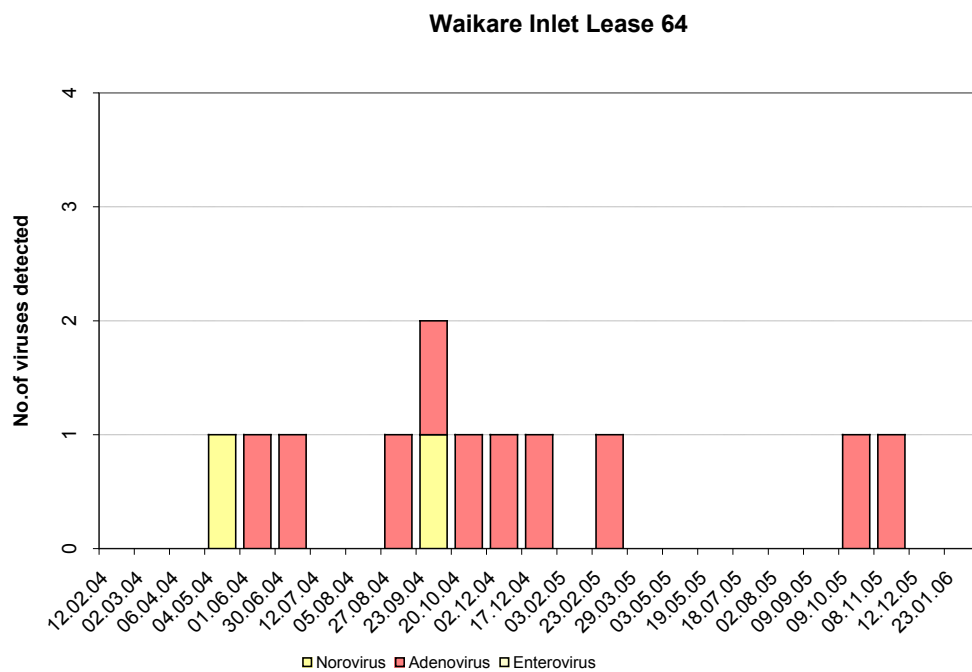


Figure 10. Waitangi Estuary

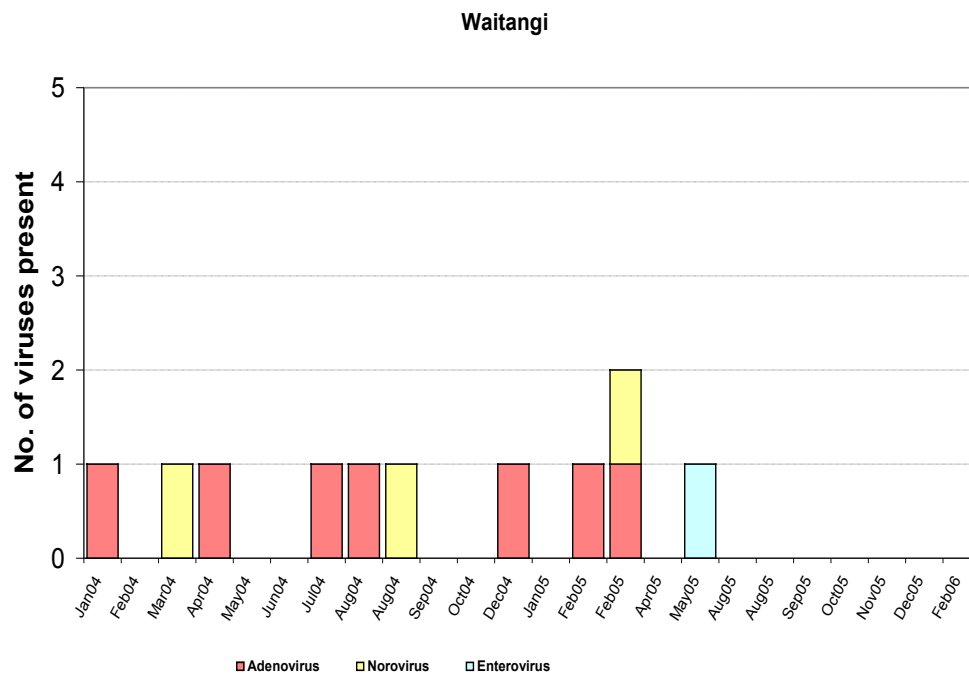


Figure 11. Te Haumi Point

