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Viruses causing lysis of the toxic bloom-forming alga *Heterosigma akashiwo* (Raphidophyceae) are widespread in coastal sediments of British Columbia, Canada

Abstract—Viruses that infect and cause lysis of the toxic alga *Heterosigma akashiwo* are abundant and widespread in the Strait of Georgia, Canada, and adjacent inlets during the summer months when blooms of this alga occur. Because viruses are subjected to many mechanisms of removal and their host is intermittently dormant, the persistence of viruses may be dependent on environmental reservoirs. We extracted pore water from sediments collected in the Strait of Georgia and screened for the presence of infectious agents that cause lysis of *H. akashiwo.* Lytic agents were widespread throughout the study region, being detected in 17 of 20 sites surveyed. Lytic agents were present in sediments ranging from highly organic to clay-rich and were retrieved from cores taken at water depths of 25–285 m. The highest concentration of lytic agents was found at the sediment-water interface; however, lytic agents were found as deep as 40 cm below the sediment-water interface. Examination of agents isolated from various sites revealed virus-like particles ~50 nm in diameter. These are similar to other virus-like particles that have been isolated that infect this alga. This suggests that the most abundant lytic agents in the sediments are viruses and that these viruses may be long-lived once buried in the sediments. The widespread presence of viral-size lytic agents that infect *H. akashiwo* is consistent with viral infection being a mortality agent of this alga in the overlying waters and suggests that they may play in important role in regulating their population dynamics.

Heterosigma akashiwo is a toxic bloom–forming raphidophyte that is responsible for extensive losses to farmed and wild fish stocks around the world (Honjo 1992). Blooms can be initiated when the water temperature is >15°C, triggering cysts to emerge from the sediments to form vegetative cells (Honjo 1993). Under appropriate conditions, which include vertical stratification of the water column, vegetative cells can form dense blooms of up to 5×10^8 cells L^{-1} (Taylor and Haigh 1993). Although initiation of *H. akashiwo* blooms has been correlated with temperature and salinity, the factors that control the intensity and duration of these blooms are poorly understood. The discovery of viruses that cause lysis of *H. akashiwo* in coastal waters of Japan and British Columbia suggests that viruses play a role in the mortality of *H. akashiwo* (Nagasaki et al. 1994; Chan et al. 1998) and therefore may play a role in bloom dynamics.

In British Columbia waters, *H. akashiwo* forms annual blooms between June and September (Taylor and Haigh 1993; Taylor et al. 1994) and can cause massive mortality of farmed salmon (Taylor 1993). Typically, the first populations of *H. akashiwo* appear in Burrard Inlet, and the blooms propagate toward the northwest along the mainland of British Columbia (Fig. 1). Propagation of the blooms is predominantly dependent on physical oceanographic conditions. It may reflect either the excystment of a seed population from the Burrard Inlet area and advection northward or the progression of excystment from different seedbeds as the waters warm (Taylor and Haigh 1993).

Viruses that infect and cause lysis of *H. akashiwo* are widespread in the Strait of Georgia during the summer months when *H. akashiwo* blooms occur (Chan et al. 1998). However, viruses are subject to various mechanisms of removal from the water column, such as adsorption to particulate matter (Suttle and Chen 1992; Noble and Fuhrman 1997), grazing by protists (Suttle and Chen 1992; Gonzalez and Suttle 1993), ultraviolet decay (Suttle and Chen 1992; Noble and Fuhrman 1997), or decay by dissolved substances (Noble and Fuhrman 1997). Consequently, viruses must be able to persist when their host is intermittently dormant. It is possible that the viruses persist by infecting overwintering cysts, or perhaps they persist in other environmental reservoirs.

A potential reservoir for viruses is in sediments, where they may accumulate when attached to sinking particles or inside infected, dying host cells. In this way, viruses in sediments may also serve as a biomarker for historical blooms and would provide us with a means for examining viruses in the environment independent of the host's dynamics. Their accumulation would also suggest the potential for these viruses to have a role in controlling future populations of *H. akashiwo* if they were resuspended into the water column. In this study, we have surveyed sediments from the Strait of Georgia and adjacent inlets to determine the abundance and distribution of viruses that infect *H. akashiwo.* The presence of viruses in sediments would potentially provide a historical account of the extent of *H. akashiwo* populations in overlying waters.

Study area and sampling—Marine sediment samples were collected from three regions in the Strait of Georgia, British Columbia, Canada (Figs. 1, 2) on each of three cruises on the CCGS Vector (16–23 July 1998, 17–22 August 1999, and 31 July–4 August 2000). Undisturbed sediment cores were collected by use of a gravity corer (Pedersen et al. 1985) in 1998 and 1999 and a triple-barrelled gravity corer (Rigosha & Co.) in 2000. Immediately after retrieval, samples were taken from the core at depths where there were visible changes in sediment characteristics, such that all sediment types and interfaces between them were sampled. The sediment-water interface was sampled with a wide-bore se-

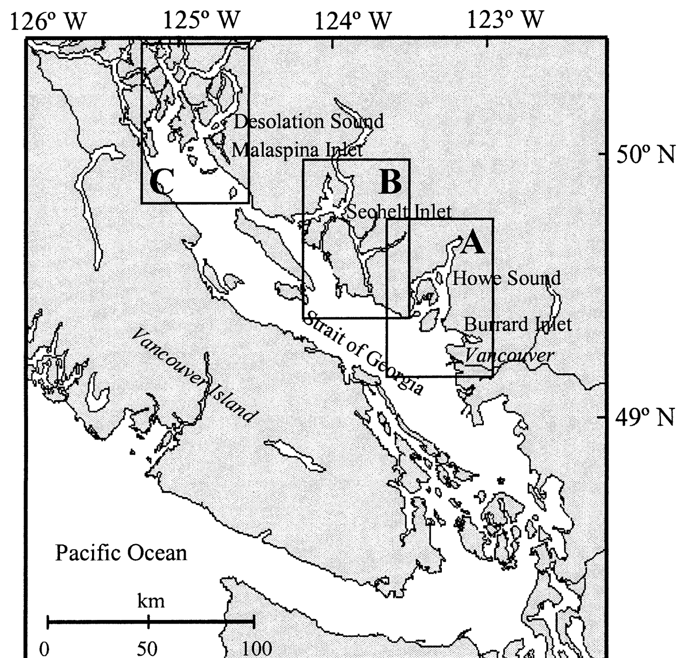

Fig. 1. Strait of Georgia, British Columbia, Canada, showing the three regions sampled during research cruises in 1998, 1999 and 2000. (A) Burrard Inlet/Howe Sound, (B) Sechelt Inlet, and (C) Malaspina Inlet/Desolation Sound.

rological pipette to minimize disruption to the sediment surface. Subsequent samples were obtained from deeper within the core by carefully pushing the core from the core barrel and slicing the sediment at the desired depth. Sediment was sampled from the center of the core, leaving 2 cm of sediment around the periphery to minimize contamination of the sample with sediment that had been smeared during coring.

In 1998, 10 cm^3 of sediment were extracted in 5 ml of phosphate-buffered saline (PBS) (Sambrook et al 1989). The samples were centrifuged at 3,000 $\times$ g for 50 min, to remove coarse sediment. The supernatants were stored at 4°C until analysis. This protocol was adjusted in 1999 and 2000 to increase the volume of extracted sediment and remove fine particulates from the sediment extract that were not sedimented by centrifugation. This revised protocol was to extract 20 cm^3 of sediment in 20 ml of PBS (1999) or 20 ml of 0.01 M sodium pyrophosphate (2000) (Maranger and Bird 1996; Danovaro and Serresi 2000). The samples were then centrifuged at 4,000 $\times$ g for 5 min at 4°C, to separate coarse sediment material from pore water. The supernatant was decanted and filtered through glass-fiber filters (nominal pore size = 1.2 μm) and polyvinylidine difluoride filters (0.45 μm pore size), and stored at 4°C prior to screening for lytic agents, which was conducted within 1 month of collection.

Screening for lytic activity and determining abundance of lytic agents—H. akashiwo strain 522 (Northeast Pacific Culture Collection), originally isolated from English Bay, Vancouver, Canada, was used to screen the sediment extract and determine the abundance of lytic agents. In all experiments, 5-ml cultures of *H. akashiwo* were grown in F/2-enriched seawater (Guillard 1975) (30 ‰) supplemented with 10 nM sodium selenite under continuous or 14 : 10 h light : dark cycle illumination of 260 μmol m^{-2} s^{-1} photosynthetically active radiation at 20°C. Growth of the cultures was estimated by directly measuring in vivo chlorophyll fluorescence (Turner Designs fluorometer) over time.

Pore-water samples were screened for the presence of lytic agents by addition of duplicate 500-μl aliquots of extracted pore water to 5-ml cultures and incubated under the conditions described above. Control cultures to which no sample was added were also maintained. Cultures were monitored daily for lysis until 10 d in stationary growth and were scored as positive for lytic activity if the fluorescence decreased by an order of magnitude relative to the controls. Lytic activity was confirmed by propagating all cultures. This was achieved by adding 1% (v/v) of each screened culture to an exponentially growing 5-ml culture and monitoring the cultures as before. The detection limit for the first assay can be calculated by assuming that a minimum of one virus is required to cause lysis of a culture and result in a positive score during screening. Because 2 $\times$ 500 μl aliquots

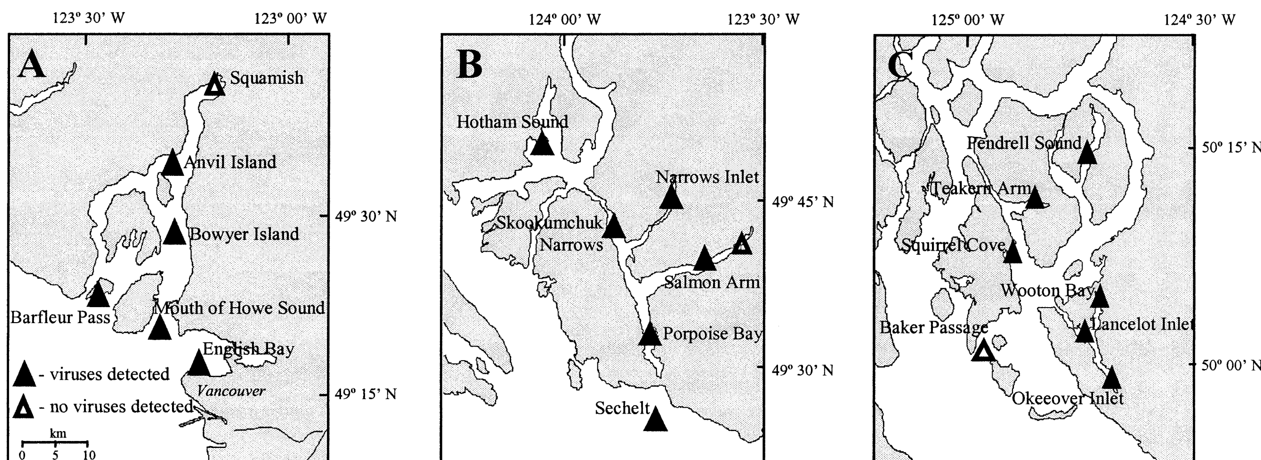

Fig. 2. Detailed maps of sampling sites in (A) Burrard Inlet/Howe Sound, (B) Sechelt Inlet, and (C) Malaspina Inlet/Desolation Sound, from sampling cruises in 1998, 1999, and 2000.

of sediment extract were initially screened and 1 ml of extract was derived from 0.5–1 cm^3 of sediment, the lower detection limits are 1–2 lytic agents cm^{-3} of sediment.

The abundance of lytic agents in samples that screened positive for lytic activity was determined with a most probable number (MPN) assay (Suttle and Chan 1995), also by use of the culture conditions described above. Tenfold dilutions of each extract were added to exponentially growing 5-ml cultures of *H. akashiwo,* with eight subsamples at each dilution. Growth in the cultures was monitored by in vivo fluorescence and compared with control cultures, to which nothing was added. Lytic activity was scored as described for the initial screening. All experimental cultures were propagated by addition of 1% (v/v) of each screened culture to an exponentially growing 5-ml culture and monitoring cell growth by in vivo fluorescence. The cultures were monitored as before. The number of cultures in which lysis occurred or did not occur was scored, and the concentration of lytic agents determined by a BASIC program (Hurley and Roscoe 1983).

Examination with transmission electron microscopy (TEM)—A selection of the lytic agents in 1999 was examined by TEM. An aliquot (50 μl) of culture lysate was added to 5 ml of exponentially growing culture. This was repeated a minimum of five times to dilute out nonreplicating viruses and other agents. The resulting lysates were filtered (0.2 μm) to remove cellular debris. Lytic agents were collected onto glow-discharged, carbon- and Formvar-coated 400-mesh electron microscope grids by centrifugation in a swinging bucket rotor (Beckman SW40) at 115,000 *g*, 20°C for 3 h (Suttle 1993). The grids were stained for 15 s with 1% phosphotungstic acid and viewed with a Zeiss 10C-TEM at an accelerating voltage of 80 kV. Sizes of particles were determined from the negatives by use of a scale loupe (calibrated with a 2160 waffle-grating replica).

Geographic distribution—Lytic agents infecting *H. akashiwo* were widely distributed in the sediments of the Strait of Georgia (Fig. 2). Of the 20 sites surveyed, cores from 17 sites contained detectable lytic agents in at least 1 yr. Within the Howe Sound/English Bay area, lytic agents were detected at five of six locations (Fig. 2A). The only location where they were not detected was near the mouth of the Squamish River, in an area of high sediment deposition. In both Sechelt Inlet (Fig. 2B) and Malaspina Inlet/Desolation Sound (Fig. 2C) regions, six of seven sites tested positive (Fig. 2B,C). Lytic agents were not detected at the head of Salmon Arm (Fig. 2B) or in Baker Passage (Fig. 2C). Lytic agents were present in sediments recovered from the shallowest (English Bay, 25 m) and deepest (Anvil Island, 285 m) depths examined (Fig. 2A). Lytic agents were recovered in sediments that were highly organic (Malaspina Inet, Hotham Sound, and Sechelt Inlet), as well as sediments that were very rich in clay (English Bay and Lancelot Inlet).

Distribution in sediment cores—Extracts from Malaspina Inlet were assayed to determine the concentration of lytic agents at different depths in the sediments. At all three sites, the abundance of lytic agents decreased with depth in the

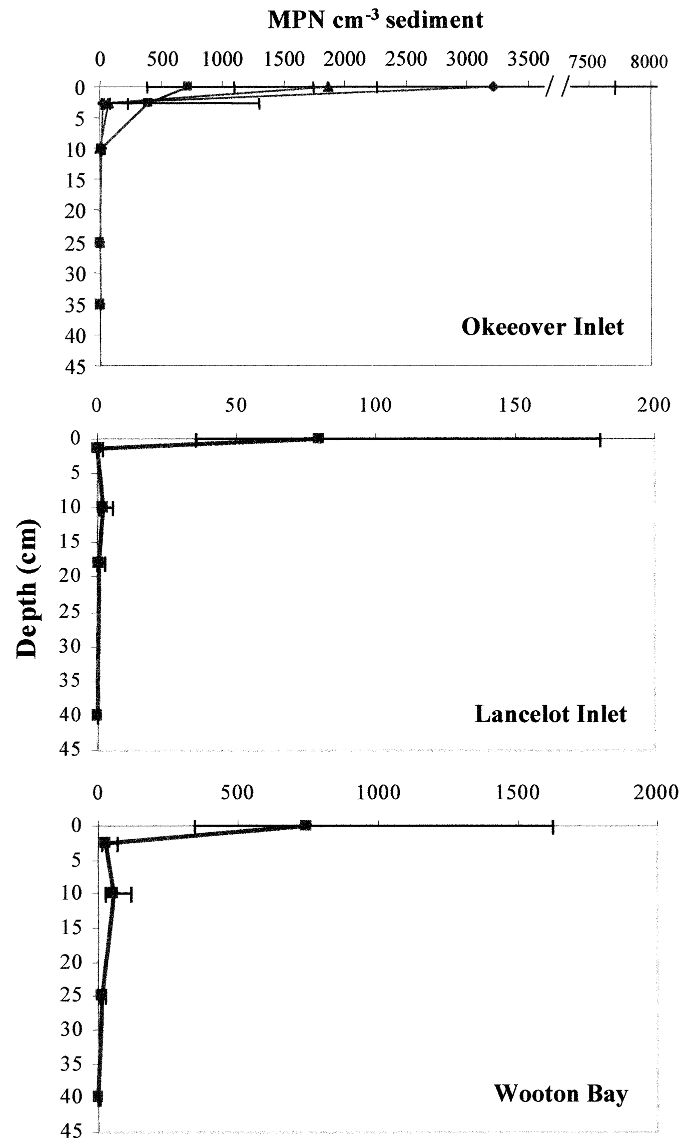

Fig. 3. Abundance of lytic agents throughout sediment cores retrieved from Malaspina Inlet in 1999. (A) Okeeover Inlet (triplicate cores), (B) Lancelot Inlet, and (C) Wooton Bay. (error bars, 95% confidence intervals.).

sediment (Fig. 3). Lytic agents were found as deep as 40 cm below the sediment-water interface but were near the detection limit. Consequently, there is a large error associated with these estimates of abundance. In some areas, lytic agents were detected in samples collected below depths in which lytic agents were undetectable (Table 1; Bowyer Island and Porpoise Bay). In others, agents were only found close to the sediment-water interface (Table 1; English Bay, Skookumchuk Narrows, and Okeeover Inlet) or at the sediment-water interface (Barfleur Pass, Pendrell Sound, Squirrel Cove, and Teakern Arm; data not shown).

The highest concentration of lytic agents was found at the sediment-water interface in Okeeover Inlet in 1999, with an average of 1,940 ($\pm$830 SE) agents cm^{-3} sediment from three cores (Fig. 3A). The highest abundance found in an

Table 1. Distribution of lytic agents in sediment cores collected in 1998, 1999, and 2000. + indicates that lytic agents were detected; − indicates that there were <1 lytic agent cm^{-3} of sediment. The station depth recorded below the site name is the average depth sampled at the location during the various years.

	Howe Sound/Burrard Inlet (A)						Sechelt Inlet Area (B)							Malaspina Inlet Area (C)					
	Bowyer Island (246 m)		Anvil Island (285 m)		English Bay (25–50 m)		Skookumchuk Narrows (186 m)			Salmon Arm (240 m)		Porpoise Bay (207 m)		Okeeover Inlet (34 m)			Wooton Bay (49 m)	Lancelot Inlet (56–207 m)	
Depth (cm)	1998	2000	1998	2000	1998	2000	1998	1999	2000	1998	1999	1998	2000	1998	1999	2000	1999	1998	1999
0	+	+	−	−	+	+	+	+	−	+	−	−	−	+	+	+	+	+	+
	−	−	+	−	+	−	+		−	+	−	+	−	+	+	+	+	+	+
	−		+		+	−	+	−	−	+	−		−	+				+	
10	+	−	+	−	−	−		−	−	+	−	+	−	+	+	+	+	+	+
					−		+			+									
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20		−		−		−								+		+		+	
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50								−											

individual core from this site was 3,210 (±95% confidence interval 1467–7629) agents cm^{-3} sediment. Overall, the highest concentrations of lytic agents were detected in sediment samples from Malaspina Inlet. Some sites were sampled in 1998, 1999, and 2000 to determine whether lytic agents could be consistently detected at specific sites and depths (Table 1). Because the extraction buffers were different in 1998/1999 and 2000 and concentrations of lytic agents were not determined for all 3 yr, absolute numbers cannot be compared. However, depth distributions within sites in Malaspina Inlet were consistent from year to year (Table 1), with the exceptions of samples from deep within the sediment from Okeeover Inlet, where samples from 1998 and 1999 tested positive and samples from 2000 tested negative. However, the MPN assays from 1999 showed that the concentration of lytic agents was near the detection limit for the assay (1.2 lytic agents cm^{-3} at 25 cm, <1 lytic agent cm^{-3} at 35 cm). The distributions of lytic agents in the Howe Sound/English Bay and Sechelt Inlet regions in 1998 and 1999/2000 were also variable (Table 1). However, the concentrations of lytic agents in these samples were also near the detection limits.

Reproducibility was also examined within the same site and time at one location. Three cores were collected from Okeeover Inlet in 1999, and sediment extracts were made from five discrete depths in all three cores. The extracts were assayed to determine the concentration of lytic agents at each depth in each core. The depth-distribution pattern was the same in all three cores, although the concentrations were slightly different (Fig. 3A).

Nature of the lytic agents—Six agents recovered from the 1999 sediment extracts were examined by use of TEM. These agents were isolated from different sites and different depths within the sediment (Okeeover Inlet, 0 and 10 cm; Wooton Bay, 0, 2.5, and 10 cm; and Mouth of Salmon Inlet, 0 cm), which indicates that they were the most abundant lytic agents in the sediments. Their infectivity could be propagated after passing lysate from an infected culture through a 0.2-μm filter. Each of the samples was dominated by virus-like particles (VLP) ~50 nm in diameter. The particles were similar in all samples examined. They were spherical and uniformly electron dense, with no obvious core or tail.

Discussion—Viruses rely on a host for reproduction; therefore, viruses that exclusively infect photosynthetic organisms will not be produced in sediments below the photic zone. Instead, their accumulation in sediments is likely mediated by other means such as sinking of infected cells or of particles with attached viruses. VLP have been reported in marine sediments (Steward et al. 1996; Drake et al. 1998; Danovaro-Serresi 2000) and lake sediments (Maranger and Bird 1994). The hosts these viruses infect, and therefore their origins, are not known, although a large proportion of these viruses likely infects bacteria in the sediments. The only study that reports finding viruses that infect photosynthetic organisms in sediments was conducted in the Gulf of Mexico (Suttle 2000). That study found infectious cyanophages throughout sediments collected at 47 m to a depth of 30 cm. On the basis of sedimentation data and Pb^{210} dating, it was estimated that these infectious cyanophages persisted in sediments for ~100 yr.

Lytic agents that infect *H. akashiwo* were widely distributed in sediments collected around the Strait of Georgia. They were associated with sediments in each of the three regions studied, which are areas where blooms of *H. akashiwo* have been documented (Taylor and Haigh 1993; Taylor et al. 1994). Two of the three sites where lytic agents were not detected were at the head of Salmon Inlet and the head of Howe Sound. This distribution may reflect the distribution

of historical blooms of *H. akashiwo,* as blooms tend to occur near the entrance to Sechelt Inlet (Taylor pers. comm.), and there is a strong outflow from the Squamish River at the head of Howe Sound. Sediments at the third site, Baker Passage, were composed of coarse sand and gravel, which suggests that this was an unfavorable depositional environment. The abundance of lytic agents detected in the sediments was around three orders of magnitude higher than that found in water column samples from the same region. The highest concentration of lytic agents detected in the water column was 18 lytic agents ml^{-1} at the Bowyer Island site in 1998, whereas the majority of water column samples contained ~0.001 lytic agents ml^{-1} (Chan et al. 1998). Further studies are required to determine the relationships between the concentration of viruses in the water column, depth of sediment surface, and depositional/preservational environment in the abundance of agents that accumulate at the sediment/water interface.

In many of the areas sampled, the concentration of lytic agents was near the detection limit at the sediment surface. It is possible that undetected viruses exist throughout these cores and in cores where no viruses were detected. It would be possible to increase the concentration of viruses in the extracted pore water by decreasing the volume of extraction buffer added to the sediments; however, it is not known whether this would affect elution efficiency. The detection of lytic agents is also dependent on their rate of decay. To estimate the decay rate in our samples, we performed the MPN assay on some samples from Malaspina Inlet 6 months after the initial MPN. During this time, the concentration of lytic agents decreased in one sample from 1,868 to 6 lytic agents cm^{-3} and from 71 lytic agents cm^{-3} to below detection limits in another. This suggests that decay is >99% in 6 months.

The observed rapid decay in infectivity indicates that viruses were likely more abundant than reported, because not all samples were assayed immediately after extraction. Clearly, the buffers used did not adequately preserve the infectivity of the viruses once they have been extracted from the sediments. Preservation in the cores may have been dependent on their attachment to particles, because attachment of viruses to particles has been shown to provide protection for viruses by increasing the stability of the viral capsid and preventing aggregate formation (reviewed in Gerba 1984). Preservation may also be dependent on pH, oxygen, and other chemical factors that change after collection and extraction of the sediments. The preservation of infectious viruses in sediments has also been demonstrated for cyanophages: the turnover rate of cyanophages in the water column is ~4 d (Suttle and Chan 1994), whereas cyanophages persist in marine sediments for up to 100 yr (Suttle 2000).

Because of extraction efficiency, loss during filtration, and decay of infectivity, the concentrations of agents reported in this study are minimum estimates. As well, only one strain of *H. akashiwo* was used to screen for the presence of agents, and strain-specific host ranges have been demonstrated for viruses known to infect *H. akashiwo*: HaVs (Nagasaki et al. 1999; Tarutani et al. 2000) and HaNIV (Lawrence et al. 2001). Consequently, there are undoubtedly agents that only infect other strains of *H. akashiwo* that were undetected, and the actual concentration of infectious agents in the sediments is therefore much higher.

At sites where the concentration of viruses could be determined throughout the core, such as in Malaspina Inlet, their abundance decreased with depth into the sediment. This decrease likely reflects a loss of infectivity of viruses over time (depth). If decay rates were known, the decrease in abundance could be used to estimate deposition rates. It is also possible that the decrease reflects a lower rate of viral deposition in the past and may indicate that the frequency or intensity of blooms was less. It is also possible that factors such as burst size or shifts in the viral community/host community alter the concentration of detectable lytic agents in the sediments and therefore contribute to the observed patterns.

The concentration of lytic agents in samples near Bowyer Island (Howe Sound) and Porpoise Bay (Sechelt Inlet) (Table 1) were ~2 viruses cm^{-3}, which is near the detection limit. Therefore, the sporadic detection with sediment depth may be the result of being close to the detection limit rather than evidence of higher virus concentrations deeper in the sediments. Fine-scale depth distributions may be present in the samples; however, our sampling method limits our detection to a resolution of 1 cm.

The number of positive samples in 1998 was higher than in other years, possibly because the sediment extracts prepared in 1998 were two times more concentrated in comparison to the 1999 and 2000 samples. Also, the 1999 and 2000 samples were filtered prior to assay and likely resulted in the loss of some lytic agents. Results from repeat sampling at the site in Okeeover Inlet in 1999 were similar, which indicates that extraction methods within a year were reproducible.

All of the samples examined with TEM contained ~50-nm-diameter VLP that were 0.2-μm filterable, which suggests that most of the agents detected in this study are likely viruses. These are much smaller than viruses that belong to the family Phycodnaviridae, which infect other algae (>100 nm) (Van Etten and Ghabrial 1991) and HaV, a virus that infects *H. akashiwo* (200 nm) (Nagasaki and Yamaguchi 1997). It is, however, similar to the size of HaNIV, (Lawrence et al. 2001) and another virus that has been isolated from water samples collected in the Strait of Georgia (Tai et al. unpubl. data), both of which infect *H. akashiwo.*

The presence of viral-size lytic agents that infect *H. akashiwo* in sediments provides strong evidence that viral infection of *H. akashiwo* occurs in overlying waters and has occurred historically. We need to determine whether the agents accumulated in sediments could exert further control on future blooms if the viruses are resuspended and entrained into the overlying water and to determine the ecological relevance of the presence of viruses that infect *H. akashiwo* in marine sediments. Regardless, the evidence presented here demonstrates that sediments are a reservoir of viruses and that viruses play a role in the mortality of *H. akashiwo* populations in the Strait of Georgia.

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References

CHAN, A. M., M. BAUR, W. MAH, AND C. A. SUTTLE. 1998. Preliminary characterization of a lytic pathogen for *Heterosigma akashiwo* and its distribution and abundance in coastal waters of British Columbia, Canada. Eighth International Symposium on Microbial Ecology, Halifax, Nova Scotia.

DANOVARO, R., AND M. SERRESI. 2000. Viral density and virus-to-bacterium ratio in deep-sea sediments of the Eastern Mediterranean. Appl. Environ. Microbiology **66:** 1857–1861.

DRAKE, L. A., K. H. CHOI, A. G. E. HASKELL, AND F. C. DOBBS. 1998. Vertical profiles of virus-like particles and bacteria in the water column and sediments of Chesapeake Bay, USA. Aquat. Microb. Ecol. **16:** 17–25.

GERBA, C. P. 1984. Applied and theoretical aspects of virus adsorption to surfaces, p. 131–168. *In* A. I. Laskin [ed.], Advances in applied microbiology. Vol. 30. Academic.

GONZALEZ, J. M., AND C. A. SUTTLE. 1993. Grazing by marine nanoflagellates on viruses and virus-sized particle: Ingestion and digestion. Mar. Ecol. Prog. Ser. **94:** 1–10.

GUILLARD, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. *In* W. L. Smith, and M. H. Chanley [eds.], Culture of marine invertebrate animals. Plenum.

HONJO, T. 1992. Harmful red tides of *Heterosigma akashiwo.* NOAA Tech. Rep. **111:** 27–32.

———. 1993. Overview on bloom dynamics and physiological ecology of *Heterosigma akashiwo,* p. 33–41. *In* T. J. Smayda and Y. Shimizu [eds.], Toxic phytoplankton blooms in the sea. Elsevier.

HURLEY, M. A., AND M. E. ROSCOE. 1983. Automated statistical analysis of microbial enumeration by dilution series. J. Appl. Bacteriol. **55:** 159–164.

LAWRENCE, J. E., A. M. CHAN, AND C. A. SUTTLE. 2001. A novel virus (HaNIV) causes lysis of the toxic bloom-forming alga, *Heterosigma akashiwo* (Raphidophyceae). J. Phycol. **37:** 1–7.

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MARANGER, R., AND D. F. BIRD. 1996. High concentrations of viruses in the sediments of Lac Gilbert, Quebec. Microb. Ecol. **31:** 141–151.

NAGASAKI, K., M. ANDO, S. ITAKURA, I. IMAI, AND Y. ISHIDA. 1994. Viral mortality in the final stage of *Heterosigma akashiwo* (Raphidophyceae) red tide. J. Plankton Res. **16:** 1595–1599.

———, K. TARUNTANI, AND M. YAMAGUCHI. 1999. Cluster analysis on algicidal activity of HaV clones and virus sensitivity of *Heterosigma akashiwo.* J. Plankton Res. **21:** 2219–2226.

———, AND M. YAMAGUCHI. 1997. Isolation of a virus infectious to the harmful bloom causing microalga *Heterosigma akashiwo* (Raphidophyceae). Aquat. Microb. Ecol. **13:** 135–140.

NOBLE, R. T., AND J. A. FUHRMAN. 1997. Virus decay and its causes in coastal waters. Appl, Environ. Microbiol. **63:** 77–83.

PEDERSEN, T. F., S. J. MALCOLM, AND E. R. SCHOLKOVITZ. 1985. A lightweight gravity corer for undisturbed sampling of soft sediments. Can. J. Earth Sci. **22:** 133–135.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor.

STEWARD, G. F., D. C. SMITH, AND F. AZAM. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Seas. Mar. Ecol. Prog. Ser. **131:** 287–300.

SUTTLE, C. A. 2000. Cyanophages and their role in the ecology of cyanobacteria, p. 563–589. *In* B. A. Whitton and M. Potts [eds.], The ecology of cyanobacteria. Kluwer Academic.

———. 1993. Enumeration and isolation of viruses, p. 121–133. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Aquatic microbial ecology. Lewis.

———, AND A. M. CHAN. 1994. Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. Appl. Environ. Microbiol. **60:** 3167–3174.

———, AND ———. 1995. Viruses infecting the marine Prymnesiophyte *Chrysochromulina* spp.: Isolation, preliminary characterization and natural abundance. Mar. Ecol. Prog. Ser. **118:** 275–282.

———, AND F. CHEN. 1992. Mechanisms and rates of decay of marine viruses in seawater. Appl. Environ. Microbiol. **58:** 3721–3729.

TARUTANI, K., K. NAGASAKI, AND M. YAMAGUCHI. 2000. Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton *Heterosigma akashiwo.* App. Environ. Microbiol. **66:** 4916–4920.

TAYLOR, F. J. R. 1993. Current problems with harmful phytoplankton blooms in British Columbia waters, p. 699–703. *In* T. J. Smayda and Y. Shimizu [eds.], Toxic phytoplankton blooms in the sea. Elsevier.

———, AND R. HAIGH. 1993. The ecology of fish-killing blooms of the chloromonad flagellate *Heterosigma* in the Strait of Georgia and adjacent waters, p. 705–710. *In* T. J. Smayda and Y. Shimizu [eds.], Toxic phytoplankton blooms in the sea. Elsevier.

———, ———, AND T. F. SUTHERLAND. 1994. Phytoplankton ecology of Sechelt Inlet, a fjord system on the British Columbia coast. II. Potentially harmful species. Mar. Ecol. Prog. Ser. **103:** 151–164.

VAN ETTEN, J. L., AND S. A. GHABRIAL. 1991. Phycodnaviridae, p. 137–139. *In* R. I. B. Francki, C. M. Fauget, D. L. Knudson, and F. Brown [eds.], Classification and nomenclature of viruses. Suppl. 2. Springer-Verlag.

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