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Effects of temperature and light on stability of microalgal viruses, HaV, HcV and HcRNAV

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Abstract: Viruses infectious to the fish-killing raphidophyte *Heterosigma akashiwo* (Hada) Hada (*Heterosigma akashiwo* Virus (HaV)) and the bivalve-killing dinophyte *Heterocapsa circularisquama* Horiguchi (*Heterocapsa circularisquama* Virus (HcV) and *Heterocapsa circularisquama* RNA Virus (HcRNAV)) have recently been isolated and characterized. The discovery of these microalgal viruses allows for their assessment as potential microbial agents for controlling harmful algal blooms (HABs). To assess the possibility of their practical use as anti-HAB agents, however, it is necessary to estimate how stable their infectivity is both *in situ* and *in vitro*. In the present study, we measured the effects of light and temperature on the stability of these viruses. All three viruses were susceptible to cool white fluorescent illumination. Although significant loss of infectivity at 4°C in the dark for one week was detected in HaV and HcV, the infectivity of HcRNAV was stable for more than one year under the same storage conditions. Furthermore, we succeeded in developing cryopreservation methods for HcV and HcRNAV, which would be useful for their long-term storage.

Key words: algal virus, stability, cryopreservation

Introduction

Heterosigma akashiwo (Hada) Hada (Raphidophyceae) is a harmful bloom-forming microalgae that often causes mortality of caged fishes such as salmon and yellowtail, especially in coastal waters of the subtropical, temperate, and subarctic areas of the world (Honjo 1993; Smayda 1998). *Heterocapsa circularisquama* Horiguchi (Dinophyceae) is a bloom-forming dinoflagellate that specifically kills bivalves and is distributed in the central and western part of Japan (Horiguchi 1995; Matsuyama 1999). Damage to fisheries caused by these harmful bloom-forming microalgae has been enormous in Japan. Various ideas including physical, chemical, and biological methods for eliminating HABs have been proposed and studied, e.g. water circulation, sonic system, porous clay, copper-sulfate, algicidal bacteria (Shirota 1992; Imai 2000; Ishida & Sugawara 1994; Hiroishi et al. 2002). A practical method for controlling HABs, however, has not been established so far due to the problems of cost, scale, effect, and safety.

Recently, studies on algal viruses have led to the idea of controlling HABs by utilizing their algicidal activity. In the last decade, a *H. akashiwo* virus (HaV) harboring a double-stranded DNA (dsDNA) genome (Nagasaki & Yamaguchi 1997; Nagasaki et al. 1999), a *H. circularisquama* virus (HcV) harboring a dsDNA genome (Tarutani et al. 2001; Nagasaki et al. 2003), and a *H. circularisquama* RNA virus (HcRNAV) harboring a single stranded RNA genome (Tomaru et al. 2004a) were isolated and characterized. Each of these viruses have high replication rates and specific infection specificities, and can be produced at a relatively low cost without using specialized equipments (Nagasaki 1998; Nagasaki et al. 1999; Tarutani et al. 2001; Tomaru et al. 2004a); thus, fulfilling the essential requirements as microbiological agents for controlling HABs.

To assess the possibility of their practical use for controlling HABs, above all, it is important to understand the ecological properties of the viruses. Recently, evidence showing intimate relationships between these viruses and their host microalgae has accumulated (Nagasaki et al. 1994, 2004; Tarutani et al. 2000; Tomaru et al. 2004b). In the case of HaV, a specific increase in the proportion of virus-har-

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boring cells within the host population and in the abundance of viruses released to the environment occurred in the termination stage of *H. akashiwo* blooms (Nagasaki et al. 1994; Tarutani et al. 2000; Tomaru et al. 2004b). HcV infection of *H. circularisquama* was also detected in natural bloom samples (Tomaru & Nagasaki 2004). As for HcRNAV, specific recruitment of viruses both to the water column and sediments was found during the duration of a *H. circularisquama* bloom (Nagasaki et al. 2004).

In addition, the development of techniques for long-term storage of viruses is also necessary for their practical use as biological agents. Regarding HaV, a cryopreservation technique using dimethyl sulfoxide (DMSO) was previously established and it is appropriate for preserving small volumes of virus suspension (Nagasaki & Yamaguchi 1999). Information on preservation techniques for microalgal viruses, however, is still limited, and techniques that are applicable for large-scale storage are required. In the present research, to develop preservation techniques for algal viruses, we examined the effects of water temperature and light on the stability of HaV, HcV and HcRNAV, and also tried a cryopreservation technique on HcV and HcRNAV.

Materials and Methods

Hosts and viruses

In the present study, we used three algal viruses, HaV, HcV and HcRNAV. Each virus culture was maintained with its suitable host as shown in Table 1. Algal cultures were grown in modified SWM3 medium (Chen et al. 1969; Itoh & Imai 1987) enriched with 2 nM Na_2SeO_3 (Imai et al. 1996) under a 12 hL : 12 hD cycle of 130 to 150 μmol photons $m^{-2}s^{-1}$ with cool white fluorescent illumination (FL40S D EDL D65, Toshiba) at 20°C. The titer of infectious viruses was measured by means of the extinction dilution method (=MPN method: Suttle 1993; Imai et al. 1998a, b; Nagasaki et al. 2004).

Light and temperature

Viruses were inoculated to their suitable host cultures in the exponentially growing phase, and incubated for 48 to 72 h under the conditions mentioned above; then, the resultant algal lysates were centrifuged at 4,490×*g*, 4°C, for 5 min. As for the large dsDNA viruses (HaV and HcV listed in Table 1), the supernatants were used in the following experiments without filtration. Since filtration of the dsDNA viral suspensions through a 0.2 μm nominal pore-size membrane filter reduces the titration of the viruses, we did not filter the lysates of HaV and HcV. In the case of HcRNAV, the supernatants filtered through a 0.2 μm nominal pore-size membrane filter (Nuclepore) were used as viral suspensions. Aliquots (3 ml) of each viral suspension were poured into test tubes equipped with a sealable cap. The viral suspensions thus prepared were incubated under light conditions (12 hL : 12 hD cycle of 130 to 150 μmol photons $m^{-2}s^{-1}$ with cool white fluorescent illumination (FL40S D EDL D65, Toshiba)) at 20°C, or under dark conditions at 20°C, 10°C, or 4°C.

Cryopreservation

For the cryopreservation tests of HcV and HcRNAV, crude lysates of HcV08, HcRNAV34, and HcRNAV109 (see Table 1) were used. Fifty ml of a HcV-infected algal lysate at 72 h post infection was added with 10% w/v of polyethylene glycol (PEG) 6000 (Wako) and incubated for 30 min in the dark at 4°C. The lysate was centrifuged at 11,500×*g*, 4°C, for 10 min, and the pellet was resuspended in 1.15 ml of the supernatant (final volume); then, the suspension was mixed with an equal volume of the cryoprotectant Cell-Banker-2 (Nippon Zenyaku Kogyo) and preserved at −80°C or in liquid nitrogen. Also to verify the preservability of HcRNAV in host cells (not released from host cells), 50 ml of a *H. circularisquama* culture 26 h post infection was centrifuged at 4,490×*g*, 4°C, for 10 min, and the cells were concentrated to 1 ml; then, the cell suspension was mixed with an equal volume of Cell-Banker-2 (Nippon Zenyaku Kogyo) and preserved in liquid nitrogen. After 7 d (HcV and HcRNAV) and 30 d (HcV) of storage at each temperature, the samples were thawed at room temperature. Titration was conducted by means of the extinction dilution method (Suttle 1993; Imai et al. 1998a, b; Nagasaki et al. 2004).

Table 1. Viral strains and host strains used for virus replications.

Viral strain	Host species	Host strain	Reference
HaV01	*Heterosigma akashiwo*	H93616	Nagasaki & Yamaguchi 1997
HaV53	*Heterosigma akashiwo*	H93616	Tarutani et al. 2000
HcV01	*Heterocapsa circularisquama*	HU9433-P	Nagasaki et al. 2003
HcV03	*Heterocapsa circularisquama*	HU9433-P	Tarutani et al. 2001; Nagasaki et al. 2003
HcV05	*Heterocapsa circularisquama*	HU9433-P	Nagasaki et al. 2003
HcV08	*Heterocapsa circularisquama*	HU9433-P	Nagasaki et al. 2003
HcV10	*Heterocapsa circularisquama*	HU9433-P	Nagasaki et al. 2003
HcRNAV34	*Heterocapsa circularisquama*	HU9433-P	Tomaru et al. 2004a
HcRNAV109	*Heterocapsa circularisquama*	HCLG-1	Tomaru et al. 2004a

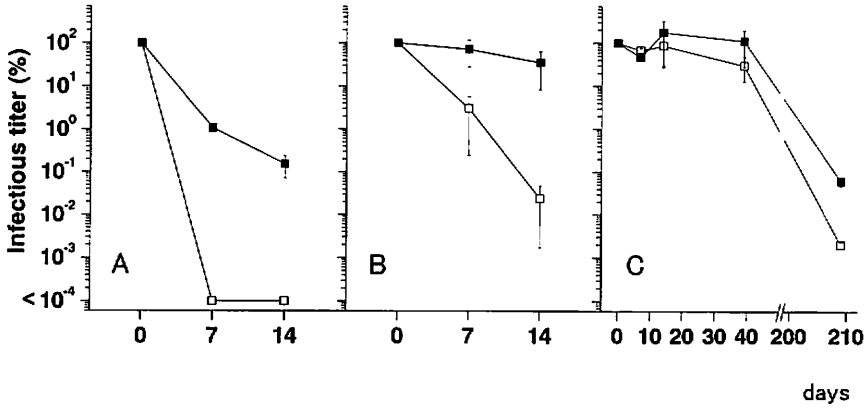

Fig. 1. Changes in relative infectious titer of viruses under light conditions (12 hL : 12 hD cycle: open squares) and dark conditions (closed squares); (A) HaV (n=2 (HaV01, 53)), (B) HcV (n=5 (HcV01, 03, 05, 08, 10)), (C) HcRNAV (n=2 (HcRNAV34, 109)) at 20°C. Error bars indicate maximum and minimum value of each plot (n=2) or standard deviation (n>2).

Results and Discussion

Effects of light

The titer of HaV suspension stored under the light conditions became lower than the detection limit (<3.01 infectious titer ml^{-1}) within 7 d, and even under the dark conditions >99% of the titer was lost within 14 d (Fig. 1A). The titer of HcV also showed a considerable decrease under the light conditions, but the decrease in titer under the dark conditions at 20°C was relatively moderate, where as high as 65% of the initial titer was maintained for 14 d (Fig. 1B). These results suggest that cool white fluorescent illumination causes a significant decay of HaV and HcV. In contrast, the decrease in titers of HcRNAV was more gradual than those of HaV and HcV; the initial titers were maintained for 39 d but a decrease of 3–4 orders of magnitude was detected 209 d after the onset of the experiment (Fig. 1C), when the titer under the light conditions was lower than under the dark conditions by one order of magnitude. These results suggest that the light conditions used in this study have little effect on HcRNAV infectivity for the first month. Longer preservation under the light conditions, however, could reduce the infectivity of HcRNAV. Solar UV light reacting with DNA, damaging bases, breaking strands, and cross-linking DNA is considered one of the major causative agents of virus decay in marine environments (Suttle & Chen 1992; Noble & Fuhrman 1997), and 434 nm blue light is also able to damage DNA (Peak et al. 1985). Considering that the cool white fluorescent illumination used in this study also contains some light in the ultraviolet range, a similar mechanism of viral decay is possible.

Effect of temperature

Even under the dark conditions, the titer of HaV at all temperatures rapidly decreased to <1% within 14 d, and became lower than the detection limit after 120 d of incubation (Fig. 2A). Thus, HaV is considered to be highly sensitive to temperature, and storage at temperatures between 4°C and 20°C appears difficult. Also, in the case of HcV, its titer at 4°C rapidly decreased to <1% (within 7 d), and reached 0.0002% after 28 d of incubation (Fig. 2B). In contrast, the ratio of decrease in titer was more moderate at 10°C and 20°C; especially at 10°C, 0.002% of the initial titer was maintained even after 120 d of incubation. Among the 3 virus species tested, HcRNAV was the most stable. The titer of HcRNAV showed a gradual decrease at 20°C, but the initial titer was almost completely maintained both at 10°C and 4°C for more than a year (Fig. 2C).

Although the intimate relationship between loss of virus infectivity and temperature effects is not fully understood, in general viruses are relatively more stable at lower than at higher temperatures (Kondo 1989). For example, within algal viruses, a dsDNA virus infecting *Chlorella*-like green alga (*Chlorovirus*) can be stored at 20–25°C for at least one month (K. Nishida personal communication), at 4°C for at least 1 year without detectable loss of infectivity, and its infectivity is rapidly lost above 45°C (Van Etten et al. 1991). In the case of MpRNAV, a dsRNA virus infecting *Micromonas pusilla* (Butcher) Manton et Parke (Prasinophyceae), its infectivity is also stable for more than a year in the dark at 4°C, but is lost above 40°C (Brussaard et al. 2004). These data indicate that HaV and HcV are more sensitive to temperature effects compared to other algal viruses even at lower temperature conditions.

Cryopreservation

Fifty ml of HcV suspension (3.85×10^4 infectious titer ml^{-1}) was concentrated by using PEG, and the resultant titer of the concentrate (1.15 ml) was 3.85×10^5 infectious titer ml^{-1}; thus, 23% of the initial infectious titer was recovered. The titer of HcV concentrate decreased to 0.03–0.2% and 1.1–1.2% when preserved at −80°C and −196°C (in liquid nitrogen), respectively (Table 2). Nagasaki & Yamaguchi (1999) reported that loss of HaV infectivity occurred during the initial stage of cryopreserva-

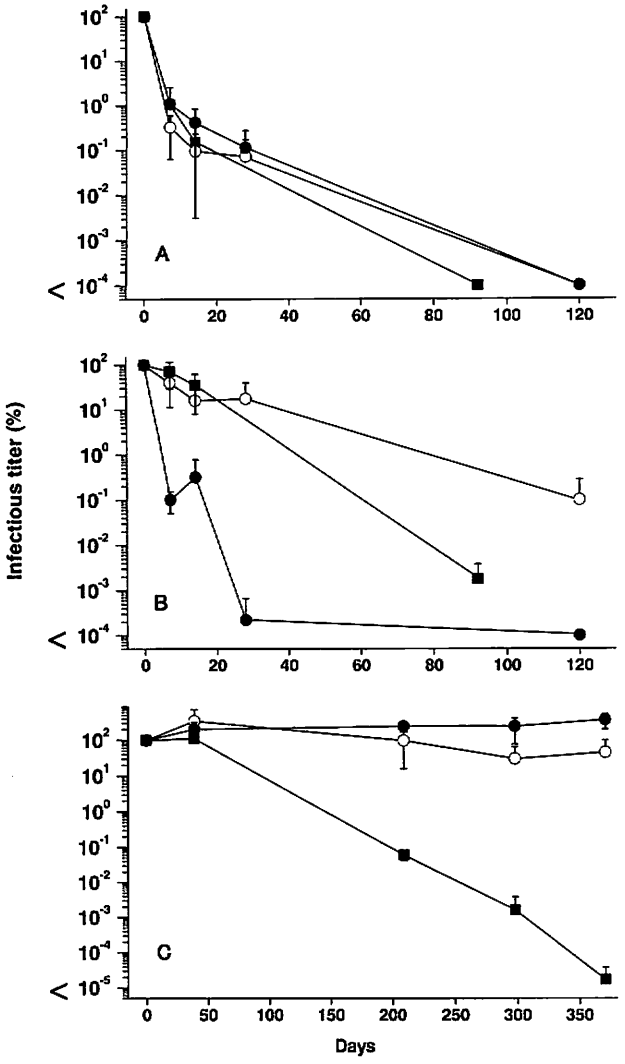

Fig. 2. Changes in relative infectious titer of viruses under dark conditions at temperatures of 20°C (closed squares), 10°C (open circles) and 4°C (closed circles); (A) HaV (n=2 (HaV01, 53)), (B) HcV (n=4 (HcV03, 05, 08, 10) at 10°C and 4°C, n=5 (HcV01, 03, 05, 08, 10) at 20°C), (C) HcRNAV (n=2 (HcRNAV34, 109)). Error bars indicate maximum and minimum value of each plot (n=2) or standard deviation (n>2).

tion using cryoprotectants (DMSO), but no marked decrease occurred after that. Although the viruses after cryopreservation should be stable under very low temperature conditions, considering the low efficiency of this preservation method, pre-concentration of HcV particles prior to freezing appears necessary.

In contrast, the titer of HcRNAV34 and HcRNAV109 after 7 d of cryopreservation in liquid nitrogen increased to 200% and 610%, respectively (Table 2). In this method, HcRNAV-infected host cells of 26 h post infection were cryopreserved. Since most *H. circularisquama* cells at 24 h post HcRNAV-infection harbored crystalline arrays and randomly aggregated clusters of the viruses within the cytoplasm (Tomaru et al. 2004a), the apparent increase in HcRNAV titer following cryopreservation is presumably caused by collapse of virus harboring cells and diffusion of aggregated viruses.

Table 2. Effects of cryopreservation on HcV and HcRNAV infectious titer. Each viral suspension was cryopreserved by adding 50% of cryoprotectant Cell-Banker-2 (Nippon Zenyaku Kogyo).

Strain	Cryopreservation condition	Infectious titer ml^{-1}		
		0 d	7 d	30 d
HcV08	−80°C	3.85×10^{5}	1.16×10^{2}	6.02×10^{2}
HcV08	Liquid nitrogen	3.85×10^{5}	4.52×10^{3}	4.16×10^{3}
HcRNAV34	Liquid nitrogen	3.85×10^{7}	7.70×10^{7}	—
HcRNAV109	Liquid nitrogen	1.90×10^{7}	1.16×10^{8}	—

Application for virus storage

The decay rates of HaV and HcV were moderate under the dark conditions. In terms of the applicability of virus preservation, however, it is necessary to assess factors affecting the stability of these viruses other than just temperature and light conditions. Possible causative agents of virus decay are solar radiation, presence of particles larger than 0.2 μm, and dissolved organic matter, including extracellular nucleases and proteases (Suttle & Chen 1992; Noble & Fuhrman 1997). More intensive purification of virus particles and/or inactivation of dissolved enzymes may lead to longer and more stable storage of HaV and HcV. On the other hand, HcRNAV was more stable under various experimental conditions than HaV or HcV. HcRNAV was well preserved at 4–10°C in the dark for at least a year with no significant loss of titer, offering a simple method of large-scale storage for practical applications. Although HcRNAV suspensions used in the present study were passed through a 0.2 μm nominal pore-size membrane filter (see materials and methods), the relationship between the higher stability of HcRNAV relative to HaV and HcV and the filtration treatment was unclear in this experiment.

Frequent transfer of the virus lysate to an actively growing host culture is often required to maintain virus infectivity. However, the operation not only requires much time and labor but is accompanied with the risks of unexpected genetic change of the virus culture, contamination by bacteria, or mislabeling of the virus strains. In general, cryopreservation is more reliable for long-term virus storage and HaV has been shown to be cryopreservable with DMSO in liquid nitrogen (Nagasaki & Yamaguchi 1999). Although the cryopreservation method using DMSO is not suitable for HcV (data not shown), results of the present study showed that HcV and HcRNAV were preservable in liquid nitrogen by using cryoprotectants (Cell-Banker-2). In the authors' laboratories, HcV and HcRNAV have been kept infectious for more than 2 yrs and 1 yr, respectively, using this technique.

Ecological implications for HaV and HcV

In spite of the authors' continued efforts to isolate viruses infecting *H. akashiwo* or *H. circularisquama* from natural environments, those harboring dsDNA (HaV or HcV) have been isolated (both from water column and sediments) only during their hosts' blooming periods (Tarutani et al. 2000, 2001; Tomaru et al. 2004a, b). Considering the fragility of these dsDNA viruses, it is probable that in the natural environment they lose their infectivity rapidly. There are several possible explanations for the mechanism of how these viruses keep their infectivity to allow re-appearance annually *in situ*; 1) the viruses are preserved in the water column or sediments at extremely small abundances that cannot be detected by the MPN method, 2) some virus particles are kept stable by adsorbing onto some substances (presumably in sediments) (Bitton & Mitchell 1974; Kapuscinski & Mitchell 1980), 3) some virus particles are lysogenic in their hosts' dormant cells (in sediments), 4) these viruses have other host organisms but appear at undetectable abundances.

Ecological implications for HcRNAV

Nagasaki et al. (2004) reported the dynamics of HcRNAV in Ago Bay, where the appearance of HcRNAV at $\sim 10^3$ infectious titer per unit of seawater (ml) or wet sediment (g) was observed during a *H. circularisquama* bloom in Ago Bay, Japan, 2001. Then, although viruses in the water column decreased to below the detection limit after 36 days following the host's bloom, those in the sediment exhibited a more gradual decrease in abundance (Nagasaki et al. 2004). Assuming that HcRNAV loses infectivity when exposed to light, though it is less sensitive compared to the dsDNA viruses examined in the present study, and also assuming that viruses suspended in seawater are more vulnerable to the effects of solar light (Noble & Fuhrman 1997; Suttle & Chen 1992), their decay due to solar radiation might be one of the causes of the rapid disappearance of viruses in the water column, as well as that due to hydrographic diffusion. In some RNA viruses, inactivation by UV irradiation has been reported (Thurston-Enriquez et al. 2003; Butkus et al. 2004). Hence, viruses buried in sediments are considered to be better protected from solar light. According to the field data given by Nagasaki et al. (2004), the titer of viruses supplied to the sediment during the host's bloom showed a decrease of 2 orders of magnitude within one month following bloom termination with a more gradual decrease in the following two months. Our speculation is that the decrease in viral titer in sediments within the first one month was largely due to hydrographic diffusion, and the titer measured subsequent to the first decrease might be of viruses adsorbing to sediment particles more tightly. In the laboratory experiments, even under dark conditions, higher temperature (20°C) led to considerable decay of viruses. Considering that the temperatures of the overlying waters in Ago Bay range from 9–26°C through the course of a year (N. Hata personal communication), a temperature effect might be one of the causes of the subsequent gradual decrease in viral titer of sediments.

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Literature Cited

Bitton, G. & R. Mitchell 1974. Effect of colloids on the survival of bacteriophages in seawater. *Water Res.* **8**: 227–229.

Brussaard, C. P. D., A. A. M. Noordeloos, R. A. Sandaa, M. Heldal & G. Bratbak 2004. Discovery of a dsRNA virus infecting the marine photosynthetic protist *Micromonas pusilla*. *Virology* **319**: 280–291.

Butkus, M. A., P. L. Michael, J. A. Starke, K. Moon & M. Talbot 2004. Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection. *Appl. Environ. Microbiol.* **70**: 2848–2853.

Chen, L. C. M., T. Edelstein & J. McLachlan 1969. *Bonnemaisonia hamifera* Hariot in nature and in culture. *J. Phycol.* **5**: 211–220.

Hiroishi, S., I. Imai & T. Ishimaru 2002. *Prevention and Extermination Strategies Harmful Algal Blooms*. Kouseishakouseikaku, Tokyo, 150 pp. (in Japanese)

Honjo, T. 1993. Overview on bloom dynamics and physical ecology of *Heterosigma akashiwo*, p. 33–41. In *Toxic Phytoplankton Blooms in the Sea* (eds. Smayda, T. J. & Y. Shimizu). Elsevier, Amsterdam.

Horiguchi, T. 1995. *Heterocapsa circularisquama* sp. nov. (Peridiniales, Dinophyceae), a new marine dinoflagellate causing mass mortality of bivalves in Japan. *Phycol. Res.* **43**: 129–136.

Imai, I. 2000. Relationship between marine phytoplankton and its algicidal bacteria. *Gekkan Kaiyo* **21**: 169–177. (in Japanese)

Imai, I., S. Itakura, Y. Matsuyama & M. Yamaguchi 1996. Selenium requirement for growth of a novel red tide flagellate *Chattonella verruculosa* (Raphidophyceae) in culture. *Fisheries Sci.* **62**: 834–835.

Imai, I., M. C. Kim, K. Nagasaki, S. Itakura & Y. Ishida 1998a. Detection and enumeration of microorganisms that are lethal to harmful phytoplankton in coastal waters. *Plankton Biol. Ecol.* **45**: 19–29.

Imai, I., M. C. Kim, K. Nagasaki, S. Itakura & Y. Ishida 1998b. Relationships between dynamics of red tide-causing raphidophycean flagellates and algicidal micro-organisms in the coastal sea of Japan. *Phycol. Res.* **46**: 139–146.

Ishida, Y. & Y. Sugawara 1994. *Prevention and Control of Red*

Tide Microalgae by Microorganisms. Kouseishakouseikaku, Tokyo, 126 pp. (in Japanese)

Itoh, K. & I. Imai 1987. Raphidophyceae, p. 122–130. In *A Guide for Studies of Red Tide Organisms* (ed. Japan Fisheries Resource Conservation Association). Shuwa, Tokyo. (in Japanese)

Kapuscinski, R. D. & R. Mitchell 1980. Processes controlling virus inactivation in coastal waters. *Water Res.* **14**: 363–371.

Kondo, A. 1989. Preservation method, p. 48–60. In *Virus Experiment* (ed. National Institute of Health of Japan). Maruzen, Tokyo. (in Japanese)

Lawrence, J. E., A. M. Chan & C. A. Suttle 2001. A novel virus (HaNIV) causes lysis of the toxic bloom-forming alga *Heterosigma akashiwo* (Raphidophyceae). *J. Phycol.* **37**: 216–222.

Matsuyama, Y. 1999. Harmful effect of dinoflagellate *Heterocapsa circularisquama* on shellfish aquaculture in Japan. *Jpn. Agric. Res. Q.* **33**: 283–293.

Nagasaki, K. 1998. Possible use of algicidal viruses as microbiological agents against harmful algal blooms. *Microb. Environ.* **13**: 109–113. (in Japanese with English abstract)

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

Nagasaki, K. & M. Yamaguchi 1999. Cryopreservation of a virus (HaV) infecting a harmful bloom causing microalga, *Heterosigma akashiwo* (Raphidophyceae). *Fisheries Sci.* **65**: 319–320.

Nagasaki, K., M. Ando, S. Itakura, I. Imai & Y. Ishida 1994. Viral mortality in the final stage of *Heterosigma akashiwo* (Raphidophyceae) red tide. *J. Plankton Res.* **16**: 1595–1599.

Nagasaki, K., K. Tarutani & M. Yamaguchi 1999. Growth characteristics of *Heterosigma akashiwo* virus and its possible use as a microbiological agent for red tide control. *Appl. Environ. Microbiol.* **65**: 898–902.

Nagasaki, K., Y. Tomaru, K. Nakanishi, N. Hata, N. Katanozaka & M. Yamaguchi 2004. Dynamics of *Heterocapsa circularisquama* (Dinophyceae) and its viruses in Ago Bay, Japan. *Aquat. Microb. Ecol.* **34**: 219–226.

Nagasaki, K., Y. Tomaru, K. Tarutani, N. Katanozaka, S. Yamanaka, H. Tanabe & M. Yamaguchi 2003. Growth characteristics and intra-species host specificity of a large virus infecting the dinoflagellate *Heterocapsa circularisquama*. *Appl. Environ. Microb.* **69**: 2580–2586.

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

Peak, J. G., M. J. Peak, R. S. Sikorski & C. A. Jones 1985. Induction of DNA-protein crosslinks in human cells by ultraviolet and visible radiations: action spectrum. *Photochem. Photobiol.* **41**: 295–302.

Shirota, A. 1992. Overview of research and development for red tide mitigation strategy. *Gekkan Kaiyo* **24**: 3–16. (in Japanese)

Smayda, T. J. 1998. Ecophysiology and bloom dynamics of *Heterosigma akashiwo* (Raphidophyceae), p. 113–131. In *Physiological Ecology of Harmful Algal Blooms* (eds. Anderson, D. M., A. D. Cembella & G. M. Hallegraeff). Springer, Berlin.

Suttle, C. A. 1993. Enumeration and isolation of viruses, p. 121–137. In *Handbook of Methods in Aquatic Microbial Ecology* (eds. Kemp, P. F., E. Sherr & J. J. Cole). Lewis Publishers, Boca Raton.

Suttle, C. A. & F. Chen 1992. Mechanisms and rates of decay of marine viruses in seawater. *Appl. Environ. Microb.* **58**: 3721–3729.

Tarutani, K., K. Nagasaki, S. Itakura & M. Yamaguchi 2001. Isolation of a virus infecting the novel shellfish-killing dinoflagellate *Heterocapsa circularisquama*. *Aquat. Microb. Ecol.* **23**: 103–111.

Tarutani, K., K. Nagasaki & M. Yamaguchi 2000. Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton *Heterosigma akashiwo*. *Appl. Environ. Microbiol.* **66**: 4916–4920.

Thurston-Enriquez, J. A., C. N. Haas, J. Jacangelo, K. Riley & C. P. Gerba 2003. Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Appl. Environ. Microbiol.* **69**: 577–582.

Tomaru, Y. & K. Nagasaki 2004. Widespread occurrence of viruses lytic to the bivalve-killing dinoflagellate *Heterocapsa circularisquama* along the western coast of Japan. *Plankton Biol. Ecol.* **51**: 1–6.

Tomaru, Y., N. Katanozaka, K. Nishida, Y. Shirai, K. Tarutani, M. Yamaguchi & K. Nagasaki 2004a. Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*. *Aquat. Microb. Ecol.* **34**: 227–238.

Tomaru, Y., K. Tarutani, M. Yamaguchi & K. Nagasaki 2004b. Quantitative and qualitative impacts of viral infection on *Heterosigma akashiwo* (Raphidophyceae) population during a bloom in Hiroshima Bay, Japan. *Aquat. Microb. Ecol.* **34**: 227–238.

Van Etten, J. L., L. C. Lane & R. H. Meints 1991. Viruses and viruslike particles of eukaryotic algae. *Microb. Rev.* **55**: 586–620.