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NOTE

Effects of temperature and salinity on diatom cell lysis by DNA and RNA viruses

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ABSTRACT: In estuarine and coastal environments, microbes are exposed to significant changes in the environment within short time periods. To examine the effects of water temperature and salinity on host–virus interactions, we used 2 strains of the marine planktonic diatom *Chaetoceros tenuissimus* and 4 viruses that exhibit contrasting host specificities. We found that the time necessary for a given virus to lyse half the diatoms within a culture (CR_{50}), measured as the number of days required for chlorophyll *a* fluorescence intensity of host cells to decrease by >50%, was significantly affected by changes in both water temperature and salinity. In several host–virus combinations, environmental suitability for the growth of the host and the CR_{50} of the virus were significantly correlated, but no correlation was observed for other combinations. The CR_{50} values for different viral strains varied significantly depending on the combination of temperature and salinity tested. Moreover, optimum conditions for host cell lysis were highly diverse among virus species and isolates. The varied environmental optima of viruses might allow them to partition use of the same host species in natural environments.

KEY WORDS: Water temperature · Water salinity · Host–virus interactions · Diatom lysis

INTRODUCTION

Viral infection is one of the significant factors shaping microbial dynamics in aquatic environments. In nature, both the host and viruses stably coexist in close proximity (Suttle 2007, Flores et al. 2013). To understand the relationships between hosts and their cognate viruses, the effects of various environmental factors on host–virus interactions need to be characterized (Mojica & Brussaard 2014). Among the diverse environmental parameters, water temperature and salinity are major factors regulating phytoplankton growth, as well as light and nutrient levels (Sarthou et al. 2005). Rapid changes in water temperature and salinity, for example due to a heavy rainfall in coastal and brackish water environments, can thus affect the growth and reproduction of phytoplankton (Balzano et al. 2011, Turner & Rabalais 2013). Such physicochemical factors are considered to affect successful virus infections and host cell lysis. Indeed, the sensitivity of *Heterosigma akashiwo* (Raphidophyceae) to viruses changes with changes in water temperature (Nagasaki & Yamaguchi 1998). For instance, positive cell lysis of *H. akashiwo* strain H93616 by HaV01 and HaV08 occurs at water temperatures of 15–30°C and 20–30°C, respectively. However, *H. akashiwo* strain NM96 shows resistance to both viruses at 30°C (Nagasaki & Yamaguchi 1998). Water temperature is similarly significant for relationships between the bloom-forming marine planktonic diatom *Chaetoceros tenuissimus* and its infectious viruses, and diverse combinations of host and virus strains have been observed (Tomaru et al. 2014). Salinity is also an important factor affecting virus infections. Evilevitch et al. (2008) reported that the efficiency of viral DNA injection into its host cells

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is affected by osmotic pressure. Although there are several previous reports on the effects of salinity on host–virus interactions in prokaryotes and *Archaea*, knowledge regarding these effects is limited, especially for eukaryotic microalgae (Mojica & Brussaard 2014). In this study, we investigated how water temperature and salinity levels regulate host–virus interactions using *C. tenuissimus* strains and 4 distinct infectious viruses. Our study emphasizes the need for further studies of physiochemical parameters and their effects on resource partitioning to understand the ecology of microalgal host–virus systems.

MATERIALS AND METHODS

Host diatoms, virus strains, and culture conditions

The axenic clonal diatom strains used in this study were *Chaetoceros tenuissimus* strains NIES-3714 (the original strain name, used in previous reports, was '2-6') (Tomaru et al. 2011) and NIES-3715 (original strain name: 2-10) (Shirai et al. 2008). The virus strains used in this study were CtenDNAV type-I and type-II and CtenRNAV type-I and type-II. All diatom and viral strains were isolated from the western coast of Japan (Shirai et al. 2008, Tomaru et al. 2011, Kimura & Tomaru 2015). The basic properties of these viruses are summarized in Tables S1 and S2 in the Supplement at www.int-res.com/articles/suppl/a079p079_supp.pdf. Algal cultures were maintained and grown in modified SWM3 medium (Imai et al. 1996) under a 12/12-h light-dark cycle of ca. 110–150 µmol photons m^{-2} s^{-1} using cool white fluorescent illumination at 15°C.

Pre-conditioning of the host culture

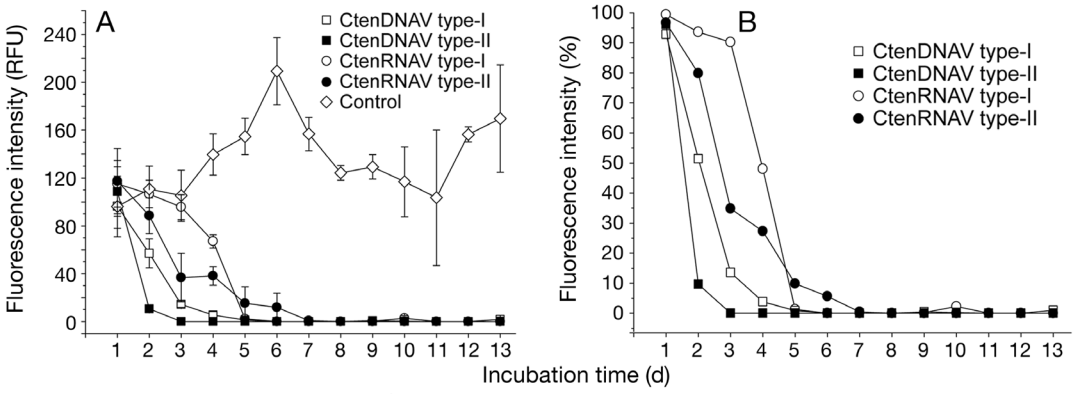
The experiments were conducted at 5 temperatures (10, 15, 20, 25 and 30°C) and 6 salinities (10, 15, 20, 25, 30, and 35 PSU) in temperature gradient growth chambers (TG-100-AD, Nippon Medical & Chemical Instrument) under the lighting conditions stated above. The salinity of the seawater was adjusted to 10–30 PSU by dilution with distilled water. A salinity of 35 PSU was achieved by concentrating the natural seawater in a drying oven at 50°C. Seawater was enriched with elements of sea water medium (SWM-3) after the salinity adjustments. Cells were pre-conditioned to the experimental conditions through stepwise transfer of stock cultures to each temperature and salinity regime. If the transferred cells grew under the experimental regime, then the culture was conditioned at that regime for at least 7 d. The cultures were then re-transferred under each experimental regime for at least 7 d again. The acclimated stock cultures were then used for further experiments.

Viral inoculation and measurement of algal growth or lysis

Viral inoculation experiments were conducted at 5 temperatures and 6 salinities. The pre-conditioned host cell cultures were grown to 1.0×10^6 cells ml^{-1} at each combination of temperature and salinity and then diluted 2-fold with the medium. For each virus to be tested, 190 µl diluted host culture was transferred in triplicate to 96-well microculture plates (Falcon; Becton, Dickinson and Company) and incubated for 24 h at the appropriate temperature. The virus suspension (10 µl, final viral concentration: 1.0×10^5 infectious units ml^{-1}) was then added to each well to obtain triplicates of each virus, host, temperature, and salinity combination. Diatom cultures grown in media without virus were used as uninfected controls. Culture plates were incubated under the specified lighting conditions for up to 2 wk. Fluorescence intensity (FI) decreases as cells are lysed. Therefore, algal growth or lysis was monitored by measuring *in vivo* chlorophyll *a* FI (the excitation and detection wavelength were 430 and 670 nm, respectively) each day using a microplate reader (ARVOsx1420, PerkinElmer). The cultures were scored as infected when the FI of an infected culture decreased to <50% of that of the control, and the period (days) needed for algal lysis following inoculation was recorded as CR_{50}.

Statistical analysis

A 2-way analysis of variance (ANOVA) was used to estimate the effects of temperature, salinity, and their combinations on the viral infections. The similarity of the degrees of the host cell lysis due to viral inoculations (CR_{50}) among the host–virus combinations was analysed using the Kolmogorov-Smirnov (KS) test. The CR_{50} values for samples in which detection did not occur were defined as 14 d in the analysis, which was the maximum time interval for the observation in the present experiment. To compare the host growth rate and CR_{50}, correlation analysis was used. The host growth rates used in this study were estimated from the growth rate regression models of the *C. tenuissimus* strains (Tomaru et al. 2014).



C NIES-3714 vs CtenDNAV type-I

Salinity \ Temp.	10°C	15°C	20°C	25°C	30°C
35	10.6 0.7	7.2 0.2	2.5 0.0	1.5 0.0	0.6 0.0
30	5.0 0.1	2.6 0.0	1.5 0.1	0.9 0.1	0.6 0.0
25	5.4 0.5	2.4 0.0	1.5 0.0	1.2 0.1	0.5 0.0
20	5.8 1.2	2.0 0.2	1.5 0.0	0.6 0.0	1.3 0.1
15	11.4 0.5	2.6 0.1	1.4 0.0	1.1 0.1	1.6 0.0
10	-	7.3 0.1	1.5 0.0	1.6 0.0	4.6 0.0

D NIES-3714 vs CtenDNAV type-II

Salinity \ Temp.	10°C	15°C	20°C	25°C	30°C
35	11.0 0.7	-	-	-	-
30	4.7 0.4	2.8 0.0	1.6 0.0	1.4 0.1	5.7 1.5
25	5.1 0.7	2.7 0.1	1.5 0.0	1.5 0.1	2.9 0.6
20	8.8 0.2	2.1 0.2	1.2 0.1	0.8 0.1	2.4 0.1
15	10.4 0.4	2.6 0.1	1.8 0.0	1.5 0.0	3.7 0.1
10	-	7.5 0.2	4.2 0.6	4.6 0.0	4.7 0.1

E NIES-3715 vs CtenDNAV type-I

Salinity \ Temp.	10°C	15°C	20°C	25°C	30°C
35	9.5 0.3	8.0 1.3	3.1 0.1	1.6 0.1	0.6 0.0
30	3.2 0.8	3.5 0.1	1.7 0.3	1.4 0.0	0.8 0.1
25	3.9 0.5	3.2 0.1	2.2 0.2	1.5 0.0	0.9 0.2
20	3.7 0.2	2.9 0.2	1.9 0.3	1.5 0.0	0.5 0.0
15	4.7 0.1	3.3 0.2	2.0 0.2	2.4 0.1	1.2 0.1
10	2.8 2.1	5.2 0.6	1.9 0.2	2.5 0.1	3.0 0.0

F NIES-3715 vs CtenDNAV type-II

Salinity \ Temp.	10°C	15°C	20°C	25°C	30°C
35	6.7 4.2	7.0 0.4	1.8 0.1	1.6 0.0	1.8 0.2
30	4.1 0.6	3.0 0.0	1.5 0.0	0.7 0.1	1.4 0.1
25	4.5 0.4	2.5 0.3	1.5 0.1	1.2 0.1	2.0 0.3
20	4.9 0.3	3.2 0.3	1.4 0.0	1.5 0.1	1.1 0.1
15	6.4 0.2	3.9 0.4	1.5 0.0	1.6 0.0	1.4 0.1
10	9.2 0.5	6.0 0.3	2.8 0.6	5.5 0.1	3.9 0.5

G NIES-3715 vs CtenRNAV type-I

Salinity \ Temp.	10°C	15°C	20°C	25°C	30°C
35	3.8 0.2	3.8 0.1	1.4 0.0	2.5 0.1	4.3 0.2
30	2.2 0.3	2.1 0.0	1.1 0.2	2.4 0.1	5.5 0.0
25	2.8 0.1	1.9 0.2	1.8 0.0	3.2 0.3	8.9 1.1
20	3.0 0.3	1.8 0.1	2.9 0.0	3.3 0.9	5.6 0.2
15	4.2 0.2	2.2 0.2	4.0 0.1	6.0 0.6	6.3 0.0
10	3.9 0.9	4.5 0.2	4.9 0.5	9.7 1.3	5.6 2.4

H NIES-3715 vs CtenRNAV type-II

Salinity \ Temp.	10°C	15°C	20°C	25°C	30°C
35	10.4 0.1	7.5 0.2	1.7 0.2	1.5 0.1	0.6 0.1
30	7.2 0.2	3.3 0.1	1.3 0.2	1.2 0.1	0.6 0.0
25	11.2 0.4	3.5 0.0	1.5 0.0	1.5 0.0	0.6 0.0
20	10.1 0.6	3.3 0.2	2.2 0.0	1.5 0.0	0.5 0.0
15	12.1 0.3	5.4 0.8	2.7 0.4	2.7 0.1	1.2 0.0
10	9.9 0.3	7.7 1.4	2.8 0.2	3.1 0.3	3.1 0.4

RESULTS AND DISCUSSION

The FI for each host–virus combination at each temperature/salinity combination (Figs. 1A, S1–S4 in the Supplement) was transformed to a CR_{50} score (Figs. 1B, S5–S8 in the Supplement). In many cases, the FI of the host culture decreased after the viral inoculations, indicating cell lysis and therefore virus infection. The CR_{50} of all host–virus combinations was significantly affected by temperature, salinity, and the temperature–salinity interaction (2-way ANOVA, $p < 0.01$). Thus, both water temperature and salinity affected diatom growth and the extent of successful viral infection, which resulted in host cell lysis. Host cell lysis resulting from viral infection was not detected in host strain NIES-3714 at 10°C and at a salinity of 10, possibly because of the low growth of the host strain under these conditions (Fig. 1C,D) (Tomaru et al. 2014). Under conditions that permit better growth of strain NIES-3714, for example a temperature of 15–30°C and a salinity of 35, host cell lysis was detected after CtenDNAV type-I inoculation, but not after CtenDNAV type-II inoculation (Fig. 1C,D). Furthermore, the CR_{50} matrix of CtenRNAV type-I against host strain NIES-3715 was significantly different from that of other host–virus combinations (Fig. 1E–H, Table 1). The CR_{50} matrix of CtenDNAV type-II against host strain NIES-3714 was also significantly different from that of CtenDNAV type-I against host strain NIES-3714 and Cten RNAV type-II against host strain

Fig. 1. Change over time in (A) chlorophyll *a* fluorescence intensity (FI) in the diatom *Chaetoceros tenuissimus* strain NIES-3715 inoculated with 4 strains of virus (CtenDNAV types I and II and CtenRNAV types I and II), together with results for the control group; and (B) FI values of infected diatoms expressed as percentages of values for the control group. Results are shown for culture plates incubated at 20°C and 30 PSU. (Panels C–H) Algal lysis by the 4 strains of virus in *C. tenuissimus* strains NIES-3714 and NIES-3715 at different combinations of temperature and salinity. Algal lysis was defined as occurring when the FI of an infected culture decreased to <50% of the control value (CR_{50}). Upper scores in each cell show mean CR_{50} values in days; lower scores are the standard deviation for 3 replicates. Black, dark grey, grey, and light grey cells show CR_{50} scores of <1, 1–2, 2–3, and >3, respectively. '-' indicates that values were not detected

Table 1. p-values determined using Kolmogorov-Smirnov test of CR_{50} values for algal lysis (see Fig. 1 legend) in the diatom *Chaetoceros tenuissimus* strains NIES-3714 and NIES-3715 following inoculation by 4 strains of virus (CtenDNAV types I and II and CtenRNAV types I and II), for different host–virus combinations

	Host strain NIES-3714		Host strain NIES-3715			
	CtenDNAV		CtenDNAV		CtenRNAV	
	type-I	type-II	type-I	type-II	type-I	type-II
NIES-3714/DNAV-I	–	0.0009	0.0644	0.2557	<0.0001	0.9613
NIES-3714/DNAV-II		–	0.0513	0.0906	0.0062	0.0001
NIES-3715/DNAV-I			–	0.8742	<0.0001	0.0401
NIES-3715/DNAV-II				–	<0.0001	0.3460
NIES-3715/RNAV-I					–	<0.0001

NIES-3715 (Table 1). These results of the CR_{50} matrix patterns indicated that the optimal range of conditions for virus-mediated diatom cell lysis depends on host–virus combinations and/or the virus species. Therefore, both water temperature and salinity might influence host cell lysis and control the influx and dominance of virus species in natural environments.

In many microalgal host–virus systems, intense host culture lysis and higher potential viral infectivity are expected under temperature conditions of optimum cell replication (Nagasaki et al. 2003). In the present study, host cell lysis (Fig. 1) and the environmental suitability for the host growth rate, which was calculated using the cubic equations of the host strains for each water temperature and salinity condition (Fig. S9 in the Supplement) (Tomaru et al. 2014), significantly correlated in most of the host–virus combinations ($p < 0.01$) (Fig. 2A–D,F), i.e. the CR_{50} was shorter when host cell replication was higher. However, this was not necessarily the case for the host strain NIES-3715 when inoculated with CtenRNAV type-I (Fig. 2E). Considering the distribution of the CR_{50} values with regard to temperature and salinity, the effect for the host strain NIES-3715 inoculated with CtenRNAV type-I may be explained by differences in the optimal conditions for maximum host growth and lytic activity of the virus. Therefore, both water temperature and salinity can alter virus-mediated host cell lysis, independent of the environmental suitability for host growth.

Although the CR_{50} could be an indicator of potential viral effects on host cells, it is influenced by the combined effects of viral adsorption, proliferation, and lysis. Moreover, viral infectivity might be affected by temperature and salinity, which directly affect the molecular structures of the virus or components of the host cell surface, such as viral capsid and receptor proteins (Selinger et al. 1991, Evilevitch et al. 2008). To understand how temperature and salinity affect

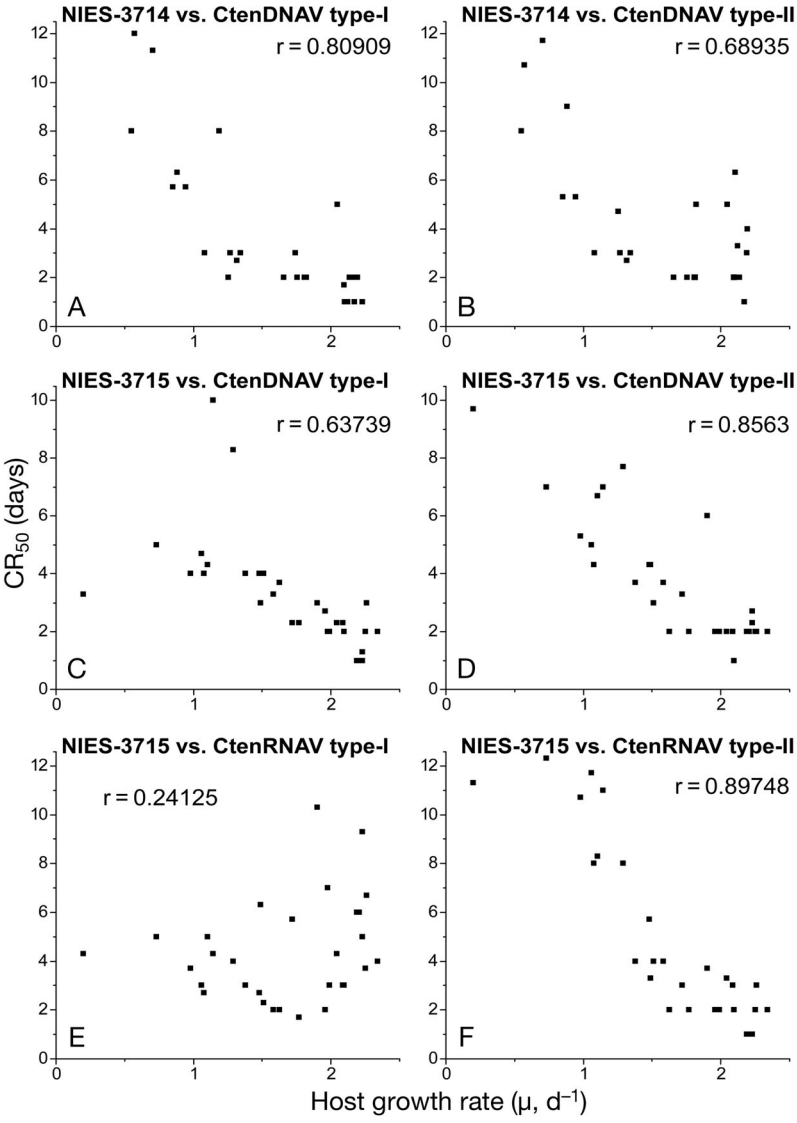


Fig. 2. Results of correlation analyses between the growth rate of host *Chaetoceros tenuissimus* (strains NIES-3714 and NIES-3715) at different combinations of water temperature and salinity (see Fig. S9 in the Supplement) (Tomaru et al. 2014) and CR_{50} values for algal lysis (see Fig. 1 legend) following inoculation by 4 strains of virus (CtenDNAV types I and II and CtenRNAV types I and II)

viral infectivity, further analysis of virus infection processes from adsorption to lysis under various environments should be conducted in the future.

Because all the viruses in this study were isolated from western Japan, individual host diatom strains might experience selection pressure exerted by infection by individual virus strains within a year. However, each virus seemed to have an optimal set of environmental conditions for infection/replication, resulting in host cell lysis, which was different from optimal conditions for rival viruses (Fig. S10 in the Supplement). The present study showed that the optimal water temperature and salinity conditions for the CR_{50} did not necessarily correspond to that of the environmental suitability for the host growth (Fig. 2E). This might be one of the survival strategies for the virus, i.e. having maximum proliferation under non-optimal host growth conditions and avoiding competition with other viruses. Therefore, these environmental optima could cause the viruses to partition the use of the same host species. This is similar to the observations made in prokaryotes, with several studies reporting that successful phage infections occur in non-optimum host growth environments (Wang et al. 2007, Zachary 1976).

The effects of salinity on prokaryotic phage infection have been well documented (Mojica & Brussaard, 2014); however, information on eukaryotic host–virus systems is lacking. In this study, we showed that both water temperature and salinity affect the interaction between microalgal hosts and their cognate viruses. In coastal and estuarine environments, viral niche partitioning and coexistence of host–virus interactions could be directly controlled by physical and chemical factors as well as by host specificity of the viruses (Flores et al. 2013). In our study, we also showed that the optimum conditions for host cell lysis are highly diverse among virus species and isolates. In the future, increases in water temperature and precipitation due to global warming might change the relationships between diatoms and their infectious viruses. Therefore, further studies of physiochemical parameters and their effects on resource partitioning are necessary to understand the ecology of microalgal host–virus systems.

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LITERATURE CITED

Balzano S, Sarno D, Kooistra WH (2011) Effects of salinity on the growth rate and morphology of 10 *Skeletonema* strains. J Plankton Res 33:937–945

Evilevitch A, Fang LT, Yoffe AM, Castelnovo M and others (2008) Effects of salt concentrations and bending energy on the extent of ejection of phage genomes. Biophys J 94: 1110–1120

Flores CO, Valverde S, Weitz JS (2013) Multi-scale structure and geographic drivers of cross-infection within marine bacteria and phages. ISME J 7:520–532

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

Kimura K, Tomaru Y (2015) Discovery of two novel viruses expands the diversity of single-stranded DNA and single-stranded RNA viruses infecting a cosmopolitan marine diatom. Appl Environ Microbiol 81:1120–1131

Mojica KD, Brussaard CP (2014) Factors affecting virus dynamics and microbial host–virus interactions in marine environments. FEMS Microbiol Ecol 89:495–515

Nagasaki K, Yamaguchi M (1998) Effect of temperature on the algicidal activity and the stability of HaV (*Heterosigma akashiwo* virus). Aquat Microb Ecol 15: 211–216

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

Sarthou G, Timmermans KR, Blain S, Treguer P (2005) Growth physiology and fate of diatoms in the ocean: a review. J Sea Res 53:25–42

Selinger RLB, Wang Z, Gelbart WM, Ben-Shaul A (1991) Statistical-thermodynamic approach to fracture. Phys Rev A 43:4396–4400

Shirai Y, Tomaru Y, Takao Y, Suzuki H, Nagumo T, Nagasaki K (2008) Isolation and characterization of a single-stranded RNA virus infecting the marine planktonic diatom *Chaetoceros tenuissimus* Meunier. Appl Environ Microbiol 74:4022–4027

Suttle CA (2007) Marine viruses—major players in the global ecosystem. Nat Rev Microbiol 5:801–812

Tomaru Y, Shirai Y, Toyoda K, Nagasaki K (2011) Isolation and characterisation of a single-stranded DNA virus infecting the marine planktonic diatom *Chaetoceros tenuissimus*. Aquat Microb Ecol 64:175–184

Tomaru Y, Kimura K, Yamaguchi H (2014) Temperature alters algicidal activity of DNA and RNA viruses infecting *Chaetoceros tenuissimus*. Aquat Microb Ecol 73: 171–183

Turner RE, Rabalais NN (2013) Nitrogen and phosphorus phytoplankton growth limitation in the northern Gulf of Mexico. Aquat Microb Ecol 68:159–169

Wang F, Wang FP, Li Q, Xiao X (2007) A novel filamentous phage from the deep-sea bacterium *Shewanella piezotolerans* WP3 is induced at low temperature. J Bacteriol 189:7151–7153

Zachary A (1976) Physiology and ecology of bacteriophages of the marine bacterium *Beneckea natriegens*: salinity. Appl Environ Microbiol 31:415–422

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