

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

Kei Kimura, Yuji Tomaru\*

\*Corresponding author: tomaruy@affrc.go.jp

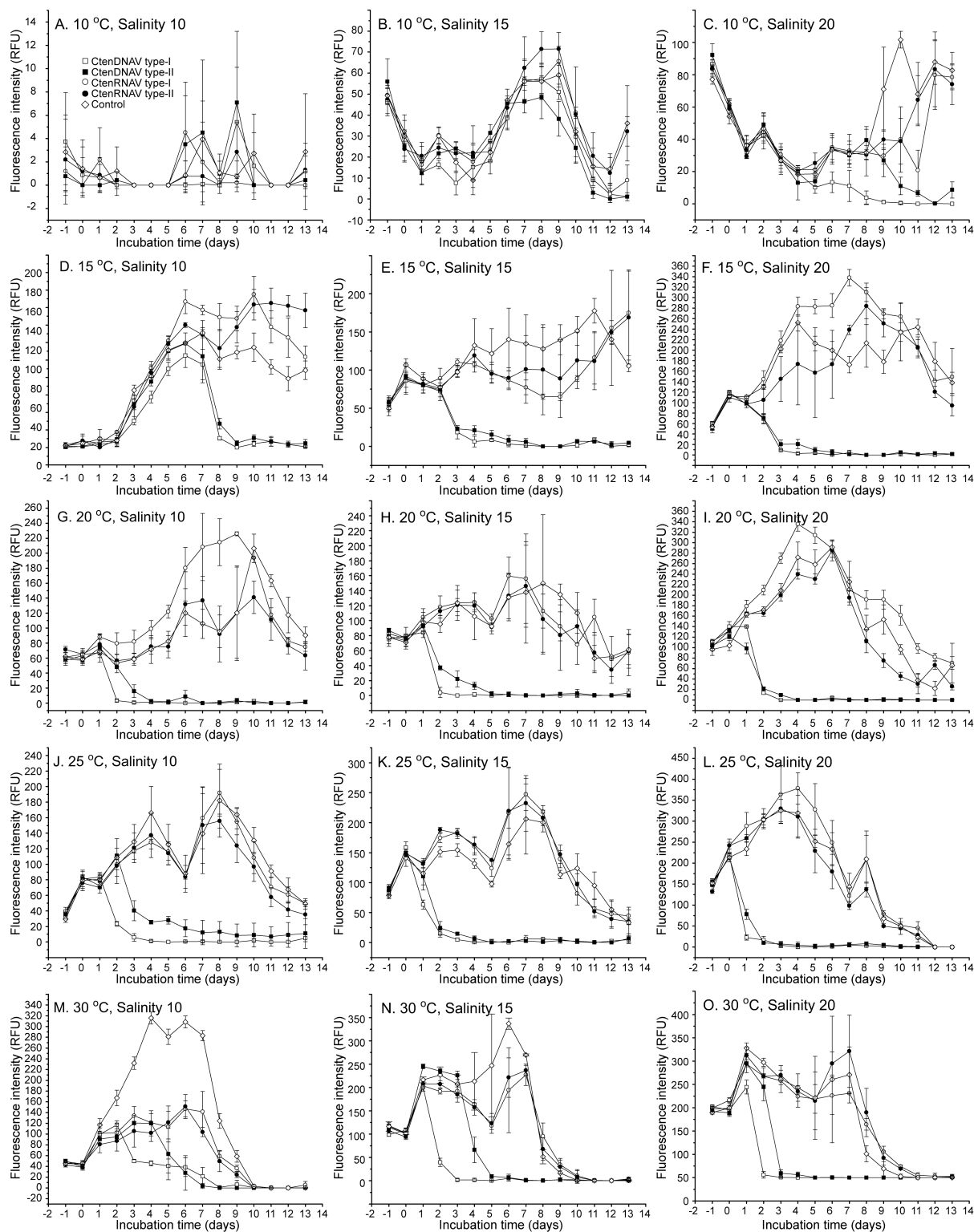
*Aquatic Microbial Ecology* 79: 79–83 (2017)

Table S1. Basic characteristics of host diatom strains.

Species name	Strain No.	Original strain name	Isolation field	Isolation year	Reference
<i>Chaetoceros tenuissimus</i>	NIES-3714	Cten 2-6	Hiroshima Bay	2002	Tomaru et al. 2011
<i>Chaetoceros tenuissimus</i>	NIES-3715	Cten 2-10	Maiko Coast	2002	Shirai et al. 2008

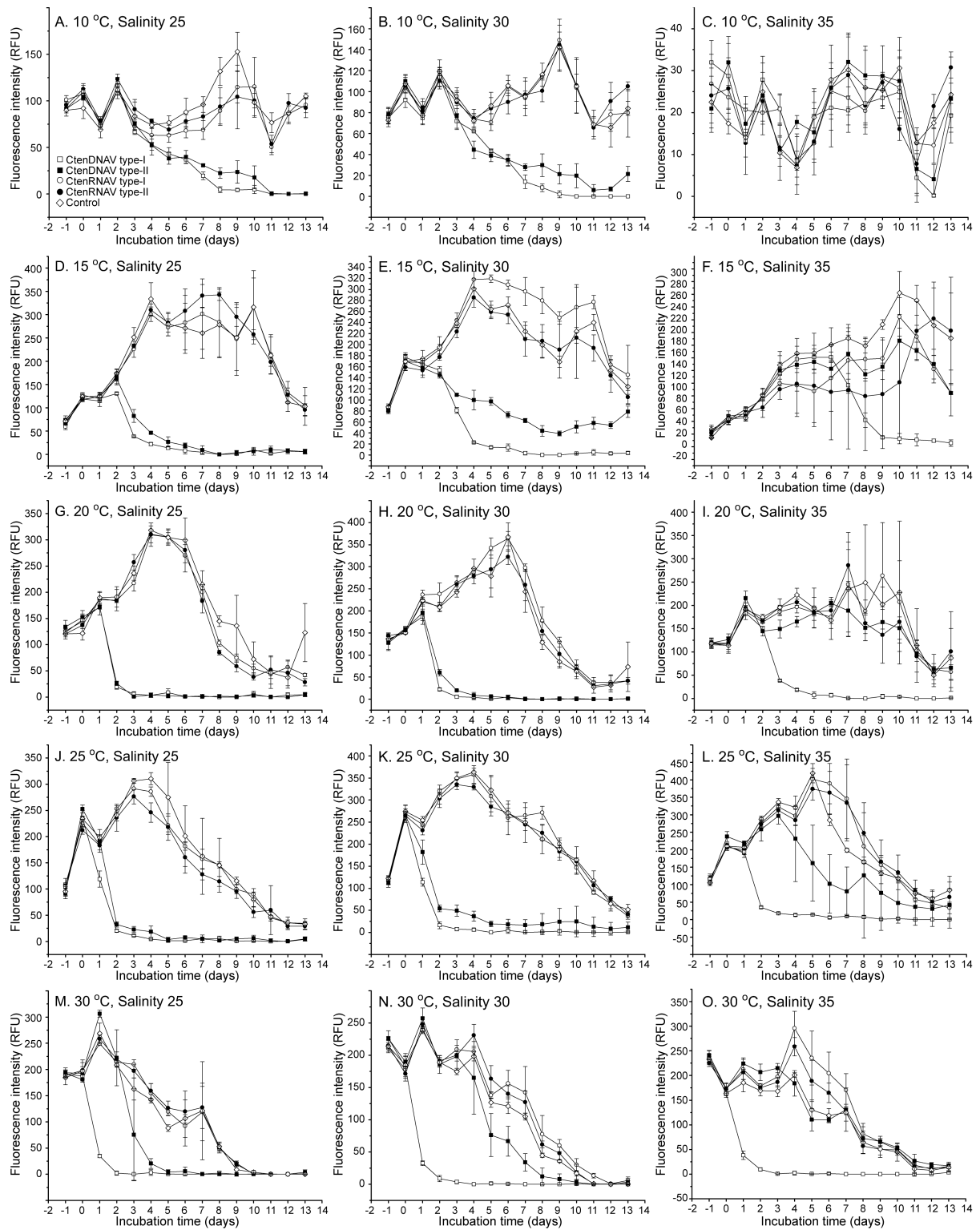
Table S2. Basic characteristics of viral strains.

Virus	Host species and strain No.	Isolation field	Isolation year	Particle size (nm)	Particle assembly	Major proteins (kDa)	Latent period (hr)	Burst size (infectious units cell <sup>-1</sup> )	Genomic structure	Genome length (nt)	Database	Reference
CtenDNAV type-I	<i>Chaetoceros tenuissimus</i> NIES-3714	Ariake Sound	2005	37	nucleus	38.5	96	320	covalently closed circular	5639	AB597949	Tomaru et al. 2011
CtenDNAV type-II	<i>Chaetoceros tenuissimus</i> NIES-3715	Hiroshima Bay	2010	37	nucleus	39.0	< 24	1737	covalently closed circular	5570	AB971658	Kimura and Tomaru 2015
CtenRNAV type-I	<i>Chaetoceros tenuissimus</i> NIES-3715	Ariake Sound	2004	31	cytoplasm	33.5, 31.5, 30.0	< 24	1.0 × 10 <sup>4</sup>	linear	9431	AB37547	Shirai et al. 2008
CtenRNAV type-II	<i>Chaetoceros tenuissimus</i> NIES-3715	Hiroshima Bay	2010	35	cytoplasm	32.2, 29.0, 26.1	24-48	287	linear	9562	AB971661	Kimura and Tomaru 2015



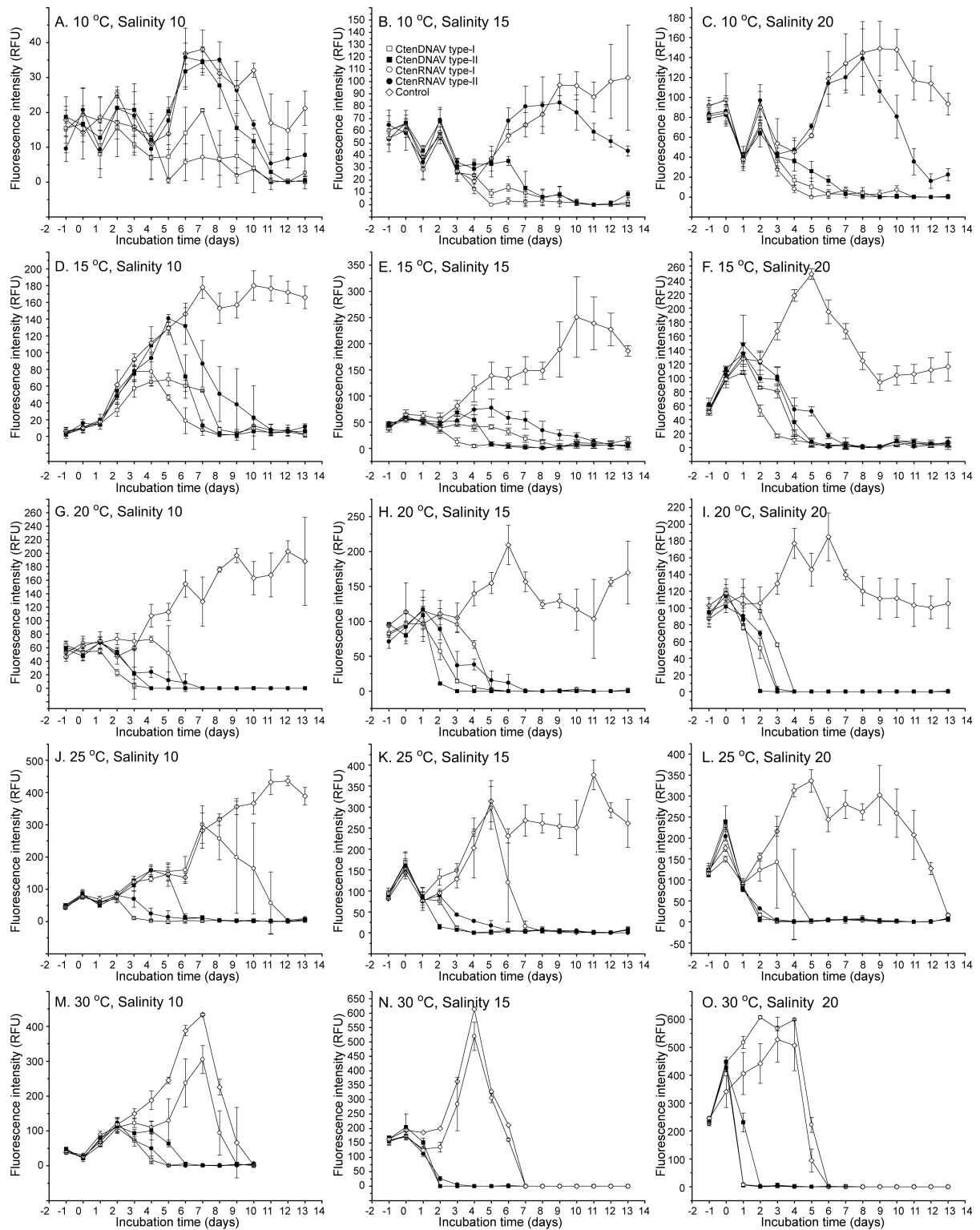
NIES-3714 vs all viruses (Salinity10-20)

**Fig. S1.** Change in chlorophyll *a* fluorescence intensity (FI) in the 2-6 host strain inoculated with each virus. Culture plates were incubated at 10–30°C and 10–20 practical salinity units (PSU).



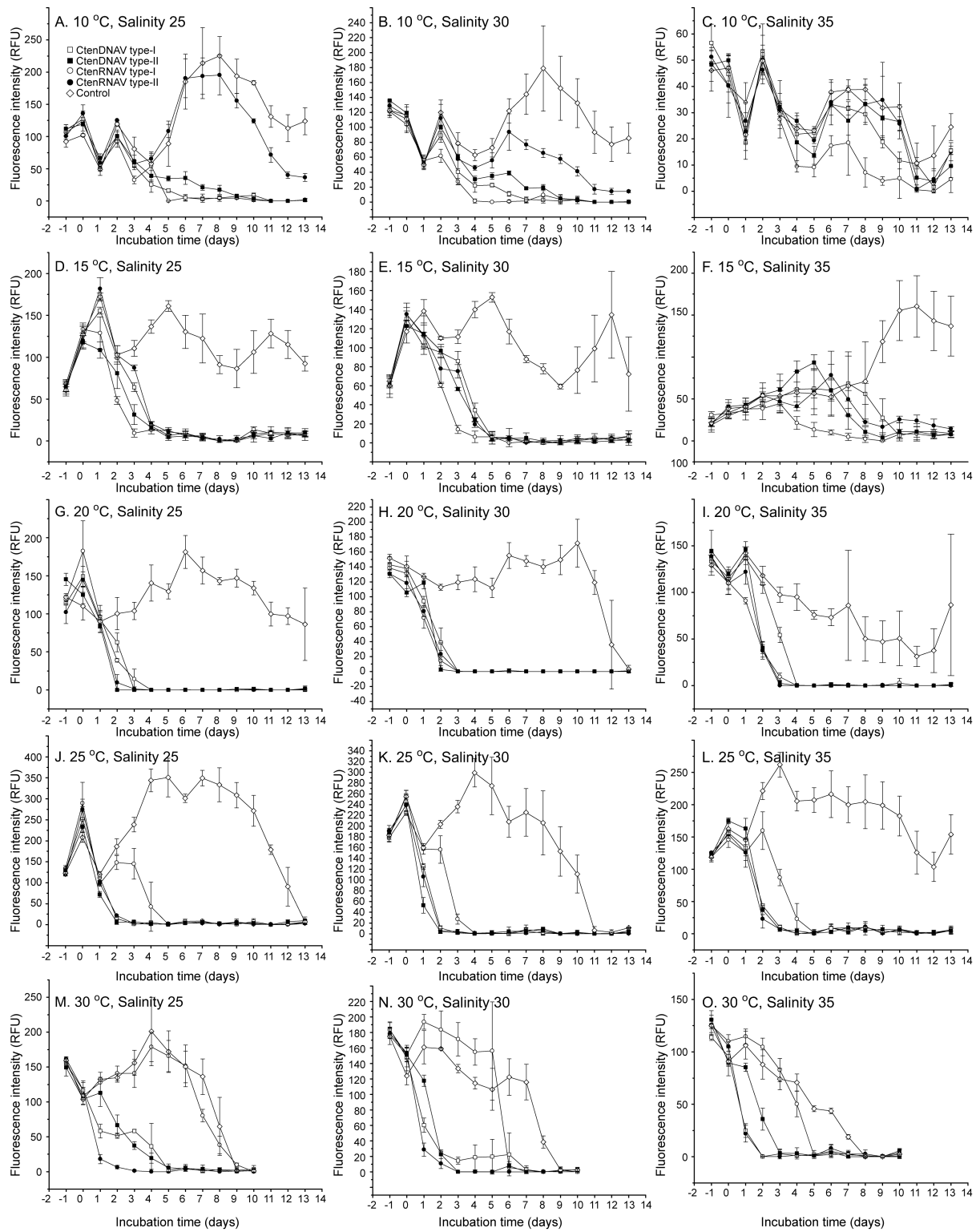
### NIES-3714 vs all viruses (Salinity25-35)

**Fig. S2.** Change in FI in the 2-6 host strain inoculated with each virus. Culture plates were incubated at 10–30°C and 25–35 practical salinity units (PSU).



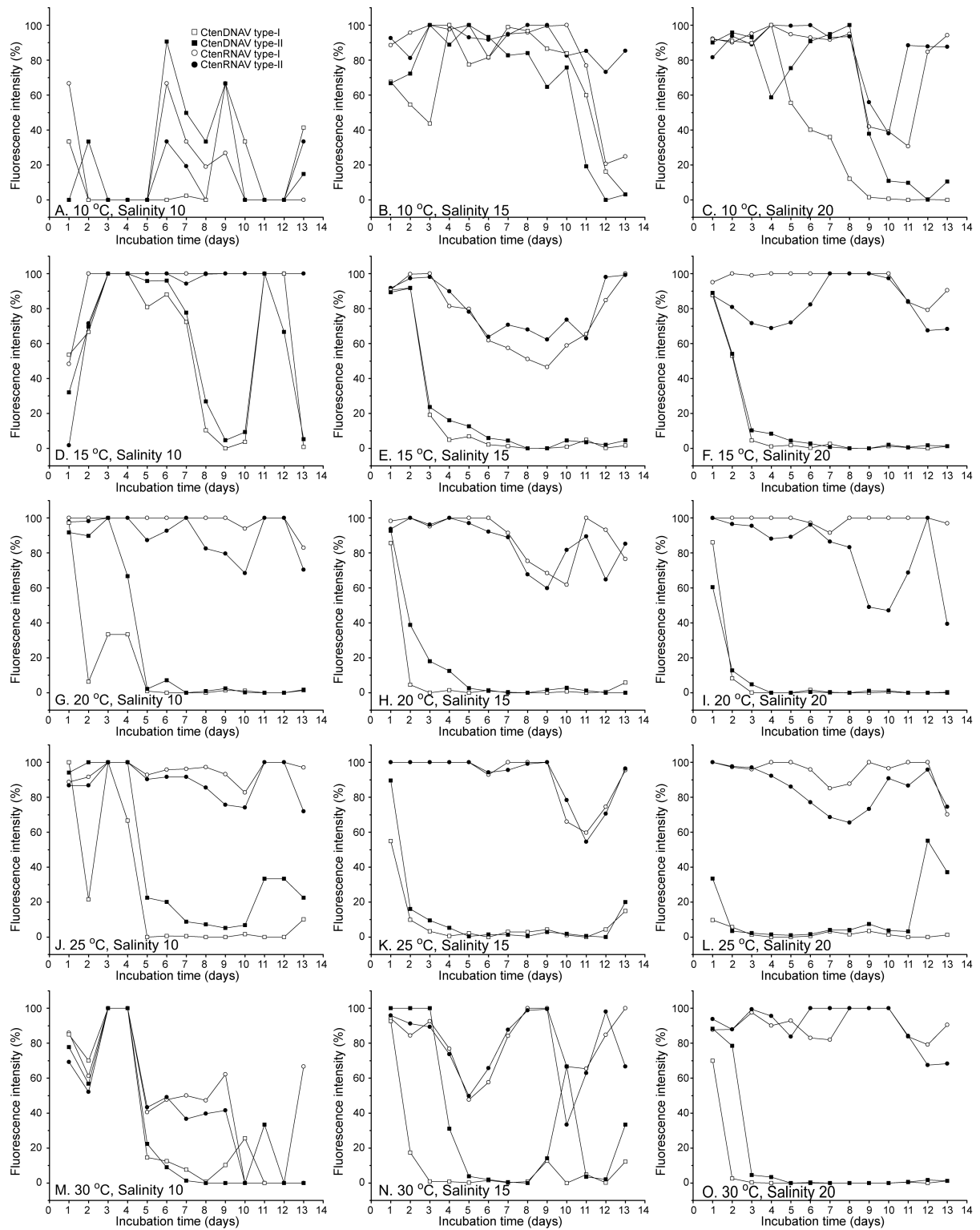
NIES-3715 vs all viruses (Salinity10-20)

**Fig. S3.** Change in FI in the 2-10 host strain inoculated with each virus. Culture plates were incubated at 10–30°C and 10–20 practical salinity units (PSU).



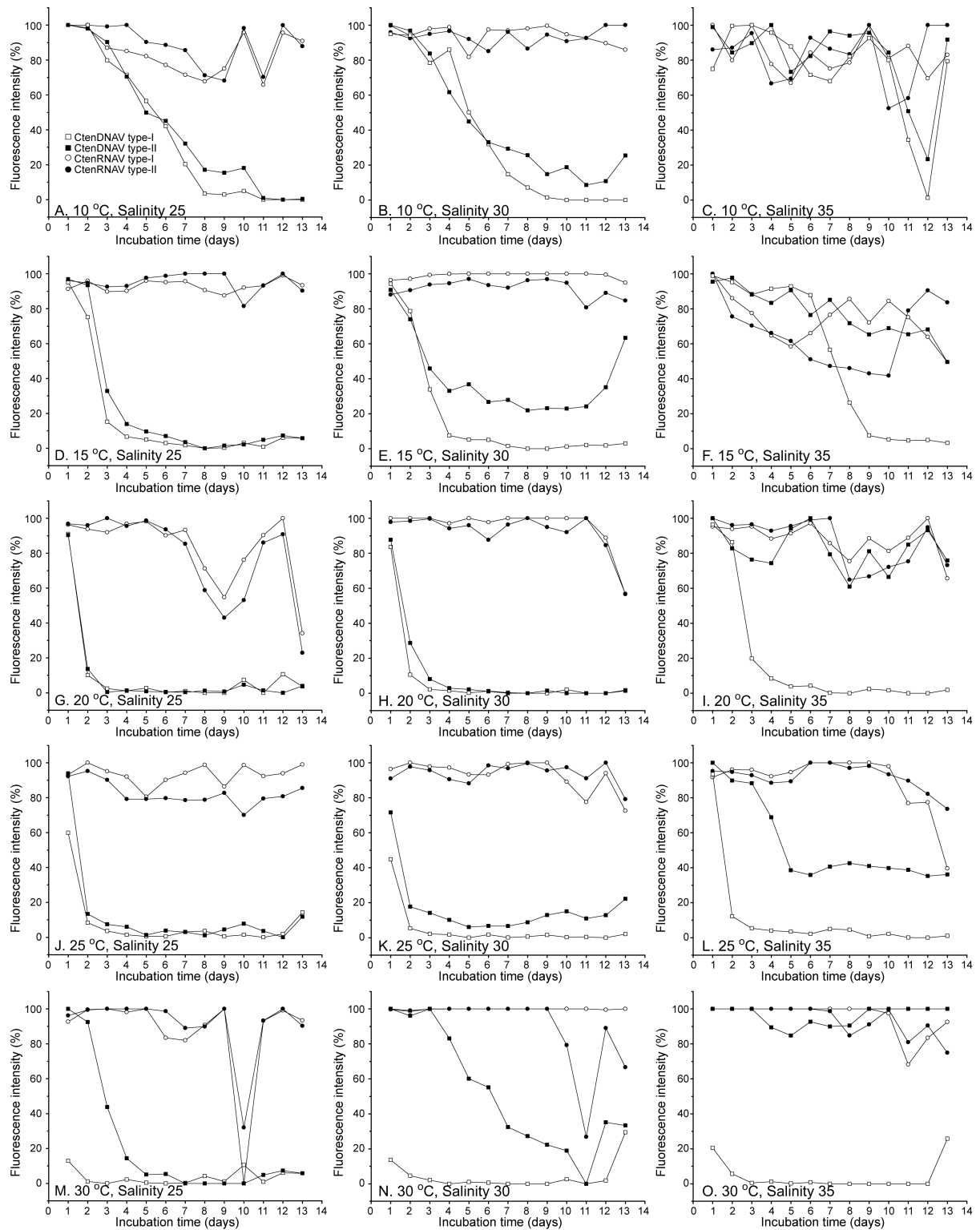
#### NIES-3715 vs all viruses (Salinity25-35)

**Fig. S4.** Change in FI in the 2-10 host strain inoculated with each virus. Culture plates were incubated at 10–30°C and 25–35 practical salinity units (PSU).



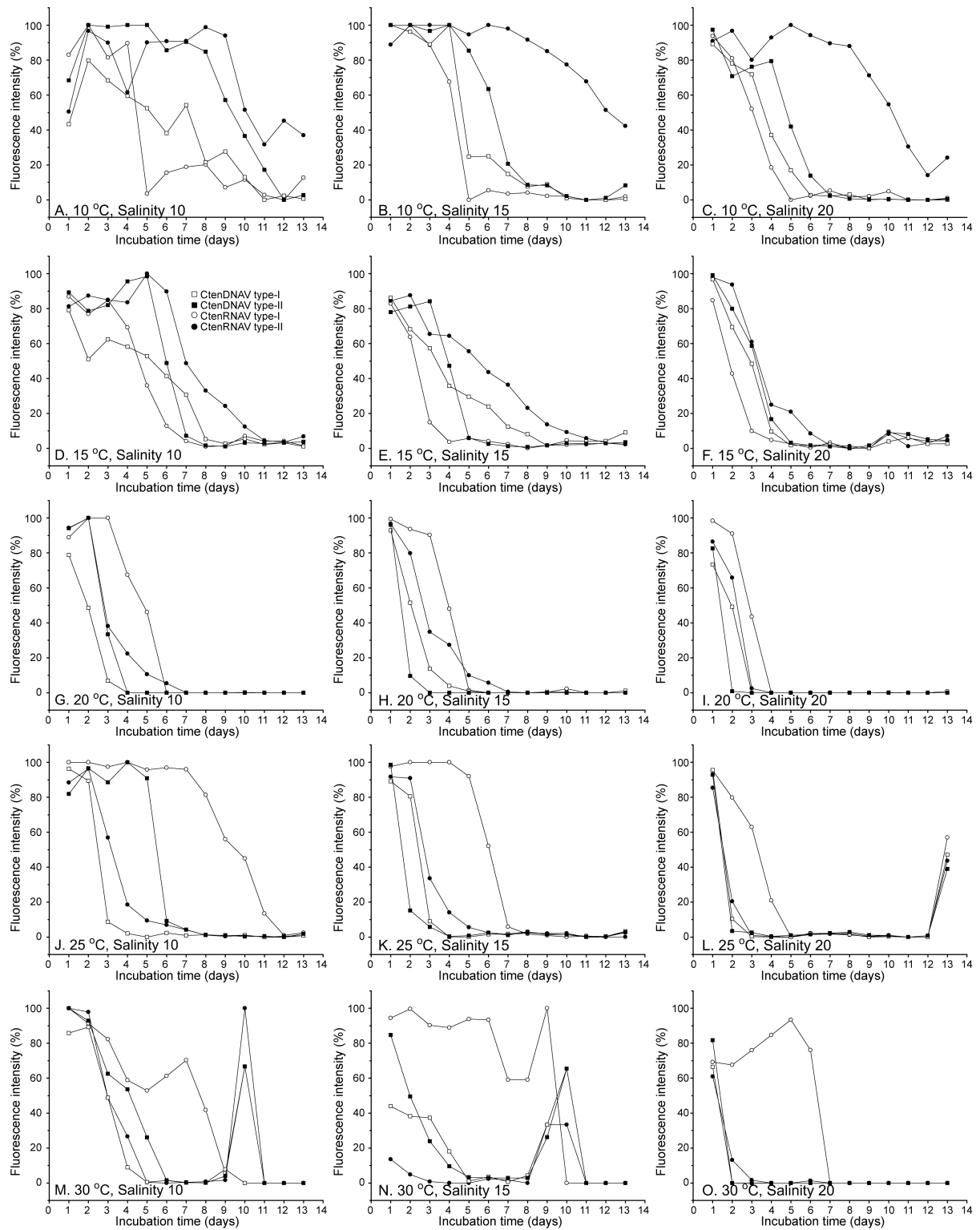
### NIES-3714 vs all viruses (Salinity10-20)

**Fig. S5.** Change on the FI percentage of the control in the 2–6 host strain inoculated with each virus. These culture plates were incubated at 10–30°C and 10–20 practical salinity units (PSU).



#### NIES-3714 vs all viruses (Salinity25-35)

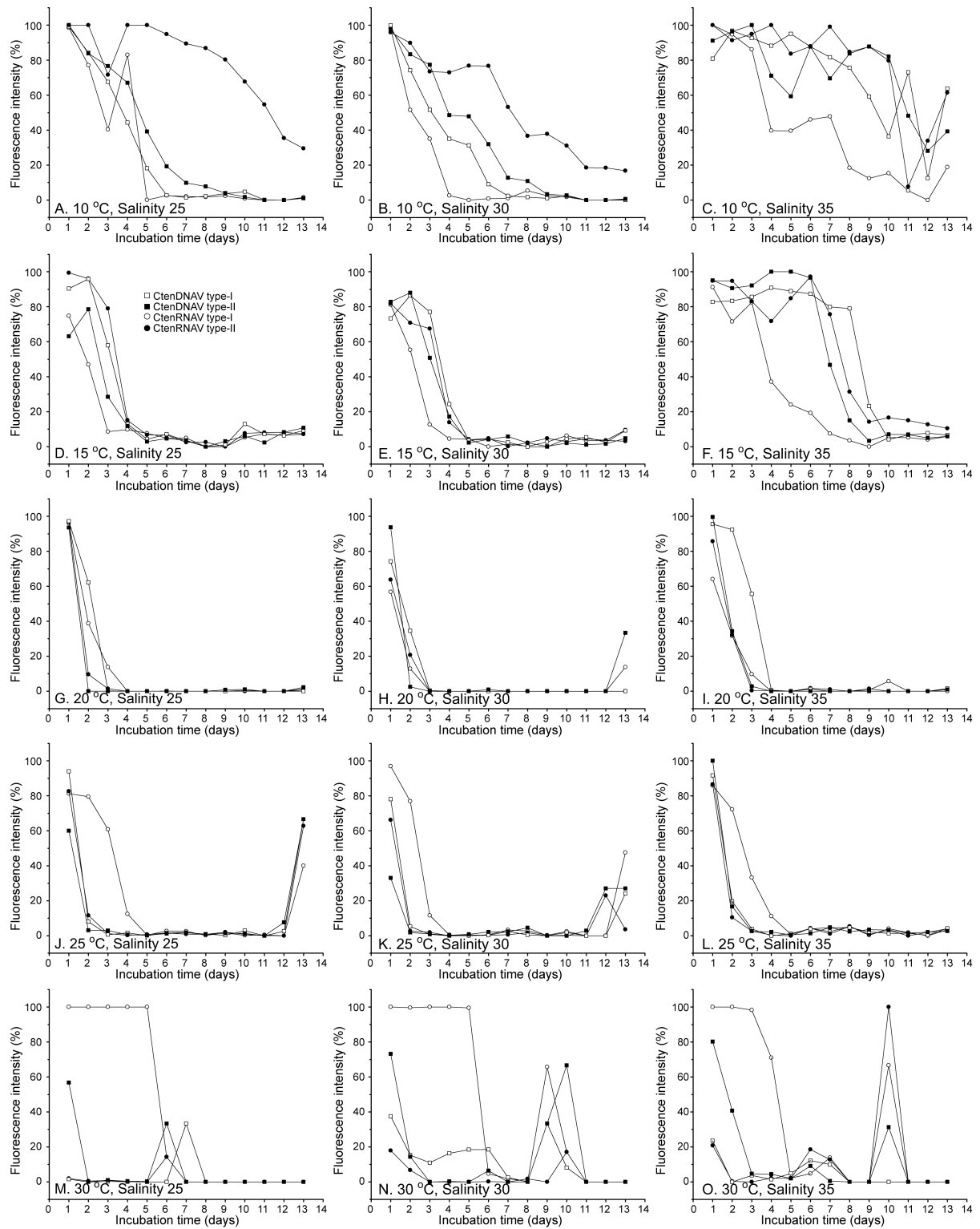
**Fig. S6.** Change in the FI percentage of the control in the 2-6 host strain inoculated with each virus. These culture plates were incubated at 10–30°C and 25–35 practical salinity units (PSU).



NIES-3715 vs all viruses (Salinity10-20)

**Fig. S7.** Change in the FI percentage of the control in the 2-10 host strain inoculated with each virus. Culture plates were incubated at 10–30°C and 10–20 practical salinity units (PSU).





### NIES-3715 vs all viruses (Salinity 25-35)

**Fig. S8.** Change in the FI percentage of the control in the 2-10 host strain inoculated with each virus. These culture plates were incubated at 10–30°C and 25–35 practical salinity units (PSU).

A NIES-3714					
Temp. Salinity	10°C	15°C	20°C	25°C	30°C
35	0.70	1.19	1.74	2.16	2.23
30	0.85	1.27	1.75	2.10	2.11
25	0.94	1.34	1.81	2.14	2.13
20	0.88	1.31	1.81	2.17	2.19
15	0.57	1.08	1.66	2.10	2.20
10	-0.09	0.55	1.25	1.82	2.05

B NIES-3715					
Temp. Salinity	10°C	15°C	20°C	25°C	30°C
35	1.14	1.29	1.63	1.99	2.23
30	1.08	1.38	1.77	2.10	2.21
25	1.06	1.51	1.96	2.25	2.23
20	0.98	1.58	2.09	2.34	2.19
15	0.73	1.48	2.04	2.26	1.98
10	0.20	1.10	1.72	1.90	1.49

**Fig. S9.** Growth rates ( $\mu$ ,  $d^{-1}$ ) of *Chaetoceros tenuissimus* cultures, strains NIES-3714 (A) and NIES-3715 (B), as functions of temperature and salinity. Growth rates were calculated using the cubic equations of the host strains NIES-3714 and NIES-3715 for each condition (Tomaru et al., 2014) and are shown in each cell. Highlighted black-, dark grey-, light grey-, and white-coloured cells show that the growth rate was  $>2.0$ ,  $>1.5$ ,  $>1.0$ , and  $<1.0$ , respectively.

The cubic equations for each strain are as follows:

$$\text{NIES-3714: (divisions } d^{-1}) = -5.705439 + 0.019532T^2 + -0.000392T^3 + 0.714763S + -0.021578S^2 + 0.000191S^3 + -0.014554TS + 0.000284TS^2$$

$$\text{NIES-3715: (divisions } d^{-1}) = -7.673658 + 0.489995T + -0.000301T^3 + 0.658279S + -0.017466S^2 + 0.000209S^3 + -0.022216TS + 0.000541T^2S$$

T: Water temperature

S: Salinity

**A** CtenDNAV type-I vs CtenRNAV type-I

Temp. Salinity	10 °C	15 °C	20 °C	25 °C	30 °C
35	<0.01	0.03	<0.01	<0.01	<0.01
30	0.14	<0.01	0.10	<0.01	<0.01
25	0.07	0.01	0.09	0.01	0.01
20	0.02	<0.01	0.03	0.08	<0.01
15	0.07	0.04	0.01	0.01	<0.01
10	0.56	0.30	0.02	0.01	0.20

**B** CtenDNAV type-II vs CtenRNAV type-II

Temp. Salinity	10 °C	15 °C	20 °C	25 °C	30 °C
35	0.27	0.27	0.40	0.03	0.01
30	0.02	0.03	0.31	<0.01	<0.01
25	<0.01	0.03	0.13	0.04	0.02
20	<0.01	0.75	<0.01	0.06	0.01
15	<0.01	0.06	0.04	<0.01	0.06
10	0.22	0.23	0.99	<0.01	0.27

**Fig. S10.** Comparisons of  $CD_{50}$  values of each virus under each temperature and salinity combination. To analyse differences in the  $CR_{50}$  values at each temperature and salinity combination between CtenDNAV type-I and CtenRNAV type-I and between CtenDNAV type-II and CtenRNAV type-II, Student's *t*-test was used. P-values are shown on each cell, (A) CtenDNAV type-I vs. CtenRNAV type-I and (B) CtenDNAV type-II vs. CtenRNAV type-II. Red- and blue-coloured cells indicate that the  $CD_{50}$  values of DNA and RNA viruses were lower than those of the other, respectively. Highlighted dark-, normal-m and light-coloured cells show P-values <0.01, <0.05, and >0.05, respectively. For example, highlighted dark-red coloured boxes indicate that DNA viruses have advantages in terms of replication and dominance at specific water temperature and salinity conditions relative to the RNA viruses.

## LITERATURE CITED

- 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
- 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
- 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