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Stable coexistence in marine algal host-virus systems

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ABSTRACT: All microalgal host-virus systems isolated to date are lytic: the viruses lyse the infected hosts within hours after infection. Moreover, current models of phytoplankton host-virus interactions predict rapid extinction of both host and virus. Nevertheless, marine phytoplankton and their respective viruses do coexist in marine ecosystems. To investigate this apparent paradox we performed a series of experiments which show that phytoplankton populations always recover after virus-induced lysis and that endemic viral infections may promote survival of the host population. We hypothesize that phenotypic plasticity of algal susceptibility to viral infection makes such coexistence of host and virus possible.

KEY WORDS: Phytoplankton · Marine viruses · Coexistence · Viral ecology

INTRODUCTION

Viruses infecting, killing and lysing phytoplankton are ubiquitous in marine ecosystems. They are recognized as active members of marine plankton communities, and they influence many important biogeochemical and ecological processes (Fuhrman 1999, Suttle 2000, Wommack & Colwell 2000). Several case studies have reported that viruses are involved in termination of algal blooms (Bratbak et al. 1993, 1995, Nagasaki et al. 1995, Nagasaki & Yamaguchi 1997, Castberg et al. 2001), but they may also control algal populations by keeping them at non-blooming levels (Zingone et al. 1999, Larsen et al. 2001).

All phytoplankton host-virus systems isolated to date are lytic (Mayer & Taylor 1979, van Etten & Meints 1999, Sandaa et al. 2001) and current models, which are based on our present conception of these systems (Bratbak et al. 1998), suggest rapid extinction of both the alga and its virulent virus. However, Zingone et al. (1999) reported that both *Micromonas pusilla* and viruses infecting this phytoplankter could be found together in water samples collected from the Gulf of Naples (Mediterranean Sea) during all seasons. Similar observations were made by Tarutani et al. (2000) who reported that *Heterosigma akashiwo* persisted in Hiroshima Bay together with its virus. The explanation proposed by Tarutani et al. (2000) was that host and virus persisted due to clonal successions of specific host-virus systems. Although this may explain the persistence of a species over short time periods, extinction of host clones should still occur if the model is assumed to be also valid for clonal host-virus systems. The solution could be that virus-resistant clones, and corresponding host-range virus mutants (Waters & Chan 1982), evolve and extinguish at high rates. However, severe mutational constraints makes such an 'arms race' between host defenses and counter-defenses of the parasite unlikely as an ecological regulating mechanism (Lenski 1984, Lenski & Levin 1985).

The aim of this study was to examine the recovery of algal cultures following viral infection and to evaluate the ecological implications of this process. Viruses depend on available host organisms for reproduction implying that both viruses and algae share a common interest in avoiding host extinction. We demonstrate here that coexistence of algae and viruses is possible, and we attribute this to an efficient feedback mechanism that reduces the infection rate when the host abundance becomes low due to viral lysis.

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MATERIALS AND METHODS

Cultures. The algal species used in this study were *Phaeocystis pouchetii* (Hariot) Lagerheim (Prymnesiophyceae) strain AJ01, *Pyramimonas orientalis* McFadden, Hill & Wetherby (Prasinophyceae) strain IFM, *Chrysochromulina ericina* Parke et Manton (Prymnesiophyceae) strain IFM and *Emiliania huxleyi* (Lohmann) Hay et Mohler (Prymnesiophyceae) strain BOF92 obtained from the culture collection at the Department of Fisheries and Marine Biology, University of Bergen, Norway. The respective viruses used were the *Phaeocystis pouchetii* virus PpV-01 (Jacobsen et al. 1996), *Pyramimonas orientalis* virus PoV-01B (Sandaa et al. 2001), *Chrysochromulina ericina* virus CeV-01B (Sandaa et al. 2001) and *Emiliania huxleyi* virus EhV-99B1 (Castberg et al. 2002). The algae were grown in f/2 -Si medium (Guillard 1975), based on aged and autoclaved seawater (N:P ratio 24:1). Algal cultures were grown at a L:D cycle of 16:8 h at 15°C, except *P. pouchetii* which was grown at 8°C. The light intensity applied was 40 to 50 (mol quanta m^{-2} s^{-1}.

Regrowth experiments. Exponentially growing cultures (100 ml) of each algal species were inoculated in triplicate with 4 different viral concentrations: 3×10^4, 3×10^5, 3×10^6, and 10^7 ml^{-1} (final concentration). The host concentration was in all cases ca. 10^5 ml^{-1}. Samples for enumeration of algae and viruses were collected at regular intervals for 4 to 7 wk.

To test the infectivity of the viruses, the hosts' resistance to viral infection, and if viral production continued in the recovered cultures, we infected fresh algal cultures and initiated test experiments when the cultures were in the recovery phase. For the infectivity test, we centrifuged 10 ml of each algal culture for 10 min at 5000 × *g* to remove most of the algae and debris, filtered the supernatants through 0.2 µm-pore size syringe filters (Schleicher & Schuell) and added 5 ml of the filtrates to 50 ml of exponentially growing host cultures. Samples for enumeration of algae and viruses were collected every 2 d for 12 d. The susceptibility of the recovered host algae to viral infection was tested by adding 10 ml stock virus lysate to recovered cultures in exponential growth. Samples for enumeration of algae and viruses were collected every 2 d for 12 d. To test if the recovered host algae continued to produce viruses, 10 ml of recovered cultures in exponential growth phase were diluted in 90 ml of fresh f/2 medium. Samples for enumeration of algae and viruses were collected at regular intervals for 20 to 22 d.

Inhibition experiment. The possible effect of filterable cues (i.e. any molecule or particle that passes through a 0.02 µm filter and inhibits viral production) on viral lysis was examined by adding virus-free lysate (VFL) to cultures prior to addition of the respective virus. Virus-free lysate was obtained by filtration of stock virus lysate (VL) through 0.02 µm anodisc filters (Millipore). VL and VFL were added to exponentially growing algal cultures in duplicate as follows: (1) 10% v/v VFL and, 10 min later, VL to a total viral concentration of 10^7 ml^{-1}; (2) 10% v/v VFL and, 10 min later, VL to a total viral concentration of 10^5 ml^{-1}; (3) VL to a total viral concentration of 10^7 ml^{-1} and 10% v/v fresh medium; (4) VL to a total viral concentration of 10^5 ml^{-1} and 10% v/v fresh medium. Samples for enumeration of algae and viruses were collected at regular intervals for 7 to 9 d.

Long-term infection experiment. To investigate the long-term stability of the host-virus interaction in the

Table 1. Equations, parameters and initial values used to simulate *Phaeocystis pouchetii*, PpV and inhibitor concentration over the first 300 d after initiation of agal recovery following viral lysis (see Fig. 7). a.u.: arbitrary units

Differential equations and initial values

Uninfected hosts: A

$$\frac{dA}{dt} = \mu A - \frac{S_1}{1+I} S_2 \alpha V A \qquad A_0 = 1 \times 10^3 \text{ cells ml}^{-1}$$

Viruses: V

$$\frac{dV}{dt} = m \frac{S_1}{1+I(t-\tau)} S_2 \alpha V(t-\tau) A(t-\tau) - \frac{S_1}{1+I} \alpha V A - \lambda_V V \qquad V_0 = 1 \times 10^8 \text{ ml}^{-1}$$

Inhibitor: I

$$\frac{dI}{dt} = k \frac{S_1}{1+I(t-\tau)} S_2 \alpha V(t-\tau) A(t-\tau) - \lambda_I I \qquad I_0 = 100 \text{ a.u.}$$

Parameters

Symbol	Values	Unit	Explanation
μ	0.015	h^{-1}	Algal specific growth rate
m	400	virus $cell^{-1}$	Virus released per lytic event (burst size)
τ	15	h	Time between infection and lysis of a cell (lytic cycle)
α	1.7×10^{-6}	ml $host^{-1}$ h^{-1}	Viral adsorption coefficient
S_1	0.05	Dimensionless	Fraction of collisions that lead to adsorption in the absence of inhibitor
S_2	0.07	Dimensionless	Fraction of adsorptions that lead to infection
λ_V	0.005	h^{-1}	Viral decay rate
λ_I	0.005	h^{-1}	Inhibitor decay rate
k	1, 2.5, 5, 10, 15, 20×10^{-5}	a.u.	Inhibitor released per lytic event

recovered cultures duplicate, cultures (1 l) of exponentially growing *Phaeocystis pouchetii* (2.5×10^5 ml^{-1}) were inoculated with 100 ml fresh lysate to a concentration of 1.5×10^6 virus particles ml^{-1}, and the evolution of *P. pouchetii*, PpV and bacteria were followed. Uninfected *P. pouchetii* cultures (duplicate) were used as controls. Samples for enumeration of *P. pouchetii*, PpV and bacteria were taken at regular intervals for ca. 1 yr.

Enumeration of algae, virus and bacteria. All analyses were performed with a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm and with the standard filter set-up. For enumeration of algal cells, fresh samples were run for 1 to 2 min at a delivery rate of ca. 30 µl min^{-1} with the discriminator set on red fluorescence. Viral and bacterial counts were performed on fixed samples (0,5% glutaraldehyde, final concentration) diluted from 1:10 to 1:1000 in TE-buffer (10 mM Tris, 1 mM EDTA, pH = 8) and stained for 10 to 15 min at 80°C with SYBRGreen-I (Molecular Probes) at a final concentration of 10^{-4} of the commercial solution (Marie et al. 1999). The samples were analyzed for 1 min at a flow rate of ca. 30 µl min^{-1} with the discriminator set on green fluorescence as described in Marie et al. (1999) and Brussaard et al. (2000).

Nutrient analysis. To test if cultures were phosphate-limited, the concentration of soluble reactive phosphorus (SRP) was measured according to Koroleff (1976).

MODEL

In current models of phytoplankton host-virus interaction, viral production depends on the probability S_1 that a virus-host collision will lead to adsorption of the virus (Bratbak et al. 1998). We modify this probability by introducing the expression:

$$\frac{S_1}{1+I}$$

where I is an inhibiting factor given in arbitrary units (a.u.) so that at an a.u. of 1 the probability is reduced to 50% of its value when no inhibitor is present (S_1). A fixed amount k of inhibitor is assumed to be produced per lytic event, and the inhibitor decay is assumed to be a first-order process with fixed rate constant (λ_I). The equations, parameters and initial values used for simulation are given in Table 1. The model was formulated in the Stella programming package running under Windows 95. A Runge-Kutta second-order procedure with time step 1 h was used.

RESULTS

All infected cultures recovered after viral lysis (Fig. 1). Within a few days after addition of the respective virus, algal abundances decreased and reached minimum densities within 1 to 2 wk. In the same period, the viral abundances increased and reached densities of 10^8 to 10^9 ml^{-1}. Following this lysis period, the number of algae grew exponentially until they entered a stationary phase, while the viral populations remained stable compared to the algae. The recovery generally occurred earlier when higher initial virus-to-host ratios were applied. This effect was most obvious in the *Emiliania huxleyi* cultures (Fig. 1A; sign test using all values in the regrowth phase until onset of the stationary phase, $p < 0.01$), but the difference was also significant in the *Pyramimonas orientalis* and *Phaeocystis pouchetii* cultures (Fig. 1C,D; sign test, $p < 0.01$ for both). The

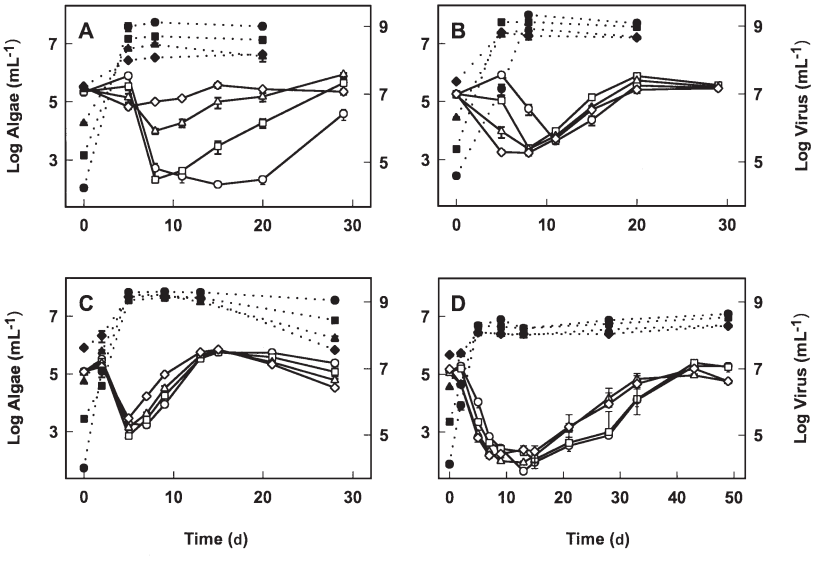

Fig. 1. Abundances of algae (○) and viruses (●) after addition of the respective virus at Day 0. (A) *Emiliania huxleyi* and EhV-99B1, (B) *Chrysochromulina ericina* and CeV-01B, (C) *Pyramimonas orientalis* and PoV-01B, (D) *Phaeocystis pouchetii* and PpV-01. Four different initial virus:host ratios were applied, namely 100 (◆, ◇), 10 (▲, △), 1 (■, □) and 0.25 (●, ○). Symbols represent average of 3 replicates. Error bars: SE

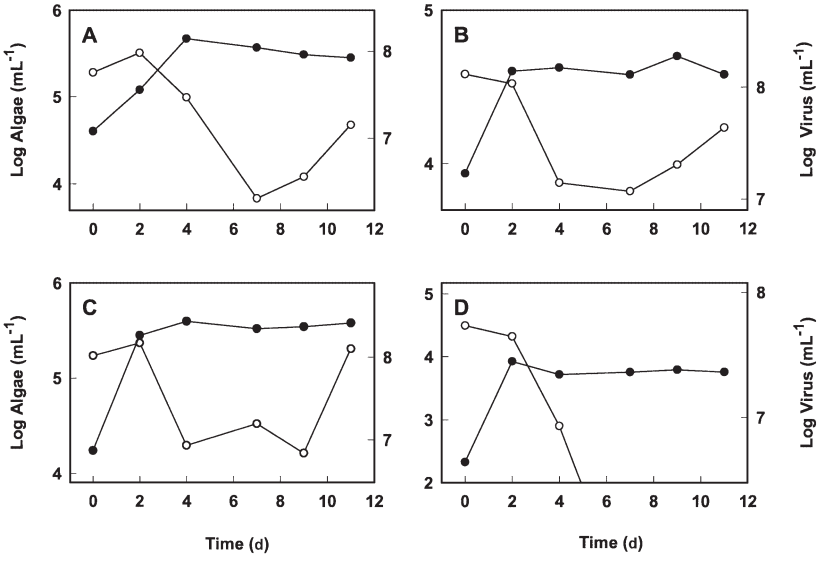

Fig. 2. Abundances of algae (○) and viruses (●) after addition of the respective virus from recovered cultures to stock algal cultures. (A) *Emiliania huxleyi* and EhV-99B1, (B) *Chrysochromulina ericina* and CeV-01B, (C) *Pyramimonas orientalis* and PoV-01B, (D) *Phaeocystis pouchetii* and PpV-01

Chrysochromulina ericina cultures did not lyse at the same time (Fig. 1B) and the onset of the recovery phase cannot therefore be compared for this species.

When viruses from recovered cultures were added to exponentially growing host cultures >80% of the host populations were lysed within 1 wk, and a concomitant 6- to 30-fold increase in the viral abundance was detected (Fig. 2). In contrast, no abrupt lysis was detected when viruses from fresh lysates were added to recovered cultures (Fig. 3). All cultures showed net growth during the experimental period of 10 d, although the concentration of *Chrysochromulina ericina* decreased at the end of the period. The viral abundance increased relatively slowly in all cultures except the *Pyramimonas orientalis* culture, where the viral abundance slowly decreased. Fig. 4 shows the development of algae and viruses in recovered cultures diluted 10× in fresh medium. Net increases in both algal and viral abundance were detected in all cultures except the *P. orientalis* culture, in which both increased over the first 13 d and then decreased until the end of the experiment.

Treating the cultures with virus-free lysate prior to addition of the respective virus delayed and/or reduced the extent of cell lysis in the cultures (Fig. 5). This effect was most pronounced in the *Emiliania huxleyi* cultures (Fig. 5A), where all treated cultures lysed later than non-treated cultures (sign test using all values except $t = 0$, $p < 0.01$). The minimum algal abundances were ca. 100× higher in treated cultures inoculated with 10^7 virus ml^{-1} compared to the untreated cultures, which represented the other extreme. However, even in the cultures with the lowest level of lysis an 85% decrease in algal abundance was observed.

For the other 3 species (Fig. 5B–D) delayed lysis was observed in the cultures treated with virus-free lysate when the initial viral concentration was low (10^5 virus ml^{-1}) (sign test using all values except $t = 0$, $p < 0.05$). When the initial virus concentration was high (10^7 virus ml^{-1}), however, a significant delay was only observed in the *Phaeocystis pouchetii* cultures (sign test using all values except $t = 0$: $p < 0.05$). We observed no differences in the extent of cell lysis in any of these cultures.

The results from the long-term incubation of infected and non-infected cultures of *Phaeocystis pouchetii* are shown in Fig. 6. The uninfected control cultures (Fig. 6A) showed exponential growth ($\mu = 0.68$ d^{-1}) until *P. pouchetii* approached 10^6 ml^{-1}. This was fol-

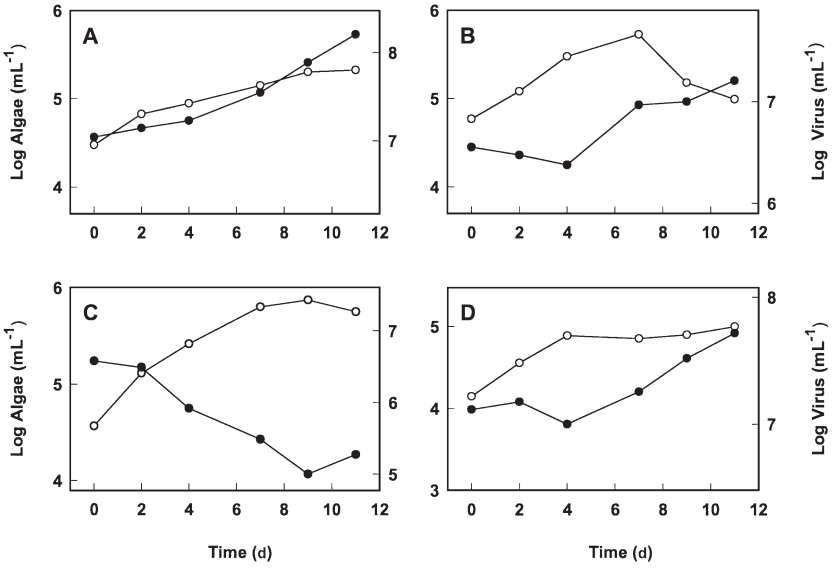

Fig. 3. Abundances of algae (○) and viruses (●) after addition of the respective virus from stock lysates to recovered algal cultures. (A) *Emiliania huxleyi* and EhV-99B1, (B) *Chrysochromulina ericina* and CeV-01B, (C) *Pyramimonas orientalis* and PoV-01B, (D) *Phaeocystis pouchetii* and PpV-01

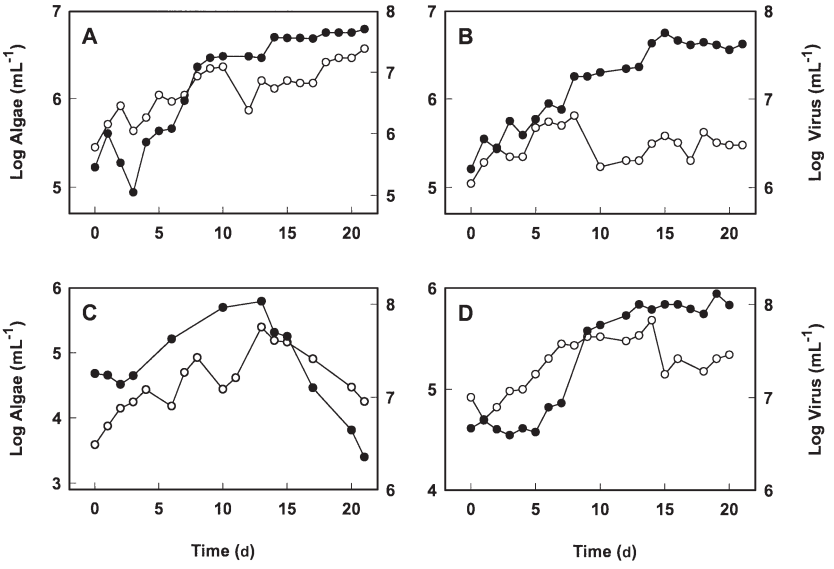

Fig. 4. Abundances of algae (○) and viruses (●) after 10× dilution of recovered cultures in fresh medium. (A) *Emiliania huxleyi* and EhV-99B1, (B) *Chrysochromulina ericina* and CeV-01B01, (C) *Pyramimonas orientalis* and PoV-01B, (D) *Phaeocystis pouchetii* and PpV-01

lowed by a stationary phase lasting a few days and then a rapid decline in algal abundance. *P. pouchetii* could not be detected after 42 d. The bacterial abundance was stable at 1.5×10^6 ml^{-1} until the stationary phase of *P. pouchetii* occurred. At this point, a phase of exponential growth began and continued until the bacterial concentration reached 1.7×10^8 ml^{-1}. Slow bacterial decline followed, which lasted throughout the remaining experimental period. PpV or any other virus infecting *P. pouchetii* was not observed in the non-infected cultures. Ten d after addition of the virus to the infected cultures the abundance of algae was reduced from 2.5×10^5 to ca. 1000 cells ml^{-1}. At the same time, the viral and bacterial concentrations increased from 1.5×10^6 to 2.1×10^8 ml^{-1} and from 1.5×10^6 to 1.1×10^8 ml^{-1}, respectively. This pattern was similar to the lysis phase of infected *P. pouchetii* cultures shown in Fig. 1. Fig. 6B shows the abundance of *P. pouchetii*, PpV and bacteria following initiation of the recovery phase. *P. pouchetii* grew exponentially ($\mu = 0.33$ d^{-1}) until it approached a density of 10^5 ml^{-1}. Then, oscillations in algal abundance with large amplitudes occurred over the next 100 to 150 d, before it stabilized at 10^4 to 10^5 ml^{-1} for the remaining experimental period. The maximum and minimum concentrations of algae were ca. 10^5 and 10^3 ml^{-1}, respectively. In the same period, PpV and bacteria underwent minor oscillations, compared to *P. pouchetii*.

Soluble reactive phosphorous (SRP) was measured in both infected and non-infected cultures 52 d after the beginning of the experiment. In the control cultures, the SRP concentrations were below the detection limit of approximately 0.02 µM, while the infected cultures had SRP concentrations of 6.5 ± 0.5 µM.

The parameters and equations in Table 1 were used as input to the simulaton model. The effect of the amount of inhibitor produced (k) on the stability properties of the system is illustrated in Fig. 7. For the model conditions defined: $k = 1 \times 10^{-5}$ a.u. per lytic event gives oscillations that increase in amplitude for the 300 d period simulated; $k = 2.5 \times 10^{-5}$ a.u. gives stable oscillations; k = 5, 10 and 15×10^{-5} a.u. give increasingly damped oscillations towards steady states; for $k = 20 \times 10^{-5}$ a.u. the inhibitor production is so large that viruses never manage to reduce the algal

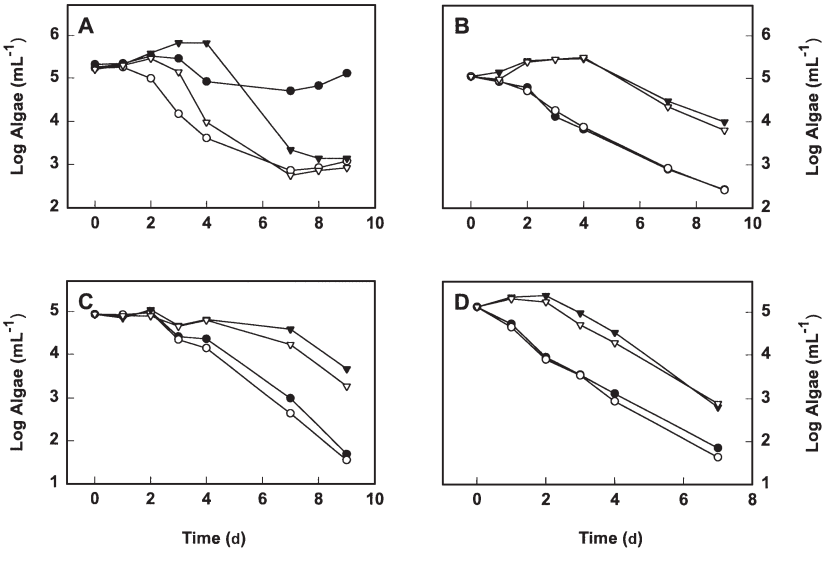

Fig. 5. Algal abundances in cultures with (●, ▼) or without (○, ▽) treatment for 10 min with 10% v/v virus-free lysate before addition of the respective virus. (A) *Emiliania huxleyi* and EhV-99B1, (B) *Chrysochromulina ericina* and CeV-01B, (C) *Pyramimonas orientalis* and PoV-01B, (D) *Phaeocystis pouchetii* and PpV-01. Initial viral concentrations 10^7 ml^{-1} (●, ○) and 10^5 ml^{-1} (▼, ▽) were applied. Symbols represent average of duplicate cultures

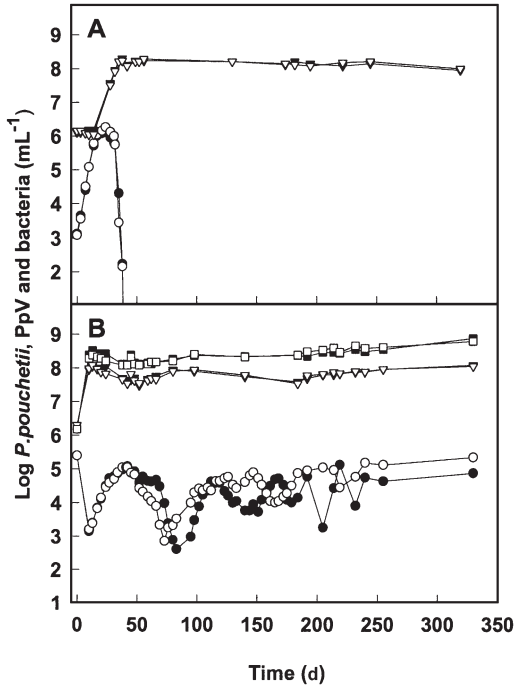

Fig. 6. Abundances of *Phaeocystis pouchetii* (●, ○), bacteria (▼, ▽) and PpV-01 (■, □) in (A) uninfected and (B) recovered cultures of *P. pouchetii*. Open and closed symbols represent duplicate cultures. PpV was never observed in uninfected cultures

population. Host, virus, and inhibitor concentrations then continue to grow until halted by some other limiting condition, not included in this model (Fig. 7).

DISCUSSION

Effect of viral inoculum on lysis and recovery

A striking pattern in our results is that addition of more virus generally resulted in delayed host lysis and in faster host recovery (Fig. 1). This effect was most pronounced in the *Emiliania huxleyi* cultures, but it was also observed in the *Pyramimonas orientalis* and the *Phaeocystis pouchetii* cultures. In the *Chrysochromulina ericina* cultures the lysis period was considerably longer in the cultures that were inoculated with less virus. For the other 3 species, the time required for mass lysis was independent of viral inoculum, or the difference was less than the sampling intervals.

Viral infectivity, algal resistance and persistency of infection

All microalgal viruses isolated so far are described as lytic with no evidence of latent infections (Mayer & Taylor 1979, van Etten & Meints 1999, Sandaa et al. 2001, Castberg et al. 2002). Thus, we focused on explanations for the recovery that are in accordance with strictly lytic host-virus systems, although the possibility of latent infections cannot be completely excluded.

A current mathematical model for lytic algal host-virus interactions suggests rapid algal extinction followed by slow viral decay (Bratbak et al. 1998). This model assumes that the virus and its host are phenotypically stable in terms of decay rates, burst sizes, diffusion coefficients, absorption coefficients, latent periods, infectivity, and growth rates. A change in 1 or more of these parameters may alter the kinetics of the host-virus interaction, and recovery of the host algal population is then 1 possible outcome. The observations made in this study—that viruses from recovered algal cultures and viruses from the original stocks had the same

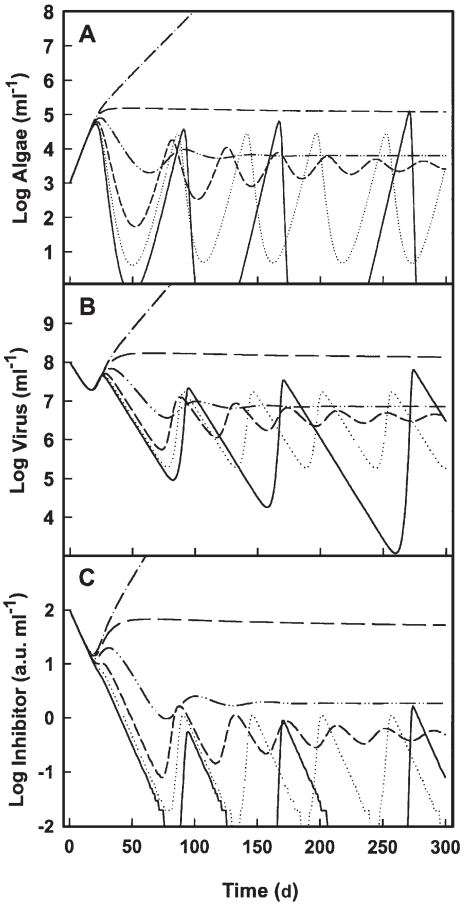

Fig. 7. Simulation of (A) *Phaeocystis pouchetii*, (B) PpV, and (C) inhibitor concentration (k) for the first 300 d after initiation of algal recovery following viral lysis. Simulation is given for 6 different values of k (——: 1×10^{-5}, ...: 2.5×10^{-5}, – – –: 5×10^{-5}, — ·· –: 10×10^{-5}, — –: 15×10^{-5}, — · —: 20×10^{-5} arbitrary units [a.u.])

effect when added to fresh algal cultures—indicate that there is no phenotypic change in the viruses (Fig. 2). The alternative hypothesis is that the hosts are phenotypically plastic and develop some kind of resistance to viral infection, which allows the cultures to recover after the initial virus attack. Fig. 3 suggests that this was indeed the case, as re-inoculation of cultures in the recovery phase with fresh virus did not result in rapid lysis with concomitant viral increase. Instead, the typical pattern was slow viral accumulation simultaneously with algal growth, indicating that the infection rate (and the resulting lysis rate) was lower than the growth rate. In the *Pyramimonas orientalis* culture, the viral abundance decreased over the first 9 d before it increased. This does not necessarily mean that no viral production occurred, but rather that the sum of decay and adsorption rate was higher than the production rate.

When recovered cultures were diluted in fresh medium the abundance of both algae and viruses increased in all cultures during the incubation period of 3 wk (Fig. 4). This shows that algal hosts and their infective viruses are able to coexist at high abundances in the same culture, and that they can grow simultaneously. This occurred in all cultures, with the exception of the *Pyramimonas orientalis* culture in which PoV declined after an increase in the first 2 wk. This may indicate that PoV particles are more unstable than the other virus particles examined in this study, or that a lower susceptibility of *P. orientalis* to viral infection causes a net decrease in viral production.

Inhibiting effect of filtered lysate

Mass lysis was delayed and also, in some cases (e.g. *Emiliania huxleyi* cultures), the extent of cell lysis was reduced when the algal cultures were treated with virus-free lysate for 10 min before addition of the respective virus. This effect may be explained by a mechanism similar to that allowing survival of phage-infected *Shigella dysenteriae* (Li et al. 1961). Clones of *S. dysenteriae* carrying phage T7 in a pseudolysogenic relationship were shown to change phenotype from Lac^- $Mann^-$ to Lac^+ $Mann^+$, and this change was attributed to an alteration of surface structures by phage-associated endolysin. Li et al. (1961) suggested that endolysin also removed phage receptors from the cell surface that rendered the remaining host cells resistant to infection; thus, a long-term association between host and parasite could be established without involving true lysogeny. In terms of our results, endolysin may act as a non-specific inhibitor to viral infection, and more specific inhibitors may facilitate recovery and stable coexistence. Infected cells produce an excess of viral molecules during viral reproduction, and these are released when the cells lyse (Joset & Guespin-Michel 1993). Thus, viral host recognition molecules may be released during cell lysis and compete with complete virus particles for receptor sites on the host cell surface, thereby reducing the fraction of collisions that results in adsorption.

Both specific and non-specific inhibitors may act as cues, giving information about viral activity. The resulting effect would be an inverse co-variation between viral absorption rate and viral abundance, fitting with the reported undetectable absorption of MpV in recovered *Micromonas pusilla* cultures (Suttle 2000). As more cells lyse the concentration of inhibitors would increase, the infection rate would decrease and algal extinction would be avoided. Net algal growth occurs when the infection rate becomes slower than the host's specific growth rate, and viral abundance decreases when the viral decay rate exceeds production. The inhibitors may be destroyed (e.g. by bacteria or chemically) and when the decay rate of inhibitors exceeds the production rate (proportional to viral production rate and algal lysis rate), the specific absorption rate would again begin to rise. Inhibitors would thus be a stabilizing factor that makes coexistence of algae and viruses more likely.

A stable coexistence was established for all host-virus systems but the effect of adding filtered lysate was pronounced only in the *Emiliania huxleyi* cultures. This suggests that mechanisms other than filterable inhibitors may have been involved. One such mechanism, which would be removed by filtration, could be defective interfering particles which are mutant viruses with partially deleted genomes that require a coinfection with the wild-type virus to be able to replicate within host cells (Roux et al. 1991, Frank 2002). Defective interfering particles would inhibit the production of normal wild-type viruses, and the rate of lytic infections would decrease over time.

If cells are susceptible to viral infection only during certain life stages, this may contribute to a stabilization of host-virus dynamics (Lenski 1988a). Thyrhaug et al. (2002) showed that the viral production in *Pyramimonas orientalis* cultures infected with PoV depend on the timing of infection in relation to the host's cell. According to Lenski (1988a), this constraint may have contributed to a stable host-virus interaction in recovered cultures. However, modelling indicates that this constraint is not enough to stabilize the host-virus interaction in systems characterized by low growth rate, high burst size and long infection cycles, and this mechanism is thus probably of less significance in the systems studied here (modelling results not shown). Moreover, no such cell-cycle-dependent infection was found for *Phaeocystis pouchetii* infected with PpV (Thyrhaug et al. 2002), and the mechanism appears thus not to apply to all host-virus systems.

Ecological implications

The possible effect of such a feedback mechanism is demonstrated in the population dynamics in infected and non-infected batch cultures of *Phaeocystis pouchetii*. Prolonged (at least 1 yr) coexistence of *P. pouchetii* and its virus PpV occurred in the infected cultures while the algae died off and disappeared within a few weeks in the cultures without PpV. Feedback may prevent the algae from extinction, while regeneration of nutrients caused by algal lysis prevents nutrient depletion. The modified model presented here illustrates that phenotypic plasticity of the host's susceptibility to infection may be important as a stabilizing factor in host-virus interactions. The inhibitor inserted in the model represents all kinds of decayable mechanisms that reduce the frequency of collisions which lead to adsorption. Thus, endolysin and other inhibitors that may be released when cells lyse potentially play a significant role in avoiding extinction of infected phytoplankton populations.

It is also noteworthy that the microbial community (*Phaeocystis pouchetii*, bacteria and bacteriophages) in uninfected cultures is not capable of efficient enough recycling to support the alga with nutrients: viral lysis of the alga by PpV is also required. Being infected may thus be an advantage for phytoplankton populations, as viral lysis yields nutrients that are available for all osmotrophs. The phytoplankton population experiences a continuous input of regenerated nutrients instead of being met with depletion.

Although the inhibiting effect of filtered lysate was significant in most of the algal host-virus systems, the effect in the *Emiliania huxleyi* cultures was much more pronounced than in the other host-virus systems. This may be attributed to a higher release of inhibiting factors when *E. huxleyi* cells lyse and/or to greater stability of these factors in these cultures. The stability of viral particles may vary between the cultures: both the initial recovery experiment and the dilution experiment suggest that PoV particles are more unstable than the other viruses examined, or that the decrease in susceptibility to infection was more efficient in *Pyramimonas orientalis* cultures. The degree of inhibiting effects, stability of these effects and stability of the viruses are all factors that may contribute to the regulation of host-virus dynamics in natural waters.

Parasites should evolve reduced virulence to their hosts because the most successful parasites are those that do the least harm to their hosts (Lenski & May 1994). Another hypothesis is that greater virulence is favoured when the host density is high, while reduced virulence is favoured when the host density is low (Ewald 1983, Lenski 1988b). The feedback mechanism in the algal host-virus systems presented here resembles the latter hypothesis, although it implies a mechanism corresponding to phenotypic plasticity in host susceptibility to infection rather than genetic evolution. When the host concentration is low, due to viral lysis, the concentration of inhibitor will be high and the probability of successful infections will be low (corresponding to low virulence). Likewise, when the host concentration is high, the concentration of inhibitor will be low and the probability of successful infections will be high (corresponding to high virulence). Over short time-scales, such as the duration of an algal bloom, this mechanism of regulating the level of infection is rapid and powerful, and may play an ecologically significant role.

Several mechanisms have been proposed to explain how microbial predators and parasites can coexists with their preys and hosts. These include interactions among predators, predators under biological control, genetic feedback, refuge, switching, density dependence and replication to compensate for killing (Alexander 1981). Conceptually close to the mechanism proposed here, are mechanisms reducing the strength of the predator-prey interaction when predation is severe (Hessen & VanDonk 1993). Until now, none of these have been shown to be important in regulating algal host-virus interactions, but the feedback response described in the present study may be interpreted as a special case of replication to compensate for killing. The dynamic feedback response allows coexistence of algae and viruses at both high and low densities and may therefore explain why viruses infecting both bloom-forming (Milligan & Cosper 1994, Jacobsen et al. 1996, Nagasaki & Yamaguchi 1997) and non-bloom-forming phytoplankton species (Mayer & Taylor 1979, Sandaa et al. 2001) can be readily found in natural waters. Moreover, speculations about whether parasites can be of benefit to their hosts have been raised (Palmieri 1982), and our results show that an endemic viral infection in a phytoplankton population may indeed be an ecological advantage to the host.

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