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The effect of host *Chlorella NC64A* carbon : phosphorus ratio on the production of *Paramecium bursaria Chlorella Virus-1*

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SUMMARY

1. We used the freshwater alga *Chlorella NC64A* (Division Chlorophyta) and its virus *Paramecium bursaria Chlorella virus-1* (PBCV-1) as a model system to test for potential stoichiometric constraints on a virus–host interaction.
2. Media phosphorus concentrations were manipulated to create *Chlorella NC64A* host cells with low (91 ± 23) or high (453 ± 246) C : P ratio. In contrast, the C : P ratio of PBCV-1, calculated from its biochemical composition, was 17 : 1.
3. Stoichiometric theory predicts that infection success and postinfection viral production should be depressed in high C : P cultures due to insufficient intracellular P for production of P-rich viral particles.
4. Consistent with this hypothesis, viral production was strongly affected by host C : P ratio. While host C : P ratio did not affect viral attachment or the percentage of new viral particles that were infectious, in the low C : P *Chlorella NC64A* treatment, nine times more viruses were produced per infected cell than in the high C : P treatment (158 ± 138 versus 18 ± 18), indicating that the low C : P cells were higher quality for PBCV-1 proliferation.
5. This result implies that the stoichiometric quality of algal cells can have a major effect on host–virus population dynamics.

Keywords: *Chlorella NC64A*, ecological stoichiometry, *Paramecium bursaria Chlorella virus-1*, phosphorus, viral ecology

Introduction

Ecological stoichiometry (ES) is the study of the balance of multiple chemical substances in ecological interactions and processes (Sterner & Elser, 2002). It treats trophic interactions as composite chemical reactions subject to the law of conservation of matter and the general constraints of stoichiometric combination. ES predicts that when consumers ingest food with an elemental composition that differs drastically from their own body composition, the consumer must compensate for these differences. Compensation includes metabolic adjustments to dispose of elements in excess and retain those is short supply (Sterner & Elser, 2002). Ultimately, these compensation mechanisms alter the processing efficiencies of key nutrients and affect consumer growth, reproduction and population dynamics.

Considerable evidence has accumulated to support ES in typical predator–prey interactions, particularly between zooplankton and phytoplankton (see Sterner & Elser, 2002 and references therein) but also between plants and insects (Elser *et al.*, 2000; Frost & Elser, 2002; Woods *et al.*, 2002). In a classic example, the growth of the crustacean zooplankter *Daphnia*, which is strongly homeostatic in its elemental composition, is negatively affected by poor quality food with high carbon : phosphorus (C : P) ratio. Sterner (1993) fed daphniids equal quantities of either low C : P (C : P = 153 : 1, all ratios atomic) or high C : P

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(C : P = 2266 : 1) *Scenedesmus acutus* var. *obliqous* (Rabenhorst). The average growth rate of the daphniids fed the high C : P phytoplankton was significantly less then those fed the low C : P food (0.1 day^{-1} versus 0.45 day^{-1}). Additionally, *Daphnia* reproduction was greatly reduced in the individuals fed high C : P algae. While much experimental work on stoichiometric effects on zooplankton production has been performed (Sterner & Schulz, 1998) very little is known about whether similar effects occur when the predator consuming the prey is a virus, rather then a metazoan.

We propose that stoichiometric theory can be extended to host–virus interactions because, like zooplankton, viruses are relatively homeostatic in composition and must process host biomass during replication. The simple structure of viruses (a genome and a protein capsid) prohibits much variation in their composition, including elemental composition. For example, the substitution of one nucleotide can completely inhibit replication (Kang, Dunigan & Van Etten, 2005) and there is no apparent mechanism for element or molecule storage in viruses. Although viruses are not technically 'predators', because they do not literally 'consume' their host cells, we would argue that, despite very different mechanisms, both herbivory and viral infections must obey the simple rules of mass conservation and there is likely to be only a limited supply of key resources needed by the virus inside a host. Viruses are abundant components of aquatic ecosystems (Suttle, 2005) but viral ecology is still in its infancy and virus–host interactions have not been analysed within a formal stoichiometric framework. Therefore, we designed a set of experiments to test whether the predictions of stoichiometric theory are supported in the context of host–virus interactions. Specifically we asked: does host C : P ratio affect viral infection success in the freshwater algae–virus model system of *Chlorella NC64A* and the virus, *Paramecium bursaria Chlorella virus-1* (hereafter PBCV-1)?

The infection cycle of PBCV-1 is similar to many bacteriophages. It attaches irreversibly to the external cell wall of *Chlorella NC64A* host cells. Following enzymatic digestion of the cell wall, viral DNA and associated molecules enter the host cell (Meints *et al*., 1984). Transcription of host DNA is inhibited almost immediately and early viral mRNAs are translated on cytoplasmic ribosomes to proteins necessary for viral DNA replication (Schuster *et al*., 1986). Replication is followed by the translation of late mRNAs into proteins used to assemble viral capsids (Meints & Van Etten, 1986). Finally, new viral particles are released via host cell lysis 4–10 h after the onset of infection (Van Etten *et al*., 1983a; Van Etten & Meints, 1999). Thus, a successful infection by PBCV-1 can be divided into three 'stages': (i) viral attachment, (ii) viral production, which in this case includes DNA replication, early and late protein production and viral assembly, and (iii) 'offspring fitness', which is the ability of the newly produced viral particles to cause infections.

In this study we assess how increasing the C : P ratio of *Chlorella NC64A* affects PBCV-1 infection success at each of the stages listed above. As stoichiometric constraints due to insufficient phosphorus supply are most likely to effect the production of phosphorus-rich viral particles, we predict that the effects will be greatest for viral production rather than attachment or infectivity of new viral particles.

Methods

Algae host–virus model system

Chlorella NC64A is a freshwater, unicellular, non-motile alga (Van Etten *et al*., 1983a; Van Etten, Lane & Meints, 1991; Van Etten & Meints, 1999). This green alga (Division Chlorophyta) is normally found in a symbiotic relationship with *Paramecium* spp. but has been cultured exosymbiotically for over 35 years (Van Etten *et al*., 1983a). *Paramecium bursaria Chlorella virus-1* (PBCV-1) is a large (190 nm in diameter), polyhedral virus in the family Phycodnaviridae (Van Etten & Meints, 1999). The virus weighs 1×10^9 Da and has a double-stranded DNA genome that is 333-kbp long (Yonker *et al*., 1985; Girton & Van Etten, 1987).

Chlorella NC64A culturing techniques

Chlorella NC64A cells were cultured in modified Bold's basal media (MBBM) as previously described in Van Etten *et al*. (1983b) and Van Etten *et al*. (1991). MBBM was supplemented with tetracycline (100 μg mL^{-1}) prior to *Chlorella NC64A* inoculation to inhibit bacterial growth. Cultures were grown in small (250 mL) flasks incubated at 25 °C with continuous light (30 $\mu E \cdot m^{-2}\ s^{-1}$) and shaking (150 rpm).

Concentrations of K_2HPO_4 and KH_2PO_4 in the alga media were manipulated to produce the two different C : P treatments. In the low C : P treatment (Low C : P hereafter), the growth medium had a phosphorus concentration of 50 000 μg P L^{-1}, while in the high C : P treatment (High C : P hereafter), the medium had a phosphorus concentration of 500 μg P L^{-1}. *Chlorella NC64A* cultures were acclimated to the different C : P treatments for >1 month before being used in any further experiments.

Chlorella NC64A elemental composition

Chlorella NC64A carbon (C) and nitrogen (N) contents were determined by filtration of a known culture volume onto pre-combusted glass fibre filters (GF/F) with a nominal pore-size of 0.7 μm. After drying at 60 °C, filter C and N contents were determined by a Perkin-Elmer model 2400 CHN analyser (Wellesley, MA, U.S.A.). *Chlorella NC64A* phosphorus (P) content was determined using the acid–molybdate technique after a persulphate digestion of cells on a P-free polycarbonate filter (A.P.H.A., 1992). All elemental ratios (C : P, N : P and C : N) are reported as molar ratios (±1 SE). *Chlorella NC64A* elemental ratios were determined in 18 replicate cultures of each C : P treatment. A *t*-test with an α-value of 0.05 was used to compare mean elemental ratios in the two treatments.

Chlorella NC64A growth rates

Chlorella NC64A growth in the two C : P treatments was analysed daily by counting cells with a CASY particle counter (Schaffe Systems, Inc., Reutlingen, Baden-Württemberg, Germany). The CASY system calculated *Chlorella NC64A* concentrations (cells mL^{-1}) by averaging four replicate counts of particle concentrations within a 2–8 μm size range. A repeated-measures analysis of variance (RM ANOVA) was used to compare *Chlorella NC64A* cell concentrations in the Low C : P and High C : P treatments. In addition, CASY counts were also used to compare daily *Chlorella NC64A* growth rates in the two C : P treatments using the following equation:

$$\text{Growth rate} = \ln(N_i/N_o)/(t)$$

where N_i and N_o are CASY counts (cells mL^{-1}) on consecutive days and $t = 1$ day. Daily growth rates in the Low C : P and High C : P cultures were calculated and compared using a *t*-test.

PBCV-1 elemental composition

The small size of viral particles makes elemental composition difficult to determine directly. Therefore, the C : N : P ratios of PBCV-1 were calculated from biochemical composition using published values of viral DNA, lipid and protein content (Skrdla *et al.*, 1984; Van Etten *et al.*, 1991; Van Etten & Meints, 1999) and the percentage of carbon, nitrogen and phosphorus in these major biomolecules. The C, N and P contents of proteins, phospholipids and the four DNA nucleotide bases were obtained from Sterner & Elser (2002). We calculated the C, N and P contents of glycoproteins, phosphoproteins and the methylated DNA nucleotide bases A and C from their molecular structures.

Viral abundance

Paramecium bursaria Chlorella virus-1 abundance was determined by plaque assays following Van Etten *et al.* (1983b) in which a series of plates were made with each plate representing a different dilution of the original sample (i.e. 1 : 10, 1 : 100, etc.). A solution composed of 100 μL of the diluted sample, 300 μL of concentrated (4.0×10^8 cells mL^{-1}) *Chlorella NC64A* cells and 2.5 mL of melted soft MBBM agar (0.75% agar) was laid over a MBBM plate (1.5% agar). The plate was then incubated inverted for 3–4 days before the plaques that formed on the lawn of host *Chlorella NC64A* were counted. As viruses are host specific, each plaque was assumed to represent one initial infecting virus in the sample (Gerba & Goyal, 1982). Therefore any other viruses (such as bacteriophages) present in the cultures would not be counted using the plaque assay technique because they are not capable of infecting the host *Chlorella NC64A* cells. Viral abundance in the original sample, recorded as plaque forming units mL^{-1} (PFU mL^{-1}), was determined from the dilution plate producing between 30 and 300 plaques (Atlas, 1995) using this equation:

$$\text{PFU mL}^{-1} = \frac{(\text{number of plaques counted})}{(\text{initial sample volume})} \times (\text{dilution factor}).$$

Viral attachment assays

The ability of PBCV-1 to attach to the host *Chlorella NC64A* cells was measured by determining the percentage of viruses attached to host cells over the first 100 min of infection. Experiments modelled after Meints *et al*. (1984) were conducted as follows: four replicates of Low C : P and High C : P *Chlorella NC64A* cultures (each replicate consisted of 2.5 mL of culture at a density of 1×10^7 cells mL^{-1}) were infected with a solution of PBCV-1 at a multiplicity of infection (MOI) equal to five, meaning there were five viruses for every potential host cell. Every 20 min, a 200-μL sample from each replicate was added to 3.3 mL of cold MBBM and centrifuged for 6 min at 3200 ***g***. The pellet formed by centrifugation contained *Chlorella NC64A* cells and the viruses attached irreversibly to these host cells. The viruses in the supernatant were free, or unattached, and their abundance was determined by plaque assays on the supernatant. We then calculated the percentage of viruses that were unattached at the time of each sub-sampling. Effects of C : P treatment were assessed using a RM ANOVA to evaluate treatment, time and time–treatment interactions.

Viral production assays

The effect of host C : P ratio on viral production was assessed by determining the number of new viruses produced during a single viral infection cycle. Eight replicates from the Low C : P and High C : P *Chlorella NC64A* cultures (2.5 mL of 1×10^7 cell mL^{-1}) were inoculated with the solution of PBCV-1 at a MOI of five. Infected cultures were incubated for 40 min to insure viral attachment and then centrifuged for 6 min at 3200 ***g***. After pouring off the supernatant, the pellet of *Chlorella NC64A* and attached viruses was resuspended in 230 mL of fresh MBBM media containing the appropriate phosphorus concentration. Every hour for 10 h after initial infection, a 100-μL sub-sample was removed and free viral abundance was determined by plaque assays. Additionally, another 100 μL was removed, diluted and fixed (to a final concentration of 2% filtered formaldehyde) for later direct microscopic enumeration of total viral abundance. Average viral abundances determined by plaque assays (PFU mL^{-1}) in the Low C : P and High C : P treatments were compared using RM ANOVA.

The number of new viruses produced per infected host cell (burst size) was determined by dividing the total number of new viruses produced during the 10-h experiment by the number of host cells lysed (determined by haemocytometer counts) over the same time period. A *t*-test was used to compare the burst sizes for the two C : P treatments. To further assess the effect of host C : P content on viral production, viral burst size was regressed against *Chlorella NC64A* C : P ratio for each replicate. Both variables were log-transformed prior to analysis to satisfy the assumptions of regression analysis.

'Offspring fitness': infectivity of new viral particles

The effect of host C : P ratio on the ability of newly produced viruses to cause infection was assessed by comparing infectious viral abundance to total viral abundance. Plaques produced on MBBM plates represent only the viruses that are able to cause infections, whereas direct counts determined by epifluorescence microscopy should more closely represent all the viruses present, including bacteriophage that may infect any bacteria in the cultures. Viral direct counts were conducted by enumeration of virus-like particles (VLP) stained with Yo-Pro-1^{TM} (Molecular Probes, Portland, OR, USA) on an epifluorescence microscope, as described in Xenopoulos & Bird (1997) and Hennes, Suttle & Chan (1995). The percent of total viruses (VLP) that were infectious (PFU) was calculated for each C : P treatment (six Low C : P and six High C : P replicates) and their means were compared using a *t*-test.

Results

Chlorella NC64A

Manipulating media phosphorus concentrations altered *Chlorella NC64A* C : P and N : P ratios (Fig. 1a,b). *Chlorella NC64A* cultures grown in the high-P medium had an average C : P ratio of 91 ± 23 (all ratios are molar ratios ±1 SE), whereas the average C : P ratio in low-P cultures was 453 ± 246 (Fig. 1a, *t*-test, $P < 0.0001$, $n = 18$). N : P ratios of *Chlorella NC64A* in the two C : P treatments also differed significantly (Fig. 1b, Low C : P cultures = 9 ± 2; high C : P cultures = 41 ± 21, *t*-test, $P < 0.0001$, $n = 18$) while, C : N ratios did not differ significantly (Fig. 1c, *t*-test, $P = 0.718$, $n = 18$).

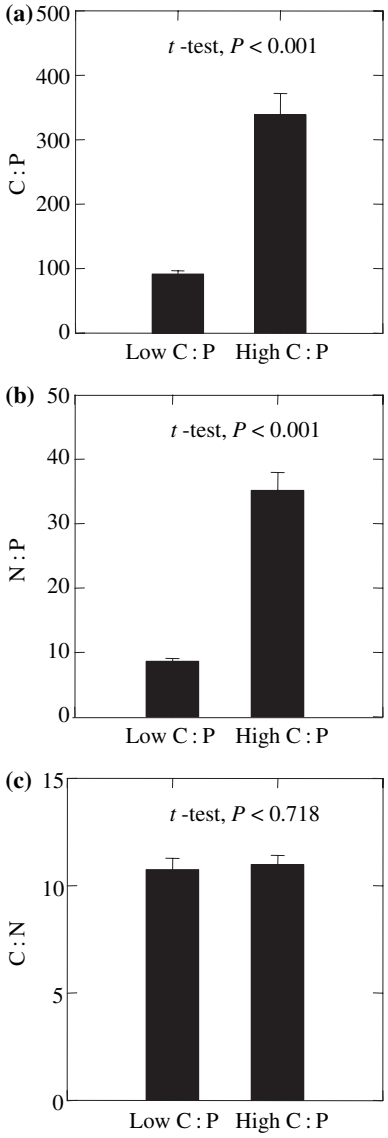

Fig. 1 Molar elemental ratios of *Chlorella NC64A* cells in the two experimental treatments differing in their media phosphorus concentrations. (a) C : P ratios, (b) N : P ratios and (c) C : N ratios. Error bars in all figures indicate ±1 SE.

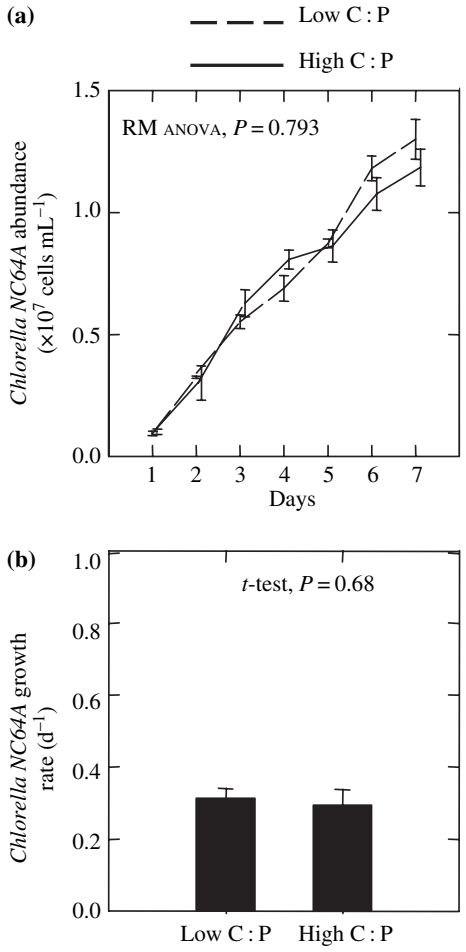

Fig. 2 Growth dynamics of *Chlorella NC64A* cells in the two C : P treatments. (a) Daily changes in *Chlorella NC64A* cell abundance in the two treatments was measured by CASY counts. Low C : P (dashed line), High C : P (solid line). Note: The High C : P treatment is slightly offset along the *x*-axis for clarity. (b) *Chlorella NC64A* average daily growth rates in the Low C : P and High C : P treatments. In both figures, the error bars indicate ±1 SE.

Despite the difference in its elemental composition, *Chlorella NC64A* growth was similar in the Low C : P and High C : P cultures when analysed either as daily *Chlorella NC64A* cell concentrations (Fig. 2a, RM ANOVA, Treatment effect $F_{1,1} = 0.079$, $P = 0.793$; Time × Treatment $F_{1,1} = 2.7$, $P = 0.036$) or as average growth rates (Fig. 2b, *t*-test, $P = 0.68$, $n = 24$). *Chlorella NC64A* cells in both C : P treatment grew at a rate of 0.4 day^{-1} and, therefore, doubled about every 1.7 days.

PBCV-1 stoichiometry

According to published sources, PBCV-1 is 23% DNA, 8% lipids and 64% protein by mass (Skrdla *et al.*, 1984; Van Etten *et al.*, 1991; Van Etten & Meints, 1999). These three groups of biomolecules account for 95% of the 1×10^9 Da viral mass. The 333-kbp genome has a G + C content of 40%, with 1.9% of the C nucleotides and 1.5% of the A nucleotides methylated (Schuster *et al.*, 1986; Van Etten & Meints, 1999). The lipid component of PBCV-1 is composed of three different phospholipids that are probably membrane lipids (Skrdla *et al.*, 1984). There are three groups of

Table 1 Calculated elemental composition of *Paramecium bursaria Chlorella virus-1* (PBCV-1). C : N : P ratios of PBCV-1 were calculated from biochemical composition using published values of viral DNA, lipid and protein content and the percentage of carbon, nitrogen and phosphorus in these major biomolecules.

Biomolecules	Composition*			PBCV-1 percent % of total†	PBCV-1 weight mass (Da)‡	C : N : P§
	% C	% N	% P			
DNA						
Nucleotide Base G	33	15	8.7	4.6	4.60E + 07	
Nucleotide Base C	33	15	8.7	4.51	4.51E + 07	
Methylated Base C	36	15	8.7	0.088	8.80E + 05	
Nucleotide Base A	33	15	8.7	6.78	6.78E + 07	
Methylated Base A	36	15	8.7	0.1	1.00E + 06	
Nucleotide Base T	33	15	8.7	6.9	6.90E + 07	
Lipids						
Phospholipids	65	1.6	4.2	8	8.00E + 07	
Proteins						
Glycoproteins	31	6.8	0	26.3	2.63E + 08	
Phosphoproteins	42	8	4	1.92	1.92E + 07	
Proteins	53	17	0	35.78	3.58E + 08	
Total				94.978	949980000	17 : 5 : 1

*The composition of biomolecules determined from either, Sterner & Elser (2002) or calculated in this study (see text).
†The percentage of PBCV-1 composed of biomolecules determined from values in Skrdla *et al.* (1984); Van Etten *et al.* (1991) and Van Etten & Meints (1999).
‡The weight of each biomolecule in PBCV-1 determined from percentage of each biomolecule present in PBCV-1 times the total viral mass (1×10^9 Da).
§To determine the C : N : P of PBCV-1, the masses of carbon, nitrogen and phosphorus in each of the four biomolecule components of PBCV-1 were first determined by multiplying the mass contribution of each biomolecule by its % C, % N or % P present in each of the biomolecules. Then the C : N : P ratios of PBCV-1 were calculated by dividing the appropriate total masses of carbon, nitrogen and phosphorus present in each virus.

proteins present in PBCV-1, including proteins, glycoproteins and phosphoproteins. Forty-one per cent of the total viral proteins are glycoproteins while 3% are phosphoproteins (Skrdla *et al.*, 1984). Based upon these previously published values and calculations of the elemental composition of major biomolecules, we estimate that PBCV-1 has a C : N : P stoichiometry of 17 : 5 : 1 (Table 1). This ratio was generated by multiplying the mass of each of the biomolecules in PBCV-1 by the percent of each biomolecule that is contributed by carbon, nitrogen or phosphorus to get the mass of each element in PBCV-1.

Viral attachment assays

The C : P ratio of host *Chlorella NC64A* cells did not affect the attachment of PBCV-1 to alga host cells (Fig. 3, RM ANOVA, Treatment effect $F_{1,1} = 0.928$, $P = 0.373$; Time × Treatment $F_{1,1} = 0.583$, $P = 0.678$). In both treatments, more than 90% of the viruses initially added were irreversibly attached to *Chlorella NC64A* cells within the first 20 min after inoculation.

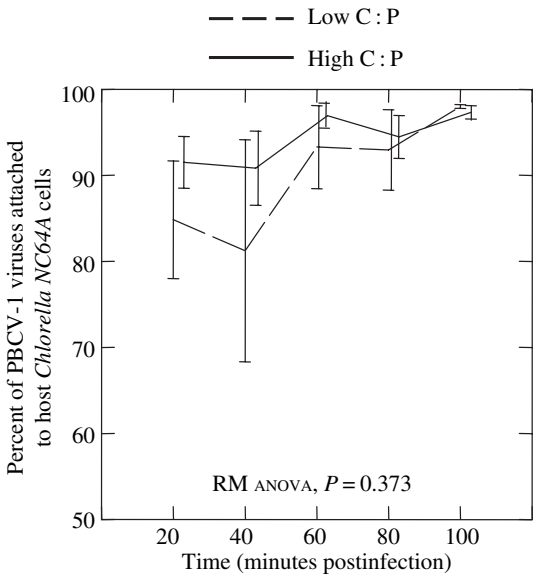

Fig. 3 Per cent of initial *Paramecium bursaria Chlorella virus-1* inoculum attached to host *Chlorella NC64A* cells over the first 100 min of an infection. Low C : P (dashed line), High C : P (solid line). Error bars indicate ±1 SE. Note: The High C : P treatment is slightly offset along the *x*-axis for clarity.

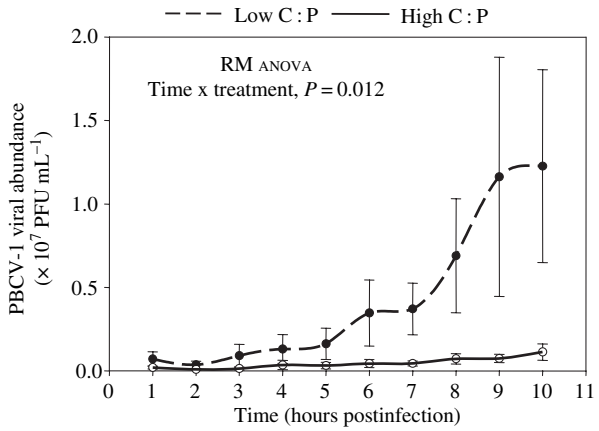

Fig. 4 Treatment means of how viral abundance (PFU mL^{-1}) in infected *Chlorella NC64A* cultures changes during the 10-hour viral production assay. Low C : P (dashed line), High C : P (solid line). Error bars indicate ±1 SE in the replicate samples.

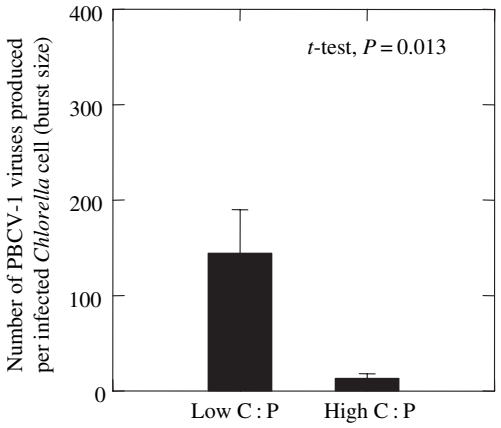

Fig. 5 The average number of new viruses produced per infected host cell (burst size) in the two C : P treatments determined by dividing the number of virus produced over the 10 experiment hours by the number of host cells lysed over the same time period. Error bars indicate ±1 SE.

Viral production assays

Viral abundance increased in both infected Low C : P and High C : P *Chlorella NC64A* cultures over the course of the experiment. However, the increase was considerably greater in the Low C : P treatment (Fig. 4, RM ANOVA, Treatment $F_{1,1} = 2.57$, $P = 0.140$; Time × Treatment, $F_{1,9} = 2.53$, $P = 0.012$). Differences in viral production between the C : P treatments were also examined based on burst size, which is the number of viruses produced per infected host cell. Each infected Low C : P *Chlorella NC64A* cell produced 158 ± 138 new viruses compared with only 18 ± 18 viruses produced in each infected cell in the High C : P treatment (Fig. 5, *t*-test, $P = 0.013$, $n = 8$). Viral production per infected cell was nine-fold higher in the Low C : P *Chlorella NC64A* treatment than in the High C : P treatment. Additionally, regression analysis of viral burst size and *Chlorella NC64A* C : P ratios indicated that burst size decreased significantly with host cell C : P ratio (Fig. 6, $r^2 = 0.63$, $F_{1,14} = 24.0$, $P < 0.0001$).

'Offspring fitness': infectivity of new viral particles

New virus particles from Low C : P and High C : P *Chlorella NC64A* treatments did not differ substantially in their ability to cause infections (Fig. 7, *t*-test, $P = 0.49$, $n = 6$). The percent of total viruses (VLP) forming plaques (PFU) was 37 ± 38 in the Low C : P cultures and 24 ± 10 in the High C : P cultures.

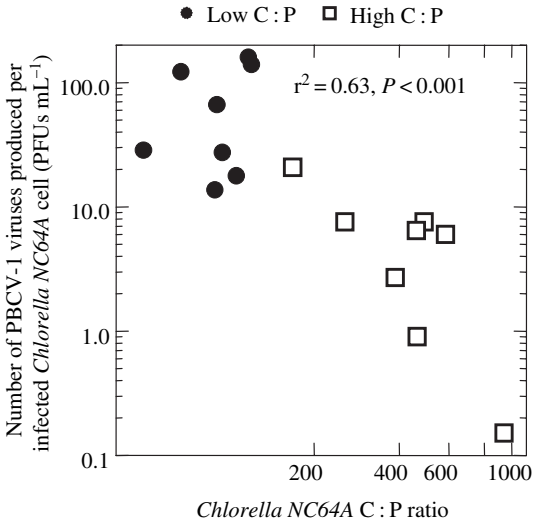

Fig. 6 The number of viruses released per infected cell (burst size) as a function of host C : P ratio in each replicate. Note that both axes are log transformed. Low C : P (closed circles), High C : P (open squares).

Typical percentages of total viruses causing infection in this *Chlorella NC64A* system range from 25% to 50% (Van Etten *et al.*, 1991).

Discussion

Our results support stoichiometric theory, which predicts that the proliferation of P-rich PBCV-1 viruses should be greater in Low C : P *Chlorella*

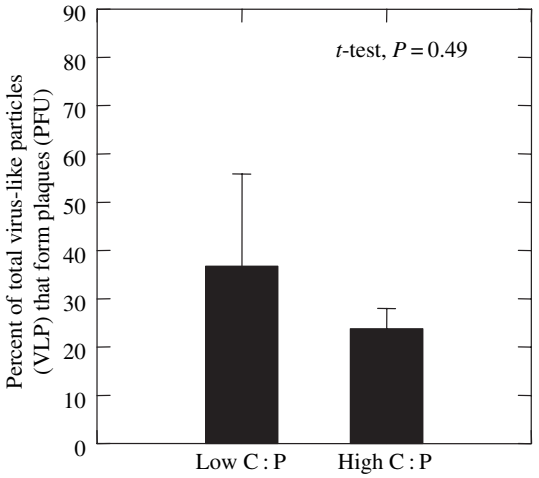

Fig. 7 The percentage of total *Paramecium bursaria Chlorella virus-1* particles that cause infections in a plaque assay. The infectivity of the new virus particles produced was tested by comparing the number of counted virus-like particles (VLP) and plaque forming units (PFU) in the *Chlorella NC64A* C : P treatments. Error bars indicate ±1 SE.

NC64A cells (91 : 1). Low C : P host cells were substantially higher quality 'prey' for viruses in that they enhanced viral production. However, only viral production was inhibited by high host C : P ratio while host attachment and the infectivity of new viral particles were not affected by host C : P. Our results indicate that viral dynamics are likely to be strongly affected by host C : P ratio due to the latter's effect on burst size.

The lack of effect of host C : P ratio on viral attachment to host cells in the *Chlorella NC64A* system (Fig. 3) is not surprising in that PBCV-1 attaches irreversibly to receptor sites on the external surface of *Chlorella NC64A*'s cell wall (Van Etten & Meints, 1999). These receptor sites are composed of the sugar portions of a polysaccharide-like molecule (Meints *et al*., 1988). As polysaccharides contain no phosphorus (Norland, Fagerbakke & Heldal, 1995), phosphorus availability and, therefore, host C : P ratios are unlikely to affect the production of these receptor molecules. Thus, the lack of effect of host C : P on viral attachment makes sense in that the site of viral attachment is unlikely to be affected by host P status.

Viral production assays indicated that production was strongly affected by host C : P ratio. Overall, infected *Chlorella NC64A* in the High C : P treatment produced 91% fewer viruses than in the Low C : P treatment (Figs 4 & 5). However, because of the complexity of PBCV-1's infection cycle and the nature of the plaque assay, these differences in the two C : P treatments may be due to several mechanisms, including differences in host growth rate, internal viral production and the infectivity of new viral particles. Our experiments shed light on each of these alternative explanations of the apparent enhancement of viral production in low C : P cells.

Previous studies have shown that *Chlorella NC64A* growth rate can affect the number of new PBCV-1 viruses produced per infected cell, with more viruses produced by cells in late log-phase growth (Van Etten *et al*., 1983a). Therefore, one possible explanation for more viral production in the Low C : P treatment could be higher host growth. However, *Chlorella NC64A* in our low C : P and high C : P treatments grew at similar rates (Fig. 2) and all experiments were conducted with cells in late log-phase growth. Thus, host growth rate was not the cause of differences in the number of viruses produced in the Low C : P and High C : P *Chlorella NC64A* treatments.

As plaque assays only measure infectious viruses, the observed difference in the production assay experiment could be the result of host C : P affecting the ability of newly produced viral particles to cause infection. We assessed the percentage of infectious new viral particles produced by comparing total viruses (VLPs) to the number of infectious viruses (PFUs) in the two C : P treatments. If host C : P ratio affected the ability of new viral particles to cause infections then there should have been a significant difference in the proportion of total viruses creating plaques in the two C : P treatments, despite the inclusion of bacteriophage in the total viral abundance counts. However, in both treatments plaques represented approximately 30% of total sample viral abundance (Fig. 7). Therefore, a difference in the infectivity of new viral particles cannot explain the observed treatment effect on viral production.

Differences in viral production between the two C : P treatments (Figs 4 & 5) were most likely due to a decrease in the ability of the infecting virus to produce new viruses via replication and assembly. Further evidence supporting this idea is provided by the significant relationship between burst size and the C : P ratio of the host cells in each of the experimental replicates (Fig. 6). As viruses have high nucleic acid : protein ratios (Bratbak *et al*., 1998b), the synthesis of new virus particles requires a substantial

amount of phosphorus because nucleic acids are phosphorus-rich (approximately 9% phosphorus by weight, Elser *et al.*, 1996). Because of their obligate intracellular parasitic nature, viruses can only use materials, including chemical elements such as P, present in the host cell at the onset of infection (Hurst & Lindquist, 2000). Thus, it seems reasonable to suggest that fewer viruses would be produced in the high C : P *Chlorella NC64A* cells because these host cells contain less available phosphorus for viral production, including viral DNA replication and mRNA transcription.

The design of our experiments precludes detailed investigation into the mechanisms behind the observed affect of host C : P ratio on viral production. We hypothesise that phosphorus-limitation of nucleotide synthesis may be involved because the degradation of host cell DNA accounts for only 10–25% of the nucleotides necessary for PBCV-1 replication (Van Etten *et al.*, 1983b, 1984, 1991). The remaining nucleotides must be synthesised *de novo* (Van Etten *et al.*, 1991). Further research is necessary to test this hypothesis directly. However, despite unknown mechanisms, our data suggest that important stoichiometric constraints are operating in this virus–host interaction and that aquatic viral ecology may benefit from the incorporation of stoichiometric theory.

Potential ecological implications

Viruses are abundant and dynamic members of aquatic ecosystems and are thought to be ecologically significant (reviewed in Wilhelm & Suttle, 1999; Wommack & Colwell, 2000; Suttle, 2005). They are an order of magnitude more numerous than bacterial cells, making them the most abundant biological entity in aquatic environments (Wommack & Colwell, 2000; Suttle, 2005). The abundance of viruses in aquatic environments has been intensively investigated since their 'discovery' in 1989 (Bergh *et al.*, 1989). Their abundance varies both temporally and spatially (Wommack & Colwell, 2000; Suttle, 2005) and is generally correlated with bacterial abundance (Cochlan *et al.*, 1993), chlorophyll *a* concentrations (Maranger & Bird, 1995), or mixed layer depth (Frederickson, Short & Suttle, 2003). This study suggests that host quality could also affect viral production/abundance and should be incorporated into future studies of viral abundance and distribution patterns.

The increase in viral replication with host nutrient content observed in this study is consistent with results from the limited number of marine studies. For example, in studies of *Phaeocystis pouchetii* (Hariot) and *Emiliania huxleyi* (Lohmann), nutrient status was manipulated by either nitrogen or phosphorus enrichment (Bratbak, Egge & Heldal, 1993; Wilson, Carr & Mann, 1996), which resulted in a measurable increase in viral abundance. However, these authors offered little explanation of the effect. Wilson *et al.* (1996) suggested that decreased viral abundance in P-depleted *Synechococcus* sp. cultures was the result of the virus entering a lysogenic infection cycle, where the viral DNA is incorporated into the host's DNA until an environmental cue induces a lytic infection. However, there is no evidence that PBCV-1 establishes any kind of lysogenic/latent infection in *Chlorella NC64A* (Kang *et al.*, 2005). No studies of the effects of nutrients on viral production have been conducted with freshwater phytoplankton species that experience more severe nutrient limitations (Hecky & Kilham, 1988) and, therefore, vary more in their natural elemental composition (Sterner & Elser, 2002). For example, in a survey of 270 lakes, the average seston C : P ratio was 307 but data ranged from 55 to 1630 (Elser *et al.*, 2000). Incidentally, the host C : P ratios in this study (91 and 453) fall well within this natural range, adding credence to the potential ecological relevance of our results.

To our knowledge, this is the first study to apply the theory of ES to virus–host interactions. Our data show that the C : P ratio of *Chlorella NC64A* strongly affects the success of a PBCV-1 infection through viral production. C : P ratio did not affect viral attachment or infectivity. These findings suggest that, despite the different 'consumption' mechanisms involved in virus–host interactions compared with conventional predator–prey interactions, ES still applies in host–virus interactions. It seems likely that host quality impacts viral proliferation and therefore the ecology of virus–phytoplankton interactions across nutrient gradients. Future research in aquatic viral ecology should incorporate the nutrient content of host cells in order to understand both the distribution and ecological significances of viruses in aquatic ecosystems.

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