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Predators catalyze an increase in chloroviruses by foraging on the symbiotic hosts of zoochlorellae

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Virus population growth depends on contacts between viruses and their hosts. It is often unclear how sufficient contacts are made between viruses and their specific hosts to generate spikes in viral abundance. Here, we show that copepods, acting as predators, can bring aquatic viruses and their algal hosts into contact. Specifically, predation of the protist *Paramecium bursaria* by copepods resulted in a >100-fold increase in the number of chloroviruses in 1 d. Copepod predation can be seen as an ecological “catalyst” by increasing contacts between chloroviruses and their hosts, zoochlorellae (endosymbiotic algae that live within paramecia), thereby facilitating viral population growth. When feeding, copepods passed *P. bursaria* through their digestive tract only partially digested, releasing endosymbiotic algae that still supported viral reproduction and resulting in a virus population spike. A simple predator–prey model parameterized for copepods consuming protists generates cycle periods for viruses consistent with those observed in natural ponds. Food webs are replete with similar symbiotic organisms, and we suspect the predator catalyst mechanism is capable of generating blooms for other endosymbiont-targeting viruses.

chloroviruses | predator–prey interactions | virus dynamics | copepod foraging | *Paramecium bursaria* endosymbionts

Chloroviruses are large dsDNA (290–370 kb) viruses that infect endosymbiotic chloroella-like green algae (zoochlorellae). Zoochlorellae occur within a wide range of hosts, including ciliates (e.g., *Paramecium bursaria*; hereafter paramecium), basal metazoans (e.g., *Hydra*), and higher metazoans (e.g., corals), found in many aquatic systems. Chloroviruses are common in freshwater habitats throughout the world (1, 2). They are sometimes quite rare, but at other times, they show major spikes in abundance (up to 10^5 infectious particles per 1 mL) (3–5).

Zoochlorellae generally number 300–600 within an individual paramecium (6). Interestingly, the zoochlorellae are resistant to virus infection when they exist as endosymbionts, because the viruses are excluded from the paramecium host. However, if released from the paramecium, the zoochlorellae are readily infected and give rise to 10^2 to 10^3 infectious particles per cell (7). These zoochlorellae do not grow efficiently in the indigenous waters that support their symbiotic hosts, but the chloroviruses that infect them are occasionally found at very high titers in freshwater environments (2). For example, in an urban lake, multiple chlorovirus types fluctuate in abundance throughout the year, with a peak during the late spring and another during late fall, along with faster oscillations at roughly bimonthly and monthly periods (5). It is not known how these chloroviruses reach and infect zoochlorellae and then, replicate to these high titers. We do know, however, that chloroviruses can attach to the external surfaces of paramecia, putting them in a good position to encounter zoochlorellae if the paramecium cell is ruptured (8). In addition to the protection provided to zoochlorellae inside the paramecium, low virus and paramecium densities suggest that the chance of an encounter between algae and chloroviruses is low. The occurrence of a large increase in chloroviruses may thus require a catalyzing mechanism that increases the collision rate by removing the physical barriers separating chloroviruses and their

algal hosts. Here, we report that cyclopid copepods (*Eucyclops agilis*; hereafter cyclops) foraging on paramecia can break down the physical barriers between chloroviruses and their hosts, zoochlorellae inside the paramecium, facilitating virus amplification. Furthermore, we evaluate the potential of this process to explain the cyclical nature of virus abundance in freshwater ponds and lakes.

Results

Microcosms of paramecia were constructed with locally acquired *Chlorella variabilis* Syngen 2–3-infecting chlorovirus concentrations at an initial density of about 10^3 pfu/mL. We applied treatments to break down the physical barrier between the chlorovirus outside the paramecium and zoochlorellae inside the paramecium and assessed the potential for predator-catalyzed viral reproduction. In untreated controls without cyclops or physical treatment, paramecium densities increased slightly over 3 d (Fig. 1A, white circles), but there was no increase in chlorovirus titers (Fig. 1B, white circles). A sonication treatment, however, ruptured nearly all of the paramecium cells (Fig. 1A, black circles) and produced a chlorovirus increase of about 10^2 pfu above initial concentrations (Fig. 1B, black circles), indicating that exposing the zoochlorellae can initiate virus replication. Cyclops that are natural predators of paramecium were allowed to forage on the paramecium for 3 d (Fig. 1, white squares). This exposure produced a drop in paramecium density similar to the sonication treatment and an increase in chlorovirus concentration that approached but did not quite reach the levels in the sonication treatment, indicating that the predators can fulfill the role of breaking down the barrier between virus and host and catalyze virus replication.

Significance

Reproduction and growth of viruses depend on successful encounters with appropriate hosts. However, some hosts are difficult to encounter. In particular, chloroviruses cannot reach their target zoochlorellae hosts, because zoochlorellae are endosymbionts, living inside the cell of a protist that protects the zoochlorellae from the chlorovirus. The protist host is subject to predation, and we show that copepods foraging on zoochlorellae-bearing protists can disrupt the mutualism and pass endosymbiotic zoochlorellae through their guts, exposing them to chloroviruses. In this way, predators can catalyze the virus population growth by breaking down physical barriers between viruses and their endosymbiont hosts.

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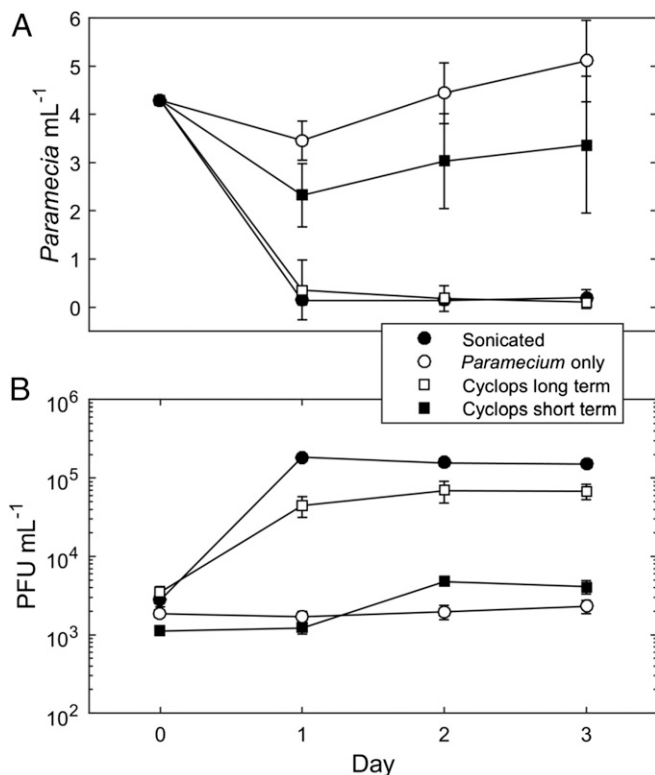


Fig. 1. Time series of (A) *P. bursaria* and (B) virus densities (pfu per 1 mL) in microcosms over a 3-d experiment. Points are means \pm SD; $n = 5$ for all treatments.

An additional set of cyclops was allowed to forage on paramecia for a shorter period (~ 2.5 h) before they were removed. This foraging incubation (Fig. 1, black squares) resulted in a modest drop in paramecium density along with a slight increase in virus titer. That set of cyclops, which was then removed and serially washed (three times) in virus-free water and placed into virus-free microcosms, produced a large increase in chloroviruses in the new microcosms (Fig. 2, black circles). In contrast, control dishes that contained an equivalent aliquot of rinse solution had no virus (Fig. 2, black squares). This result shows that the way that the virus–host barrier is broken down is by passing the zoochlorellae through the predator’s gut, because no additional foraging occurred in the “cyclops transferred dishes” that could release zoochlorellae directly into the water. The algae that pass through the gut of the copepod are apparently vital to the extent that, for any virus to replicate, the host cell must have some level of vitality. In addition, this result indicates that at least some zoochlorellae that pass through the gut of the cyclops still support virus replication.

The mechanism of this catalysis was rapid ingestion of paramecia followed by defecation of ruptured but not fully digested paramecia that still contained viable zoochlorellae (Movie S1). The cyclops (1.2–1.4 mm in length) engulfed the entire paramecium in quick bites (Movie S1). After ingesting paramecia, the cyclops defecated a pellet of packed zoochlorellae. Some of the freshly produced fecal pellets were transferred to a microcosm with virus-free water, and virus amplification in this dish matched the virus amplification produced by the cyclops transferred to their virus-free incubations (Fig. 2, white circles). This amplification required a ready source of infectious virus particles, which indicates that, in addition to the zoochlorellae, at least some viable viruses also passed through the cyclops.

To determine how many viruses might be available, 20 paramecia were serially rinsed (three times) and disrupted by either

sonication or exposure to 0.25% Triton X-100. Plaque assay of these disrupted cells indicated there was an average of 225 pfu on the surface of the paramecia used in this study, creating strong potential for de novo virus replication after the virus and the zoochlorellae pass through the cyclops gut.

Additional evidence for predator-activated catalysis is provided by a positive correlation between the overall amount of foraging and the magnitude of virus replication (Fig. 3). Our observations suggest that about one fecal pellet was produced per three paramecium consumed. Across the long-term foraging incubations, the virus concentration increased with the number of fecal pellets found in the dishes, which is an indication of cumulative cyclops foraging during the experiment. Including the viral concentrations of control samples, the slope of a linear regression of virus density on minimum paramecia consumption (minimum pellet density $\times 3$) suggests that $\sim 5,700$ pfu were produced per paramecium consumed. Assuming ~ 450 zoochlorellae per paramecium and a burst size of ~ 100 pfu per algal cell (3), the expected rate of production per paramecium would be $\sim 45,000$ pfu if all zoochlorellae remained viable and were encountered by an infectious virus particle. The maximum potential yield of virus per paramecium also can be calculated from the sonicated microcosms, where 28–30 of the initial 30 paramecia were ruptured at the beginning of the experiment. The average virus concentration rose to 1.65×10^5 pfu mL^{-1} (Fig. 2) by day 1, which indicates a yield of roughly 38,500 pfu per paramecium above the starting concentration (1.65×10^5 pfu mL^{-1} per 4.29 paramecia per 1 mL), in line with the theoretical yield. Our data, therefore, indicate that roughly 12–15% of the theoretical yield can be accomplished through the predator catalysis mechanism.

To determine whether our predator-activated model could generate the kinds of infectious chlorovirus fluctuations observed in nature (Fig. 4A), we incorporated the model into an ordinary differential equation (ODE) model of predator–prey dynamics (Materials and Methods) (9). The connection between the predator and virus production is through the overall foraging rate F , which is embedded in predator–prey modeling as the product of predator density and the per capita foraging rate, f_{pc} , written as a type II functional response: $f_{pc} = aN / (1 + ahN)$, where a is the area of capture (the space cleared of prey by a predator per unit time), h is the handling time, and N is the prey density. We parameterized the model using data from a literature compilation describing the interactions between cyclops and various protists (10, 11) to predict the size and timing of virus blooms that would arise through the predator-activated mechanism.

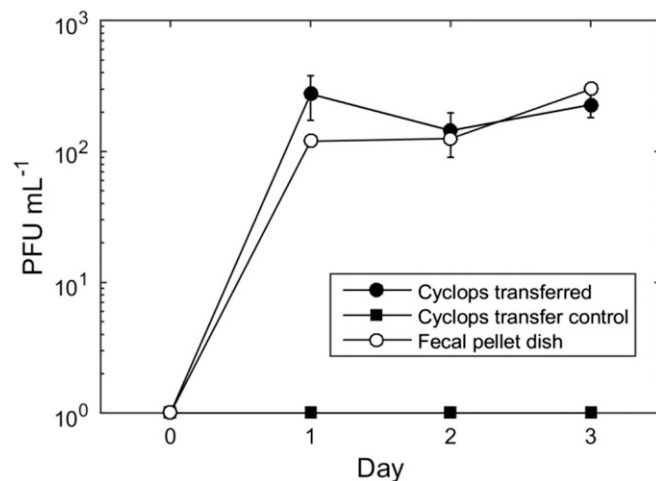


Fig. 2. Time series of virus densities (pfu; mean \pm SD) for cyclops incubations rinsed and transferred to new dishes. No viruses were detected in the transfer controls; these values were plotted as one to facilitate log comparisons.

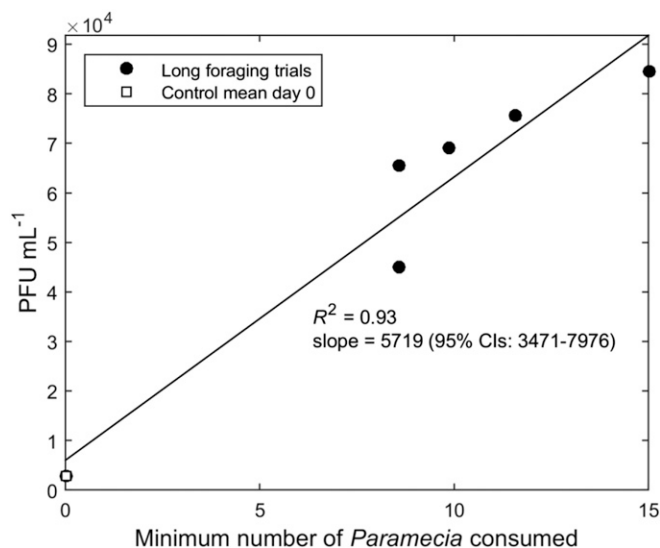


Fig. 3. Virus density (pfu) in relation to minimum total foraging over the first 2 d in the long-term foraging dishes (black circles) given by a tally of cyclops-generated fecal pellets containing *P. bursaria* on the bottom of the microcosm and assuming a ratio of 3:1 paramecium consumed per pellet produced. Also shown is the baseline pfu on day 0 of the controls (white square). Linear regression through all points gives the pfus per disrupted *P. bursaria* (~5,700). 95% CI, 95% confidence interval.

Model solutions suggest typical predator–prey cycles (Fig. 4*B*), where spikes in virus abundance follow the peak in cyclops abundance (Fig. 4*C*). This outcome is expected, because virus population growth depends on the overall foraging rate, which is high when cyclops abundance is high. The period of the cycles (time between successive peaks) varies depending on the area of capture (Fig. 4*D*). We varied this parameter in the model across the observed range of this parameter in the literature for cyclops foraging on ciliates (4–60 mL predator⁻¹ d⁻¹) (11) and found that the cycle period varied between roughly 10 and 120 d (Fig. 4*B*), broadly consistent with cycle periods in field surveys of Syngen virus abundance in freshwater lakes (5) (Fig. 4*A*).

Discussion

Our results reveal a previously unknown mechanism, wherein predators catalyzed an increase in virus population by reducing the physical barriers that separate viruses from their hosts and increasing virus–host contacts (Fig. 5). This mechanism can explain how viruses that cannot efficiently reach their hosts can fluctuate and achieve high abundances in nature. In short, predation by the cyclopoid copepod *E. agilis* on the ciliate *P. bursaria*, which maintains a symbiotic relationship with certain eukaryotic green algae, including *Chlorella* species, that support *Chlorovirus* replication, caused virus activation that resulted in significantly higher virus titers in the water column.

Our study can be contrasted with recent findings of copepod-facilitated virus dispersal. *Emiliania huxleyi* virus (EhV) can be transmitted by certain zooplankton grazing on the EhV-infected free-living algal host, *E. huxleyi*, or when feeding on EhV alone (12). Calanoid copepods that consume *E. huxleyi* or the EhV physically move in the water column between feeding and defecating, thereby acting as vectors of the virus, because the fecal pellets release infectious viruses. This study was novel in that it showed that zooplankton facilitate virus dispersal. However, it did not address the biological process that we address in this manuscript, which is virus activation, or the instigation of virus replication by virtue of exposing the virus host to the virus. The virus in our study was unable to infect its algal host until the host

was freed from its symbiotic host, making it a qualitatively different finding than that of the EhV/*E. huxleyi* studies.

The predator–catalyst mechanism adds a new dimension to the role that predators can play in disease dynamics (13, 14). Copepods are now known to activate virus reproduction and facilitate dispersal of viruses (12), with attendant effects on blooming algae (15). Copepods, however, may display some avoidance of infected prey, including EhV-infected *E. huxleyi*, potentially altering the rates at which activation and dispersal might occur (16). We suggest that these phenomena would not necessarily be limited to these particular trophic interactions and that predator activation and dispersal of other types of viruses would seem likely in systems where symbiotic relationships may provide a barrier to virus–host interactions. Together, these studies seem to elevate the role of zooplankton in algal virus biology, bringing a new understanding in both virus transmission and activation.

The chlorovirus replication that was observed in fecal pellets requires that the algae are vital, at least for a time. Crucially, the term “vital” need not imply that the cells will live, divide, or carry out normal physiological functions, like photosynthesis. The chloroviruses in these studies have a lytic lifestyle that results in the death of the infected cell. Our previous studies show that the onset of cell death occurs almost immediately on infection where the algal cell plasma membrane becomes depolarized, resulting in the significant loss of secondary transporters (17). The virus-infected cells score as “dead” with live/dead indicator stains; thus, they are “the living dead.” Additionally, chlorovirus-infected algae quickly lose their ability for photosynthesis (18). Perhaps one of the best indicators of vitality is whether a cell can

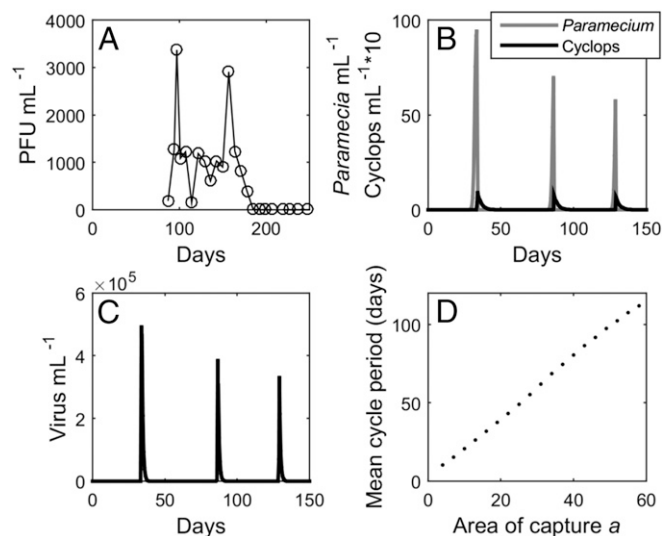


Fig. 4. Population dynamics of chloroviruses. (A) An example time series for viruses that infect *C. variabilis* NC64A from Holmes Lake (Lincoln, NE) showing peaks in abundance separated by 1–2 mo. (B) Simulated cyclops–paramecium predator–prey dynamics using our predator-activated virus model embedded into a standard ODE model for zooplankton (*Materials and Methods*) with the following parameters: $r = 2$, $k = 100$, $h = 0.0003$, $e = 0.01$, $d_c = 0.1$, $a = 50$, $v = 5,700$, and $d_v = 1.3$. (C) Cyclical virus dynamics that would ensue given the predator-activated mechanism and connecting virus production to the overall foraging rate of the cyclops on paramecium. (D) The mean of the first three cycle periods in the model solution for the same parameters, except that the area of capture (a) parameter was varied from 4 to 60, which is the approximate range of this parameter for freshwater copepods foraging on protists (11). *D* shows that the expected rate of foraging by cyclops on paramecium is expected to generate fluctuations in virus abundance that are in rough agreement with those observed in freshwater ponds, although our model does not take into account the seasonality of temperate lakes (an example is in *A*).

point they were removed, serially rinsed (three times) in 30 mL virus-free water, and transferred in 0.2 mL into a new 6-cm petri dish with 6.8 mL virus-free water. Any defecated paramecium pellets passed by the cyclops during the rinse process were transferred along with the cyclops to the new dish. Defecated pellets in the short-term foraging dishes were removed and placed in a separate microcosm with 7 mL virus-free water. Transfer controls were the same and included 0.2 mL serial rinse water without a cyclops.

Virus Assay Methods. Infectious virus was assayed by a plaque assay as described previously (4), except that *C. variabilis* Syngen 2–3 (product no. 30562; American Type Culture Collection) cells were used as the lawn.

Modeling Methods. We used a standard ODE model of predator–prey dynamics linked to virus replication dynamics to produce a predator-activated model:

$$\begin{aligned} \frac{dR}{dt} &= rR \left(1 - \frac{R}{K}\right) - \frac{aRC}{1+ahR} \\ \frac{dC}{dt} &= \frac{eaRC}{1+ahR} - d_c C, \text{ and} \\ \frac{dV}{dt} &= \frac{vaRC}{1+ahR} - d_v V. \end{aligned} \quad [1]$$

The model simulated the density of paramecia (R), cyclops (C), and viruses (V) through time t . The parameters are r (paramecium intrinsic growth rate), K (paramecium carrying capacity), a (cyclops area of capture of paramecia),

h (handling time of cyclops consuming paramecia), e (efficiency of converting consumed paramecia into new cyclops), d_c (cyclops background death rate), and d_v (virus background death rate). The parameter v is the latter portion of the predator-activated model, stipulating a rate of virus production per paramecium consumed, which is taken to be 5,700 as empirically estimated (in the text). The virus background death rate was set at 1.3 per day, which is the median of wintertime decay rates reported for a temperate chlorovirus (25). Other parameters were taken from literature compilations for protists and cyclops (10, 11), with $r = 2$, $k = 100$, $h = 0.0003$, $e = 0.01$, $d_c = 0.1$, and $a = 50$. The model was solved using the ode45 solver in Matlab. Note that, in this model, the period of successive virus peaks is generated by the interaction between the cyclops and the ciliates, not the values of the virus production (v) or virus death rate (d_v). These virus-specific parameters, however, can affect the steepness of the rising and falling parts of the virus peaks, which we do not analyze here.

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- Jeanniard A, et al. (2013) Towards defining the chloroviruses: A genomic journey through a genus of large DNA viruses. *BMC Genomics* 14(1):158.
- Van Etten JL, Dunigan DD (2012) Chloroviruses: Not your everyday plant virus. *Trends Plant Sci* 17(1):1–8.
- Van Etten JL, Lane LC, Meints RH (1991) Viruses and viruslike particles of eukaryotic algae. *Microbiol Rev* 55(4):586–620.
- Van Etten JL, Burbank DE, Kuczmarski D, Meints RH (1983) Virus infection of culturable chlorella-like algae and development of a plaque assay. *Science* 219(4587):994–996.
- Quispe CF, et al. (2016) Three-year survey of abundance, prevalence and genetic diversity of chlorovirus populations in a small urban lake. *Arch Virol* 161(7):1839–1847.
- Kodama Y, et al. (2014) Comparison of gene expression of *Paramecium bursaria* with and without *Chlorella variabilis* symbionts. *BMC Genomics* 15(1):183.
- Van Etten JL, Burbank DE, Xia Y, Meints RH (1983) Growth cycle of a virus, PBCV-1, that infects *Chlorella*-like algae. *Virology* 126(1):117–125.
- Yashchenko VV, Gavrilova OV, Rautian MS, Jakobsen KS (2012) Association of *Paramecium bursaria* *Chlorella* viruses with *Paramecium bursaria* cells: Ultrastructural studies. *Eur J Protistol* 48(2):149–159.
- DeLong JP, Hanley TC, Vasseur DA (2014) Predator–prey dynamics and the plasticity of predator body size. *Funct Ecol* 28(2):487–493.
- DeLong JP, et al. (2015) The body size dependence of trophic cascades. *Am Nat* 185(3):354–366.
- Kalinoski RM, DeLong JP (2016) Beyond body mass: How prey traits improve predictions of functional response parameters. *Oecologia* 180(2):543–550.
- Frada MJ, et al. (2014) Zooplankton may serve as transmission vectors for viruses infecting algal blooms in the ocean. *Curr Biol* 24(21):2592–2597.
- Hall SR, et al. (2007) Eating yourself sick: Transmission of disease as a function of foraging ecology. *Ecol Lett* 10(3):207–218.
- Hall SR, Duffy MA, Cáceres CE (2005) Selective predation and productivity jointly drive complex behavior in host-parasite systems. *Am Nat* 165(1):70–81.
- Chow C-ET, Suttle CA (2015) Biogeography of viruses in the sea. *Annu Rev Virol* 2(1):41–66.
- Vermont AI, et al. (2016) Virus infection of *Emiliania huxleyi* deters grazing by the copepod *Acartia tonsa*. *J Plankton Res*, 10.1093/plankt/ftw064.
- Agarkova I, et al. (2008) Chlorovirus-mediated membrane depolarization of *Chlorella* alters secondary active transport of solutes. *J Virol* 82(24):12181–12190.
- Seaton G, Lee K, Rohozinski J (1995) Photosynthetic shutdown in chlorella NC64A associated with the infection cycle of *Paramecium bursaria* chlorella virus-1. *Plant Physiol* 108(4):1431–1438.
- Weitz JS (2016) *Quantitative Viral Ecology: Dynamics of Viruses and Their Microbial Hosts* (Princeton Univ Press, Princeton).
- Hewson I, et al. (2012) Temporal dynamics and decay of putatively allochthonous and autochthonous viral genotypes in contrasting freshwater lakes. *Appl Environ Microbiol* 78(18):6583–6591.
- Sarma SSS, Jiménez-Contreras J, Fernández R, Nandini S, García-García G (2013) Functional responses and feeding rates of *Mesocyclops pehpeiensis* Hu (Copepoda) fed different diets (rotifers, cladocerans, alga and cyanobacteria). *J Nat Hist* 47(5–12):841–852.
- Sud GC (1968) Volumetric relationships of symbiotic zoochlorellae to their hosts. *J Protozool* 15(3):605–607.
- Dziallas C, Allgaier M, Monaghan MT, Grossart H-P (2012) Act together-implications of symbioses in aquatic ciliates. *Front Microbiol* 3:288.
- Johnson PTJ, de Roode JC, Fenton A (2015) Why infectious disease research needs community ecology. *Science* 349(6252):1259504.
- Long AM, Short SM (2016) Seasonal determinations of algal virus decay rates reveal overwintering in a temperate freshwater pond. *ISME J* 10(7):1602–1612.

Supporting Information

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Movie S1. Time-lapse images (one image every 10 s) of *Eucyclops agilis* in a drop of water (~0.2 mL) consuming *Paramecium bursaria* and defecating zoochlorellae in fecal pellets. The drop of water was compressed under a petri dish lid to restrict vertical movement and facilitate keeping the camera in focus. The series of images began with three *P. bursaria* (two paramecia are seen in the initial frame as pale green ellipsoids: one at ~100° to the right of center and the other at ~250° to the left of the copepod). The last of three paramecia was consumed at movie time 0:57. The fecal pellet produced by the copepod was starting to be visible in the digestive tract at 2:50, and a pellet was finally passed at 4:08.

[Movie S1](#)