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Supplementary Materials for

The consumption of viruses returns energy to food chains

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Materials and Methods

We collected the ciliates *Halteria* (possibly *H. grandinella*) and *Paramecium bursaria* from ponds near Lincoln, NE, USA. We cultured the ciliates in protozoan medium (Carolina Biological Supply, Burlington, NC) made from filtered and autoclaved pond water with 2 g per L of autoclaved pond sediment. We inoculated media with mixed bacteria extracted from the source pond. We maintained cultures of *Halteria* and *Paramecium* in monoxenic conditions.

We obtained a concentrated chlorovirus preparation (OSYNE-ZA1) by infection of *Chlorella variabilis* Syngen 2-3 and purified the viruses by differential centrifugation and sucrose density gradient centrifugation (1). We suspended virus in VSB buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.8) for storage but then washed the virus via centrifugation using 0.1 µm autoclaved and filtered pond water for experiments.

We conducted foraging trials with *Halteria* and *Paramecium* as grazers. We created small foraging arenas by rinsing ciliates 3x in autoclaved pond water and then placing three cells in a 0.4 mL drop of water (from the final rinse) onto a 100 mm Petri dish lid. We then applied two treatments. In the virus treatment, we added 0.5 mL washed virus (2×10^7 plaque-forming units [PFUs] per mL) to the drop with ciliates. In the control treatment, we added 0.5 mL of the virus treatment, cleared of virus by passage

through a 0.1 µm syringe filter. We then covered drops with an inverted 35 mm Petri dish lid to prevent them from drying out. We conducted six replicate treatment and control trials at 22°C. We counted ciliates after one and two days, and we then used the plaque assay to enumerate infectious chlorovirus particles for the initial virus preparation, the initial rinsed ciliate stocks, and the virus treatment drops at the end of the experiment. Virus quantified by plaque assay provided the number of PFUs per mL.

We used fluorescently labeled chloroviruses to visually confirm uptake of virions by the ciliates. We stained chloroviruses with Sybr green dye (SYBR™ Green I Nucleic Acid Gel Stain - 10,000X concentrate in DMSO, ThermoFisher Scientific, Catalog number: S7563) overnight at 6°C, and then washed the virus 10x to clear the remaining stain from the suspension using centrifugation at 15,000 g x 30 min. We used a Lumascope 400 (San Diego, CA) inverted microscope to image cells at 20x and determine if virions could be detected within vacuoles. We tested for uptake in *Halteria* and two other common ciliates, *P. caudatum* and *Euplotes* sp. We were unable to image *P. bursaria* because the endosymbiotic zoochlorellae autofluorescence blocked the light emission from the virions.

To determine whether the virus and *Halteria* dynamics were consistent with expected behavior of a trophic system, we modified an ordinary differential equation (ODE) predator-prey model and fit it to the data. Because chlorovirus hosts (zoochlorellae) were not present in the system, we used a death-only equation for the chloroviruses, and because of the short time frame and lack of predators, we used a birth-only equation for the *Halteria*. Thus, we refer to this model as a trophic-interaction model, as it contains the trophic link and the production of *Halteria*:

$\frac{dV}{dt} = -aVH$ Equation S1A

$\frac{dH}{dt} = eaVH$ Equation S1B

In this model, *V* is the abundance of chloroviruses per mL, *H* is the abundance of *Halteria* per mL, *a* is the space clearance rate, and *e* is the conversion efficiency (predators produced per virus particle eaten). For the model, we considered *V* to be the total virus density, including non-infectious particles. Because chlorovirus specific infectivity is 0.2-0.4 (2), we transformed abundance measured by plaque assay to total virions by dividing by 0.3.

We fit this model to the pooled abundance data, because there were not enough time points to fit each replicate separately. We used the PottersWheel Toolbox in Matlab 2017a to conduct the fits. We

created a bootstrapped dataset by stratified random sampling across days with replacement, and then fit the model to the dataset. We repeated this 100 times. We then used the median of the fitted parameters as an estimate of the system-level parameters. We assumed that in two replicate trials, a drop in *Halteria* abundance from 3 to 2 from the start to day one was caused by (normal) transfer mortality, so in these two cases, the original abundance was adjusted to two cells.

We calculated the gross growth efficiency in this way. The conversion efficiency model parameter indicates how many new predators are made from an individual resource item (in this case the virions), so the inverse of this value indicates that it takes approximately 2.1 x 10^6 chloroviruses to produce a new *Halteria* cell. Multiplying this by the volume of a chlorovirus (~3.6 x 10^{-3} µm^3) and dividing by the volume of a *Halteria* (~1.3 x 10^3 µm^3) indicates that the *Halteria* consume about 6 µm^3 of virion biovolume to make one µm^3 of *Halteria* biovolume. The inverse of this is gross growth efficiency, about 0.17.

We estimated the daily total consumption per *Halteria* by multiplying the space clearance rate (0.2 mL per predator per day) by the resource density (varying from 10^5 to 10^7 per mL during the experiment) to get a foraging rate, *F*, per day:

$F = \frac{0.2\ mL}{pred\ day} * \frac{10^5 V}{mL} = 2 * 10^4 \frac{V}{pred\ day}$ on the low end, or

$F = \frac{0.2\ mL}{pred\ day} * \frac{10^7 V}{mL} = 2 * 10^6 \frac{V}{pred\ day}$ on the high end.

We can use this daily per capita foraging rate to scale up to the total virion uptake rate in a body of water that has typical chlorovirus and ciliate communities (around 10^2 protists per mL) (3). In a pond about 10 m on a side and one meter deep on average, the total water volume would be 10^2 m^3. To get the total virion uptake, we multiply the per capita daily rate by the density of ciliates by the water volume, converting mL to m^3 as well:

$F_{pond} = \frac{2*10^4 V}{pred\ day} * \frac{10^2 pred}{mL} * \frac{10^6 mL}{m^3} * \frac{10^2 m^3}{pond} = 10^{14} \frac{V}{day\ pond}$ on the low end, or

$F_{pond} = \frac{2*10^6 V}{pred\ day} * \frac{10^2 pred}{mL} * \frac{10^6 mL}{m^3} * \frac{10^2 m^3}{pond} = 10^{16} \frac{V}{day\ pond}$ on the high end.

References

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