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Cell volume as a determinant of virus-mediated population growth in ciliates

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“Cell volume as a determinant of virus-mediated population growth in ciliates”

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Abstract:

Many protists and other small aquatic organisms consume virus particles, a behavior known as virovory. Some species of protists, such as the ciliate *Halteria grandinella*, can grow and divide using viruses as their sole food source. Other ciliate species have previously been shown to consume large quantities of viral particles, but it is unclear if they are able to support population growth with viruses alone. Because large ciliates have a higher energy demand, we hypothesize that they will be unable to support population growth on a virus-only diet. We fed nine ciliate species a diet of chloroviruses and found that most species' populations grew better with viruses added relative to controls. We found that the increase in growth rate relative to controls was independent of cell volume. Our results indicate that virovory-driven population growth is widespread among ciliates, but that the factors which determine the impact of viruses on population growth remain unknown.

Introduction:

Viral particles saturate aquatic environments and are taken up by any species that ingests water, particularly those that rely on filter feeding. However, because of the small size of viral particles, it was previously believed that viruses had little to no meaningful nutritional value and no impact on the growth and development of the organisms that ingested them[1]. Despite this, a recent study has demonstrated that populations of the ciliate protist *H. grandinella* are capable of surviving and growing solely on chlorovirus virions[2]. The documentation of virus-mediated population growth in a wider variety of species has the potential to reshape our understanding of the role viruses play in aquatic food webs. Due to the small size of viral particles relative to protist cells, we hypothesize that smaller ciliate species will be able to utilize viral resources more efficiently, which will result in smaller species experiencing higher rates of virus-meditated population growth.

Materials and Methods:

We conducted population growth trials with nine freshwater ciliate species: *H. grandinella, Paramecium bursaria, P. caudatum, P. aurelia, Cyclidium glaucoma, Colpidium striatum, Coleps hirtus,* and two unidentified species of the genus *Euplotes*. We collected these specimens from the Spring Creek Prairie Audubon Center in Denton, NE and other nearby ponds in July and August of 2022, and grew and maintained populations of each species in pond water under lab conditions at 23°C. We conducted trials in September of the same year.

We isolated 20 individuals of each species in a Petri dish and washed them three times using 0.2 µm filtered autoclaved pond water (ACPW), also collected from Spring Creek. These washes helped clear bacteria and other contaminants from the media, ensuring that viral particles were the primary available food source. After washing, we placed three individuals of each species into 0.4 mL drops of rinse media on the underside of a 150 mm Petri dish lid. Each lid accommodated one species' six replicate drops, arranged in a circle.

We treated each drop with either a virus or control solution. The virus solution consisted of *Paramecium bursaria* chlorella virus virions (hereafter simply chlorovirus) suspended in autoclaved pond water at a titer of $3.0*10^7$ PFU/mL, and the control solution consisting of this same viral solution passed through a 0.1 µm filter to remove viral particles while retaining all the chemical properties and small particulate matter of the virus treatment. We added 0.5 µL of either virus or control solution to alternating drops and covered them with 60 mm Petri dish lids to prevent evaporation. The cover dish also flattened the drop making it easier to observe and count cells. We incubated the dishes in the dark at 23°C and counted at 24-hour intervals over the following two days.

Because the magnitude of population growth varies considerably between species, we standardized the data between species by calculating the relative increase in population size between the virus and control treatments, rather than simply comparing raw population growth rates. We accomplished this by performing pairwise comparisons of each of the three virus trials with the three control trials for each species. This produced nine estimates per species of the relative increase in population size between the two treatments. A value of one was indicative of population growth parity between treatments, and a value of greater than one was indicative of an increase in population growth in viral treatments. We gathered estimates of each species' cell volume from the literature[3], or calculated them from existing measurements of each species[4–9]. To test our hypothesis, we performed a linear least squares regression of cell volume and relative population increase in MATLAB R2022b.

Results:

Most ciliate population grew when incubated with viruses (Fig. 1). In many cases, growth was higher in populations treated with viruses than those treated with controls. Some species, notably *P. bursaria* and the smaller *Euplotes,* saw very little change in population size in either treatment. *C. hirtus* populations crashed in both virus and control treatments. *C. hirtus* was included as a control organism, as it is an obligate predator and should be unable to subsist on any small, suspended organic matter, including viruses. The largest numerical increase in population was observed in *H. grandinella*, with each viral treatment population seeing at least a 13x growth by the end of the trials (Fig. 1). However, we also observed growth in the control populations of several species, most notably *H. grandinella*. This population growth is likely the result of dissolved organic matter smaller than the 0.1 μm filter pores, and/or nutrient stores acquired prior to placement in the treatment arena.

Relative population growth was highly variable between species, however all species had mean ratios of greater than one, indicating that most species experienced at least some increased growth when given viruses as food. *P. caudatum* had the single highest virus to control growth ratio, but *C. striatum* had highest mean value for all of its ratios (Fig. 2). Our linear least squares regression determined that were was no statistically significant link between cell volume and population growth (R^2= 0.0001, F = 0.00284, p = 0.96).

Figures:

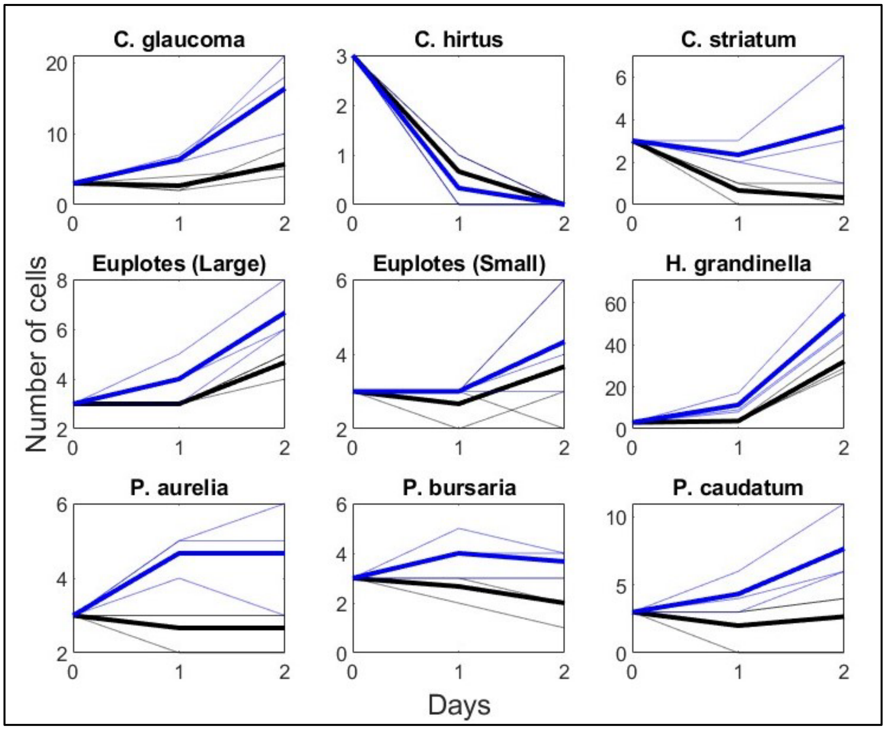

Figure 1:

The growth of virus and control population over the two-day study period. Blue lines represent viral treatment population, black lines represent control populations. Bold lines represent means for each treatment type within each species.

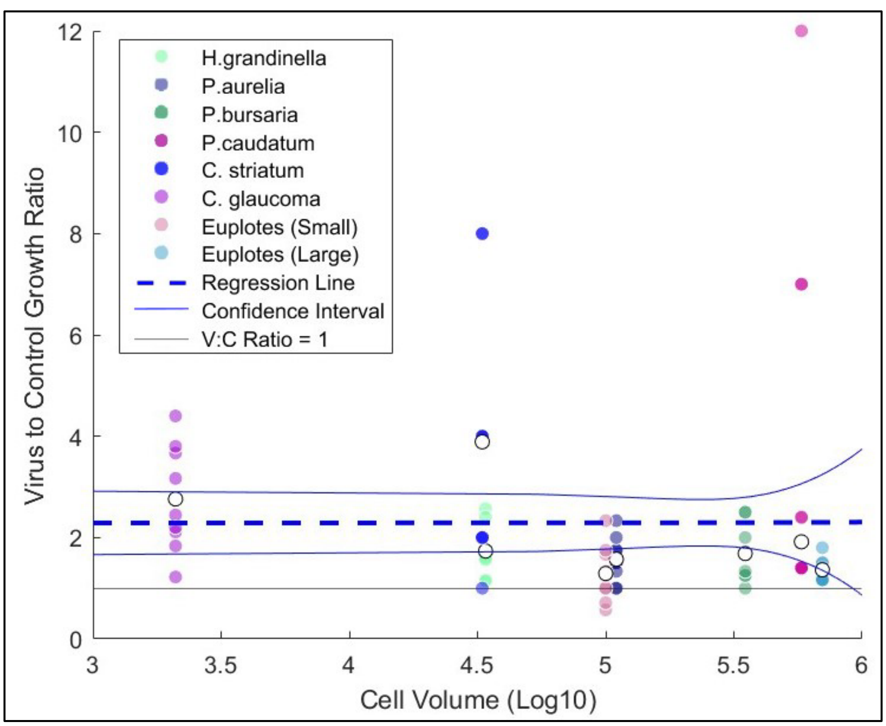

Figure 2:

Ratio of cell abundance in the virus treatment to the control treatment as a function of cell volume. The fitted regression line and confidence limits on the regression line are shown in blue. The horizontal black line represents a ratio of 1, indicative of no difference between treatments.

Discussion:

This experiment demonstrated not only that virovory is widespread across several discrete ciliate taxa, but that a wide range of species are capable of supporting population growth using viruses as their primary food source. Virovory has now been observed not only in several other ciliate taxa[4,5] but also in nanoflagellates[6], meaning that viruses are likely a common food source for many protist species. The results of this experiment suggest that if virovory is indeed common, then viruses likely have significant impacts on not only the populations of a wide array of protists but, because protists are a major food source for larger organisms, aquatic ecosystems more broadly.

These findings, when taken in conjunction with existing literature on viruses' role in the cycling of nutrients through aquatic ecosystems[2,7], begin to lay the groundwork for a more complete understanding of the important role viruses play in aquatic food webs. It is becoming increasingly clear that viruses influence the cycling of carbon, nitrogen, and phosphorous both up and down the trophic ladder[6–8], and there is evidence that different varieties of virus were consumed at different rates by protists. This indicates that different virus species likely play different roles in their ecosystems, meaning that variations in the viral profile of an environment may influence nutrients flows [5,6]. There is potential for future experiments that measure multitrophic systems to determine the impacts that virus concentration and variety have on the populations of not only the species that graze on viruses but also those that prey on virovores, or experiments that include both chloroviruses and their algae hosts in a system with a protist that grazes on both. This study only scratches the surface of the complexity of the ecological implications of virovory, but it sets the stage for a myriad of other potential explorations of the phenomenon.

While this study's findings relating virovory to population growth provide valuable insight into the ecological functions of viruses, we failed to establish any significant relationship between protist species' cell volume and ability to grow on viruses. This is likely because cell volume is not the sole determinant of virus-mediated population growth. Other potential factors include metabolic rate, space clearance rate, and surface area, among others. The protists we studied in this experiment are very morphologically and taxonomically diverse, making direct interspecies comparisons difficult. However, now that virovory has been demonstrated to impact the growth of a wide variety of species, a detailed study of the behavior within a single clade seems to be the logical next step. Such an experiment could be conducted using species from a single genus, such as *Paramecium*, and would allow for a more refined study of the impacts that specific traits have on virus-mediated population growth, as well as a more focused study of cell volume as a determinant of impactful virovory.

While this experiment's results are promising, it still has several methodological imperfections that could be improved in future iterations. Most notably, the growth of several control populations indicates that our filtration was insufficient to completely remove all non-virus food sources from the control environments. These additional nutrients may have originated from a variety of sources, including other viruses and organic particulate smaller than the 0.1 μm and 0.2 μm filter pores, detritus from viral hosts that persisted in the viral solution, or bacteria that our washes were insufficient to remove from the samples. The presence of these nutrient sources means that our experimental treatments may have been confounded by inadvertent alternate food sources.

However, the differential growth rates of various species in this experiment still present us with a path forward for the refinement of our techniques in future studies. *H. grandinella* is now an established virovore, and also experienced the greatest population growth in our current control environment. This growth under control conditions indicates that *H. grandinella* is capable of extensive growth and development using only small, dissolved particulate as a food source. This ability means that the species has the potential to be utilized in the future as a test group for evaluating the nutrient content available in control environments, allowing us to further refine our methods to eventually produce fully negative controls. These negative controls would make it possible to conduct a more complete study of virovory and its population effects in the future. Such a study would allow us to better evaluate the specific energetic and nutritional contents of individual varieties of virus, as well as the variable rates at which different protist populations are able to grow and divide when eating viruses.

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