INFLUENCE OF NITROGEN SOURCE ON MIXOPLANKTON-GIANT VIRUS INFECTION DYNAMICS

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ABSTRACT

Giant viruses are large, double-stranded DNA viruses that play an important role in marine ecosystems by killing unicellular eukaryotic plankton. Due to their size, giant viruses are hypothesized to enter and infect aquatic protists via phagocytosis, potentially disguised as bacterial prey. This study investigates the role of nitrogen source on the infection dynamics of two dictyochophyte-infecting giant virus strains, DictyXV and FloV-SA1, when infecting six mixotrophic algal strains. The viruses and algae in this study were isolated from the epipelagic zone of the North Pacific Subtropical Gyre, where mixotrophic algae consume prey to overcome nutrient limitation. We hypothesized that the algae grown under mixotrophic conditions would face increased susceptibility to viral infection, and therefore greater mortality, because they would be conditioned to graze on bacterial and viral-sized particles. First, we acclimated the parent cultures to two different conditions: dissolved N (autotrophic growth) or prey-derived N (mixotrophic growth) in seawater-based medium. Second, we infected the experimental cultures with either DictyXV or FloV-SA1, and monitored algae and virus populations using flow cytometry until ten days post-infection. These results were then analyzed to determine percent mortality and burst size, and statistical models tested whether virus strain, host strain, and N source explained the success of the viral infections. Our results indicate that FloV-SA1 infection leads to higher percent mortality than DictyXV in 11 of 12 host:medium combinations and that FloV-SA1 had a slightly larger burst size than DictyXV, suggesting it is able to replicate more efficiently inside the host cells. We found that N source variably affected the magnitude of mortality and burst size in most algae strains, and not always in the direction hypothesized. These results emphasize that N source and trophic plasticity can shape virus-mixotroph interactions, complicating our ability to predict how these interactions may contribute to marine biogeochemical cycles.

Key words: viruses, mixotrophs, nitrogen, infection

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1.0 INTRODUCTION

1.1 BACKGROUND

Phytoplankton are the foundation of marine food webs and export large amounts of organic carbon to the deep ocean through the biological C pump (Ducklow et al. 2001, Stuckel et al., 2023). Past conceptual models put forth a strict distinction between autotrophic phytoplankton that receive their energy from the sun and heterotrophic plankton that graze on smaller plankton. In the late 1990s, conceptual models began blurring those distinctions in recognition of mixotrophic plankton – phytoplankton that are both autotrophic and heterotrophic (Stoecker, 1998). Recent mathematical models have predicted that mixotrophic algae may enhance the biological C pump (Ward & Follows, 2016). Mixotrophy has ecological implications as well, as mixotrophs are less dependent on dissolved, inorganic nutrients to survive because they can acquire nutrients through predation (Ward & Follows, 2016; Ward, 2019). In subtropical ocean gyres, mixotrophic plankton outcompete strictly autotrophic or heterotrophic plankton because they can utilize ample sunlight to produce organic C and overcome nutrient limitation through the ingestion of prey (Edwards, 2019). While mixotrophs may be able to survive and even thrive in low-nutrient conditions, they still face population loss due to predation (Calbert & Landry, 2004) and viral infection (Brussaard et al., 2005).

Viruses are abundant and genetically diverse, and play a vital role in the marine food web by contributing to loss terms of phytoplankton populations, and releasing macronutrients such as N and P back to the base of the food web (Suttle, 2007). They do this through lytic infections, which entail the virus killing the host cell, causing it to “burst” open, releasing intracellular material into the water column (Rohwer and Thurber, 2009). On an ecosystem-wide scale, giant viruses in the *Nucleovytoviricota* phylum have led to the demise of eukaryotic algae blooms in the North Atlantic (Laber et al., 2018). Giant viruses often have a capsid diameter equal to or greater than 300 nm (Claverie & Abergel, 2016), whereas the average marine virus has a capsid diameter of ~50 nm (Brum et al., 2013). Since their discovery in 1999, the physical characteristics of giant viruses have challenged the boundary between viruses and cellular life (Etten and Meints, 1999). Their size may be a key factor of their success because certain giant viruses use their size to gain entry into amoeboid protists by triggering the host cell to engulf the viral particle via phagocytosis (Abergel & Claverie, 2020).

Mixotrophic plankton occupy a unique ecological niche due to their ability to take up dissolved nutrients as well as nutrients in the form of prey, which allows them to survive a wide range of environmental conditions. The relative availability of dissolved nutrients and prey varies with factors such as depth, seasonality, and geographic location, and this exerts environmental pressure on the mixotrophic community to modulate between autotophy or mixotrophy to exploit favorable environmental conditions (Millette et al. 2023). The form of nutrients most available to mixotrophs can influence their nutritional mode, either favoring autotrophy when dissolved nutrients are replete or phagotrophy when they are scarce, and this in turn may influence their susceptibility to viral infection and how many viruses can be produced during infection. Therefore, understanding how nutrient source affects virus-host dynamics is important for gathering more insight into the ecology of mixotrophic algae in the marine environment. The response of mixotrophs to infection may depend on the physiology of the host, which can vary greatly across mixotroph taxa (Li et al., 2022). By testing multiple mixotroph strains from different genera, as well as multiple virus strains, we can determine if responses to nutrient source are consistent across taxa.

Here, we focused on quantifying the virus-driven population loss of mixotrophic algae under both dissolved and prey-derived nutrient growth conditions. We challenged six dictyochophyte algae strains from three genera against two *Schizomimiviridae* viruses. All of the algae and two viruses were isolated from the North Pacific Subtropical Gyre (NPSG), where N limitation exerts a bottom-up control on ecosystem productivity (Karl et al., 1997). We varied the N source between dissolved, inorganic N (DIN) and prey-derived N, using *Prochlorococcus* as the bacterial prey because dictyochophytes are important grazers of *Prochlorococcus* in the NPSG (Frias-Lopez et al., 2009), (Li, 2022). We hypothesized that mixotrophic algae grown in a nitrogen-deplete medium, but supplied with prey cells as an alternative source of nutrition, would have higher percent mortality because they would be forced to consume particles in the size range of bacteria and giant viruses to get the nitrogen they require to grow. To test this hypothesis, we used two closely related giant viruses. These giant viruses, DictyXV and FloV-SA1, were isolated on dictyochophyte algae from the *Dictyochophyceae clade-X* and *Florenciella* genera in 2014 and 2019.

2.0 METHODS

2.1 TYPES OF MEDIA

The xenic algae cultures were grown in either full K medium to promote autotrophy (Keller et al., 1987), or K medium with the exclusion of dissolved, inorganic N (DIN) and the addition of prey cyanobacterium *Prochlorococcus* (K-N+*Prochlorococcus*, Li et al., 2021, Li et al., 2022) to promote mixotrophy. To make the K-N+*Prochlorococcus* medium we excluded nitrate and ammonium chloride and instead added 500 μL of stationary phase *Prochlorococcus* ($2.0710^{3} - 7.94 * 10^{6}$ cells/mL) grown in Pro99 (Moore et al., 2007) to the 25 mL mixotrophic algae cultures. We estimated the DIN carry-over of NH_4Cl from Pro99 medium, as well as the amount of N contained in the added *Prochlorococcus* cells, to compare total added N in autotrophic and mixotrophic growth conditions. The autotrophic conditions contained $1.0 * 10^{-3}$ mM N and the mixotrophic conditions contained 16-24 μM N. The autotrophic growth conditions contained greater total N by two orders of magnitude, but the carrying capacity of the autotrophic vs. mixotrophic growth conditions were not systemically higher (Figure 4-7), which suggests that the interaction between strain physiology and N source may have a greater influence on population growth than total N.

2.2 TYPES OF VIRUS TREATMENTS AND MAINTENANCE

There were three treatments each algal cultures was subjected to: two virus treatments — DictyXV or FloV-SA1— and one control treatment without virus. For the virus treatments, we added raw lysate to the algae cultures at a volumetric ratio of 0.5 : 25 mL, with concentrations of the virus inoculum listed in Table 2. Virus inoculum for the experiments were prepared on *Florenciella* str. UHM3003 grown on K-N+*Prochlorococcus* medium. To prepare the virus inoculum, 25 mL of UHM3003 culture was grown for 3-4 days to early exponential phase, then infected with 300 µL of either DictyXV or FloV-SA1 lysate. The cells were infected over a period of 4-5 days until the culture cleared, then the raw lysate was placed in 4°C for storage. The inoculum was stored for 6-18 days before use. For the experiment, the timing of infections was internally consistent for each algae strain, either beginning in late exponential or early stationary phase to control for growth phase when tracking population dynamics.

Table 1. Estimated mean virus-like particles (VLP) of DictyXV and FloV-SA1 in the two N-source treatments.

	Autotrophic condition		*Mixotrophic condition*	
Algae Strain	DictyXV (VLP/mL)	FloV-SA1 (VLP/mL)	DictyXV (VLP/mL)	FloV-SA1 (VLP/mL)
UHM3003	1.41E+06	1.30E+06	1.41E+06	1.30E+06
UHM3011	1.41E+06	1.30E+06	1.41E+06	1.30E+06
UHM3054	1.59E+06	2.05E+06	1.55E+06	1.60E+06
UHM3070	1.67E+06	2.04E+06	1.67E+06	2.04E+06
UHM3071 (1)	1.39E+06	2.46E+06	1.67E+06	2.04E+06
UHM3071 (2)	1.13E+06	1.19E+06	1.13E+06	1.19E+06
UHM3074	1.67E+06	2.04E+06	1.67E+06	2.04E+06

2.3 ALGAE CULTURE MAINTENANCE AND SAMPLE COLLECTION

Parent cultures of the six algae strains were transferred into either full K or K-N+*Prochlorococcus* medium on May 28, 2024 at a volumetric ratio of 300 µL : 25 mL. This gave the cultures 20-93 days to acclimate to either medium. Parent cultures with longer acclimations were transferred into fresh medium each month, and if grown under prey-derived

N, given fresh bacterial prey as described in section 2.1. We acclimated the parent cultures so that when the first experiment began, the experimental cultures would not have a period of adjustment to new growth conditions. At the beginning of each experiment, 300 μL of algae parent culture was transferred into 25 mL of either full K or K-N+Prochlorococcus medium. Each treatment included three biological replicates, totaling 18 experimental cultures per host strain (2 growth conditions × [2 virus + 1 control treatments] × 3 biological replicates). These cultures were sampled daily throughout the entirety of the experiments performed.

To sample the 25 mL cultures, 490 μL of culture was subsampled into corresponding cryovials labeled with the day of the experiment, algae strain, treatment type, and replicate number. This was done in the laminar flow hood following sterile technique to lower contamination risk. The samples were then fixed in a chemical fume hood by adding 10 μL of electron microscopy grade ice cold glutaraldehyde (25% concentration), and pipetting up and down 5 times to mix the fixative and algae culture together for a final concentration of 0.5% glutaraldehyde. The fixative helped preserve the structure of the algal cell walls and virion capsids to prevent denaturation during the freeze-thaw steps taken to store and process the samples, so they can be accurately counted using flow cytometry.

After fixing the cultures, they were incubated at 4°C for 15 minutes, then immediately flash frozen into liquid N_2 and placed in storage at -80°C until they were processed on the flow cytometer (FCM). When the samples were counted on the FCM, they were taken out of the -80°C freezer and thawed at room temperature (20°C). Once thawed, the samples were counted the day-of.

2.4 FCM OVERVIEW AND CALIBRATION

The flow cytometer (CytoFLEX S; Beckman Coulter Life Sciences, Indianapolis, IN, USA) was calibrated to three different flow rates: 60, 30, and 10 µL/min, and the algae samples were run at 20-30 µL/min and the virus samples were run at 10 µL/min (Table 2). We selected acquisition speeds based on the concentration of particle events in the sample; the higher the concentration of particles, the slower the flow rate should be. High event and flow rates can lead to higher abort rates, wherein the instrument detects, but fails to record a particle event because its optical properties cannot be distinguished from other particles. The abort rates for our experiment were <5%. We distinguished populations of events using side scatter area (SSC-A) and fluorescence, chlorophyll autofluorescence (680 nm) for algae populations and SYBR Green DNA stain (520 nm) for virus populations (Table 3).

Table 2. CytoFlex FCM acquisition speeds and durations used for counting mixotrophs and virus-like particles.

	Speed (µL/min)	Duration (min)
Mixotroph acquisition speed (Jun. 17-23, 2025)	30	2
Mixotroph acquisition speed (Jun. 24-Nov. 3, 2025)	20	1
Virus acquisition speed (Jun. 21-Nov. 3, 2025)	10	1

Table 3. CytoFlex FCM gain settings used for counting mixotrophs and virus-like particles.

	FSC	SSC	Sybr Green	Chloro-phyll	V-SSC	Hoechst-Violet	Violet-610	Violet-660	PE	R-Phyco-cyanin
Mixotroph gain settings (Jun. 17-19, 2025)	72	42	84	300	1	115	298	528	500	N/A
Mixotroph gain settings (Jun. 20-Nov. 3, 2025)	100	100	84	200	50	50	N/A	50	100	50
Virus gain settings (Jun. 21-Nov. 3, 2025)	100	100	300	N/A	200	N/A	N/A	N/A	500	N/A

To determine the population of mixotrophic algae and prey cyanobacteria, 200 µL of sample was pipetted into the wells of a 96-well plate and detected using side-scatter and autofluorescence. For virus counts, the samples were stained with the DNA-specific SYBR Green I dye to distinguish for the virus-like particles (VLP) based on DNA content and side scatter (Brussaard et al., 2004). We prepared fresh working stocks (2.5x final concentration into 0.02 µm-filtered TE buffer, pH 8.0) of SYBR Green dye each day. To stain the samples, 5 µL of fixed sample was diluted into 195 µL of the SYBR Green working stock. Because of the significant dilution of the sample, we could not accurately count VLPs when concentrations were lower than ~10^4 VLP/mL (1-3 days post-infection, DPI), but the dilution greatly improved our ability to count VLPs when they reached maximum concentrations (~10^8 VLP/mL). To promote stain penetration, samples were incubated at 80°C in a water bath for 20 minutes. A counting plate with bead standards was prepared by adding 3 µL of 0.5 µm of fluorescent beads (0.0002% final concentration, Polysciences Inc., Warington, PA USA) to each well, then 150 µL of each of the stained samples was transferred to wells of the plate and mixed. Since the beads were slightly larger than the virus (345-425 nm capsid diameter), they were used as a marker to confirm the location of the giant virus population on the flow cytograph.

2.5 SAMPLE COUNTS AND ANALYSIS

We utilized a custom python script to set gates and count mixotrophic algae populations. For virus populations gates were drawn manually for each sample to ensure minimal interference from debris from the lysing algae populations. We then calculated cell and virus concentrations, the standard deviation of cell and virus counts per treatment, and visualized this data as a time-series with a custom R script.

2.6 CALCULATIONS OF PERCENT MORTALITY

To calculate percent mortality, the following equation was used:

$$\%\, mortality \;=\frac{(max.\, algae - min.\, algae)}{(max.\, algae)}\ .$$

The data were logit-transformed for normality and analyzed with a linear model to test whether percent mortality varied by virus strain, host genus, or N source, as well as interactions between these predictors. The formula was lm(logit.mortality ~ virus.strain + host.strain + N source + virus.strain:host.strain + virus.strain:N source + host.strain:N source + virus.strain:host.strain:nutrient.regime). The significance of each model term was tested with Type II F-tests.

2.7 CALCULATIONS OF BURST SIZE

To calculate burst size per replicate, the following equation was used:

$$Burst\ size\ =\ \frac{(max.\, VLP)}{(max.\, algae - min.\, algae)}.$$

The burst size data was normalized to the estimated host cell volume to control for cell size (Li et al., 2022), since larger cells with greater volume can produce more virions. This normalized burst size value provided a more accurate comparison of burst size across host strains that differ in size. The normalized burst size values were analyzed with a linear model using the same set of predictors as the percent mortality model.

3.0 RESULTS

3.1 *FLORENCIELLA* UHM3003

Florenciella UHM3003 had a higher carrying capacity in full K than in K-N+*Prochlrococcus*, suggesting the amount of DIN supported more growth and could be the preferred N source (Fig. 1). Algae populations grown under both N-source conditions dropped dramatically after being infected (Fig. 1). Virion production of both DictyXV and FloV-SA1 was higher under full K conditions, but UHM3003 showed signs of recovery to DictyXV infection about 8 days post-infection (DPI, Fig. 1). FloV-SA1 infection suppressed the UHM3003 population over the course of the experiment (10 DPI, Fig. 1). The results under full K conditions were less dramatic than DictyXV. Both virus populations reached a steady-state ~4 DPI (Fig. 1).

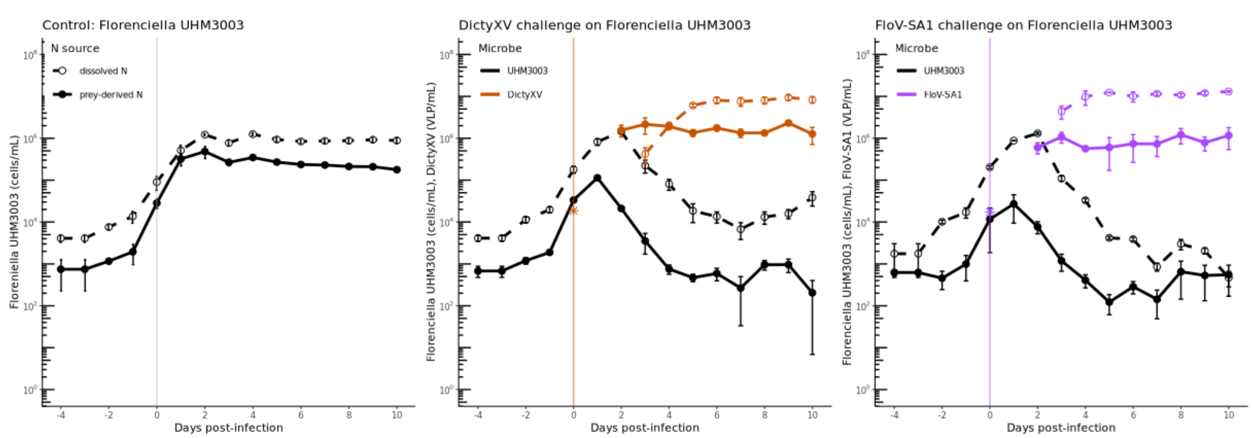

Figure 1. Time-series of control and infection treatments on Florenciella str. UHM3003 under DIN (autotrophic growth conditions, dashed line and open circle) and prey-derived N (mixotrophic growth conditions, solid line and closed circle). The line colors denote the population: algae (black), DictyXV (orange), and FloV-SA1 (purple).

3.2 *FLORENCIALLA* UHM3011

Florenciella UHM3011 also grew to a higher carrying capacity under DIN vs. prey-derived N conditions, and the UHM3011 population grown in DIN showed signs of stabilization to DictyXV infection by 3 DPI (Fig. 2). In contrast, the UHM3011 population experienced higher mortality and produced a greater number of viruses from FloV-SA1 infection in DIN conditions (Fig. 2). Both viruses suppressed UHM3011 populations under prey-derived N conditions and virus population levels reached an equilibrium ~4 DPI (Fig. 2).

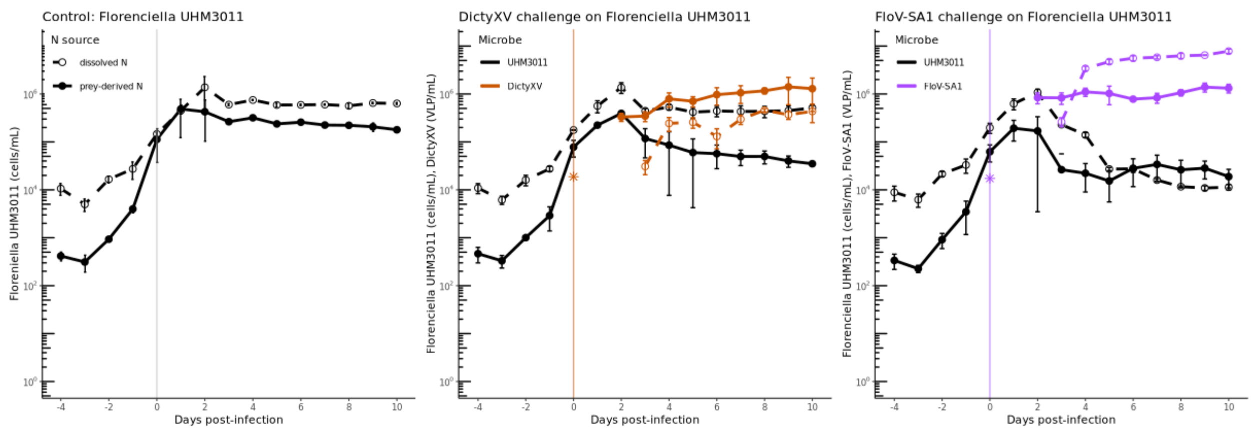

Figure 2. Time-series of control and infection treatments on *Florenciella* str. UHM3011 under DIN and prey-derived N. The line type and color follow the conventions of figure 1.

3.3 *DICTYOCHOPHYCEAE CLADE-X* UHM3054

The *Dictyochophyceae clade-X* str. UHM3054 grew at a faster rate and had a higher maximum abundance under prey-derived N conditions than DIN conditions (Fig. 3). To control for growth rate, and by extension growth phase, the DIN conditions were inoculated 8 days post initial transfer. Prey-derived N not only resulted in higher yields for UHM3054, it also resulted in a greater proportion of cells dying and more virions produced in comparison to DIN treatment (Fig. 3). Population sizes of UHM3054 collapsed to similar concentrations by 10 DPI regardless of nitrogen source (Fig. 3). DictyXV counts increased rapidly between 3 to 4 DPI under prey-derived N conditions, then leveled off at steady state (Fig. 3). In full K medium, both the maximum counts of UHM3054 and DictyXV were considerably lower (Fig. 3), suggesting autotrophic growth was unfavorable for both host and virus production. The same patterns were observed for FloV-SA1 (Fig. 3).

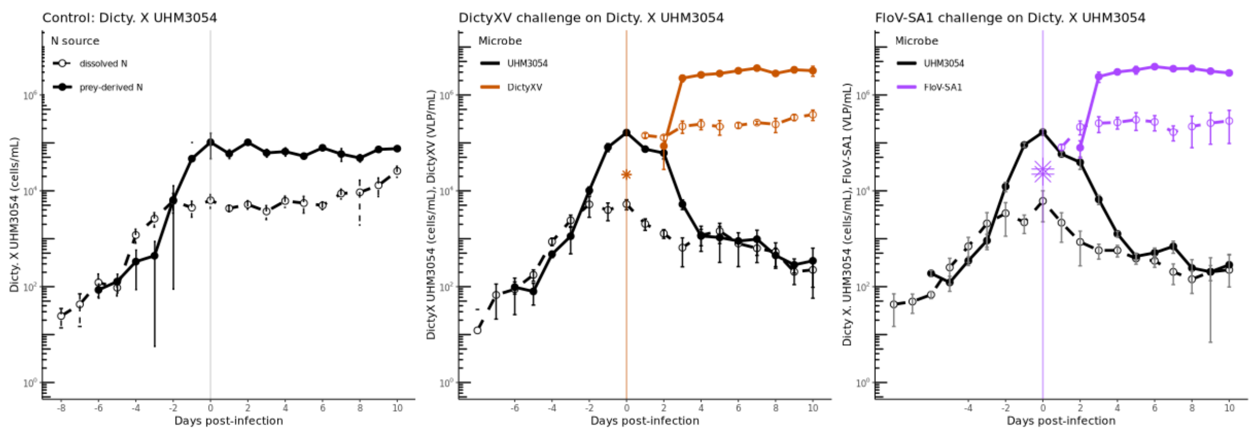

Figure 3. Time-series of control and infection treatments on *Dictyochophyceae clade-X* str. UHM3054 under DIN and prey-derived N. The line type and color follow the conventions of figure 1.

3.4 *RHIZOCHROMULINA* UHM3070

Growth on DIN resulted in lower maximum yields of *Rhizochromulina* UHM3070 compared to the growth on prey-derived N (Fig. 4). UHM3070 cells collapsed to a similar concentration after infection in all treatments. Number of DictyXV produced under prey-derived N conditions were slightly higher than the virus grown under DIN (Fig. 4). However, both had very similar rates of production (Fig. 4). FloV-SA1 displayed a similar infection pattern (Fig. 4).

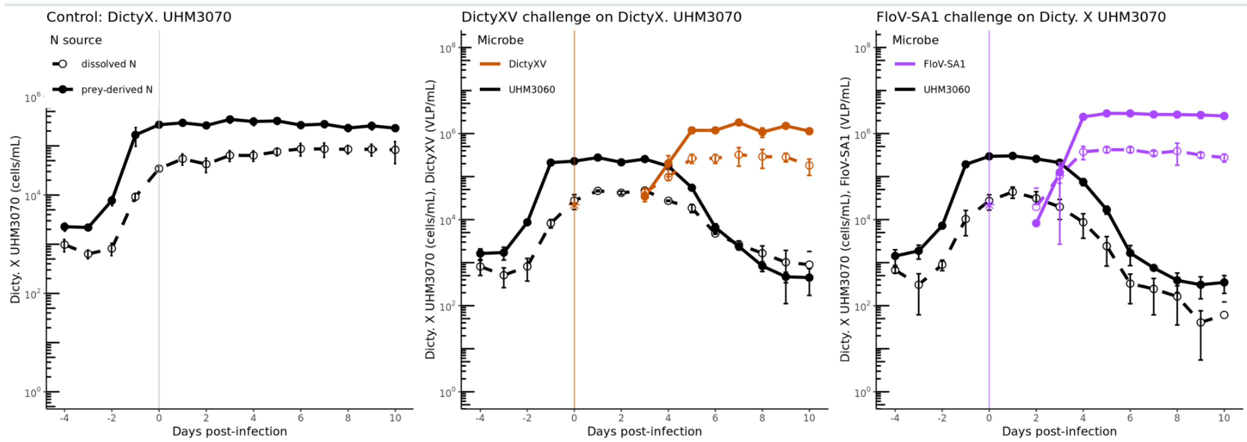

Figure 4. Time-series of control and infection treatments on *Rhizochromulina* UHM3070 under DIN and prey-derived N. The line type and color follow the conventions of Fig. 1.

3.5 *RHIZOCHROMULINA* UHM3071

DIN was also the less preferred N source for *Rhizochromulina* UHM3071 as indicated by the lower maximum cell yields (Figs. 5, 6). Despite the lower cell yields, the death of cells and production of virions was greater with DIN (Figs. 5, 6). Under the preferred, prey-derived N source, the population of the host remained relatively stable, barely showing signs of decline after infection with either virus (Figs. 5, 6). This may provide an example of how a host having access to its preferred N source significantly affects its ability to resist viral infection. DictyXV was not able to replicate to a detectable concentration under prey-derived N conditions (Fig. 5, 6). DictyXV could replicate under the DIN conditions, and had a steady rise in population before leveling off 4 DPI (Figs. 5, 6). In the first time-series experiment, FloV-SA1 was able to replicate under prey-derived N conditions, but had a much smaller impact on the overall algae population (Fig. 5). We were able to reproduce results showing a failed DictyXV infection under prey-derived N conditions, notably, the FloV-SA1 infection also failed in the second time-series experiment (Fig. 5, 6).

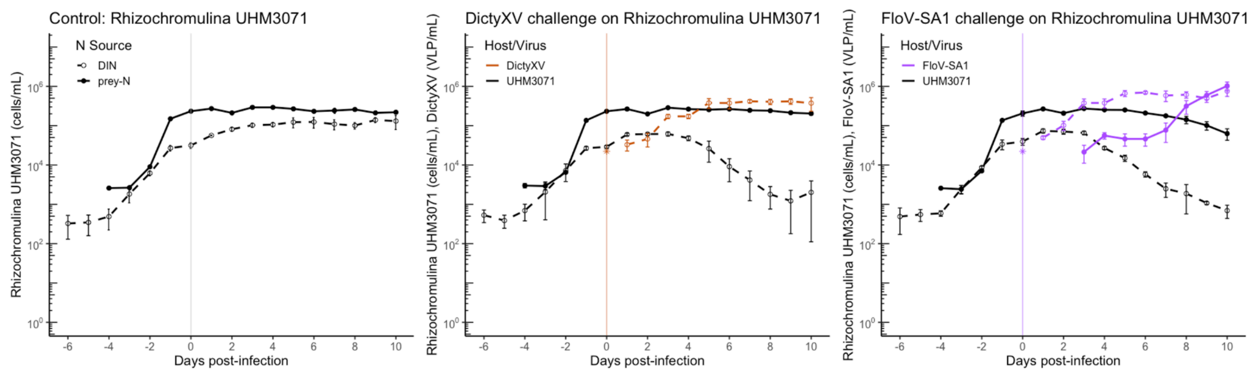

Figure 5. Time-series of control and infection treatments on Rhizochromulina str. UHM3071 under DIN and prey-derived N. The line type and color follow the conventions of figure 1.

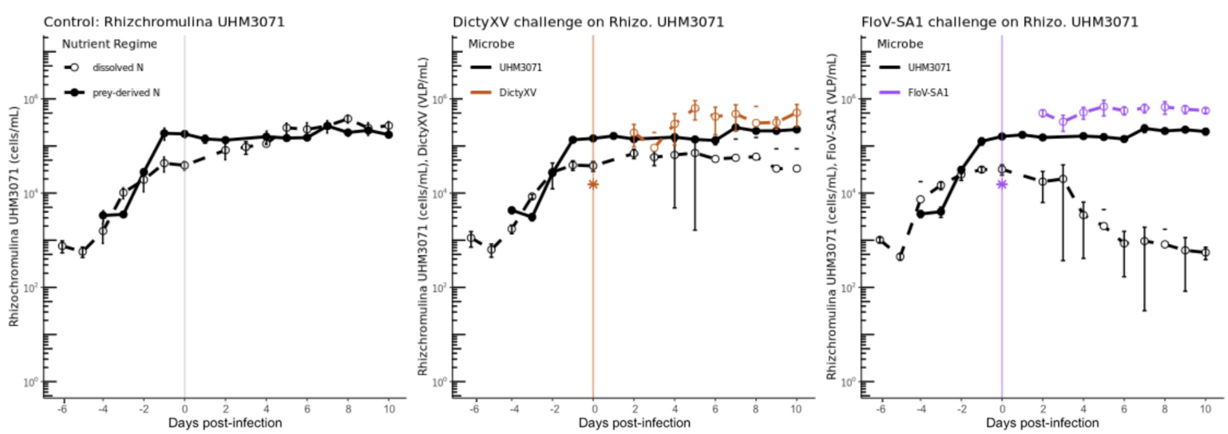

Figure 6. Repeated time-series of control and infection treatments on *Rhizochromulina* str. UHM3071 under DIN and prey-derived N. The line type and color follow the conventions of figure 1.

3.6 *RHIZOCHROMULINA* UHM3074

DictyXV grown under full K had slightly higher counts than virus grown under K-N+Pro (Fig. 7). They had similar growth rates however, with a steep overall slope of growth. FloV-SA1 growth rates were almost the exact same for the two different mediums (Fig. 7).

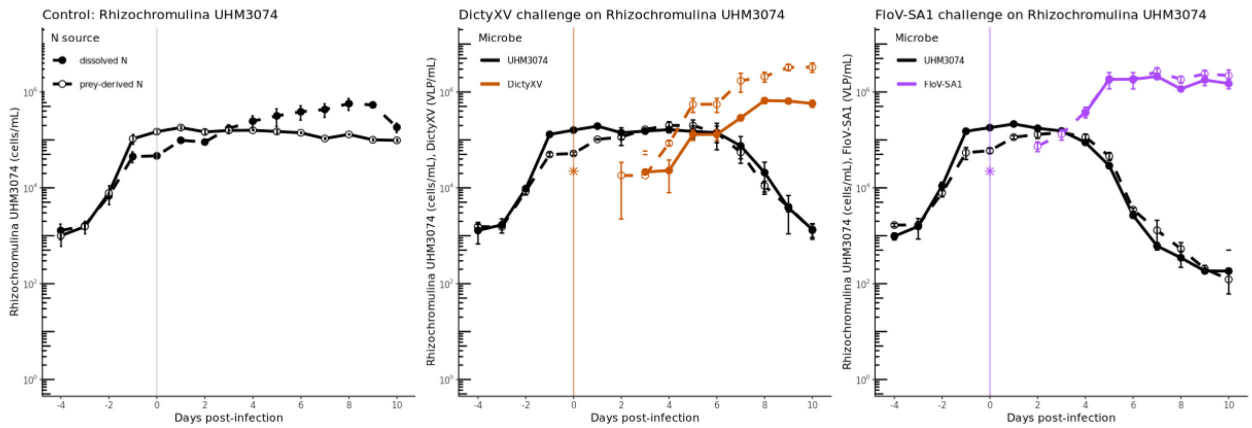

Figure 7. Time-series of control and infection treatments on Rhizochromulina str. UHM3074 under DIN and prey-derived N. The line type and color follow the conventions of figure 1.

3.7 PERCENT MORTALITY

Our most robust result was that FloV-SA1 led to higher percent mortality in 11 out of 12 of the medium and host permutations ($F_{1,60} = 61$, $p < 1e\text{-}10$, Fig. 8, Table 4). In 3 out of 6 algae strains, the differences in percent mortality between the two viruses were less distinct under prey-derived N versus DIN conditions (Fig 8). In contrast to our hypothesis, N source did not have consistent effects on percent mortality. Different strains exhibited higher, lower, or unchanged mean mortality when grown mixotrophically. One host, *Rhizochromulina* str. UHM3071, showed dramatically improved survival in mixotrophic versus autotrophic conditions (percent mortality in DictyXV: 40.5% [mixotrophic] vs. 94.1% [autotrophic], FloV-SA1: 60.8% [mixotrophic] vs. 99.0% [autotrophic], reproduced 2x, Fig. 8), which was the opposite of the prediction.

Across all strains and N sources, the main effect of host strain ($F_{5,60} = 115.03$, $p < 2.2e{-16}$) indicates that mortality varied significantly among hosts, confirming they differ in their overall susceptibility to infection. The main effect of N source ($F_{1,60} = 16.30$, $p = 0.00016$)

suggests a consistent difference in mortality between N sources, with higher average mortality under dissolved N, contrary to the initial hypothesis.

There was no significant virus:host strain interaction ($F_{5,60} = 1.61$, $p = 0.17$)), indicating that, on average, the viruses did not exhibit host-specific performance. However, the virus:N source interaction ($F_{1,60} = 12.84$, $p = 0.00068$) supports that the relative effect of each virus depends on nitrogen conditions, generally with reduced virus-driven mortality differences under prey-derived N. The host:N source interaction ($F_{5,60} = 53.91$, $p < 2.2e{-16}$) reveals that host responses to N source varied. Finally, the three-way virus:host:N source interaction ($F_{5,60} = 3.46$, $p = 0.0083$) provided some evidence for viral specialization on hosts that is contingent on N source.

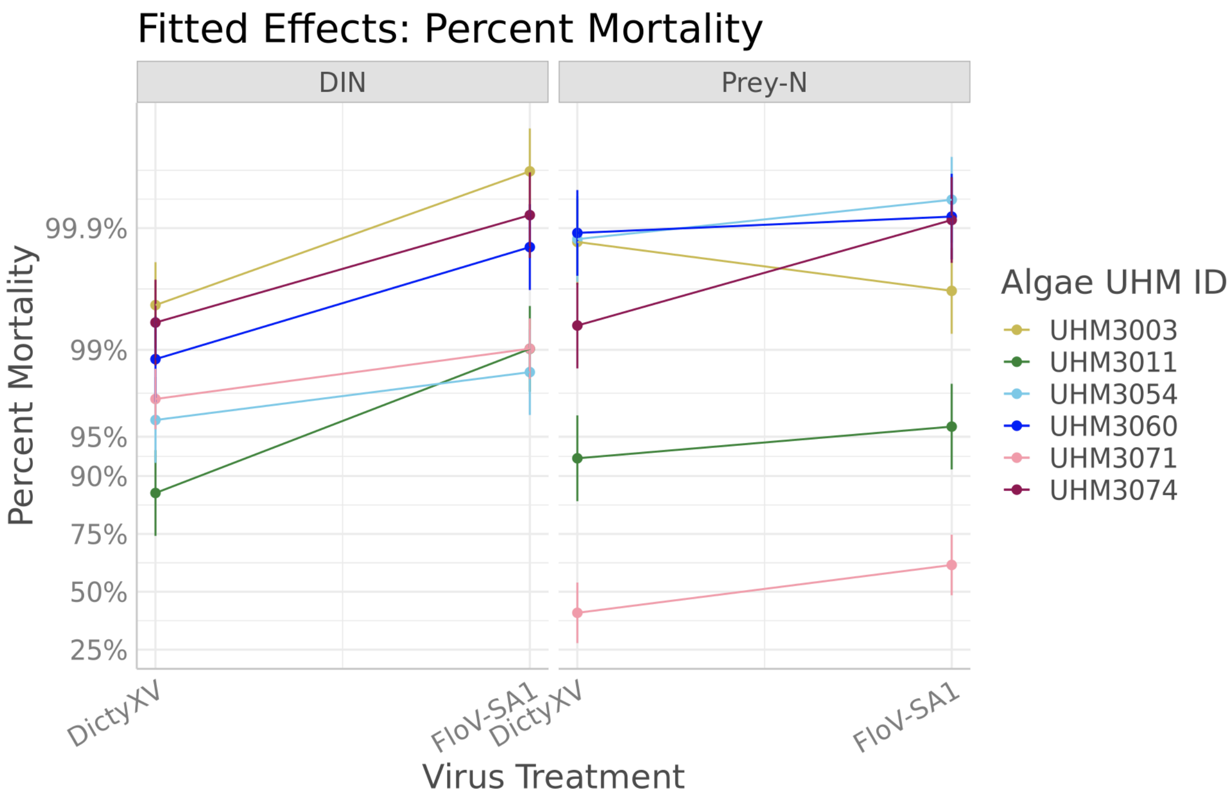

Figure 8. The fitted effects plot from the linear model of logit-transformed percent mortality values of DictyXV and FloV-SA1 (left to right) under different N sources (panels), showing DIN on the left panel and prey-derived N on the right panel. The different colors represent the different host strains (yellow = *Florenciella* str. UHM3003, green = *Florenciella* str. UHM3011, light blue = *Dictyochophyceae clade-X* str. UHM3054, dark blue = *Rhizochromulina* UHM3070, *Rhizochromulina* str. UHM3071, *Rhizochromulina* str. UHM3074.

Table 4. Variation in percent mortality explained by virus, host strain, N source, virus: host strain, virus:N source, host strain N source, and virus:host strain:N source. These factors were fixed effects in the ANOVA model.

Term	Sum of Squares	Degrees of Freedom	F-value	p-value
Virus	30.31	1	61.05	1.02e-10
Host strain	285.54	5	115.03	< 2.2e-16
N source	8.09	1	16.30	0.00016
Virus:Host strain	4.01	5	1.61	0.17
Virus: N Source	6.37	1	12.84	0.00068
Host strain: N Source	133.82	5	53.91	< 2.2e-16
Virus: Host strain: N Source	8.58	5	3.46	0.0083

3.8 BURST SIZE

On average the burst size of FloV-SA1 was greater than DictyXV $F_{1,60} = 9.54$, $p = 3.04 \times 10^{-3}$; Fig. 9, Table 5). This trend was most pronounced in *Florenciella* str. UHM3003 under prey-derived N conditions (Fig. 8), although there was a weaker trend for increased burst size of FloV-SA1 for most host strain:medium combinations. These results suggest that the replication efficiency of FloV-SA1 may be greater than DictyXV, which may contribute to its greater impact on host mortality.

The ANOVA results indicate a significant effect of host strain ($F_{5,60} = 7.91$, $p = 8.96 \times 10^{-6}$) on burst size, demonstrating that host species differ in how many viruses are produced before lysis. There was also an effect of N source ($F_{1,60} = 11.54$, $p = 1.22 \times 10^{-3}$), indicating that N source influences burst size, with a trend toward higher virus production before lysis under dissolved N conditions. Finally, the host strain:N source interaction was significant ($F_{5,60} = 11.3$, $p = 1.06 \times 10^{-7}$), indicating that the effect of N source on viral replication varies among the host strains.

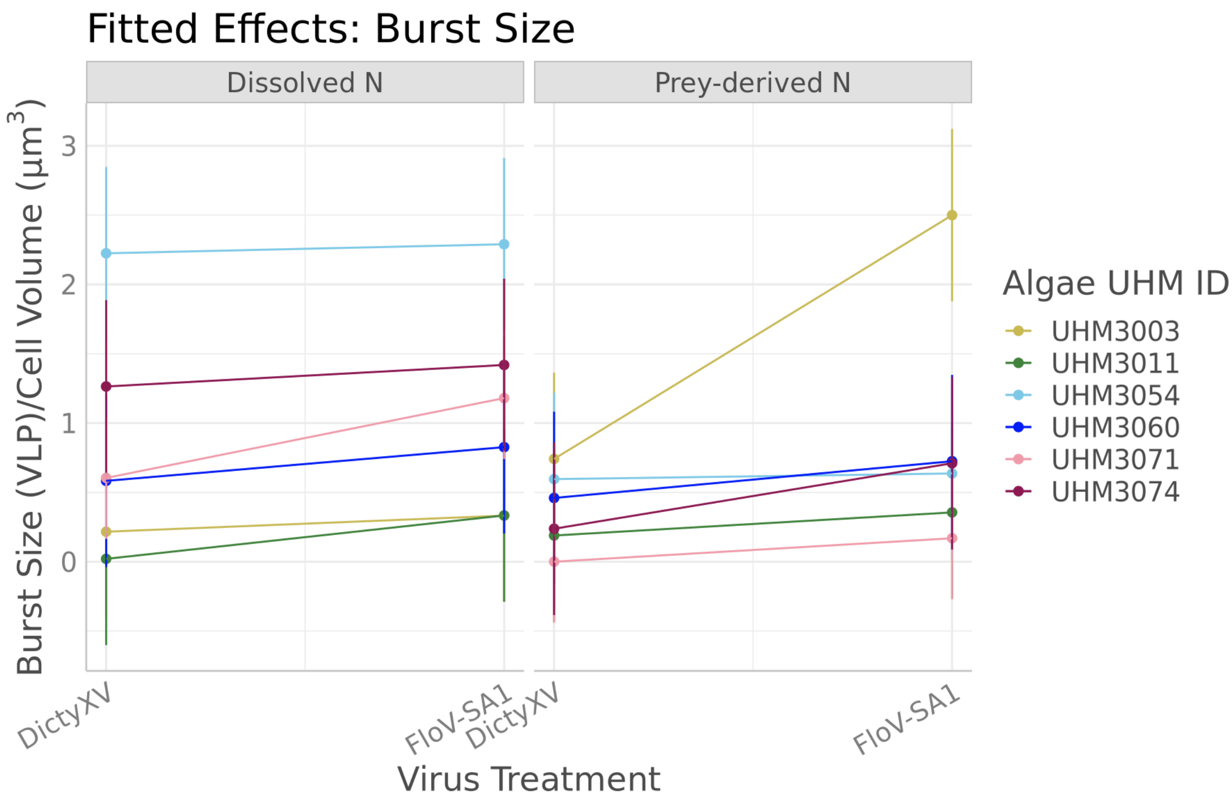

Figure 9. Fitted effects plot from the linear model of burst size normalized to estimated host cell volume. Burst size values for DictyXV and FloV-SA1 are shown left to right within different N-source growth condition panels, showing DIN on the left panel and prey-derived N on the right panel. The colors represent algal strain as described in Figure 8.

Table. 5. Variation in burst size explained by virus, host strain, N source, virus: host strain, virus:N source, host:strain N source, and virus:host strain:N source. These factors were fixed effects in the ANOVA model. The significance codes are the same as Table 4.

Terms (Burst Size)	Sum of Squares	Degrees of Freedom	F-value	p-value
Virus	2.77	1	9.54	3.04E-03
Host strain	11.48	5	7.91	8.96E-06
N source	3.35	1	11.54	1.22E-03
Virus:Host strain	1.37	5	0.94	0.460762
Virus: N Source	0.11	1	0.37	0.547076
Host strain: N Source	16.4	5	11.3	1.06E-07
Virus: Host strain: N Source	2.26	5	1.56	0.186588

4.0 DISCUSSION

Our study investigated the production of viruses on mixotrophic algae under varying N sources. We found that FloV-SA1 had greater virulence than DictyXV, as shown by its increased host mortality and larger burst size. However, the susceptibility to infection of hosts grown in DIN versus prey-derived N conditions varied with host strain, indicating that the role of N source in infection dynamics was complex. We found that mixotrophic conditions did not, in all instances, lead to higher mortality. Instead, interactions between the host and virus were specific to the strain of algae.

The higher virulence of FloV-SA1 is likely connected to its larger burst size. Burst size is an indicator of the impact viruses can have on phytoplankton populations, with larger burst sizes correlating with a more rapid decline in the population of the host algae (Edwards et al., 2021). Our study's results align with this idea and suggest that FloV-SA1's high efficiency of replication may be why it causes greater host mortality. Small variations in virus genotype, such as additional peptidases observed in cyanophages, may lead to greater efficiency in the breakdown of host proteins (Ke et al., 2025). A similar genetic mechanism relating to increased efficiency of viral take-over may underlie the the increased host mortality and burst size of FloV-SA1, giving this virus a competitive advantage over the other.

Our results indicate that the effect of N source on host mortality varies depending on the host strain, and that there was no universal response to infection. Some hosts, such as *Rhizochromulina* UHM3071, had noticeably higher survivorship under mixotrophic conditions, while other hosts showed no significant differences between N sources. We hypothesized that mixotrophic hosts would show greater mortality due to their use of phagocytosis that would lead to engulfing viral particles. Our data did not consistently support this, which suggests that N source affects infection via other mechanisms that are currently unknown.

Our findings contribute to a broader understanding of virus-host interactions in marine ecosystems. FloV-SA1 has a higher virulence and this suggests that certain giant viruses can control phytoplankton populations to a greater extent than others, which can have implications for the movement and storage of carbon in the oceans. Mixotrophic algae are both primary producers and can consume other organisms, so viral infections of these algae could impact both carbon cycling through the lysis of carbon-fixing microorganisms and removal of grazers of smaller planktonic prey. Additionally, the wide range of results in host responses is representative of the complexity of virus-host interactions. While our study provides insights into these interactions, further studies should clarify the genetic mechanisms that influence host mortality and how these genetic mechanisms are differentially expressed.

5.0 CONCLUSION

This study provides new insight into how giant viruses impact mixotrophic algae and how N source influences the susceptibility of a host algae to infection. The effect of N source showed no universal pattern. Our hypothesis was that mixotrophic conditions would lead to higher mortality, however our results suggest otherwise. We conclude that nutrient-host-virus interactions are complex and specific to the strain of algae. However, infection by the FloV-SA1 virus led to increased mortality and burst size across most algae strains.

Our findings help further our understanding of the ecological role of giant viruses in oceanic systems, especially in oligotrophic environments such as the North Pacific Subtropical Gyre. Giant viruses play a critical role in controlling phytoplankton communities and influencing the cycle of nutrients through the ocean. If we can understand the causes of virus driven mortality and virus production, we can better predict how phytoplankton and virus populations will respond to changing environmental conditions, such as the expansion of oligotrophic ocean gyres due to climate change (Polovina et al., 2008). Although N source did not have consistent effect on infection dynamics across hosts, it did alter the magnitude of mortality and burst size in most cases, implying that the impact of viruses on their hosts will change as the supply of dissolved nutrients changes across environmental conditions. Further research is needed to investigate how environmental factors, host metabolic strategies, and virus strain diversity shape virus-host interactions in the nutrient limited surface ocean.

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