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Variability in Protist Grazing and Growth on Different Marine *Synechococcus* Isolates▽

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Grazing mortality of the marine phytoplankton *Synechococcus* is dominated by planktonic protists, yet rates of consumption and factors regulating grazer-*Synechococcus* interactions are poorly understood. One aspect of predator-prey interactions for which little is known are the mechanisms by which *Synechococcus* avoids or resists predation and, in turn, how this relates to the ability of *Synechococcus* to support growth of protist grazer populations. Grazing experiments conducted with the raptorial dinoflagellate *Oxyrrhis marina* and phylogenetically diverse *Synechococcus* isolates (strains WH8102, CC9605, CC9311, and CC9902) revealed marked differences in grazing rates—specifically that WH8102 was grazed at significantly lower rates than all other isolates. Additional experiments using the heterotrophic nanoflagellate *Goniomonas pacifica* and the filter-feeding tintinnid ciliate *Eutintinnis* sp. revealed that this pattern in grazing susceptibility among the isolates transcended feeding guilds and grazer taxon. *Synechococcus* cell size, elemental ratios, and motility were not able to explain differences in grazing rates, indicating that other features play a primary role in grazing resistance. Growth of heterotrophic protists was poorly coupled to prey ingestion and was influenced by the strain of *Synechococcus* being consumed. Although *Synechococcus* was generally a poor-quality food source, it tended to support higher growth and survival of *G. pacifica* and *O. marina* relative to *Eutintinnis* sp., indicating that suitability of *Synechococcus* varies among grazer taxa and may be a more suitable food source for the smaller protist grazers. This work has developed tractable model systems for further studies of grazer-*Synechococcus* interactions in marine microbial food webs.

Synechococcus spp. are one of the most abundant representatives of marine picophytoplankton, ranging from 10^3 to greater than 10^5 cells ml^{-1} across a range of marine ecosystems (17, 58, 59) and typically accounting for a significant fraction of primary production (12, 42, 46). *Synechococcus* spp. are an important trophic resource and hold a key position at the base of marine food webs, where grazing mortality is dominated by heterotrophic protists (9, 43). However, there is little consensus regarding the most important consumers of *Synechococcus*. Some studies have identified small, heterotrophic nanoflagellates (HNFs) as primary grazers of *Synechococcus* (10, 12, 45), while other studies counter that dinoflagellates, small (<20-μm) aloricate ciliates, or larger ciliates and appendicularians are more important than HNFs as a source of grazing mortality (19, 43). Thus, evidence exists that heterotrophic protists representing a wide range of sizes, forms, and feeding mechanisms are consuming *Synechococcus* in natural waters, yet we know little about what governs grazer-prey interactions at this crucial level of the microbial food web and how this varies among feeding guilds.

It has been suggested that the natural abundance of *Synechococcus* is regulated predominantly by predation (2, 25), which would lead to strong selection for grazing resistance (49). Indeed, a number of factors have been identified that potentially serve as constitutive or inducible mechanisms of grazing defense. Prey size and shape, metabolic activity (18, 24), starvation state of both predator and prey (5, 23), and nutritive quality (32) are all factors that influence grazability of picophytoplankton prey. Diel periodicity of prey growth may have a direct effect on grazing rates (15, 19), as well as influence the way in which other factors influence feeding. Prey motility has also been identified as an important factor influencing rates of contact, capture, and subsequent ingestion of prey by protist grazers (5, 23, 34, 53). More recently, advances in our understanding of chemical signaling suggest that dissolved cues and predator-prey cell surface interactions related to prey charge and cell surface biochemical properties play a fundamental role in governing predator-prey and food web dynamics (44, 50, 57). Despite this growing body of knowledge, our understanding of grazer-prey dynamics among heterotrophic protists and *Synechococcus* remains nascent—as does our understanding of the underlying mechanisms that ultimately determine the rate at which *Synechococcus* is consumed in natural waters.

The phylogenetic identity of prokaryotic prey may also play an important role in shaping grazing resistance, prey palatability, and ability to support grazer growth (5, 6, 60). *Synechococcus* is a physiologically and phylogenetically diverse group of phytoplankton (40, 55, 59), exhibiting a high degree of phylogenetic variability across the global oceans (59). However, it is not clear to what extent these patterns in diversity are linked to the effect of prey phylogeny on predator-prey interactions. A recent study of temporal-scale variability in the dominance of different *Synechococcus* clades in coastal waters suggests that such patterns may be linked to differential grazing pressure (51). Exploration of grazer-prey interactions among

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TABLE 1. Characteristics of *Synechococcus* isolates used in the present study[a]

Strain	Growth day^{-1} (mean ± SD)[b]	Motile	C/N molar ratio (mean ± SD)[b]	Length, μm (mean ± SD)	Width, μm (mean ± SD)	Aspect ratio (length/width)
WH8102	0.36 ± 0.05	Yes	4.4 ± 0.26	1.19 ± 0.38	0.98 ± 0.20	1.21
CC9311	0.40 ± 0.10	No	4.6 ± 0.07	1.27 ± 0.35	0.80 ± 0.09	1.59
CC9605	0.40 ± 0.09	No	4.4 ± 0.30	1.23 ± 0.29	0.91 ± 0.20	1.35
CC9902	0.37 ± 0.06	No	4.8 ± 0.27	1.38 ± 0.31	0.89 ± 0.09	1.55

[a] Size measurements are based on a minimum of 100 cells for each isolate.
[b] $n = 4$.

cultured *Synechococcus* isolates from different phylogenetic lineages is a fundamental first step in assessing the phylogeny-related variability in consumption of *Synechococcus* that likely exists in natural marine systems.

The objectives of the present study were to (i) investigate grazing on phylogenetically distinct *Synechococcus* isolates by using ecologically relevant model grazer systems, (ii) examine mechanisms potentially driving differences in grazing on *Synechococcus* (e.g., cell size, growth phase, and motility), and (iii) explore differences in the abilities of *Synechococcus* isolates to support growth of different protist grazers. Our study revealed that not all *Synechococcus* isolates are grazed at the same rate. Furthermore, isolates vary in their abilities to support growth of protist grazers. The consistency of patterns of ingestion for the different *Synechococcus* isolates among distinct grazers underscores the importance of prey phylogenetic identity in microbial food web dynamics.

MATERIALS AND METHODS

Culture conditions. Grazing experiments were conducted with four *Synechococcus* isolates from highly divergent clades, each with available whole-genome sequences (Table 1), including the motile open-ocean isolate WH8102 (clade III) and nonmotile open-ocean isolate CC9605 (clade II) and coastal isolates CC9311 (clade I) and CC9902 (clade IV) (20a). *Synechococcus* cultures were transferred and maintained under sterile conditions on SN medium (56) at 22°C with a 12-h light/12-h-dark cycle of low light intensity (~15 μE m^{-2} s^{-1}). All *Synechococcus* cultures were axenic, except CC9902, in which heterotrophic bacteria were present but at low densities relative to *Synechococcus*. Growth rates (day^{-1}) for *Synechococcus* cultures were estimated from both *in vivo* fluorescence (Turner 10AU Fluorometer) and direct counts of abundance (epifluorescent microscopy) by using the slope of the regression of ln (fluorescence or abundance) versus time. Our experiments also included four protist grazers representing a range of sizes and feeding strategies, including the suspension-feeding tintinnid ciliates *Eutintinnis* sp. (SPMC132; 175 by 30 μm) and *Metacylis* sp. (SPMC125; 60 by 55 μm), the heterotrophic dinoflagellate *Oxyrrhis marina* (SPMC107; 25 by 15 μm), and the small colorless cryptomonad *Goniomonas pacifica* (CCMP1869; 6- to 8-μm diameter). *G. pacifica* was provided by CCMP (West Boothbay Harbor, ME), while all other protist grazers were isolated from marine waters of Northern Puget Sound. Grazer cultures were grown in low light on a 12-h light/12-h-dark cycle and maintained in sterile filtered seawater with dilute trace metal additions (i.e., ciliate medium) (21a). Both ciliates and *O. marina* were kept at 15°C and fed a mixed diet of phytoplankton (i.e., for *Eutintinnis*, *Heterocapsa rotundata*, *Mantoniella squamata*, *Micromonas pusilla*, and *Nannochloropsis* sp.; for *Metacylis*, *Emiliania huxleyi*, *Isochrysis galbana*, and *M. pusilla*; and for *O. marina*, *Dunaliella tertiolecta*, *E. huxleyi*, *I. galbana*, and *Pyrenomonas salina*) as optimized in growth trials (data not shown). *G. pacifica* was kept at 22°C and maintained on rice-grain-enriched heterotrophic bacteria.

Ingestion and growth rates. We began our investigation by conducting grazing experiments with *O. marina* to identify differences in grazing among the four *Synechococcus* clades. These experiments were repeated using *G. pacifica* and *Eutintinnis* sp. to identify the extent to which differences in grazing on the various *Synechococcus* clades were consistent across grazer taxa. Each grazing experiment was accompanied by growth assays to determine the ability of *Synechococcus* to support grazer growth. Additional experiments were conducted to investigate the effect of *Synechococcus* growth phase on grazing; we also used untreated and heat-treated WH8102 and CC9311 to examine the role of prey motility as a grazing defense strategy. In all experiments, ingestion rates were calculated from rates of accumulation of *Synechococcus* in grazer food vacuoles over a short-term incubations (i.e., <2 h), and grazer growth was determined by monitoring grazer abundance in incubations for 24 to 48 h.

Prior to each experiment, maintenance prey were reduced or eliminated in grazer stock cultures by a variety of methods. *Eutintinnis* and *Metacylis* were sieved through a 10-μm Nitex screen affixed to the end of a short polyvinyl chloride (PVC) cylinder with a diameter of 5 cm. To maximize recovery of living grazers, care must be taken to keep ciliates suspended in medium at all times during the filtration process by gently rinsing with autoclaved, 0.2-μm-filtered seawater (AFSW). In this manner, prey were removed from the medium, and ciliate cultures were reduced in volume from 1 liter to approximately 250 ml. Filtered ciliates were resuspended in ciliate medium for 4 to 6 h prior to the initiation of each experiment. *O. marina* cultures were placed in the dark for 3 to 4 days, allowing grazers to remove phytoplankton prey. Since *G. pacifica* and their heterotrophic bacterial prey could not be separated, rice grains were removed 4 to 5 days prior to the experiment to allow the flagellates to graze down maintenance prey to less than 1×10^6 cells ml^{-1} and thereby minimize the contribution of heterotrophic bacteria in grazing experiments. All experiments were initiated by combining grazers and ciliate media in polycarbonate bottles to reach desired concentrations (i.e., approximately 10 *Metacylis* cells ml^{-1}, 50 *Eutintinnis* cells ml^{-1}, 200 *O. marina* cells ml^{-1}, and 1,000 *G. pacifica* cells ml^{-1}). *Synechococcus* cells from each isolate were added to triplicate experiment bottles to achieve initial concentrations of 1×10^6 cells ml^{-1}. The small volume of *G. pacifica* culture (i.e., 1 ml) added to each 50-ml treatment resulted in concentrations of heterotrophic bacteria approximately 2 orders of magnitude lower than the experimental concentration of *Synechococcus*. The stock *Synechococcus* cultures used for experiments were in the mid-exponential phase (1×10^7 to 5×10^7 cells ml^{-1}), except for experiments investigating the effect of growth stage on grazing of *Synechococcus*. The presence of small clumps of CC6905 and CC9902 in the first experiment involving *O. marina* prompted us to prefilter *Synechococcus* cultures through 3-μm-pore-size polycarbonate filters to maximize the proportion of single cells and allow for comparison of properties other than prey particle size, particularly for the larger grazers. We observed no evidence that *G. pacifica* is capable of ingesting more than 1 or 2 cells per feeding event.

After *Synechococcus* and grazers were combined, flasks were incubated at 15°C (22°C for *G. pacifica*) under low-light conditions and sampled at three regular intervals during the course of the incubation. Total incubation length from time of *Synechococcus* addition varied (i.e., 4 min for *Eutintinnis*, 30 min for *O. marina*, and 1.5 h for *G. pacifica*) and was dependent upon ingestion rates of each grazer at optimal prey concentrations, as determined by prior feeding trials (data not shown). Ingestion experiments were always initiated at the same time of day (i.e., early afternoon) to avoid the confounding effect of diel periodicity on picoplankton physiology and protist feeding rates (15, 19, 30).

Subsamples for each time point were collected and preserved with glutaraldehyde (final concentration [f.c.], 0.5%), stained with DAPI (4′,6-diamidino-2-phenylindole; f.c., 0.7 μM), refrigerated overnight at 4°C, then filtered onto polycarbonate filters of predetermined pore size (i.e., 8 μm for ciliates, 5 μm for *O. marina*, and 3 μm for *G. pacifica*). Filters were slide mounted and frozen (−20°C) for later microscopic analysis. At least 100 individual grazers per slide were examined under UV (340 to 380 nm) and blue (450 to 490 nm) excitation with LP 430 and 520 filters, respectively, to identify the number of ingested *Synechococcus* cells per grazer. These counts were used to determine grazing rates and the proportion of the grazer population feeding (i.e., grazers with ingested *Synechococcus*/total grazers counted). Ingestion rates were determined according to the mean number of cells ingested per grazer (cells grazer^{-1} unit of

time^{-1}). All three time points were used in calculation of grazing rates, except for experiments in which a nonlinear change in cell ingestion over time (e.g., decrease in feeding at later time points) was observed. In these situations, only the first two time points of the feeding curve were used in an effort not introduce a downward bias (i.e., underestimation) and thus achieve the most representative estimate of instantaneous feeding rates.

Population growth rates of grazers were estimated by continuing the short-term grazing incubations described above for 24 h (ciliates) or 48 h (*O. marina* and *G. pacifica*) and determining changes in grazer abundance. Growth was also monitored in starved (no added food) and positive control (prey species from maintenance diet) treatments. At the termination of each incubation, samples were preserved with acid Lugol's solution (f.c., 2%), settled in 10-ml chambers, and all grazer cells were enumerated with an inverted microscope. Growth rates (g) day^{-1} were estimated with the equation $g = 1/t \ln(N_t/N_0)$, where t is total time of incubation (day) and N_t and N_0 are final and initial abundance of the grazer, respectively. Positive grazer growth was defined as treatments in which final abundance was significantly greater than initial abundance, while positive grazer survival was defined as those where final abundance was significantly greater than in starved treatments.

Growth rates were converted to carbon units by using published carbon conversion factors. A cellular volume of 1,590 μm^3 was used for *O. marina* (41), while *Eutintinnis* sp. and *G. pacifica* abundances were converted to cylindrical and spherical biovolumes, respectively, using appropriate geometric formulas. Biovolume-to-carbon conversion factors of 100 fg C μm^{-3} and 122 fg C μm^{-3} were used for *G. pacifica* (7) and *O. marina* (41), respectively. Carbon content of 6.3 ng C cell^{-1} was used for *Eutintinnis* sp. (54). Carbon-specific growth and grazing rates were calculated with these values, which were then used to estimate grazer growth efficiencies (GGEs). Because these carbon conversion factors are not well constrained, comparisons of GGEs are best suited for evaluating differences within rather than among grazer types. Any variations in sizes of individual grazer cells over the incubation time course were not considered for growth rate estimates.

Effect of growth stage and motility. The effect of prey growth stage on grazing rates was investigated by adding WH8102 or CC9311 in the early (10^6 cells ml^{-1} stock culture density), mid (10^7 cells ml^{-1})-, and late-exponential/stationary (10^8 cells ml^{-1}) phases to *O. marina* to achieve final concentrations of 1×10^6 *Synechococcus* cells ml^{-1} in experimental bottles, and otherwise following the experimental procedures described previously. To have *Synechococcus* cultures at different growth stages available simultaneously, 0.5 to 15 ml from WH8102 and CC9311 stock cultures was added to SN medium 5 days prior to the experiment, with inoculum volume based on empirically determined *Synechococcus* growth rates (Table 2). Target abundance of *Synechococcus* cultures was confirmed by epifluorescent microscopy. The use of parallel cultures at different growth stages rather than performing a series of experiments on the same *Synechococcus* cultures as they increased in abundance was based on our experience with protist grazers and the understanding that the performance of grazer cultures tends to be more variable over time than growth rate or other characteristics observed for *Synechococcus* cultures.

The effect of motility was investigated by comparing grazing rates between heat-treated versus untreated cultures of WH8102 and CC9311 when fed upon by *O. marina* or *Eutintinnis* sp. Motility was temporarily (i.e., ~1.5 h) arrested in WH8102 by transferring 12 ml of *Synechococcus* culture at 5×10^6 cells ml^{-1} to a 15-ml polyethylene Falcon tube and fully immersing the tube in a water bath at 37°C for 6 min. CC9311 received identical treatment as a control. This approach was used to arrest motility while minimizing the effect on other cell properties that might occur with more destructive heat treatments in which higher temperatures and longer durations are used (e.g., see references 20 and 47). Loss of motility was confirmed by wet mounts viewed under an epifluorescence microscope. Heat-treated and untreated cultures of WH8102 and CC9311 were used immediately in grazing experiments with *O. marina* and *Eutintinnis* sp. following the methods described previously. Four milliliters of the remaining heat-treated cultures was transferred into 50 ml of SN medium and incubated to check viability of cells. Regrowth comparable to that of untreated controls was observed for all heat-treated cultures.

Cell size, shape, and C/N ratios. Cell size was determined for different *Synechococcus* isolates with a Leica epifluorescent microscope and glutaraldehyde-fixed samples (f.c., 0.5%). Images were captured at ×1,000 magnification with a tower-mounted Photometrics CoolSNAP camera and RSImage software (v1.9; Roper Scientific). The length and width of individual glutaraldehyde-fixed cells were measured directly with ImagePro Plus software (v5.0; MediaCybernetics). Mean values were derived from the measurements of approximately 100 *Synechococcus* cells per isolate. Measurements did not account for potential shrinkage of cells in response to glutaraldehyde fixation. Molar C/N ratios were determined at each growth phase by filtering 4 ml of *Synechococcus* culture through two stacked precombusted 13-mm GF/F Whatman filters. The use of two filters was found to be the most effective means of yielding the highest cell retention and reducing the number of cells lost in the filtrate to less than 1%. Filters were dried at 50°C and stored in a desiccator until analyzed with a Carlo Erba Flash 1112 series elemental analyzer.

Statistics and calculations. All statistical analyses, including standard least-squares regressions, analyses of variance (ANOVA), and *post hoc* means comparisons (Tukey-Kramer honestly significant difference [HSD]; $\alpha = 0.05$) were performed with the JMP 5.0.1 statistical software package (SAS Institute, Inc.). Mean values are reported in the text as ± 1 SD unless otherwise stated.

RESULTS

***Synechococcus* strain characteristics.** The mean growth rate for all *Synechococcus* strains was 0.39 ± 0.07 day^{-1}, and the rates did not differ among the four isolates (Table 1) (Tukey-Kramer HSD, $\alpha = 0.05$). Mean cell length was 1.25 ± 0.35 μm ($n = 114$), and the lengths were also similar among the isolates (ANOVA, $P = 0.27$). WH8102 was the widest of all cultured isolates (i.e., 0.98 ± 0.2 μm)—significantly wider than CC9311 (i.e., 0.80 ± 0.09 μm; Tukey-Kramer HSD, $\alpha = 0.05$)—with a mean aspect ratio (1.2) that was significantly lower than that of CC9311 and CC9902 (Tukey-Kramer HSD, $\alpha = 0.05$). WH8102 and CC9311 were well dispersed in culture, dominated by single cells, with doublets generally accounting for less than 20% of total cell abundance. The relative abundances of single cells and doublets were similar for CC9605 and CC9902, although these strains also exhibit a tendency to form aggregations, the majority of which (>80%) had a diameter of approximately 10 μm, with the remainder ranging from 25 to 50 μm. Cells in aggregates often outnumbered free-living cells for these two strains under our culture conditions, with cells in aggregates ranging from 65 to 75% of total abundance.

Ingestion of *Synechococcus*. Rates of ingestion varied among grazers, with the lowest values observed for *G. pacifica*, intermediate values for *O. marina*, and highest values for *Eutintinnis* sp., while no ingestion or survival was seen with *Metacylis* sp. (Table 2). For core experiments with *O. marina* as a grazer (i.e., experiments 1 and 2), mean ingestion was 23.7 cells grazer^{-1} h^{-1} and ranged from 1.7 to 71 cells grazer^{-1} h^{-1}, with dramatically higher (and statistically similar) rates observed for ingestion of CC9605 and CC9902 and the lowest rates observed for WH8102 (Fig. 1). Mean ingestion of *Synechococcus* by *G. pacifica* was 0.41 cell grazer^{-1} h^{-1}, ranging from 0.23 cell grazer^{-1} h^{-1} for WH8102 to >0.5 cell grazer^{-1} h^{-1} for the other three isolates (i.e., CC9311, CC9605, and CC9902) (Fig. 1). For the core experiment involving the large filter-feeding *Eutintinnis* sp. (i.e., experiment 1), mean ingestion was much higher than that for *G. pacifica* or *O. marina* (i.e., 920 cells grazer^{-1} h^{-1}), ranging from 89 to 160 cells grazer^{-1} h^{-1} for WH8102 and CC9311, respectively, to >1,200 cells grazer^{-1} h^{-1} for CC9605 and CC9902 (Fig. 1). The ingestion rates observed in the present study were comparable to those reported in other studies for ingestion of *Synechococcus* by similar protozoan grazers (Table 3). Due to formation of aggregates in CC9605 and CC9902 cultures, we focused our comparisons on WH8102 and CC9311. Across all experiments and grazers, carbon-specific ingestion rates for WH8102 were significantly lower than those for CC9311 (Fig. 2). The magnitude of this difference varied among experiments, with CC9311 ingested at rates 2-fold higher than that of WH8102 for *Eutintinnis* sp. and the second *O.*

TABLE 2. Summary of grazing and growth of *O. marina*, *G. pacifica*, *Eutintinnis* sp., and *Metacylis* sp. on different *Synechococcus* isolates[a]

Grazer expt	Prey (phase or characteristic)	Grazing assays			Grazer growth assays		
		Proportion of population feeding (mean ± SE)	Ingestion		Grazer growth		GGE (%)[e]
			No. of cells grazer^{-1} h^{-1} (mean ± SE)	μg C liter^{-1} day^{-1}[b]	Day^{-1} (mean ± SE)	μg C liter^{-1} day^{-1}[b]	
O. marina							
Expt 1	WH8102	0.35 ± 0.01	6.2 ± 1.5	3.9	0.09 ± 0.04	1.3	33
	CC9311	0.34 ± 0.06	9.6 ± 0.3	6.4	0.20 ± 0.04	3.3	51
	CC9605	0.64 ± 0.11	21.4 ± 3.3	12.8	−0.01 ± 0.07	−0.1	–
	CC9902	0.61 ± 0.01	19.0 ± 5.2	11.7	0.05 ± 0.08	0.6	6
	D. tertiolecta[c]	–	–	–	0.37 ± 0.08	7.29	–
	None[d]	–	–	–	0.07 ± 0.01	1.0	–
Expt 2	WH8102	0.41 ± 0.02	1.7 ± 0.2	2.1	0.04 ± 0.04	1.8	84
	CC9311	0.60 ± 0.04	4.5 ± 1.0	5.4	−0.04 ± 0.04	−1.9	–
	CC9605	0.87 ± 0.07	55.9 ± 10.8	72.8	0.13 ± 0.05	6.8	9
	CC9902	0.91 ± 0.02	71.2 ± 19.2	91.4	0.10 ± 0.09	5.2	6
	I. galbana[c]	–	–	–	0.21 ± 0.05	11.8	–
	None[d]	–	–	–	−0.07 ± 0.01	−2.90	–
Growth phase expt	WH8102 (early)	0.47 ± 0.07	1.2 ± 0.1	1.6	–	–	–
	WH8102 (mid)	0.46 ± 0.05	1.0 ± 0.3	1.3	–	–	–
	WH8102 (late)	0.45 ± 0.04	1.9 ± 0.2	2.6	–	–	–
	CC9311 (early)	0.77 ± 0.12	4.6 ± 1.2	6.2	–	–	–
	CC9311 (mid)	0.77 ± 0.22	4.8 ± 1.5	6.5	–	–	–
	CC9311 (late)	0.32 ± 0.01	0.8 ± 0.1	1.0	–	–	–
Heat treatment expt	WH8102 (control)	0.65 ± 0.10	4 ± 0.5	4.2	–	–	–
	WH8102 (heat treated)	0.56 ± 0.03	2 ± 0.6	2.9	–	–	–
	CC9311 (control)	0.94 ± 0.04	49 ± 10.7	58.3	–	–	–
	CC9311 (heat treated)	0.95 ± 0.02	56 ± 9.4	66.8	–	–	–
Eutintinnis sp.							
Expt 1	WH8102	0.91 ± 0.05	89 ± 12	23.3	−0.29 ± 0.07	−98	–
	CC9311	0.86 ± 0.06	160 ± 19	43.8	−0.18 ± 0.15	−66	–
	CC9605	0.85 ± 0.01	1213 ± 9	323	−0.24 ± 0.06	−84	–
	CC9902	0.85 ± 0.03	2217 ± 721	602	−0.20 ± 0.13	−72	–
	I. galbana[c]	–	–	–	0.01 ± 0.12	5.21	–
	None[d]	–	–	–	−1.40 ± 0.96	−267	–
Heat treatment expt	WH8102 (control)	0.62 ± 0.80	51 ± 1	15.2	–	–	–
	WH8102 (heat treated)	0.57 ± 0.01	47 ± 12	14.2	–	–	–
	CC9311 (control)	0.87 ± 0.10	707 ± 196	212.1	–	–	–
	CC9311 (heat treated)	0.87 ± 0.60	692 ± 202	207.6	–	–	–
G. pacifica (expt 1)							
	WH8102	0.13 ± 0.03	0.23 ± 0.07	1.1	0.05 ± 0.09	0.33	31
	CC9311	0.48 ± 0.07	0.69 ± 0.15	3.4	0.20 ± 0.05	1.55	45
	CC9605	0.54 ± 0.06	0.63 ± 0.09	2.9	0.08 ± 0.01	0.50	17
	CC9902	0.48 ± 0.01	0.56 ± 0.11	2.6	0.05 ± 0.08	0.33	13
	M. squamata[c]	–	–	–	0.06 ± 0.05	0.43	–
	None[d]	–	–	–	−0.08 ± 0.05	–	–
Metacylis sp. (expt 1)							
	WH8102	ND[f]	ND	ND	−3.92 ± 0.60	–	–
	CC9311	ND	ND	ND	−4.00 ± 0.49	–	–
	CC9605	ND	ND	ND	−5.44 ± 0.20	–	–
	CC9902	ND	ND	ND	−4.20 ± 0.20	–	–
	I. galbana[c]	ND	ND	ND	−0.12 ± 0.11	–	–
	None[d]	ND	ND	ND	−3.05 ± 0.44	–	–

[a] Not all parameters were measured in each experiment. –, not measured.
[b] Carbon-normalized values.
[c] Positive control.
[d] Starved control.
[e] GGE, gross growth efficiency. Negative values are not reported.
[f] ND, not detected.

marina experiment and approximately 3-fold higher than that for the *G. pacifica* experiment.

Synechococcus growth phase appeared to have a significant effect on the differences in ingestion of WH8102 and CC9311 by *O. marina* (Fig. 3). Cultures at early exponential (~5×10^6 cells ml^{-1}) and mid-exponential (1×10^7 cells ml^{-1}) growth phases exhibited the same pattern as observed previously, with CC9311 grazed at rates significantly (i.e., 5×) higher than WH8102. However, this pattern reversed when cultures at late-exponential/stationary growth (1×10^8 cells ml^{-1}) were used. Late-stage CC9311 appears to be considerably less palatable relative to early and mid-exponential growth, while the cells in the later stage of growth for WH8102 appear to be marginally more palatable. The proportions of *O. marina* feeding were

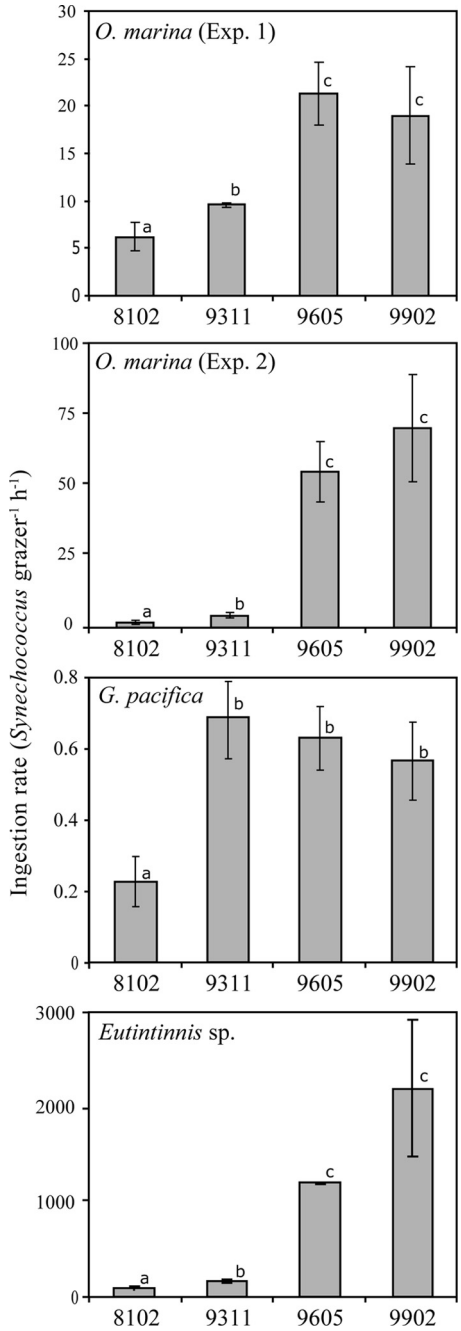

FIG. 1. Mean rates (±SE) of ingestion of different *Synechococcus* isolates recorded for the four grazing experiments conducted with *O. marina*, *G. pacifica*, and *Eutintinnis* sp. Means sharing the same letter are statistically indistinguishable (Tukey-Kramer HSD, $\alpha = 0.05$).

similar for all stages of WH8102 growth and for early and mid-exponential growth in CC9311, although the proportion feeding on late exponential CC9311 dropped to less than 50%. Although we conducted technical replicates of these experiments, we did not pursue additional experiments exploring the many possible implications of these results with further biological replicates, including the possibility of dissolved compounds being different in late growth phases of CC9311. We concentrated instead on the major differences between *Synechococcus* CC9311 and WH8102.

The consistent difference in ingestion of motile WH8102 versus nonmotile CC9311 observed for all grazers led to the hypothesis that motility of WH8102 plays a role in grazing resistance. Comparison of untreated and heat-treated (i.e., nonmotile) WH8102 and CC9311 revealed no major effect of motility on the proportion of feeding grazers or grazing rates for *O. marina* or *Eutintinnis* sp. (Fig. 4). There was a slight (~1.5×) enhancement of feeding by *O. marina* on untreated (motile) versus heat-treated (nonmotile) WH8102, although this difference was not significant. Heat treatment experiments corroborated the consistent pattern of lower ingestion rates on WH8102 than on CC9311, with an approximate 15-fold difference in rates of grazing on these isolates by *O. marina* and *Eutintinnis* sp.

Grazer growth. The ability of the different *Synechococcus* isolates to support protist growth was highly variable both within and among protist grazer species (Fig. 5). CC9311 was the only strain to support positive growth in any of the grazers (i.e., *O. marina* in experiment 1 and *G. pacifica*), although CC9311 failed to support even survival of *O. marina* in experiment 2, with CC9605 and CC9902 being the only strains to support survival in this experiment. For *Eutintinnis* sp., *Synechococcus* strains as well as *Isochrysis galbana* (control prey) supported survival, yet positive growth was not observed for any of these treatments. No grazing or survival was observed for the experiment with *Metacylis* sp. as a grazer (Table 2).

Despite the well-documented linkage between feeding and grazer growth, we did not observe a consistent positive relationship between ingestion of and growth on the various *Synechococcus* isolates. Elevated rates of ingestion of CC9605 and CC9902 appeared to support survival of *O. marina* in the second experiment, and CC9311 appeared to support positive growth of *G. pacifica* (Fig. 1 and 5), yet there were also instances in all experiments where elevated ingestion of *Synechococcus* was not accompanied by grazer growth, such as with ingestion of CC9605 and CC9902 by *G. pacifica*, *Eutintinnis*, and *O. marina* (experiment 1). Comparison of growth and grazing rates from all experiments revealed little or no relationship between these parameters, with a high degree of variability in growth associated with any level of ingestion or for a given grazer species (Fig. 6A). However, carbon-normalized growth and grazing rates from treatments in which positive growth was observed (i.e., *G. pacifica* and *O. marina*) revealed a positive relationship between growth and grazing (solid line in Fig. 6B). This figure illustrates that positive growth of a protist was always accompanied by grazing (Fig. 6B), but substantial grazing could occur with no corresponding growth (Fig. 6A). Estimates of gross growth efficiency (GGE) derived from these data ranged from 6 to 84% (Table 2), with an overall mean of 30% (hatched line in Fig. 6B). In general, WH8102 and CC9311 supported a higher level of growth per unit of ingestion than CC9605 and CC9902 (Fig. 6B and Table 2).

DISCUSSION

Differences in grazing mortality among distinct *Synechococcus* clades. We observed a persistent pattern among our graz-

TABLE 3. Ingestion and clearance rates reported for marine protist grazers feeding on picophytoplankton and picoplankton-size particles

Grazer	Prey	Prey concn (cells ml^{-1})	Method[a]	Ingestion rate (prey $grazer^{-1}$ h^{-1})	Clearance rate (nl $grazer^{-1}$ h^{-1})	Source or reference
G. pacifica (cryptomonad)	*Synechococcus*[b]	1.0×10^6	Ingestion	0.5	4.6	Present study
O. marina (dinoflagellate)	*Synechococcus*[b]	1.0×10^6	Ingestion	5.5	42.2	Present study
Eutintinnis sp. (ciliate)	*Synechococcus*[b]	1.0×10^6	Ingestion	124	219	Present study
Marine flagellates						
HNFs, marine assemblage	*Synechococcus*	7×10^3	Disappearance	0.03	7.9	12
Marine HNF (*Pseudobodo* sp.)	*Synechococcus*	1×10^3–1×10^6	Disappearance	1.0	5.1	11
HNFs, mixed population	*Synechococcus*	2×10^3–2×10^6	Disappearance	0.74	3.2	11
Mixed small (<5 μm) bacteriovores	*Synechococcus*	2.7×10^6	Disappearance	0.8	3	14
HNFs, offshore assemblage	*Synechococcus*	5.0×10^6	Disappearance	1.5	5	14
HNFs, coastal assemblage	*Synechococcus*	2.5×10^6	Disappearance	2.9	9.9	14
HNFs, coastal assemblage	*Synechococcus*	3.9×10^6	Disappearance	1.9	1.4	14
Picophagus flagellatus	*Synechococcus*	1.0×10^7	Disappearance	0.7	2.5	25
Mixed flagellates, coastal assemblage	1-μm beads	1.0×10^4	Ingestion	0.01[c]	0.9	25a
Mixed flagellates, natural assemblage	Picophytoplankton	1×10^4–3×10^4	Disappearance	NR[e]	0.3	45
Mixed estuarine flagellate assemblage	FLB[d]	NR	Ingestion	1.2[c]	3.2	47
Paraphysomonas imperforata	*Synechococcus*	1×10^7–1×10^8	Disappearance	NR	NR	60
Pteridomonas danica	*Synechococcus*	1×10^7–1×10^8	Disappearance	NR	NR	60
Marine ciliates						
Strombidium sulcatum	*Synechococcus*	1.9×10^5	Disappearance	96	515	13
Uronema sp.	*Synechococcus*	2.0×10^5	Disappearance	31	148	13
Aloricate ciliates	1-μm beads	1.0×10^4	Ingestion	0.015[c]	1.5	25a
Tintinnids	*Synechococcus*	1×10^3–1×10^4	Ingestion	0.41	NR	42
Aloricate ciliates	*Synechococcus*	1×10^3–1×10^4	Ingestion	0.13	NR	42
Mixed ciliates (natural assemblage)	Picophytoplankton	1×10^4–3×10^4	Disappearance	NR	2.0	45
Scuticociliates (estuarine)	FLB	NR	Ingestion	68[c]	140	47
Oligotrichs (estuarine)	FLB	NR	Ingestion	120[c]	260	47

[a] Method used to estimate grazing rates. Ingestion, direct observation of prey in food vacuoles; disappearance, ingestion inferred from changes in prey abundance.
[b] Mean values reported are from experiments in which *Synechococcus* strains WH8102 and CC9311 in mid-exponential growth were used as prey.
[c] Ingestion rates not reported. Values calculated based on reported clearance rates and prey concentrations.
[d] FLB, fluorescently labeled bacteria.
[e] NR, not reported.

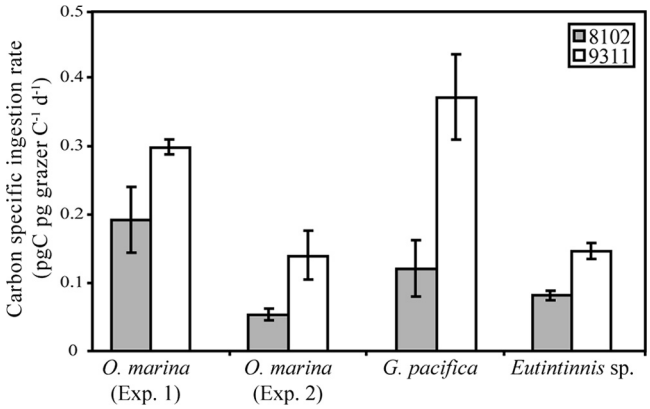

FIG. 2. Summary of mean (±SE) carbon-specific ingestion of WH8102 and CC9311 by *O. marina*, *G. pacifica*, and *Eutintinnis* sp. from all four experiments.

ing experiments in which WH8102 was grazed at consistently lower rates than CC9311. Although the magnitude of this difference was variable (i.e., 2- to 15-fold), the pattern itself was robust, being observed for all grazer taxa and experiments (Fig. 1 and 2). Grazer physiological state did not appear to influence this pattern, as evidenced by higher rates of ingestion of CC9311 by *O. marina* despite substantial differences in feeding activity (i.e., 10-fold range in absolute grazing rates) by this grazer. Lower rates of grazing upon WH8102 were also observed for carbon-normalized rates of ingestion (Fig. 2), for cells in early and mid-exponential growth (Fig. 3) and for both normal and heat-treated cells (Fig. 4).

It would seem that elevated rates of ingestion observed for CC9605 and CC9902 would also be a noteworthy pattern. However, we believe that these elevated ingestion rates are due in part to formation of aggregates by CC9605 and CC9902, providing particles of a size more efficiently captured by the larger grazers. Indeed, large clumps of these cells were visible inside *Eutintinnis* sp. loricae and packed inside *O. marina* food vacuoles. The absence of this effect

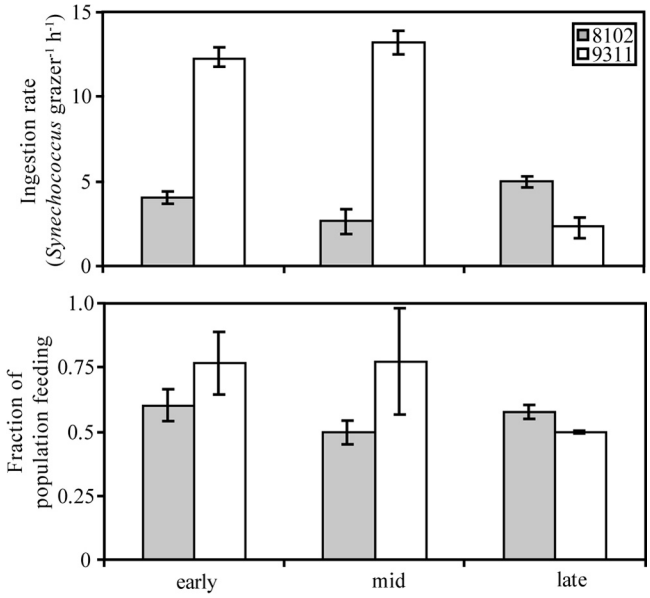

FIG. 3. Ingestion rates (upper panel) and proportion of population feeding (lower panel) (±SE) of *O. marina* on *Synechococcus* isolates WH8102 and CC9311 at different stages of growth (i.e., early, mid-, and late exponential).

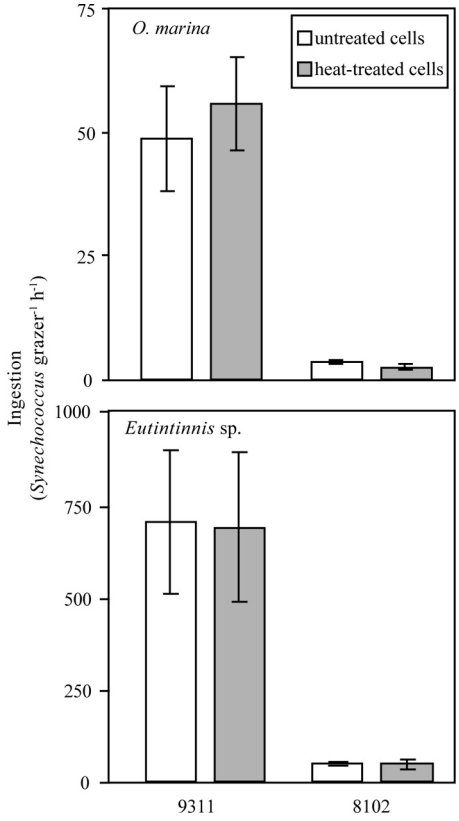

FIG. 4. Results from experiments investigating the effect of motility on grazing by comparing ingestion of untreated versus heat-treated *Synechococcus* isolates WH8102 and CC9311 by *O. marina* and *Eutintinnis* sp. Error bars represent 1 standard error.

with *G. pacifica* (Fig. 1) may be related to an upper size threshold for nanoflagellate feeding (19) that only allows ingestion of 1 or 2 cells at a time. Although aggregate formation may be a natural phenomenon in marine phytoplankton assemblages (29) and natural populations of *Synechococcus* (33), the presence of aggregates in grazing experiments confounds interpretation of grazing rates and undermines the ability to use CC9605 and CC9902 to further investigate factors regulating grazing upon *Synechococcus*. For this reason, we focused the remainder of our investigation on *Synechococcus* strains WH8102 and CC9311, which were present as well-dispersed single and double cells and which revealed a consistent difference in susceptibility to grazing among all grazers included in our study. Using these two *Synechococcus* clades and three protist grazers as model grazing systems, we investigated several factors that are commonly implicated in regulation of grazing rates, including prey cell size, cellular stoichiometry, growth phase, and prey motility.

Cell size, stoichiometry, and growth phase. It is well documented that prey size has a strong influence on grazing, with a tendency for many bacterivores to preferentially consume larger cells (24). In contrast, larger cell size may serve as a refuge from small (i.e., 3- to 5-μm) HNFs, which have an upper size threshold that may prevent ingestion of large, dividing *Synechococcus* cells (19). Regardless of the nature of this effect, similarity in size among the four *Synechococcus* isolates suggests that cell size was not a factor shaping predation rates in our experiments. It could be argued that although we did not observe statistically significant differences in size between WH8102 and CC9311, subtle differences in the sizes and shapes of these strains may indeed be relevant for a small grazer such as *G. pacifica* and thus explain reduced grazing rates on WH8102. However, the persistent difference in ingestion of WH8102 versus CC9311 across all grazer taxa indicates that cell size alone is not responsible for differences in grazing rates observed in our study.

Prey cellular stoichiometry is another factor that has been reported to influence grazing rates (16, 32). It is important to recognize that prey C/N ratio is not a mechanism for selection unto itself; rather, cellular stoichiometry may serve as proxy for other aspects of cell physiology that do influence rates of predation (e.g., metabolic activity, starvation, nutritive quality, and growth phase). As with cell size, we did not observe differences in C/N ratios among *Synechococcus* isolates, despite variability in grazing rates, suggesting that cellular stoichiometry—or factors for which stoichiometry is a proxy—did not influence grazing rates.

The difference in ingestion of WH8102 versus CC9311 was more pronounced in early and mid-exponential growth phases, with ingestion of late-exponential-phase CC9311 dropping to rates slightly lower than those of WH8102 (Fig. 3). This suggests that there are aspects of cellular physiology that contribute to the difference in grazing susceptibilities between WH8102 and CC9311 that are prevalent in earlier growth phases yet change as the cells approach stationary phase. Analyses of C/N ratios of cells at different growth stages did not reveal significant changes between the mid- and late stationary phases (data not shown), suggesting that the change in susceptibility to grazing of CC9311 may be driven by factors more

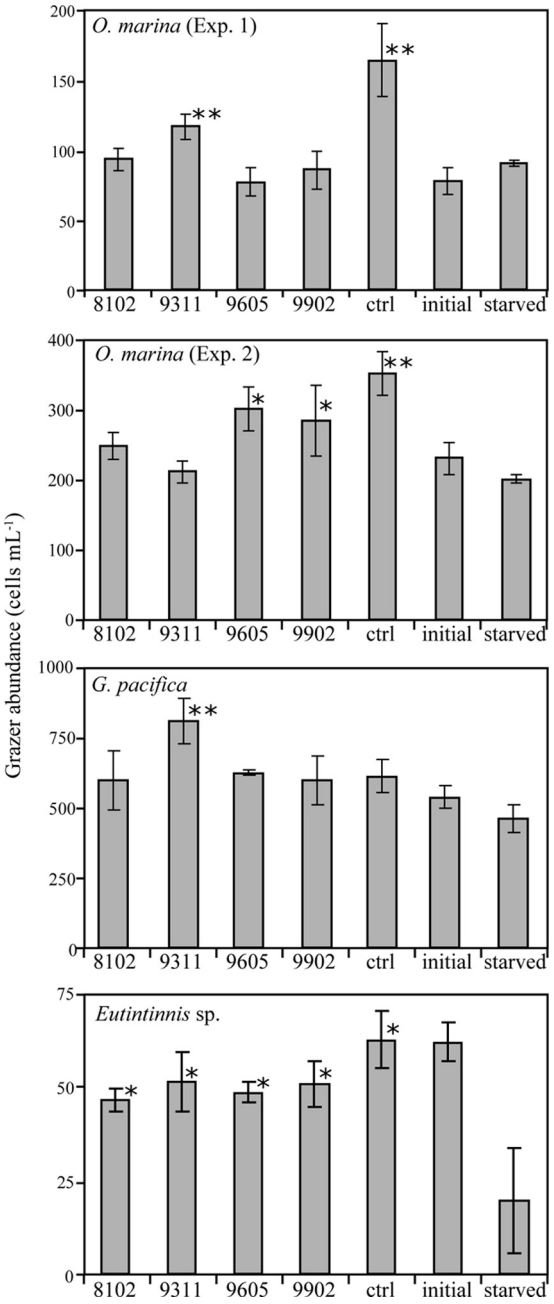

FIG. 5. Grazer abundance in growth experiments in which grazers were provided different food sources. Prey treatments supporting grazer growth (i.e., final abundance significantly greater than initial) are indicated by **, while those merely supporting survival (i.e., final abundance significantly greater than starved) are indicated by *. Incubations with *O. marina* and *G. pacifica* were 48 h with *D. tertiolecta* and *M. squamata* as positive controls (ctrl), respectively, and incubations for *Eutintinnis* were 24 h with *I. galbana* as a positive control.

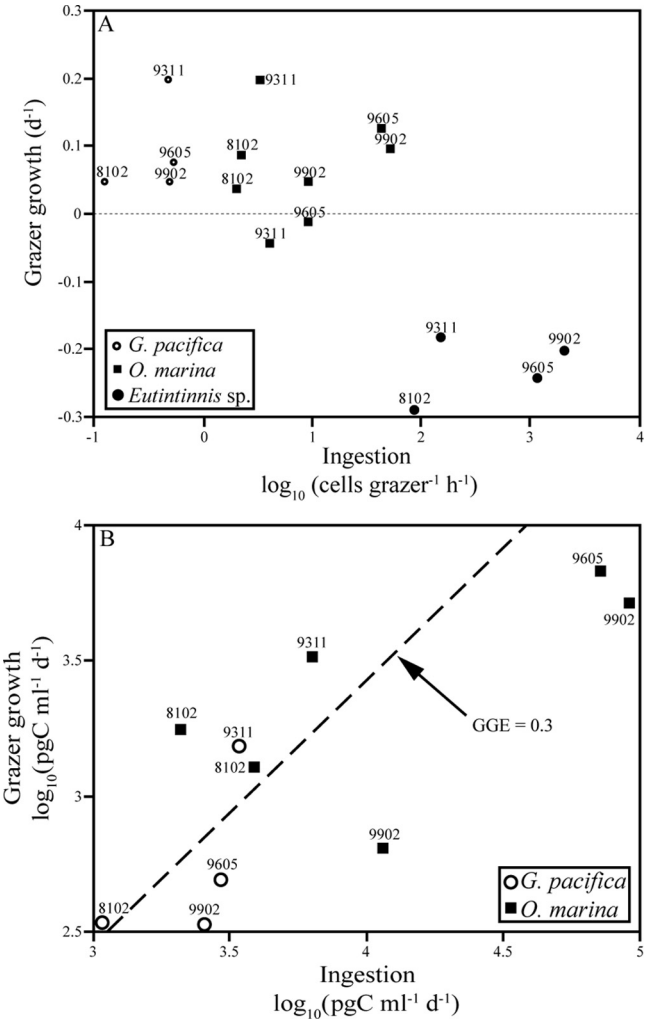

FIG. 6. Relationship between grazer growth and ingestion associated with each *Synechococcus* isolate. (A) Log-transformed ingestion rates versus grazer growth for all grazing experiments, except those investigating effects of *Synechococcus* growth phase and motility; (B) log-transformed carbon-based rates of grazer ingestion versus log-transformed growth rates from those experiments in which positive grazer growth was observed. The hatched line represents the overall mean grazer growth efficiency (GGE, 0.3).

subtle than shifts in total cell composition (e.g., cell surface characteristics). We believe that differences in grazing susceptibilities of WH8102 and CC9311 at early and mid-growth stages may provide insight into discrepancies between findings of the present study and those of others investigating grazing and growth of marine protists on *Synechococcus* (e.g., see reference 60). Using prey disappearance as an index of grazing, these authors report that marine flagellates (i.e., *Pteridomonas* and *Paraphysomonas*) consumed CC9311 at levels comparable to or slightly lower than that of WH8102. This discrepancy with our findings could be explained by the fact that the *Synechococcus* cultures used in their grazing experiments were in late-exponential-phase growth, corroborating results from our *Synechococcus* growth phase experiment. Zwirglmaier et al. (60) also report that CC9311 did not support growth in either of the flagellate grazers, whereas WH8102 supported very high growth. Because grazing experiments in the present study were conducted with *Synechococcus* at mid-exponential growth, it is difficult to determine whether the discrepancies between our findings and those of Zwirglmaier et al. (60) are the result of differences in methodology, experimental conditions, preference of individual grazers (e.g., *G. pacifica* versus *Pteridomo-*

nas) for WH8102, or some other unidentified variable. Collectively, our study and that of Zwirglmaier et al. (60) highlight the possible importance of *Synechococcus* growth status when conducting grazing experiments with heterotrophic protists, especially when comparisons across grazers or laboratories are desired.

Motility. Motility is an important factor influencing ingestion of prey by protist grazers, by either the probability of predator-prey encounters (21, 23, 31, 39) or providing an escape mechanism for captured prey (34). Our observation of consistently lower ingestion rates for motile WH8102 relative to nonmotile CC9311 for all grazers (Fig. 1 and 2) suggests that motility may confer protection from grazers. Indeed, WH8102 is capable of swimming at speeds of up to 25 $\mu m\ s^{-1}$ (8, 56), which may be adequate for escaping bacterivorous predators (3). We explored the hypothesis that reduced grazing upon WH8102 relative to CC9311 could be explained by the nonflagellar motility characteristics of WH8102.

Heat treatment has been used as a means of inhibiting motility in a number of studies investigating protist grazing on picoplankton (1, 20, 23, 47). However, such heat treatments may alter cell surface characteristics that are important in prey selection and grazing resistance (44, 57). As an alternative, we used brief, low-temperature heat treatment that inhibited motility for the duration of short-term grazing experiments, but which left *Synechococcus* cells viable, as evidenced by their ability to grow normally after heat treatment. In addition, the heat treatment method we employed did not appear to alter other cell characteristics that might be involved in prey selection, as evidenced by the similarity of ingestion rates when heat-treated and untreated cells of each strain were compared (Fig. 4). Contrary to our expectations, we did not observe differences in ingestion of heat-treated (i.e., nonmotile) versus untreated WH8102 and CC9311, indicating that motility is not a mechanism by which WH8102 avoids predation by either *O. marina* or *Eutintinnis* sp. The absence of an effect of motility on grazing has been observed in other studies involving motile bacterial prey strains and protist grazers (e.g., see reference 5).

Other factors influencing predation on *Synechococcus*. The absence of an apparent effect of size, elemental composition, or motility suggests the presence of a yet unidentified factor responsible for grazing resistance in WH8102 or, alternatively, grazing stimulus in CC9311. Recent reviews identify dissolved cues, cell surface characteristics, and other chemical defenses as important factors regulating predator-prey interactions (32, 35). Although it has been suggested that protist grazers lack the capacity for preingestion selection of prey (5, 19), other studies have observed detection, selection, and ingestion of prey that are linked to predator-prey cell surface interactions (26, 44, 52, 60), prey identity (52, 57), nutritive quality (32), or chemical defense strategies (36). Postingestion mechanisms for avoiding mortality also exist, and it has been argued that selective digestion is the most important process determining rates of picoplankton grazing mortality (4, 5).

Consistent differences in grazing rates on WH8102 versus CC9311 provide compelling evidence that preingestion selection processes are an important factor regulating grazing mortality of *Synechococcus* isolates. This is supported by a few key observations. First, all *Synechococcus* cultures in our study were grown under nutrient-replete conditions with no significant differences in C/N ratios among isolates, suggesting that isolates did not differ with respect to at least this aspect of nutritive quality or other cellular characteristics for which C/N ratios are a proxy. Second, differences in grazing between WH8102 and CC9311 transcend grazer taxa and were remarkably persistent across all experiments, which would be unlikely if postingestion phenomena such as selective digestion alone were the mechanism underlying these differences. Finally, it has been shown that cell surface interactions (27, 32, 44, 50, 57)—many of which involve cell surface characteristics such as S-layers (52)—play an important role in prey selection by protist grazers, and such mechanisms may be manifest in the interactions between *Synechococcus* and the protist grazers investigated as part of the present study. Indeed, WH8102 has an outer envelope composed of an S-layer (38) with large cell surface proteins SwmA and SwmB (37). Although these proteins are primarily involved in motility, their prominence on the cell surface may also serve as a tactile or dissolved cue that influences predation. In this manner, although not deliberate, these proteins may serve as a potential constitutive grazing deterrent. SwmA and SwmB proteins can be found in spent WH8102 medium as well as on the cell surface (37) and could account for lower rates of ingestion associated with intact WH8102 cells. Our study provides preliminary evidence that prey selection of *Synechococcus* by protist grazers is a preingestion mechanism that is probably linked to chemical interactions between predator and prey. Further investigations are necessary to identify the specific nature of these interactions.

Comparison to studies investigating grazing on *Synechococcus* by heterotrophic protists. Clearance rates for *G. pacifica* observed in present study were within the range of reported values for other marine HNFs and similar to the overall mean (i.e., 4.2 nl $grazer^{-1}\ h^{-1}$) (Table 3). Jezbera et al. (28) found that *Goniomonas* sp. ingested bacteria at rates slightly higher (i.e., 2 to 3.5 cells $grazer^{-1}\ h^{-1}$) than those reported in the present study, but also found that *Goniomonas* was able to discriminate between, and selectively graze upon, *Gamma*- versus *Alpha*proteobacteria, thus corroborating evidence from our study that small HNF grazers can discriminate between even closely related prey (i.e., *Synechococcus* WH8102 versus CC9311). Rates of ingestion of *Synechococcus* by *O. marina* were more similar to those reported for small phytoplankton (i.e., *D. tertiolecta* and *I. galbana*) (22) than picoplankton-size prey (26, 27). The relatively low rate at which *O. marina* grazes upon small particles suggests that *Synechococcus* may be at the lower end of the size range for efficient capture and ingestion by this flagellate. Grazing rates for *Eutintinnis* sp. fell well within the range of those reported for other marine ciliates, with the mean clearance rate of 219 nl $grazer^{-1}\ h^{-1}$ strikingly similar to the collective mean of reported clearance rates for marine ciliates (i.e., 213 nl $grazer^{-1}\ h^{-1}$). Our study and others listed in Table 3 collectively reveal highly variable rates of grazing by heterotrophic protists on picophytoplankton and that taxon-specific estimates remain poorly constrained.

Variable relationship between grazing and grazer growth. Growth is one of the most direct and easily measured outcomes of predator-prey interactions. As a result, grazer growth is often used as a proxy for feeding, and, in turn, evidence of feeding (through prey disappearance) is often used as an indication of potential growth. However, growth and feeding in

heterotrophic protists are often uncoupled, such that ingestion of prey does not always result in grazer growth, and grazer growth does not necessarily reflect a corresponding rate of grazing (13, 14, 60). Indeed, we observed little or no relationship between ingestion and growth of each protist grazer, with variable growth at any given level of feeding (Fig. 6A). This may be driven in part by *Synechococcus* being a relatively poor-quality food source for marine protists (5, 11, 25, 48). Comparison of carbon-normalized rates of growth and grazing also provides evidence that the suitability of *Synechococcus* as a food source may vary among different isolates (Fig. 6B). For both *G. pacifica* and *O. marina*, grazer growth on WH8102 and CC9311 consistently fell above the mean gross growth efficiency (GGE, = 0.3), while growth on CC9902 and CC9605 fell below. Although this pattern of lower GGE associated with CC9605 and CC9902 could be driven by the energetic cost of consuming aggregates of *Synechococcus*, consistently lower GGE for grazers that both ingested (i.e., *O. marina*) and did not ingest (i.e., *G. pacifica*) aggregates suggests the effect of other factors, such as the energetic expense of grazing at high rates or strain-specific differences in food quality and digestibility. In this regard, not only phylogenetic identity may play an important role in determining the susceptibility of *Synechococcus* to grazing, but also the extent to which ingested *Synechococcus* cells are able to support growth of heterotrophic protists.

Concluding remarks. *Synechococcus* strains are among the most abundant members of the marine picophytoplankton community, yet the mechanisms that govern their interactions with protozoan grazers are poorly understood. In the present study, we established model predator-prey systems involving *Synechococcus* and identified persistent differences in the ingestion of different *Synechococcus* strains among a range of protist grazers. Our study provides preliminary evidence that preingestion interactions between predator and prey play an important but poorly understood role in grazing resistance of *Synechococcus*. Our study also reveals that, despite ecological and empirical evidence for high grazing rates, *Synechococcus* may be a lower-quality component in the diets of heterotrophic protists in marine ecosystems. Additional work investigating cell surface interactions and dissolved chemical cues between *Synechococcus* and their protist grazers, as well as identifying the limitations of *Synechococcus* as a food source, will be instrumental in elucidating the role of *Synechococcus* in microbial food web dynamics and oceanic carbon flux.

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