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ORIGINAL ARTICLE

Phagotrophy by the picoeukaryotic green alga *Micromonas*: implications for Arctic Oceans

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Photosynthetic picoeukaryotes (PPE) are recognized as major primary producers and contributors to phytoplankton biomass in oceanic and coastal environments. Molecular surveys indicate a large phylogenetic diversity in the picoeukaryotes, with members of the Prymnesiophyceae and Chrysophyseae tending to be more common in open ocean waters and Prasinophyceae dominating coastal and Arctic waters. In addition to their role as primary producers, PPE have been identified in several studies as mixotrophic and major predators of prokaryotes. Mixotrophy, the combination of photosynthesis and phagotrophy in a single organism, is well established for most photosynthetic lineages. However, green algae, including prasinophytes, were widely considered as a purely photosynthetic group. The prasinophyte *Micromonas* is perhaps the most common picoeukaryote in coastal and Arctic waters and is one of the relatively few cultured representatives of the picoeukaryotes available for physiological investigations. In this study, we demonstrate phagotrophy by a strain of *Micromonas* (CCMP2099) isolated from Arctic waters and show that environmental factors (light and nutrient concentration) affect ingestion rates in this mixotroph. In addition, we show size-selective feeding with a preference for smaller particles, and determine P vs I (photosynthesis vs irradiance) responses in different nutrient conditions. If other strains have mixotrophic abilities similar to *Micromonas* CCMP2099, the widespread distribution and frequently high abundances of *Micromonas* suggest that these green algae may have significant impact on prokaryote populations in several oceanic regimes.
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Introduction

Functional groups of marine plankton have long been organized according to size, with the smallest fraction (0.2–2 μm) designated as picoplankton (Sieburth *et al.*, 1978). Early studies of photosynthetic picoplankton recognized them as an important and widely distributed group across marine habitats, but assumed they were composed exclusively of bacterioplankton (Platt *et al.*, 1983). Further research revealed that there are several clades of eukaryotic algae within this size-fraction (reviewed by Vaulot *et al.*, 2008), especially within an extended size boundary $\leqslant 3$ μm, that is considered a more natural delineation for picoeukaryotes (Massana, 2011). Photosynthetic picoeukaryotes (PPE) contribute greatly to both biomass and primary production in various marine systems; in some regions with reduced cyanobacterial populations, small-sized eukaryotic phytoplankton can comprise up to 90% of this production (Worden *et al.*, 2004; Jardillier *et al.*, 2010; Grob *et al.*, 2011). Evidence is also accumulating that mixotrophic PPE are major consumers of prokaryotes, with potential to dominate not only primary production but also bacterivory in ecosystems as diverse as subtropical gyres and Arctic seas (Zubkov and Tarran, 2008; Hartmann *et al.*, 2012; Sanders and Gast, 2012).

Culture-independent molecular surveys, often coupled with flow cytometry, demonstrate high genetic diversity for PPE across a range of pelagic and coastal marine environments (Díez *et al.*, 2004; Not *et al.*, 2007; Lepère *et al.*, 2009; Cuvelier *et al.*, 2010; Kirkham *et al.*, 2013). These phylogenetic analyses indicate that distinct clades of PPE can be associated with different water masses. For example, Prymnesiophyceae and Chrysophyceae had complementary abundance patterns associated with N:P ratios in all major ocean biomes (Kirkham *et al.*, 2013). Furthermore, Foulan *et al.* (2008) found that three clades of *Micromonas pusilla* had different relative contributions to total abundance along environmental gradients in tropical, temperate and

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polar environments suggesting niche partitioning. Despite this growing understanding of the diversity and the key roles that PPE have as primary producers and grazers of prokaryotes in marine waters, many of the major clades remain uncultured (Vaulot *et al.*, 2008; Massana, 2011). Consequently, there is relatively little information on their morphology and physiology that could aid in understanding environmental conditions that affect their growth and relative abundances (DuRand *et al.*, 2002; Foulon *et al.*, 2008; Hartmann *et al.*, 2013).

One PPE that has several strains in culture is *M. pusilla*, a small ($<2\,\mu m$) motile green alga with a single flagellum, mitochondria and chloroplast (Manton and Parke, 1960). It has a global distribution and can be abundant in marine biomes from the Arctic to the ice-edge of Antarctica (Díez *et al.*, 2004; Not *et al.*, 2004; Lovejoy *et al.*, 2007; Foulon *et al.*, 2008), and has the potential to be the dominant contributor to PPE communities in coastal and nutrient-rich environments (Not *et al.*, 2004; Lovejoy *et al.*, 2007; Not *et al.*, 2007; Li *et al.*, 2009). Molecular data indicate that *M. pusilla*, long considered a single morphospecies, actually represents several lineages, and analyses of complete genomes of two isolates support their classification as separate species (Šlapeta *et al.*, 2006; Worden *et al.*, 2009).

The aim of this study was to confirm mixotrophy in a strain of *M. pusilla* (hereafter identified as *Micromonas* CCMP2099) isolated from Arctic waters, which was suggested to be a major bacterivore by Sanders and Gast (2012). A temperate strain identified as *M. pusilla* was previously shown to be bacterivorous (González *et al.*, 1993), but there still is little eco-physiological information available for any of the clades (Foulon *et al.*, 2008; Hartmann *et al.*, 2013). To this end, we compared the effect of light and nutrients on rates of bacterivory for *Micromonas* CCMP2099, tested its ability to discriminate between prey sizes and examined autotrophic functioning (P vs I) in different light and nutrient conditions. To our knowledge, this study provides the first species-specific examination of factors potentially affecting mixotrophy in algae of this size class.

Materials and methods

Culture origin, maintenance and sizing

Cultures of *Micromonas* (CCMP2099), originally isolated from an Arctic polyna (76.283 °N, 74.75 °W) between Ellesmere Island and Greenland (Lovejoy *et al.*, 2007) and designated as *M. pusilla* by CCMP, were maintained at 4 °C in f/2 + Si media made with artificial seawater (ASW) at 32 PSU (Caron, 1993; Guillard, 1975). The cultures were non-axenic and grown under continuous irradiance from 50 W cool-white fluorescent bulbs at $\sim 50\,\mu E\,m^{-2}\,s^{-1}$.

Average cell size of *Micromonas* CCMP2099 in late exponential phase was determined by measurement of 50 Lugol's iodine-fixed cells using a calibrated ocular micrometer at $\times 1000$ magnification on a Zeiss Axiovert 10 inverted microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

Microscopic imaging of Micromonas

For confocal images, fixed primulin-stained samples of *Micromonas* were centrifuged at 16 000 *g* for 1 h to separate uningested spheres from algal cells. Centrifuged *Micromonas* were then pipeted onto slides and air-dried overnight. Images were obtained using a Leica TCS-NT confocal microscope. Illumination was provided by a Krypton/Argon Laser with excitation wavelengths of 488 and 633 nm. Emission wavelengths were 503–550 nm for the microspheres and 628–724 nm to view chloroplast autofluorescence and primulin staining. In addition, a differential interference contrast (DIC) image was captured to combine with the confocal.

For epiflourescent micrographs, samples of *Micromonas* CCMP2099 inoculated with micropsheres were fixed as described below, stained with primulin (Sigma-Aldrich, St Louis, MO, USA), concentrated on 0.8-μm pore black Poretics PC filters and excess primulin cleared with 0.1 M Tris (pH 4). Filters were mounted on slides with Vectashield with 4′,6-diamidino-2-phenylindole (DAPI) and were frozen until images were taken. Images of *Micromonas* CCMP2099 were captured with a Zeiss Axiovert 10 microscope equipped for epifluorescence and a Coolpix995 digital camera (Nikon, Tokyo, Japan). Images were post-processed to reduce overexposure of fluorescing particles in ImageJ (http://rsbweb.nih.gov/ij/). Images of a single field of view were taken with both filters and each image was separated into red, blue and green channels, and adjusted for brightness and contrast. Channels with the strongest signal for tracer particles and algal cells were selected and used to create a merged image of a specific cell.

General grazing experiment (2 × 2 factorial)

Replicate flasks with high- and low-nutrient concentrations were divided into dark or light ($\sim 50\,\mu E\,m^{-2}\,s^{-1}$) treatments for a 2×2 factorial design comparing light and nutrients on grazing activity ($n = 4$ flasks per treatment). High-nutrient treatments were full strength f/2 + Si media in ASW (that is, the maintenance media). The concentration of N and P in f/2 are 145 and 8 μM, respectively. Low-nutrient treatments were grown in f/2 + Si diluted 10-fold with ASW. These nutrient concentrations were generally greater than those reported for much of the Arctic summer, but phosphate concentrations in this range have been observed (Wheeler *et al.*, 1997). Light was supplied in the same manner and level as for culture maintenance. Culture flasks for dark treatments were wrapped in aluminum foil and placed in a cardboard container lined with

aluminum foil to exclude all light. Both light and dark treatments remained in the same incubator at 3 °C. After 1 week of acclimation to experimental conditions, fluorescently labeled particles (0.55 μm diameter, Fluoresebrite, Polysciences, Inc., Warrington, PA, USA) were added to culture flasks at tracer levels (5–10% of bacterial abundance). Bacterial and total microsphere abundances were determined from samples collected on 2.4 mm, 0.2-μm pore-size Poretics polycarbonate filters (GE Infrastructure Water & Process Technologies, Trevose, PA, USA), mounted on glass slides using Vectashield with DAPI (Vector Laboratories, Inc., Burlingame, CA, USA) and enumerated at ×1000 magnification using epifluorescence on a Zeiss Axiovert microscope. To determine ingestion, samples were taken from each flask immediately after addition of microspheres (T_0) and at 10 min intervals thereafter for ~1 h. Samples were fixed with Lugol's iodine, cleared with $Na_2S_2O_3$, with final fixation in formalin (Sherr and Sherr, 1993). Subsamples from each replicate and time point were collected onto 0.8 μm polycarbonate filters, mounted using Vectashield with DAPI and examined as described above for bacteria. At least 100 *Micromonas* cells from each replicate slide through the time course were examined for ingested microspheres. For each replicate, an average number of ingested microspheres, based on all cells examined, was determined. Random coincidental overlap of microspheres and cells was accounted for by subtracting T_0 counts from other time points. Ingestion rates of micropheres were determined from the slope of a linear regression of average ingestion vs time using all replicates. Ingestion rates of bacteria were calculated, assuming that microspheres and bacteria were ingested relative to their ratios at the beginning of the time course (Sherr and Sherr, 1993).

Size-selection experiment

Size selectivity by *Micromonas* CCMP2099 was examined with three treatments of added Fluoresebrite fluorescent microspheres: (1) small, 0.46 μm diameter; (2) large, 0.9 μm diameter; and (3) a combination of the two particle sizes. Microsphere sizes were chosen to give the largest size range that we believed to be ingestible by *Micromonas* based on previous field experiments (Sanders and Gast, 2012). In addition, the microspheres fluoresced at different wavelengths making them even easier to distinguish from each other in the combined treatment. Microspheres were presented at the same total particle concentration ($3.5 \times 10^6\,ml^{-1}$) for all treatments. These size-selection experiments were carried out under the previously described low-nutrient conditions with light. Ingestion rates were measured over a 1-h time course, sampling at 10-min intervals from replicate flasks ($n = 5$) using protocols for grazing experiments above.

Photosynthesis vs irradiance responses

Carbon fixation by *Micromonas* CCMP2099 grown in high-nutrient (f/2 + Si) or low-nutrient conditions (fivefold dilution of f/2 + Si) were measured under a range of irradiances using the method of MacIntyre et al., (1996), modified by the addition of centrifugation techniques used by Smith and Azam (1992). Photosynthesis vs irradiance (P vs I) responses were established by incubating 1-ml of *Micromonas* CCMP2099 adapted to either high- or low nutrients at different light levels (four replicate incubations per light level per nutrient treatment) with sodium [^{14}C]bicarbonate (final specific activity per sample 0.5 μCi, 18.5 kBq). Samples were incubated at PAR light levels ranging from ~3 to 150 $\mu E\,m^{-2}\,s^{-1}$ for 2 h Following incubations, samples were centrifuged at 16 000 g for 30 min, followed by aspiration of the supernatant. The algal pellet was then resuspended in 32 PSU ASW solution and fumed with 6 N HCl overnight to remove residual unassimilated ^{14}C. The samples were neutralized with 6 M NaOH and scintillation fluid was added. Radioactivity of samples was measured in a scintillation counter (Beckman LS-3801, Irvine, CA, USA), and the average counts per minute were converted to disintegrations per minute using a quench correction curve. Rates of carbon fixation were determined by the equation:

$$\frac{C_{fixed}}{h} = \frac{DIC_{sample} \times DPM_{^{14}C_{sample}}}{DPM_{^{14}C_{total}}} \times \text{incubation time (h)}$$

where,

C_{fixed} = the amount of total carbon fixed by photosynthesis,

DIC_{sample} = total dissolved organic carbon available for fixation in sample,

$DPM_{14_{Csample}}$ = disintegrations per minute for the algal pellet

$DPM_{14_{Ctotal}}$ = disintegrations per minute of total ^{14}C available for incorporation.

DIC was calculated using the program CO2SYS in Excel available at http://cdiac.ornl.gov/ftp/co2sys/. Average ^{14}C uptake of dark controls was subtracted from the light incubations to correct for incorporation not due to photosynthesis. Net primary production was normalized to a per cell basis from enumeration of *Micromonas* CCMP2099 using light microscopy fixed with Lugol's iodine (3% final concentration).

Data and statistical analysis

General and size-selection grazing experiments were analyzed first using a repeated-measures regression to identify whether there was significant variance between the replicate flasks. Following this, ingestion per cell was regressed over time to obtain a grazing coefficient and related standard error parameters. Rates were compared using an analysis of covariance (ANCOVA) of ingestions over time by treatment with pairwise comparisons

assessed using Tukey's honestly significant difference (HSD) test. The R statistical software program was used to fit P vs I curves to the Eilers–Peeters light limitation curve, which defines photosynthesis as the product of the maximal photosynthetic rate and the light limitation at a given light irradiance, I (Eilers and Peeters, 1988). Specifically,

$$\text{Light limitation} = \frac{2\times(1+\beta)\times \mathrm{I}/\mathrm{I}_{\mathrm{opt}}}{(\mathrm{I}/\mathrm{I}_{\mathrm{opt}})2 + 2\times\beta\times\mathrm{I}/\mathrm{I}_{\mathrm{opt}} + 1}$$

and

Photosynthesis $= P_{\max} \times$ Light limitation

where,

$\mathrm{I}_{\mathrm{opt}}$ = light irradiance at optimal photosynthetic rate

β = a dimensionless parameter which defines the degree of photoinhibition.

$P_{\max}$ = maximum photosynthetic rate.

Nonlinear regression analysis of P vs I data was done using nls function in the R statistical software program. Pairwise comparisons of grazing rates were performed with the multcomp package in R.

Results

Micromonas CCMP2099 ingested particles under all experimental conditions and the average number of ingestions increased at each time point over the course of the incubations (Figure 1, Supplementary Figure S1). Bacterial ingestion rates based on the time courses ranged from 0.44 to 1.32 bacteria per hour and were unlikely to be influenced by prey density, as bacterial abundances did not differ significantly in any of experimental light and nutrient conditions. The highest grazing rates were observed under low-nutrient/illuminated

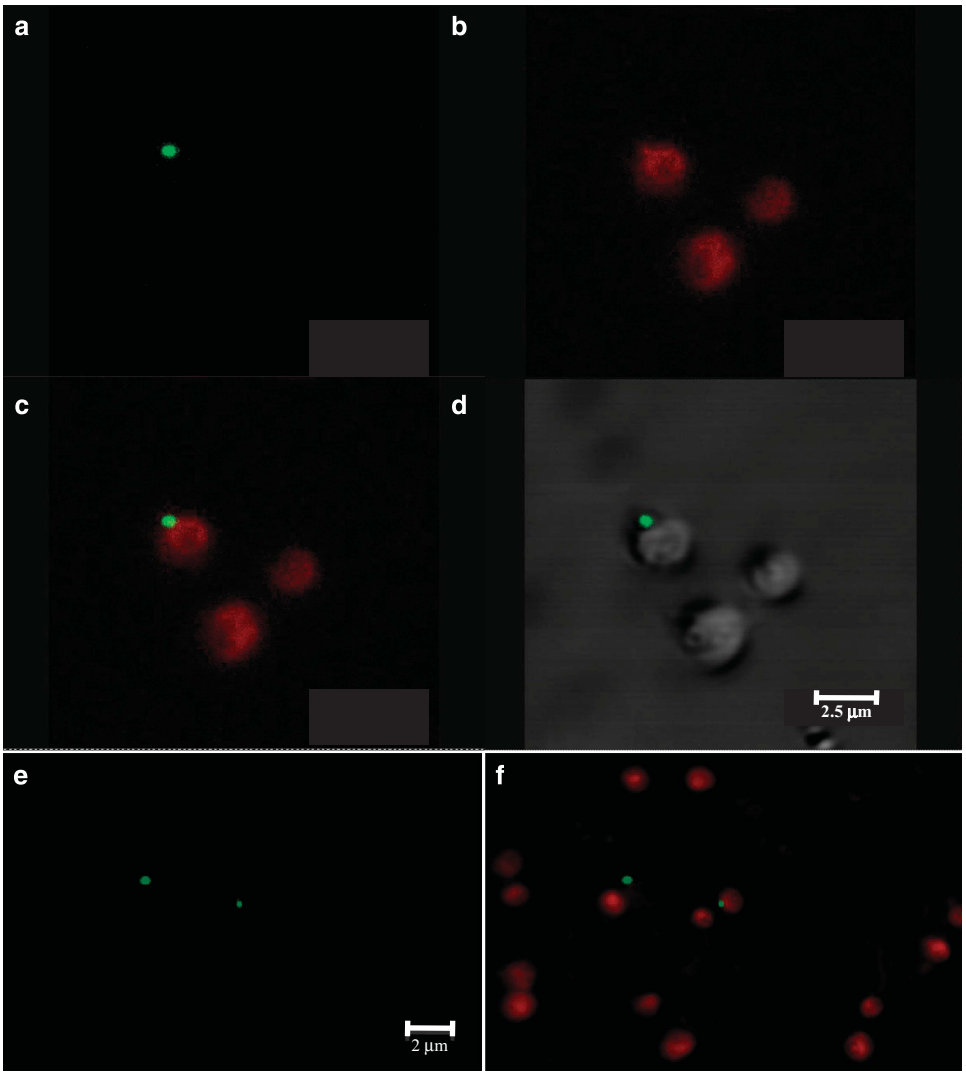

Figure 1 (**a–c**) Confocal images of three *Micromonas* CCMP2099 cells, one with an ingested microsphere. (**d**) DIC image of the same *Micromonas* cells. Scale bar in **d** applies to panels **a–d**. (**e**) Epifluorescence micrograph of fluorescent microspheres only (diameter = 0.5 µm), and (**f**) the same image showing microspheres and *Micromonas* cells, including an ingestion event. *Micromonas* cells stained with DAPI and primulin. Scale bar in panel **e** also applies to panel **f**. Images in **a–d** are from a separate feeding experiment than for panels **e** and **f**.

conditions and were three times greater than in other treatments. Ingestion rates for the remaining three treatments (low nutrient in darkness and high nutrient in light or dark) were not significantly different from each other. Light incubations did not increase ingestion rates as a single factor; higher grazing rates were induced only when nutrient levels were reduced and light was present (Figure 2).

Micromonas CCMP2099 exhibited an ability to discriminate between different particle sizes, with ingestion rates of microspheres ranging from 2.8 to 5.4 spheres per cell per hour. Small (0.46 μm) and large (0.9 μm) particles were both ingested when either was exclusively available, but small-sized particles were ingested at almost twice the rate (91% higher) of large particles (Figure 3). When both sizes of particles were offered together, *Micromonas* CCMP2099 grazing of the large-sized particles was not significantly different from background. Small particles were grazed at equivalent rates in the treatment with a mixture of microsphere sizes and in the treatment with only small particles (Figure 3).

P vs I determinations at the two nutrient levels showed similar distribution of photosynthesis with increasing light (Figure 4, Table 1). The maximum photosynthetic output was produced at ~30 μE m^{-2} s^{-1} (I_{opt}, optimal irradiance) for both nutrient treatments (Table 1) and photoinhibition occurred at higher irradiances in both treatments (Figure 4). However, the maximum photosynthetic rate was reduced by ~59% in the lower nutrient treatment; rates of photosynthetic C fixation ranged from 18 to 301 pg C per cell per hour in low-nutrient treatments, and from 13 to 519 pg C per cell per hour in high-nutrient treatments (Figure 4, Table 1). Thus, lower nutrient concentrations resulted in both an

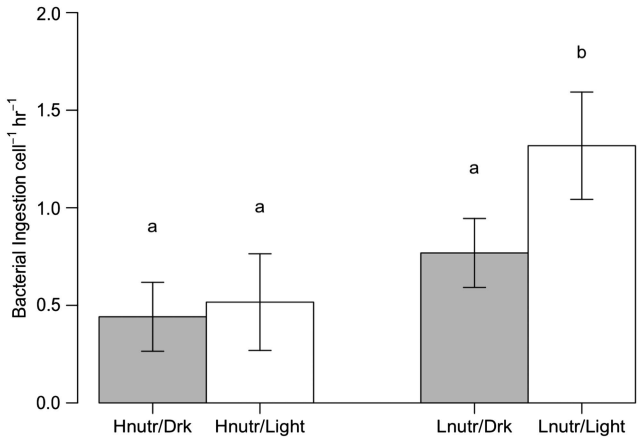

Figure 2 Bacterivory rates of *Micromonas* CCMP2099 in 2 × 2 factorial design experiment including high (f/2 media, Hnutr) and low nutrients (1:9 dilution of f/2 media with ASW, Lnutr) in either dark or light (50 μE m^{-2} s^{-1}) conditions. Means and error bars (± s.e.; $n = 4$) are a result of the deviation of the data from the slope of the line predicted by the regression of ingestions over time. Letters indicate Tukey's HSD comparisons; there are no significant differences between treatments with the same letter above the bars.

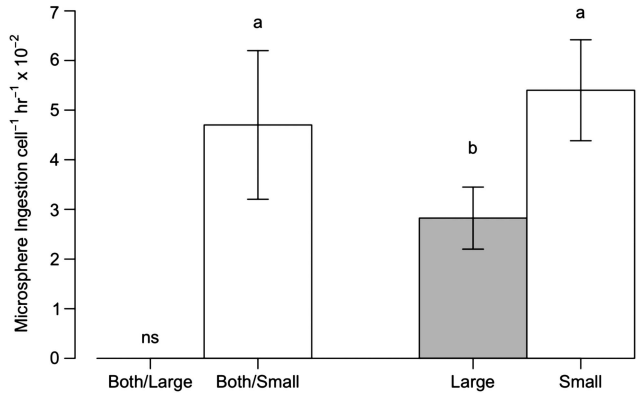

Figure 3 Ingestion rates of large (diameter = 0.9 μm) and small (diameter = 0.46 μm) microspheres when combined (Both/) or offered alone in separate treatments. Total microsphere abundance was identical in all treatments ($n = 5$; NS, not significant; error bars ± s.e.). Letters indicate Tukey's HSD comparisons, as in Figure 2.

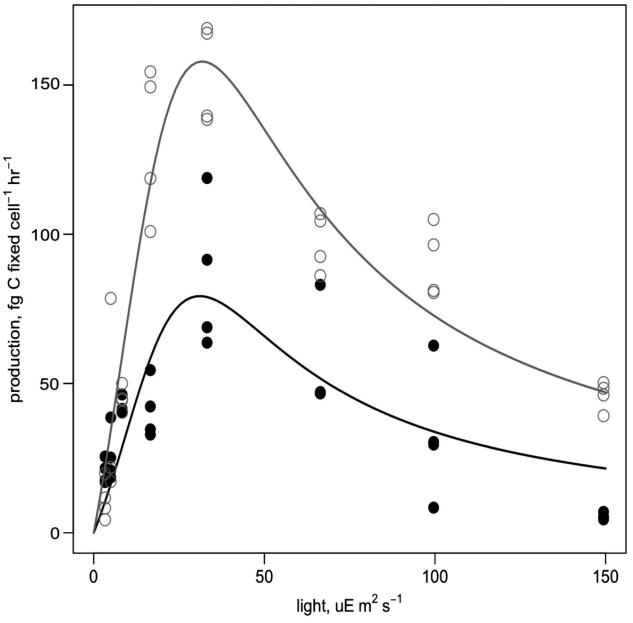

Figure 4 P vs I curves for *Micromonas* CCMP2099 grown in high (open circles, full strength) or low (closed circles, 5 × dilution with ASW) f/2 media.

Table 1 Parameters from high and low-nutrient treatments of *Micromonas* CCMP2099 photosynthesis vs irradiance responses fit to the Eilers–Peeters curve by nonlinear regression

	High nutrient			*Low nutrient*		
	Estimate	*s.e.*	*Pr(>|t|)*	*Estimate*	*s.e.*	*Pr(>|t|)*
P_{max}	157.89	8.46	***	79.2832	8.0943	***
β	−0.377	0.099	***	−0.4328	0.1695	*
I_{opt}	31.74	1.64	***	31.0514	2.9805	***
Df	29			29		

Abbreviation: Df, degrees of freedom. *$P<0.05$, ***$P<0.0001$.

increase in grazing activity (Figure 2) and a decrease in photosynthetic output (Figure 4).

Discussion

Though microbial eukaryotes occupy complex and versatile ecological niches, most were thought to engage solely in photoautotrophic or heterotrophic nutritional strategies to fulfill their energy and nutrient requirements. Mixotrophy, the combination of phototrophic production and phagotrophic ingestion of prey has received increasing amounts of attention by ecologists as its prevalence in aquatic systems has become apparent. The majority of mixotrophy studies focused on nanoplanktonic (2–20 μm) flagellates and ciliates, which are known to sometimes have greater grazing impact than heterotrophic flagellates—the presumed dominant bacterivores of planktonic systems—in marine, freshwater and even sea ice ecosystems (Sanders, 1991; Nygaard and Tobiesen, 1993; Moorthi *et al.*, 2009; Flynn *et al.*, 2013). Recent studies, especially in oligotrophic waters, have demonstrated the importance of PPE with phagotrophic capabilities. PPE comprised high proportions of the bacterivores and were responsible for up to 95% of the bacterivory in some marine systems (Unrein *et al.*, 2007; Zubkov and Tarran, 2008; Sanders and Gast, 2012). These experimental results and observations of the numerical dominance of PPE have led to the suggestion that mixotrophy sustains the functioning of oligotrophic ocean ecosystems (Hartmann *et al.*, 2012). In oligotrophic systems, it seems likely that the mixotrophic PPE are using phagocytosis for increased access to limiting nutrients. However, there are a variety of advantages that a mixotrophic strategy offers to nanoplankton, and by extension, to PPE (Sanders, 1991; Jones, 1994; Unrein *et al.*, 2013).

Bacterivory in Micromonas

Evidence of mixotrophic activity in green algal lineages has rarely been reported. However, Maruyama and Kim (2013) showed ultrastructural evidence of bacterivory in a nanoplanktonic prasinophyte, *Cymbomonas*, and González *et al.* (1993) found that a temperate strain of *M. pusilla* was capable of ingesting fluorescently labeled bacteria. The current experiments with the pan-Arctic strain of *Micromonas* CCMP2099 confirms mixotrophy reported for a *Micromonas*-like alga from field experiments in the Beaufort Sea (Sanders and Gast, 2012). Our calculations from the data presented in González *et al.* (1993) indicate an ingestion rate of 2.4 bacteria per alga per hour for that *M. pusilla* strain at 'room temperature,' which is in the same range as the Arctic strain investigated here. These data together suggest that despite the divergence of *Micromonas* CCMP2099 from numerous other *M. pusilla* lineages (Šlapeta *et al.*, 2006), other clades also may be phagotrophic. However, in experiments in the NW Mediterranean Sea, where *M. pusilla* dominated the PPE, the species was not observed to ingest fluorescently labeled bacteria (Unrein *et al.*, 2013). These contrasting results could be clade-specific or environmentally determined, and highlight the potential of distinct mixotrophic strategies between species–even in the same genus (Worden and Not, 2008; Unrein *et al.*, 2013).

Nutrient limitation increased phagotrophic behavior in some mixotrophic nanoflagellates (Nygaard and Tobiesen, 1993; Arenovski *et al.*, 1995), and reduced nutrient concentration also altered feeding rates in *Micromonas* CCMP2099 (Figure 2). The increased grazing rates of *Micromonas* in the lower nutrient conditions imply that mixotrophy functioned to obtain limiting nutrients required for physiological functions. Furthermore, increased rates of bacterivory were induced only in light treatments, suggesting that ingestion supplemented N and/or P supply in order to allow for balanced growth when photosynthesis was greatest. However, as lower nutrient conditions were achieved by dilution of complete f/2 media, other components of the media such as vitamins and trace metals were also lower and could have factored in the increased feeding rate we observed. The increase in feeding in the light vs dark (Figure 2) also suggests that phagotrophy and photosynthesis are not substitutable as carbon sources for *Micromonas* CCMP2099. The result of reduced grazing activity in the absence of light was unexpected, although reduced feeding rates by mixotrophs in the dark were reported for several freshwater species. Pålsson and Granéli (2003) found reduced bacterivory rates during night for several cryptomonads, and light-dependent phagotrophy in a mixotrophic chrysophyte was also observed in culture (Caron *et al.,* 1993). We suggest that the trophic strategy of phagotrophy in *Micromonas* CCMP2099 is to obtain some limiting dissolved nutrients rather than a mechanism for survival through the boreal winter darkness.

Size-selective grazing by heterotrophic protists is well established (for example, Verity, 1991; Hahn and Höfle, 2001). Less is known for mixotrophic nanoflagellates, though the chrysophyte, *Ochromonas*, selected for larger bacteria in several studies (Andersson *et al.*, 1986; Chrzanowski and Simek, 1990; Pfandl *et al.*, 2004). In contrast, *Micromonas* CCMP2099 selected small-sized prey, and ingested at lower rates than *Ochromonas*, most likely due to its own small size. The predator/prey size ratios for *Micromonas* CCMP2099 were only 2.4 for 0.5 μm microspheres, 2.6 for 0.46 μm microspheres, and 1.3 for 0.9 μm microspheres, based on the average diameter of 1.27 ± 0.07 μm for *Micromonas* CCMP2099. These results agree with the observation of Sanders and Gast (2012) that

the *Micromonas*-like PPE in the Beaufort Sea ingested 0.5 μm microspheres, but never ingested larger (1.0–1.2 μm) fluorescently labeled bacteria. Size-selective feeding has several implications for the microbial food web; the morphology and species structure of prey bacterial populations can be altered (Andersson *et al.*, 1986; Jürgens *et al.*, 1999), and the potential contribution of PPE such as *Micromonas* to the total grazing impact on prokaryotes may be limited to the smallest prey in a mixed-size bacterial assemblage. It also implies that cyanobacteria, typically on the large end of the size spectrum of bacteria, are less likely to be ingested by *Micromonas*.

Photosynthetic responses in Micromonas

Photosynthetic parameters observed for *Micromonas* CCMP2099 included a low optimal irradiance (I_{opt}) for maximal photosynthesis ($\sim 30\,\mu E\,m^{-2}\,s^{-1}$; Table 1). This was expected based on field observations of *M. pusilla* in the Arctic where blooms were typically located under ice at <20% of incident surface irradiance (Booth and Horner, 1997; Sherr *et al.*, 2003). Lovejoy *et al.* (2007) found that the clone used in our experiments (CCMP2099) had pan-Arctic distribution and grew best at or below 10 μM photons per $m^2\,s^{-1}$ ($\sim 10\,\mu E\,m^{-2}\,s^{-1}$) in field experiments. Other autotrophic picoplankton also appear to be adapted to photosynthesize at lower light irradiances typical of deep chlorophyll maximum layers; small-sized phytoplankton also can have a greater potential for photodamage compared with their larger counterparts (Raven, 1998). Carbon fixation by *Micromonas* CCMP2099 was reduced under low-nutrient compared with high-nutrient levels (Figure 4), which may be due to decreased energy conversion efficiencies caused by nutrient limitation (Kolber *et al.*, 1990). Conversely, grazing activity increased under reduced nutrient conditions in the light for *Micromonas* CCMP2099 (Figure 2). Increased bacterivory in low nutrients has been noted for some other mixotrophs (Nygaard and Tobiesen, 1993; Arenovski *et al.*, 1995), with the assumption that ingested bacteria supplied limiting nutrients, but photosynthetic production was not determined in those experiments. It is possible that the reduced photosynthesis and increased feeding rate *Micromonas* CCMP2099 could be an effect of the mode of nutrient supply (dissolved vs particulate) affecting the efficiency of photosynthesis; alternatively some other tradeoff may exists for combining phototrophy and heterotrophy in mixotrophic organisms (Raven, 1997).

Conclusion

Mixotrophy by PPE appears to be a widespread phenomenon, though most studies found bacterivory by Prymnesiohyceae, Chrysophyceae and Pelagophyceae, all of which have known mixotrophic species in larger size classes. Mixotrophic PPE are particularly important in oligotrophic oceans where they potentially sustain ecological functioning of the ecosystem (Hartmann *et al.*, 2012). This study is the first to directly examine species-specific factors that affect feeding by a phototrophic picoeukaryote, in particular, of a pransinophyte member of the Chlorophyta. The chlorophytes are generally considered to be predominantly phototrophic, although the ability to ingest particulate food was previously shown for another strain of *Micromonas* and for *Cymbomonas* (González *et al.*, 1993; Maruyama and Kim, 2013). *Micromonas* may be the most widely dispersed genus of PPE and can dominate the picoplankton biomass in many coastal and more nutrient-rich environments, including the Arctic where they can be the dominant bacterivore (Not *et al.*, 2005; Lovejoy *et al.*, 2007; Sanders and Gast, 2012). In the Arctic, global climate change may be leading to a picoplankton-dominated system, in which *Micromonas* could have an increasingly important role (Li *et al.*, 2009). Thus, understanding factors that affect phagotrophy and photosynthesis in this widespread group is increasing valuable (Foulon *et al.*, 2008).

Conflict of Interest

The authors declare no conflict of interest.

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