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Valeria Jimenez, John A Burns, Florence Le Gall, Fabrice Not, Daniel Vaultot. No evidence of Phago-mixotrophy in *Micromonas polaris* (Mamiellophyceae), the Dominant Picophytoplankton Species in the Arctic. *Journal of Phycology*, 2021, 57 (2), pp.435-446. 10.1111/jpy.13125 . hal-03267092

HAL Id: hal-03267092

<https://hal.sorbonne-universite.fr/hal-03267092>

Submitted on 22 Jun 2021

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No evidence of phago-mixotrophy in *Micromonas polaris*, the dominant picophytoplankton species in the Arctic †

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† This paper is dedicated to the memory of Hervé Moreau, who was coordinating this research project and passed away too young in July 2020.

keywords: phytoplankton, Arctic, phago-mixotrophy, *Micromonas*

Submitted to: Journal of Phycology

Date: November 26, 2020

Abstract

In the Arctic Ocean, the small green alga *Micromonas polaris* dominates picophytoplankton during the summer months but is also present in winter. It has been previously hypothesized to be phago-mixotrophic (capable of bacteria ingestion) based on laboratory and field experiments. Prey uptake was analysed in several *M. polaris* strains isolated from different regions and depths of the Arctic Ocean and in *Ochromonas triangulata*, known to be a phago-mixotroph, as a control. Using both fluorescent beads and fluorescently labelled bacteria as prey, we found no evidence of phago-mixotrophy in any *M. polaris* strain by flow cytometric measurement of prey ingestion in contrast to *O. triangulata*. In addition, *in silico* predictions revealed that members of the genus *Micromonas* lack a genetic signature of phagocytotic capacity.

Introduction

Polar regions are undergoing drastic changes due to climate change and global warming in particular. These changes have strong effects in Arctic marine ecosystems (Box et al. 2019, Graversen et al. 2008, Wassmann 2015) where phytoplankton production plays an essential role in food web dynamics and biogeochemical cycles (Arrigo et al. 2008, Kahru et al. 2016, Park et al. 2015). Considerable spatial and temporal changes in primary production have been observed in the last two decades (Kahru et al. 2016, Renaut et al. 2018, Tedesco et al. 2019). Rapid melting and early ice retreat increase the open areas exposed to solar radiation which in turns could result in an increase in annual net primary production along with a lengthening of the phytoplankton growing season (Kahru et al. 2016, Park et al. 2015, Renaut et al. 2018). Changes in Arctic primary production are also influenced by the increase of freshwater delivery to the upper ocean that leads to stronger water column stratification limiting the upward flux of nutrients to the surface (Brown et al. 2019, Coupel et al. 2015, Nummelin et al. 2016, Park et al. 2015, Slagstad et al. 2015, Timmermans et al. 2011, Tremblay et al. 2015).

Our ability to explain and predict the responses of Arctic phytoplankton communities to climate change is challenged by our limited understanding regarding their ecological and physiological strategies of growth and survival. Arctic phytoplankton communities experience extreme environmental conditions such as nutrient limitation, exposure to a long period of darkness (polar winter) followed by low light levels under the ice linked to snow coverage and ice thickness (Berge et al. 2015). In such unfavorable and shifting context, it has been suggested that phago-mixotrophy (ability to combine photosynthesis and bacterivory) could be a common trophic strategy among Arctic protists (Stoecker & Lavrentyev 2018). At the scale of the global ocean, phago-mixotrophy is an important, but until recently underestimated, process for energy and nutrient transfer (e.g. carbon fluxes) throughout the food web (Caron 2016, Mitra et al. 2014, Ward & Follows 2016). Phago-mixotrophic plankton are widespread in the ocean and evolutionary diverse, found in many branched of the eukaryotic tree (Selosse et al. 2017). They account for a large proportion of bacterivory in aquatic environments (Hartmann et al. 2012, Leles et al. 2019, Unrein et al. 2014). A recent study (Stoecker & Lavrentyev 2018) reviews the current evidence and importance of phago-mixotrophy in the Arctic ocean where

this trophic mode has been documented in chrysophytes (e.g. *Ochromonas* spp., *Dinobryon balticum*), cryptophytes (e.g. *Geminigera cryophila*, *Teleaulax amphioxeia*), prymnesiophytes (e.g. *Chrysochromulina* spp.), dinoflagellates (e.g. *Heterocapsa triquetra*, *Tripos arcticus*) as well as chlorophytes (e.g. *Pyramimonas* spp.).

The on-going expansion of stratification and nutrient limitation in the Arctic have been associated with an observed increase of the smaller picophytoplankton (Li et al. 2009, Ward 2015) which ranges 2-3 μm in cell diameter and is composed essentially of eukaryotes since cyanobacteria are nearly absent in polar marine ecosystems (Paulsen et al. 2016). Among the picoeukaryotic phytoplankton community, the green alga *M. polaris* (Lovejoy et al. 2007, Simon et al. 2017) dominates in the Arctic ocean in the summer months (Balzano et al. 2012, Kiliyas et al. 2014, Lovejoy et al. 2007, Marquardt et al. 2016) but is also present throughout the winter (Joli et al. 2017). Its abundance is expected to increase as the stratified oligotrophic areas expand (Benner et al. 2020, Hoppe et al. 2018, Li et al. 2009). The physiological plasticity allowing *M. polaris* to dominate the Arctic picoeukaryote community is not yet well understood. *M. polaris* was shown in the laboratory, to positively respond to a combination of temperature increase and acidification by higher growth rate and biomass production (Hoppe et al. 2018). Phago-mixotrophy would be another advantageous trait that could contribute to the success of *M. polaris* in the Arctic. Under prolonged periods of darkness or low irradiance, phago-mixotrophs could survive, despite reduced or even null rates of photosynthesis, by supplementing their carbon requirements through phagocytosis (Millette et al. 2017, Stoecker & Lavrentyev 2018, Zhang et al. 1998). Under oligotrophic conditions, phago-mixotrophy could also supply the cell with limiting nutrients (Stoecker et al. 2017).

Evidence of phago-mixotrophy in *Micromonas* has been previously obtained in laboratory and field experiments (González et al. 1993, McKie-Krisberg & Sanders 2014, McKie-Krisberg et al. 2018, Sanders & Gast 2012). More than 25 years ago, González et al. (1993) reported phago-mixotrophy in a temperate *Micromonas* strain (identified at that time as *M. pusilla*) based on a positive acid lysozyme assay and ingestion of fluorescently labelled bacteria (FLBs) measured by microscopy. More recently, the ability of Arctic pico and nanoplankton microbial communities to consume bacterioplankton has been analyzed by *in situ* experiments using FLBs and

yellow-green fluorescent microspheres (YG-beads) as prey. A *Micromonas*-like picoeukaryote, based on its shape and analysis of denaturing gradient gel electrophoresis (DGGE) band sequences, was reported to ingest a significant quantity of prey offered to it (Sanders & Gast 2012). Ingestion of beads was further tested in *M. polaris* strain CCMP2099 under laboratory conditions that compared different light levels and nutrient concentrations. The highest grazing rates were observed under light and low nutrient conditions (McKie-Krisberg & Sanders 2014) for which transcriptional response was also investigated (McKie-Krisberg et al. 2018). Despite the evidence presented, it is still unclear whether *M. polaris* is capable of ingesting bacteria because of the difficulty to distinguish whether the prey are inside the cells or just externally attached to them (Wilken et al. 2019) when using epifluorescence microscopy. Recently, association of YG-beads with *M. polaris* (strain CCMP2099) cells was found after performing feeding experiments with heat-killed cultures (Wilken et al. 2019), suggesting that beads may stick to the surface of the cells resulting in a potential over-estimation of phagocytosis.

In the present paper, we used flow cytometry to analyse prey uptake in several *M. polaris* strains isolated from different regions and depths in the Arctic Ocean, including CCMP2099 using the chrysophyte *Ochromonas triangulata* which is phago-mixotroph (Andersen et al. 2017) as a positive control. We also made predictions of the capacity of *Micromonas* to be a phago-mixotroph from an *in silico* gene-based model.

Materials and Methods

Strains and culturing conditions

Four *M. polaris* strains and one phago-mixotrophic *Ochromonas triangulata* strain were used in this study. Three of the *M. polaris* strains (RCC2306, RCC4298 and RCC2258) and *O. triangulata* strain RCC21 (previously known as *O. distigma*, Andersen et al. 2017) were obtained from the Roscoff Culture Collection (RCC, <http://www.roscoff-culture-collection.org>). The fourth *M. polaris* strain (CCMP2099) was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton and Microbiota (<https://ncma.bigelow.org>) and is also available from the RCC as RCC807. The *M. polaris* strains originate from different locations and depths in the Arctic (Table 1). All strains were non-axenic and grown under a 12h:12h light:dark cycle at $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR using L1 medium (Guillard & Hargraves 1993) made with artificial sea water (ASW, salinity 35) (Keller et al. 1987). All *M. polaris* strains were grown at 4 °C and *O. triangulata* at 20 °C. Cells were acclimated and maintained in mid-exponential growth phase before the beginning of each experiment.

Cell monitoring, feeding estimates and sample fixation

Cells and prey were counted using a Guava easyCyte (Luminex Corporation, USA) flow cytometer (FCM) equipped with a 488 nm laser recording cell counts, forward and side angle light scatters (FALS and SSC), both proxies of cell size, green (525 ± 30 nm band pass filter) and red (695 ± 50 nm band pass filter) fluorescences. Cultures under the different experimental conditions were monitored live using red autofluorescence from chlorophyll as a threshold. Flow cytometry was also used to determine the percent of cells with prey (YG-beads and FLBs) in samples fixed using a protocol modified from Sherr & Sherr (1993) (acid Lugol's iodine solution and formaldehyde 3.7%, and cleared with sodium thiosulfate 3%) with a threshold either on red fluorescence or green fluorescence. With the threshold on red fluorescence, cells that contained chlorophyll as well as green fluorescence (same signal as the prey added, YG-beads or FLBs) were considered to be cells containing prey (Figure S1). In addition, to confirm the total concentration of prey added to each flask, the sample was also run with the threshold on green fluorescence. FCM listmodes were analyzed with the Guava easyCyte Suite Software 3.1

(Luminex Corporation, USA).

For each feeding experiment, the ingestion of prey was quantified in each experimental flask by first adding prey and then sub-sampling and fixing after an incubation of 0 (T_0), 20 (T_{20}) and 40 (T_{40}) minutes. The T_0 sample accounts for the physical attachment of prey to the cell and therefore the percent of cells ingesting prey corresponds to the percent of cells with prey at T_{20} or T_{40} , minus the percent of cells with prey at T_0 .

Microscopy

Light-limited *M. polaris* (strain RCC2306) cells were fixed just after the addition of YG-beads (T_0) with glutaraldehyde (1% final concentration). Fixed cells were sedimented onto formvar coated copper grids for 30 minutes. Grids were then stained with three drops of uranyl acetate 2%, dried and examined using a JEOL JEM1400 transmission electron microscopy (TEM, Jeol, Tokyo, Japan) operating at 80 kV. Images were obtained with a Gatan Orius camera (Roper Scientific SAS, France).

Major feeding experiments

To test feeding, three different experimental designs were performed with *M. polaris* strains and another fourth with *O. triangulata* (Table 2). Feeding was primarily tested using yellow-green fluorescent polystyrene-based microspheres (YG-beads, diameter 0.5 μm , Fluoresbrite, Polysciences, Inc., Warrington, PA, USA) as prey. In some experiments fluorescently labelled bacteria (FLBs) were used. FLBs were prepared according to the protocol of Sherr *et al.* (Sherr *et al.* 1987) using the bacteria *Brevundimonas diminuta* (strain CECT313, also named *Pseudomonas diminuta*), obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain).

In experiment type 1 (*M. polaris*-EXP1) feeding was tested for each *M. polaris* strain grown under four different culture conditions. Each treatment was carried out (in duplicates for RCC2306 and RCC4298 and triplicates for RCC2258 and CCMP2099) by transferring a small volume of culture (a few ml in general), previously maintained in mid-exponential growth, to about 40 ml of L1-ASW medium (replete) or ASW without any addition (limited) in a 50 ml culture flask and then placed in the dark or left in the same light conditions as for culture

maintenance. Each treatment (light-replete, light-limited, dark-replete and dark-limited) was followed up for 15-17 days and feeding was tested with YG-beads after 7 (Feeding 1) and 14-17 (Feeding 2) days.

Experiment type 2 (*M. polaris*-EXP2) was performed with *M. polaris* strain RCC2306 and RCC2258 and was set-up the same way as EXP1 (in triplicates), but with an additional treatment (light-replete-Ab) in which 1 μ l of Penicillin-Streptomycin-Neomycin (PSN) antibiotics solution (Sigma Aldrich P4083) was added per ml of culture at the beginning of the experiment in order to minimize bacteria concentration. Moreover, the five treatments were incubated for only one week and feeding was tested with YG-beads at the end of the incubation (Day 7).

To compare feeding on YG-beads vs. FLBs, a third type of experiment (*M. polaris*-EXP3) was performed with *M. polaris* RCC4298. For each prey type (YG-beads and FLBs) feeding was tested in triplicate in mid-exponential phase cultures (light-replete).

For all experiments (*M. polaris*-EXP1 to EXP3), the initial concentration for each treatment was 5×10^5 cells ml⁻¹. The prey concentration was adjusted in order to achieve a prey to cell ratio of 1.5 to 2.5.

The experimental design of experiments EXP1 and 2 performed with *O. triangulata* was the same and only differed in their replication and number of feeding time points. *O. triangulata*-EXP1 was conducted in duplicate and with three feeding time points (T₀, T₂₀ and T₄₀), and *O. triangulata*-EXP2 in triplicates and two feeding time points (T₀ and T₄₀). Feeding was tested under two different culture conditions by transferring a small volume of culture, previously maintained in mid-exponential growth, to L1-ASW medium (light-replete) or ASW without any addition (light-limited) and incubated in the same light conditions as for culture maintenance. After one week of incubation, feeding was tested with YG-beads. The third experiment type (*O. triangulata*-EXP3) was performed in parallel with *M. polaris*-EXP3 to compare feeding on YG-beads and FLBs. For each prey type (YG-beads and FLBs) feeding was tested in biological triplicates in mid-exponential phase cultures (light-replete). In a fourth experiment type (*O. triangulata*-EXP4) feeding was tested using FLBs as prey in light-replete culture conditions. EXP4 was performed two times (EXP4a and b) and each time in duplicates.

Additional experiments

The degree of attachment of YG-beads to cells, immediately after the addition of prey (T_0) was further examined in a number of additional experiments (*M. polaris*-EXP5) performed with *M. polaris* strains RCC2306 and RCC4298. For *M. polaris* strain RCC2306 the quantification was done in cultures grown under light-replete, light-limited, dark-replete and dark-limited conditions, and for *M. polaris* strain RCC4298 with cultures grown under these four conditions plus light-replete-Ab.

The effects of fixation on the attachment of YG-beads to cells (*M. polaris*-EXP6) was measured by simultaneously comparing feeding in experiments performed with *M. polaris* (strain RCC2306) and run in the flow cytometer live or after fixation with Lugol's iodine solution and glutaraldehyde (0.25% final concentration). For this experiment, *M. polaris* (strain RCC2306) in mid-exponential (Light-replete) feeding was measured at two time points (T_0 and T_{40}).

Feeding on three different YG-bead sizes (0.5, 1, and 2 μm in diameter) (*M. polaris*-EXP7) was measured in *M. polaris* (strain RCC2306) incubated for one week in light-limited conditions (duplicates). Feeding was measured independently for each bead size using two feeding time points (T_0 and T_{40}).

Changes in the number of cells with YG-beads was measured by continuously running a live sample for 20 minutes immediately after the addition of YG-beads (*M. polaris*-EXP8). Samples were quantified on the FACSCanto (BD Biosciences, USA) flow cytometer with the same configuration as the Guava. For this experiment, cultures of *M. polaris* (strain RCC2306), previously incubated for one week in light-limited conditions, were used. Two ratios of beads to cells were tested (ratio of 1:1 and 2:1) in duplicates.

The percent of cells potentially containing food vacuoles (EXP9) was quantified in *M. polaris* (strain RCC2306) and *O. triangulata*, stained with the probe LysoSensor Green DND-189 (Thermo Fisher Scientific), that accumulates in acid cellular compartments like food vacuoles, at a final concentration of 1 μM . After the addition of LysoSensor, cells were incubated in the dark for 8 minutes and measured for 2 minutes using the Guava easyCyte (Luminex Corporation, USA) flow cytometer (triggered on green fluorescence). Cells with higher green fluorescence, after incubation with LysoSensor, were considered as potentially containing food vac-

uoles (Figure S2). The cells used for this test came from light-limited cultures (duplicates), from *O. triangulata*-EXP1 and *M. polaris*-EXP2, on which feeding experiments were performed.

Data analysis

Data processing, graphics and statistical analyses were performed using the R software (R Core Team 2014) using in particular the package set *tidyverse*. Pairwise comparisons were performed with the *t.test* function to calculate p-values based on Student (assuming equal variances) and Welch (assuming unequal variances) t-test.

Trophic mode predictions from genome and transcriptome analysis

Predicted peptides from whole genome (van Baren et al. 2016, Worden et al. 2009) or transcriptome data (Keeling et al. 2014) were downloaded from publicly available databases as detailed in Table S1. Because computational predictions are based on presence/absence information, predicted peptides from independent transcriptome assemblies of the same strain were concatenated to include as much information about each strain as possible. Computational prediction of phagocytotic, photosynthetic, and prototrophic capabilities were completed as in Burns et al. (2018a). This involved scoring a set of 14,095 protein profile hidden Markov models (HMMs) that were derived by clustering all proteins in 35 reference eukaryote genomes of known trophic mode against all proteins from each test genome or transcriptome. HMM profiles with a full sequence e-value $\leq 10^{-5}$ and a single domain e-value $\leq 10^{-4}$ to any protein in a test genome or transcriptome were marked as "present" for that organism. Predictive models of trophic modes were built by grouping the reference eukaryotes by known trophic mode and discovering HMMs from the set of 14,095 proteins that had differential presence/absence patterns between groups. Those HMMs whose presence/absence patterns differed according to trophic mode were annotated against SwissProt and grouped by gene ontology (GO) terms. GO categories were scored per reference organism and a best predictive set of GO terms was selected for each trophic mode using machine learning algorithms, forming the core of the predictive trophic mode models. Each test genome/transcriptome was scored for the predictive GO categories of the trophic mode models using its HMM presence/absence vector. Final prediction probabil-

ities for each test genome/transcriptome were calculated against the reference trophic mode models using a probability neural network. To visualize the prediction output, which exists in four dimensions with three degrees of freedom (phagocytosis, photosynthesis, prototrophy, and a fourth dependent dimension for absence of each trophic mode), predictions were normalized such that the sum of the three predictions plus the probability of not fitting each trophic mode equaled 1 using the relation: $1 - (p_{phago} + p_{proto} + p_{photo})/3$. The fractional independent probabilities of each trophic mode and the dependent absence number were mapped to 4-dimensional color space and projected onto a circle using scripts adapted from the R package "pavo" (Maia et al. 2013). Scripts are available at <https://github.com/burnsajohn/predictTrophicMode>.

Results

Feeding experiments

M. polaris feeding was analyzed in four strains (CCMP2099, RCC2306, RCC4298 and RCC2258, Table 1) using a slight modification of the protocol described in Sherr & Sherr (1993). We determined the percentage of cells feeding on YG-beads or FLBs using flow cytometry to quantify the proportion of cells with prey. Compared to epifluorescence microscopy, which is low throughput allowing examination of at most 100 to 200 cells per sample (e.g. McKie-Krisberg & Sanders 2014), flow cytometry allows screening of a large number of cells per sample (typically several thousand). It also circumvents ambiguities that arise with microscopy when cells and prey randomly overlap during the filtration process (Wilken et al. 2019). To validate our approach we used the phago-mixotrophic *O. triangulata* strain RCC21 as a positive control. A range of experiments were performed to test different parameter combinations (Table 2).

M. polaris strains were grown under a combination of 2 light regimes and 2 nutrient concentrations (*M. polaris*-EXP1: light-nutrient replete, light-nutrient limited, dark-nutrient replete and dark-nutrient limited). Experiments took place over a period of 15 to 17 days. Feeding was examined with YG-beads after 7 (Feeding 1) and 14-17 days (Feeding 2). Clear negative growth effects under darkness and nutrient limitation conditions were observed for all strains. Overall, for all 4 strains under dark conditions, growth ceased between day 4 and day 7 and thereafter cell concentration remained stable (Figure 1). For cultures grown under low nutrient conditions (nutrient-limited), a decrease in growth rate was observed after one week (Figure 1). Additional signs of the effect of darkness and nutrient limitation were observed in FALS (proxy of cell size) and chlorophyll fluorescence: for example FALS decreased for cells in the dark (Figure S3). In all feeding experiments we observed that cells at time T_0 , immediately following addition of the beads, already had a number of beads associated with them. However, no significant difference was observed between the percent of cells with YG-beads at T_0 vs. T_{20} or T_{40} whatever the growth phase or the culture condition (Figures 2A and S4, Table S2).

We questioned whether the absence of feeding on YG-beads could have been due to the presence of bacteria in the cultures. In order to address this issue, we performed a second series

of experiments in which we included a fifth condition by adding antibiotics to a light-nutrient replete culture (*M. polaris*-EXP2). This was only performed with two of the *M. polaris* strains (RCC2258 and RCC2306) and a single feeding experiment was conducted after one week. No feeding was detected under any of the culture conditions (Figure S5).

We then compared feeding on YG-beads vs. FLBs as prey (*M. polaris*-EXP3) since the prey type may influence feeding behavior. Again no feeding was observed when using either YG-beads or FLBs (Figure 2B). In contrast, in the four experiments performed with *O. triangulata* (EXP1 to EXP4) we observed feeding on YG-beads and FLBs that ranged from 7 to 14 and 21 to 27 percent of cells feeding on each prey type respectively, suggesting that *Ochromonas* clearly preferred FLBs over YG-beads ((Figure 2B, Table S2).

The percentage of cells with 0.5 μm YG-beads at T_0 was linearly related to bead concentration (Figure 3, $R^2 = 0.93$). Cell size did not seem to have an influence since *Micromonas* ($\simeq 1.5 \mu\text{m}$) and *Ochromonas* ($\simeq 5 \mu\text{m}$) fitted the same curve (Figure 3). The number of cells with YG-beads did not change over time as demonstrated by monitoring live cells of *M. polaris* (strain RCC2306) in the presence of YG-beads by flow cytometry over a 20 minutes period (Figure S6).

The association of beads and cells did not seem to be linked to bead size. We still observed association of 1 and 2 μm YG-beads with cells at T_0 , even though the 2 μm beads are close in size to *M. polaris* cells and no differences were observed between the percent of cells with YG-beads at T_0 and T_{40} (Figure S7 and Table S3). Fixation does not seem to impact the association of beads at T_0 as we observed this co-association when samples were run live or fixed with Lugol's solution or glutaraldehyde (Table S4). External attachment of YG-beads to cells of *M. polaris* (strain RCC2306) was visualized by TEM (Figure S8).

Since the observation of acidic food vacuoles has been proposed as an evidence of phagotrophy (Carvalho & Graneli 2006, Wilken et al. 2019), light-nutrient limited cultures of *M. polaris* (from EXP-2, which did not feed on YG-beads) were stained with the acidotropic LysoSensor fluorescent probe. No significant difference was observed in green LysoSensor fluorescence between unstained and stained cells, whereas for *O. triangulata* (EXP1 light-nutrient limited) green fluorescence increased 3.5 times after staining, suggesting the presence of food vacuoles

in the latter species (Figure S2 and Table S5).

Trophic mode predictions

Phagocytotic, photosynthetic, and prototrophic capacity of protists can be predicted based on their genome or transcriptome composition (Burns et al. 2018a). We used this approach to analyze gene composition of a number of microalgae including *Micromonas* (Table S1). The predictions confirm that known phago-mixotrophs like *Dinobryon sp.*, *Pedinelalles sp.*, *O. tri-angulata*, and *Prymnesium parvum* have and express a battery of genes consistent with their observed lifestyle coherent with the capacity for phagocytosis, photosynthesis, and prototrophy (Figure 4). Presumed photo-autotrophs like members of the genus *Ostreococcus* lack genes consistent with the capacity for phagocytosis, but have genes consistent with the capacity for photosynthesis and prototrophy (Figure 4). Similarly, all members of the genus *Micromonas* are predicted to be photo-autotrophs as they contain genes consistent with photosynthesis and prototrophy, but lack genes consistent with the capacity for phagocytosis (Figure 4).

Discussion

We examined feeding of *M. polaris* on prey in a series of experiments with four different strains (CCMP2099, RCC2306, RCC4298 and RCC2258) grown under different light and nutrient conditions using flow cytometry to monitor prey uptake (Table 2). In none of these experiments (Figures 2, S5 and S4, Table S2), significant differences were detected between the number of *M. polaris* cells associated with prey at T_0 and other time points (T_{20} or T_{40}). We also tested different fixation methods vs. live cells and three different diameters of beads (0.5, 1, and 2 μm in diameter) without detecting any suggestion of active uptake by *M. polaris*. No evidence of phago-mixotrophy was found when using the acidotropic LysoSensor dye in *M. polaris* light-nutrient limited cultures. Trait-based computational analysis of available genomes and transcriptomes confirmed that *Micromonas* lack genes consistent with the capacity for phagocytosis. These data are in contrast to what was observed for the known phagotroph *O. triangulata* (strain RCC21) that always displayed evidence of prey uptake when using similar approaches to the one we used for *M. polaris* strains and fits the computational profile of a phago-mixotroph. None of our evidence is consistent with the consideration of *M. polaris* as a phago-mixotroph.

In each of our experiments, there was a considerable number of *M. polaris* cells at T_0 associated with prey, immediately following addition of prey to the cultures and before time had elapsed for prey ingestion. The percentage of cells with 0.5 μm YG-beads at T_0 appears to be linearly related to the bead concentration (Figure 3), suggesting the association is the result of a physical property of the cells surface rather than an active behavior that the cells execute. The external attachment of YG-beads to *M. polaris* cells was also visualized by electron microscopy (Figure S8). Such passive associations of cells with beads have recently been observed in *Micromonas* by Wilken et al. (2019) using flow cytometry. They observed that the proportion of cells associated with beads at T_0 was much larger for heat-killed vs. live cells and that in live cells it increased with time for cultures left in the dark compared to light conditions. This contrasts with our data since we observed less attachment under dark conditions (Figure 2A). However in the Wilken et al. (2019) study cells were put in the dark for a maximum of 44 h (less than 2 days, their Figure 3a and b) while in our case we performed the first feeding experiment after 7 days (Figure 1). Therefore in our experiment the number of cells corresponding to our

first feeding experiment was much higher in the light than in the dark (Figure 1) and since we adjusted the bead concentration as a function of the cell concentration, the bead concentration was also much higher in the light vs. the dark condition. This explains why, based on Figure 3 (linear relationship between % of cells with beads and bead concentration), we found a much higher % of cells with beads under the light condition. External attachment of particles or bacterial cells to phytoplankton cell surfaces may be enhanced by phycosphere properties (Bell & Mitchell 1972, Seymour et al. 2017) which mainly consist of polysaccharides released by the cells (Mühlenbruch et al. 2018, Mykkestad 1995, Passow 2002a). The "stickiness" properties of abundant exopolysaccharides have mainly been studied in diatoms (Engel 2000, Passow 2002b, Seymour et al. 2017). Bacteria colonization of this sticky phycosphere occurs in both live and compromised cells and is a function of the probability of random encounters of phytoplankton and bacteria which is influenced by both bacteria concentration and motility (Seymour et al. 2017).

Our experimental conditions were very similar to those reported by McKie-Krisberg & Sanders (2014). We used the same *M. polaris* strain (CCMP2099), dark and light conditions, ASW as medium, 0.5 μm beads, Lugol's iodine fixation and short term incubation (40 min.). The main methodological difference is that we used flow cytometry analysis of cell suspensions instead of epifluorescence microscopy of filtered samples. Our approach has many advantages over epifluorescence microscopy: it allows counting of a much larger number of cells (typically several thousand vs. 100-200); it is faster; it results in less potential biases related to individual operator interpretation; there is no ambiguity linked to food particles randomly overlapping with cells during filtration. The latter problem is demonstrated in McKie-Krisberg & Sanders (2014): their differential interference contrast and confocal microscopy images (Figure 1 c-d in their paper) aimed at demonstrating a YG-bead inside a *M. polaris* cell are inconclusive as the bead is at the edge of the cell (probably externally attached) which closely resembles the TEM images obtained in the present study (Figure S8). The two other papers that have reported phago-mixotrophy in *Micromonas* (González et al. 1993, Sanders & Gast 2012) may have suffered from the same problem, i.e. initial attachment of prey to cells. Moreover in the Sanders & Gast (2012) paper on natural communities the identity of the potential grazer was only "ten-

tatively identified as *Micromonas*” from the presence of a DGGE band with a *Micromonas* sequence. A study that examined gene expression of *M. polaris* strain CCMP2099 under nutrient stress conditions that reportedly influence grazing rate failed to find differential expression of any gene linked to the process of phagocytosis in *M. polaris* (McKie-Krisberg et al. 2018). The authors propose that *M. polaris* may constitutively express phagocytosis proteins to support low-level grazing. However, a study on the model phagocyte *Dictyostelium discoideum* suggests that an increase in phagocytosis can indeed be linked to differential expression of genes involved in the process (Sillo et al. 2008). An alternative hypothesis regarding the gene expression results from *M. polaris*, supported by the data presented here, is that members of the genus *Micromonas* are not phagocytotic and therefore have no mechanism for differential expression of genes linked to phagocytosis. Sets of proteins identified as part of the phagosome compartment are broadly distributed among phagocyte and non-phagocyte organisms and only a small subset of those proteins are indicative that a species has the capacity for phagocytosis (Burns et al. 2018b). Computational models show that members of the genus *Micromonas* lack those indicative proteins, reinforcing our hypothesis that *Micromonas* is not a phago-mixotroph.

It is now acknowledged that phago-mixotrophy is a widespread trait in planktonic communities and has profound implications for marine ecosystem functioning (Flynn et al. 2019, Stoecker et al. 2017). In particular phago-mixotrophy is believed to provide a competitive advantage to photosynthetic organisms under otherwise limiting environmental conditions (e.g. low light, low nutrients). Bacterial phagocytosis has been found everywhere across the eukaryotic tree of life (Selosse et al. 2017), but most laboratory studies on phago-mixotrophy have focused on a few model organisms such as the chrysophyte *Ochromonas* spp. (e.g. Lie et al. 2018, Terrado et al. 2017, Wilken et al. 2020), the haptophytes *Prymnesium parvum* (e.g. Brutemark & Granéli 2011, Liu et al. 2015) and *Chrysochromulina* spp. (Hansen & Hjorth 2002, Jones et al. 1993), and several taxa of dinoflagellate such as *Alexandrium* spp. (e.g. Hansen 2011, Jeong et al. 2010, Lee et al. 2016). Among green algae in addition to the works on *Micromonas* mentioned previously, only a few studies have been performed with 6 other species described as phago-mixotrophs: *Pyramimonas gelicola* (Gast et al. 2014), *Pyramimonas tychoireta* and *Mantoniella antarctica* (McKie-Krisberg et al. 2015), *Cymbomonas*

tetramitiformis (Burns et al. 2015, Maruyama & Kim 2013), *Nephroselmis rotunda* and *N. pyriformis* (Anderson et al. 2018). None of these species fall however in the picoplankton size range. Interestingly none of the picoplanktonic Mamiellophyceae (in addition to *Micromonas*) for which the trait-based computational analysis was performed (*Bathycoccus*, *Ostreococcus*, *Mantoniella*, including *M. antarctica*) showed evidence for phago-mixotrophy. This may suggest that the whole Mamiellophyceae class (or at least the orders Mamiellales and Bathycoccales, since we have no data for orders Dolichomastigales and Monomastigales). Despite being primary players in many environments (Tragin & Vaulot 2018), including oligotrophic waters (Vannier et al. 2016), green picoeukaryotes are likely to rely on other strategies to thrive. Knowing the nutrition modes of this group will be important for modelling marine ecosystems since phago-mixotrophs increase the transfer of biomass to higher trophic levels resulting in larger organism mean size and sinking carbon fluxes (Ward & Follows 2016).

The evidence presented in this paper indicating that *M. polaris* is unlikely to be phago-mixotroph has profound impacts in present and future predictions of Arctic primary production, because of the importance and predicted increasing concentrations of this species in the Arctic Ocean (Li et al. 2009). If indeed *M. polaris* is not a phago-mixotroph, the question of how it survives during the long Arctic winter (Joli et al. 2017, Vader et al. 2015) and how it is able to develop during the Spring bloom that starts with very low light condition under the snow-covered ice (Arrigo et al. 2012) remains open.

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Acknowledgments

This work was supported by ANR contract PhytoPol (ANR-15-CE02-0007). We thank Robert Sanders and Rebecca Gast for hosting VJ and training her on techniques to determine mixotrophy in phytoplankton. We also thank Dominique Marie for help with flow cytometry, Christian Jeanthon for help and advice with fluorescently labelled bacteria, Sophie Le Panse from the Merimage microscopy platform at the Roscoff Marine Station for assistance with the transmission electron micrographs and the Roscoff Culture Collection for providing of the algal strains.

Author contributions statement

DV and VJ conceived the study. VJ and FLG collected and processed the samples. VJ, JB and DV analyzed the data. VJ, JB and DV drafted the manuscript. VJ, DV, FLG, FN and JB edited the final version of the paper.

Additional information

Data availability

Protocols are available at protocols.io at <https://www.protocols.io/edit/mixotrophy-quantification-of-the-percent-of-phytop-be2vjge6/steps>. Scripts for trophic mode prediction and visualization are available at <https://github.com/burnsajohn/predictTrophicMode>.

Competing interests

The authors declare no competing interests.

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- Figure S1 Examples of flow cytograms for *M. polaris* and the positive control *O. triangulata*. Flow cytometry was used to determine the percent of cells with prey (YG-beads and FLBs) in fixed samples using a protocol modified from Sherr & Sherr (1993). Data collection was performed with threshold on red (695 ± 50 nm band pass filter) or green fluorescence (525 ± 30 nm band pass filter). Cells that displayed red autofluorescence from chlorophyll as well as green fluorescence were considered to be containing prey (cells with YG-beads in green, cells with FLBs in orange and cells without prey in blue). In addition, to confirm the total concentration of prey added to each experimental flask, the same sample was also run with the threshold on green fluorescence (YG-beads and FLBs in grey and black respectively).
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- Figure S5 Percent of *M. polaris* cells with YG-beads (*M. polaris*-EXP2) for each strain and treatment. The color of the bars represents the time point (in minutes) after the addition of YG-beads (0 minutes; light grey, 20 minutes; dark grey, 40 minutes; black). Replete Ab correspond to nutrient replete conditions with antibiotics.

- Figure S6 Changes with time in the number of *M. polaris* (strain RCC2306) cells with YG-beads measured by continuously running a live sample for 20 minutes immediately after the addition of YG-beads. Two ratios of beads to cells were tested, 1:1 and 2:1, each in duplicate.
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Table 1. List of algal strains used in this study with isolation region, coordinates, depth (m) and growth temperature (°C).

Species	Strain	Origin	Lat Long	Depth	Temperature
<i>M. polaris</i>	CCMP2099	Arctic, Baffin Bay	76°N 75°W	55	4
	RCC2306	Arctic, Beaufort Sea	71°N 132°W	70	4
	RCC4298	Arctic, Greenland Sea	82°N 20°E	20	4
	RCC2288	Arctic, Beaufort Sea	70°N 135°W	0	4
<i>O. triangulata</i>	RCC21	Atlantic, Bay of Biscay	48°N 4°W	-	20

Table 2. Experimental scheme. Replete correspond to cultures grown in Artificial Sea Water (ASW) with L1 medium components added and limited to cultures grown in ASW without any addition. Ab correspond to cultures for which 1 μ l of Penicillin-Streptomycin-Neomycin (PSN) antibiotics solution was added to 1 ml of culture. The time points on which the percent of cells with prey was measured is indicated (T_0 , T_{20} and T_{40} , where the subscript corresponds to minutes). LR: Light nutrient replete, LL: Light nutrient limited, DR: Dark nutrient replete, DL: Dark nutrient limited, LR-Ab: Light nutrient replete with antibiotics.

Code	Conditions	Prey	Genus	Time points (mins)	Goal
EXP1	LR, LL, DR, DL	YG-beads	<i>Micromonas</i> , <i>Ochromonas</i>	0, 20, 40	Basic experiment with beads
EXP2	LR, LL, DR, DL, LR-Ab	YG-beads	<i>Micromonas</i> , <i>Ochromonas</i>	0, 20, 40	Test effect of antibiotics
EXP3	LR	YG-beads, FLBs	<i>Micromonas</i> , <i>Ochromonas</i>	0, 40	Compare beads vs FLBs
EXP4	LR	FLBs	<i>Ochromonas</i>	0, 40	Test FLBs on <i>Ochromonas</i>
EXP5	LR, LL, DR, DL, LR-Ab	YG-beads	<i>Micromonas</i>	0	Test attachment of preys at T_0
EXP6	LR	YG-beads	<i>Micromonas</i>	0, 40	Test effect of fixation
EXP7	LL	YG-beads	<i>Micromonas</i>	0, 40	Test effect of bead size
EXP8	LR	YG-beads	<i>Micromonas</i>	0 to 20	Time course of bead attachment
EXP9	LL	YG-beads	<i>Micromonas</i> , <i>Ochromonas</i>		Test Lysosensor (food vacuoles)

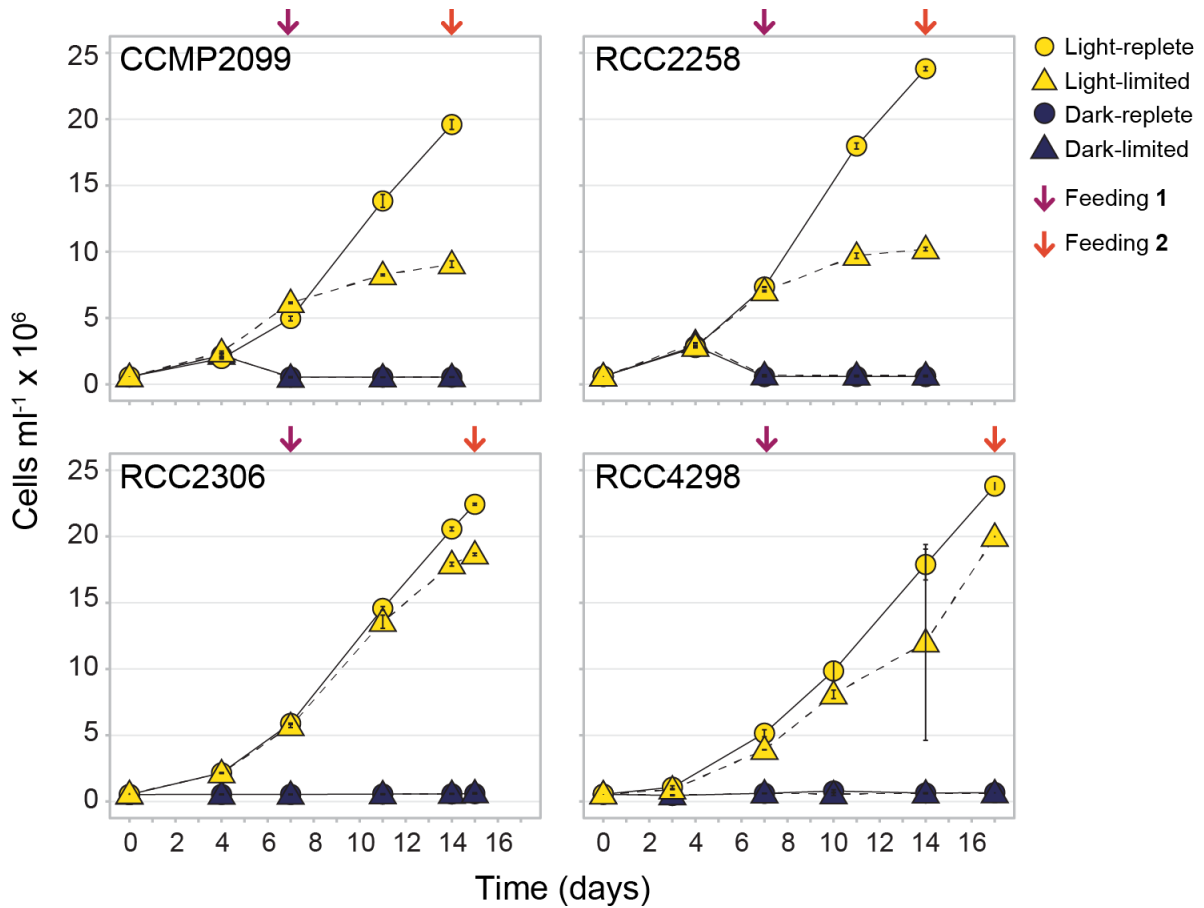


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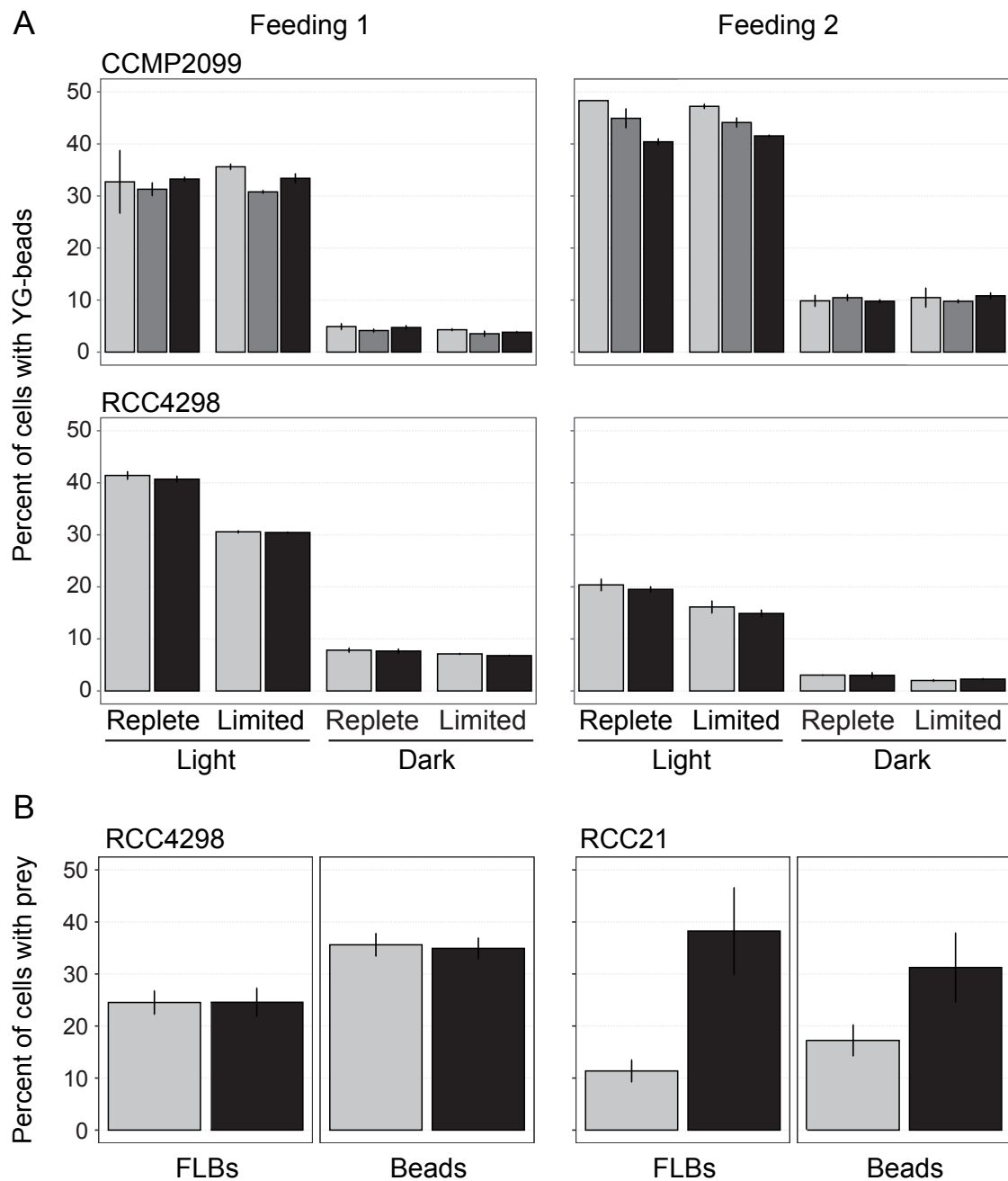


Figure 2. A. Percent of *M. polaris* strains CCMP2009 and RCC4298 cells with YG-beads (*M. polaris*-EXP1) for different treatments. Two feeding experiments were performed. **B.** Percent of *M. polaris* strain RCC4298 and *O. triangulata* strain RCC21 cells with FLBs and YG-beads (EXP3). The color of the bars corresponds to the time after the addition of YG-beads (0 minutes; light grey, 20 minutes; dark grey, 40 minutes; black). Error bars correspond to standard deviation.

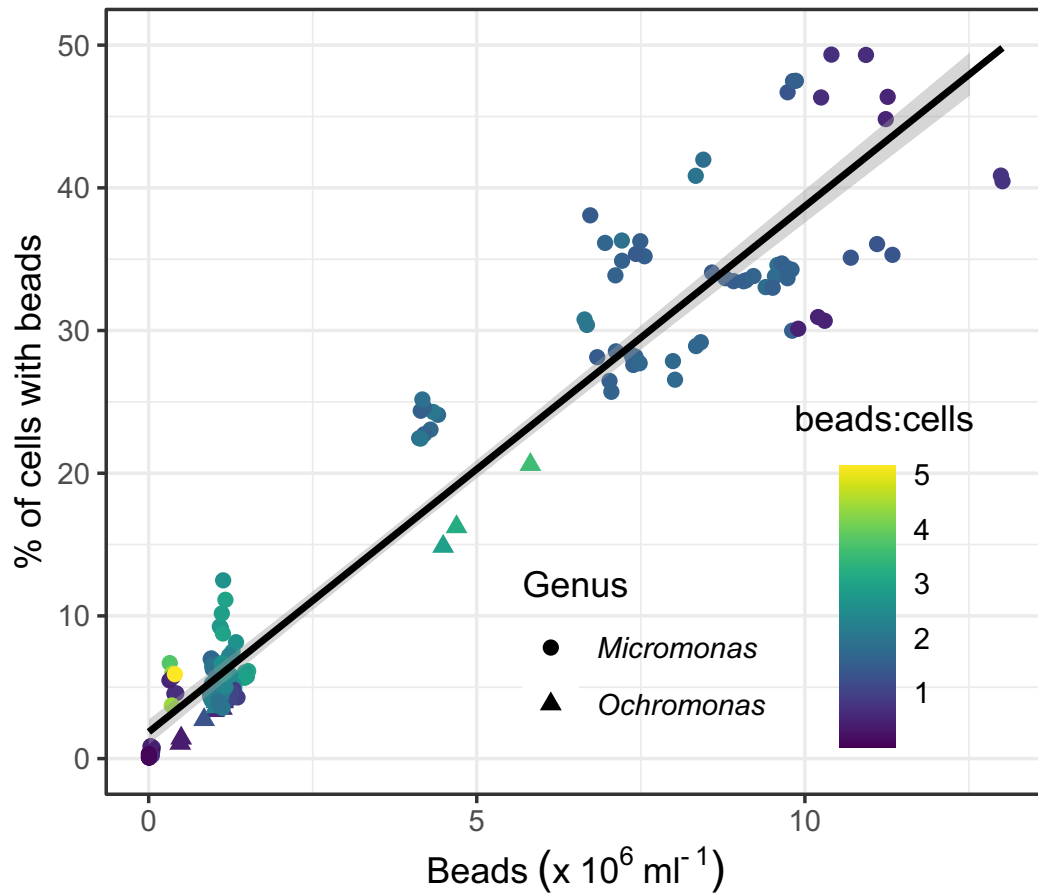


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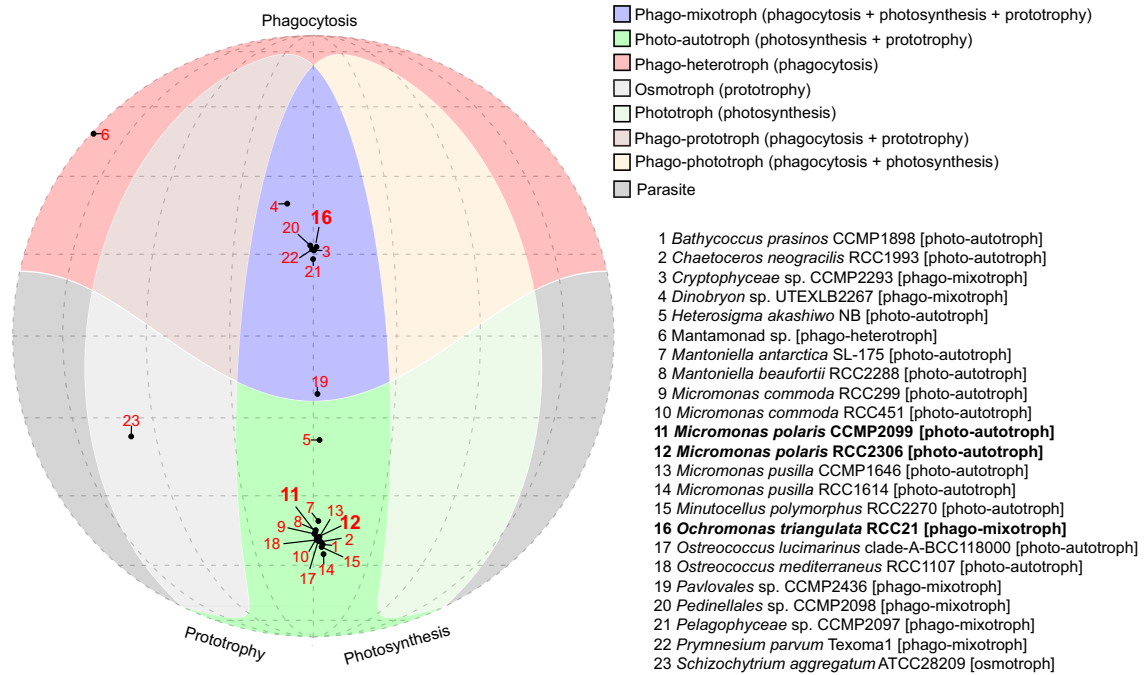


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Supplementary Material

Table S1. List of strains used for transcriptome analysis. MMETSP corresponds to the Marine Microbial Eukaryote Transcriptome Sequencing Project (Keeling et al. 2014). METDB corresponds to the micro-eukaryotic marine species transcriptomes database available from <http://metdb.sb-roscoff.fr/metdb/>.

Sequence Source	Species	Strain	Database	Reference
Transcriptome	<i>Bathycoccus prasinus</i>	CCMP1898	MMETSP	MMETSP1399
Transcriptome	<i>Chaetoceros neogracilis</i>	RCC1993	MMETSP	MMETSP1336
Transcriptome	Cryptophyceae sp.	CCMP2293	MMETSP	MMETSP0986-89
Transcriptome	<i>Dinobryon</i> sp.	UTEXLB2267	MMETSP	MMETSP0019-20
Transcriptome	<i>Dinobryon</i> sp.	UTEXLB2267	MMETSP	MMETSP0812
Transcriptome	<i>Heterosigma akashiwo</i>	NB	MMETSP	MMETSP0416
Transcriptome	<i>Mantoniella antarctica</i>	SL-175	MMETSP	MMETSP1106
Transcriptome	<i>Mantoniella beaufortii</i>	RCC2288	MMETSP	MMETSP1326
Genome	<i>Micromonas commoda</i>	RCC299	NCBI	GCF_000090985.2
Transcriptome	<i>Micromonas commoda</i>	RCC451	MMETSP	MMETSP1400
Transcriptome	<i>Micromonas polaris</i>	CCMP2099	MMETSP	MMETSP0802
Transcriptome	<i>Micromonas polaris</i>	CCMP2099	MMETSP	MMETSP1390
Transcriptome	<i>Micromonas polaris</i>	RCC2306	MMETSP	MMETSP1327
Transcriptome	<i>Micromonas pusilla</i>	CCMP1646	MMETSP	MMETSP1080
Transcriptome	<i>Micromonas pusilla</i>	RCC1614	MMETSP	MMETSP1402
Transcriptome	<i>Minutocellus polymorphus</i>	RCC2270	MMETSP	MMETSP1322
Transcriptome	<i>Ochromonas triangulata</i>	RCC21	METDB	METDB-00278
Transcriptome	<i>Ostreococcus lucimarinus</i>	BCC118000	MMETSP	MMETSP0939
Transcriptome	<i>Ostreococcus mediterraneus</i>	RCC1107	MMETSP	MMETSP0938
Transcriptome	Pedinellales sp.	CCMP2098	MMETSP	MMETSP0990-93
Transcriptome	Pelagophyceae sp.	CCMP2097	MMETSP	MMETSP0974-77
Transcriptome	Pavlovales sp.	CCMP2436	MMETSP	MMETSP0082-85
Transcriptome	<i>Prymnesium parvum</i>	Texoma1	MMETSP	MMETSP0006-08
Transcriptome	<i>Prymnesium parvum</i>	Texoma1	MMETSP	MMETSP0814-15
Transcriptome	<i>Prymnesium parvum</i>	Texoma1	MMETSP	MMETSP1083
Transcriptome	<i>Schizochytrium aggregatum</i>	ATCC28209	MMETSP	MMETSP0965

Table S2. Summary of experimental conditions and results for all experiment performed with *M. Polar* and *O. triangulata* strains. The percent of cells with prey (mean±sd) is indicated for each time point after the addition of prey (T_0 , T_{20} and T_{40} , where the subscript corresponds to minutes). The last four columns correspond to Student and Welch T_0 vs. T_{20} and T_{40} p -values.

Species	strain	EXP type	Treatment	Prey	Feeding	Replication	Ratio prey to cells	T_0	T_{20}	T_{40}	$T_{40}-T_0$	Student T_0 vs. T_{20}	Welch T_0 vs. T_{20}	Student T_0 vs. T_{40}	Welch T_0 vs. T_{40}
<i>Ochromonas triangulata</i>	RCC21	EXP1	Light-replete	YG-beads	1	2	0.6	4.0±0.1	9.3±0.3	12.3±0.3	8.3	0.001	0.016	0.001	0.011
<i>Ochromonas triangulata</i>	RCC21	EXP1	Light-limited	YG-beads	1	2	0.6	3.4±0.1	7.6±0.0	9.9±0.4	6.5	0.000	0.011	0.002	0.016
<i>Ochromonas triangulata</i>	RCC21	EXP2	Light-replete	YG-beads	1	2	1.3	3.3±0.8		9.4±2.2	6.2			0.066	0.130
<i>Ochromonas triangulata</i>	RCC21	EXP2	Light-limited	YG-beads	1	2	2.3	3.8±0.2		15.7±0.5	11.9			0.001	0.006
<i>Ochromonas triangulata</i>	RCC21	EXP3	Light-replete	FLBs	1	3	1.6	11.4±2.1		38.3±8.3	26.9			0.006	0.025
<i>Ochromonas triangulata</i>	RCC21	EXP3	Light-replete	YG-Beads	1	3	3.2	17.2±3.0		31.2±6.7	14.0			0.029	0.050
<i>Ochromonas triangulata</i>	RCC21	EXP4a	Light-replete	FLBs	1	2	2.1	4.6±0.1		25.1±0.1	20.5			0.000	0.000
<i>Ochromonas triangulata</i>	RCC21	EXP4b	Light-replete	FLBs	1	2	2.8	7.2±0.8		33.4±1.4	26.2			0.002	0.006
<i>Micromonas polaris</i>	CCMP2099	EXP1	Dark-replete	YG-beads	1	3	2.3	4.9±0.6	4.1±0.3	4.7±0.4	-0.2	0.134	0.154	0.707	0.710
<i>Micromonas polaris</i>	CCMP2099	EXP1	Dark-limited	YG-beads	1	3	2.2	4.3±0.2	3.5±0.6	3.8±0.2	-0.5	0.092	0.126	0.044	0.047
<i>Micromonas polaris</i>	CCMP2099	EXP1	Light-replete	YG-beads	1	3	1.7	32.7±6.1	31.3±1.3	33.3±0.4	0.5	0.712	0.727	0.886	0.893
<i>Micromonas polaris</i>	CCMP2099	EXP1	Light-limited	YG-beads	1	3	1.5	35.6±0.6	30.8±0.3	33.4±0.9	-2.2	0.000	0.001	0.024	0.032
<i>Micromonas polaris</i>	CCMP2099	EXP1	Dark-replete	YG-beads	2	3	2.8	9.8±1.1	10.5±0.6	9.8±0.3	-0.1	0.451	0.464	0.922	0.925
<i>Micromonas polaris</i>	CCMP2099	EXP1	Dark-limited	YG-beads	2	3	2.8	10.5±1.9	9.8±0.3	10.8±0.6	0.3	0.551	0.579	0.775	0.784
<i>Micromonas polaris</i>	CCMP2099	EXP1	Light-replete	YG-beads	2	3	0.7	48.3±1.7	44.9±1.9	40.4±0.6	-7.9	0.082	0.083	0.002	0.009
<i>Micromonas polaris</i>	CCMP2099	EXP1	Light-limited	YG-beads	2	3	1.5	47.2±0.5	44.1±0.9	41.6±0.2	-5.7	0.007	0.015	0.000	0.000
<i>Micromonas polaris</i>	RCC2258	EXP1	Dark-replete	YG-beads	1	3	2.1	4.5±0.2	4.9±0.4	4.5±0.5	-0.0	0.242	0.255	0.899	0.901
<i>Micromonas polaris</i>	RCC2258	EXP1	Dark-limited	YG-beads	1	3	2.0	3.8±0.2	4.1±0.6	4.1±0.3	0.3	0.458	0.479	0.219	0.221
<i>Micromonas polaris</i>	RCC2258	EXP1	Light-replete	YG-beads	1	3	1.6	34.0±0.9	34.1±1.6	33.1±2.3	-0.9	0.979	0.979	0.557	0.574
<i>Micromonas polaris</i>	RCC2258	EXP1	Light-limited	YG-beads	1	3	1.6	32.6±2.3	34.2±0.4	32.0±2.1	-0.6	0.315	0.365	0.756	0.757
<i>Micromonas polaris</i>	RCC2258	EXP1	Dark-replete	YG-beads	2	3	2.4	5.3±0.5	4.9±0.9	5.3±0.1	-0.1	0.499	0.513	0.849	0.816
<i>Micromonas polaris</i>	RCC2258	EXP1	Dark-limited	YG-beads	2	3	2.6	6.3±0.4	6.5±0.0	6.3±1.1	-0.1	0.522	0.555	0.936	0.938
<i>Micromonas polaris</i>	RCC2258	EXP1	Light-replete	YG-beads	2	3	0.5	30.6±0.4	30.5±0.5	29.8±0.3	-0.8	0.795	0.795	0.065	0.065
<i>Micromonas polaris</i>	RCC2258	EXP1	Light-limited	YG-beads	2	3	1.4	35.5±0.5	36.6±1.2	33.8±2.4	-1.7	0.211	0.244	0.313	0.360
<i>Micromonas polaris</i>	RCC2306	EXP1	Dark-replete	YG-beads	1	2	2.6	4.3±0.6		4.2±0.2	-0.1			0.853	0.865
<i>Micromonas polaris</i>	RCC2306	EXP1	Dark-limited	YG-beads	1	2	2.5	4.6±0.0		3.7±0.2	-0.9			0.035	0.115
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-replete	YG-beads	1	2	1.7	29.0±0.2		29.0±0.1	-0.0			0.832	0.833
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-limited	YG-beads	1	2	1.7	27.2±0.9		27.7±0.3	0.4			0.579	0.615
<i>Micromonas polaris</i>	RCC2306	EXP1	Dark-replete	YG-beads	2	2	2.9	6.0±0.3		6.0±0.7	0.1			0.883	0.891
<i>Micromonas polaris</i>	RCC2306	EXP1	Dark-limited	YG-beads	2	2	2.9	5.9±0.3		5.5±0.2	-0.3			0.296	0.323
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-replete	YG-beads	2	2	0.5	45.6±1.1		46.5±0.1	0.9			0.377	0.458
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-limited	YG-beads	2	2	0.8	40.7±0.3		40.5±0.2	-0.2			0.593	0.596
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-replete	YG-beads	1	2	2.6	7.8±0.5		7.7±0.5	-0.2			0.740	0.740
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-limited	YG-beads	1	2	2.3	7.1±0.2		6.8±0.1	-0.3			0.147	0.150
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-replete	YG-beads	1	2	1.9	41.4±0.8		40.7±0.6	-0.7			0.429	0.433
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-limited	YG-beads	1	2	2.0	30.6±0.3		30.5±0.1	-0.1			0.598	0.618
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-replete	YG-beads	2	2	0.5	3.0±0.1		3.0±0.6	-0.0			0.928	0.935
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-limited	YG-beads	2	2	0.5	2.0±0.2		2.3±0.2	0.3			0.293	0.302
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-replete	YG-beads	2	2	0.2	20.4±1.2		19.5±0.6	-0.9			0.452	0.482
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-limited	YG-beads	2	2	0.2	16.1±1.2		14.9±0.7	-1.2			0.340	0.365
<i>Micromonas polaris</i>	RCC2258	EXP2	Dark-replete	YG-beads	1	3	2.5	5.0±0.1	4.7±0.1	5.1±0.5	0.1	0.046	0.053	0.826	0.834
<i>Micromonas polaris</i>	RCC2258	EXP2	Dark-limited	YG-beads	1	3	2.2	4.4±0.1	4.5±0.1	4.3±0.1	-0.1	0.772	0.772	0.339	0.340
<i>Micromonas polaris</i>	RCC2258	EXP2	Light-replete	YG-beads	1	3	1.6	33.6±0.2	33.5±0.1	33.2±0.1	-0.4	0.691	0.697	0.030	0.068
<i>Micromonas polaris</i>	RCC2258	EXP2	Light-replete-AntiB	YG-beads	1	3	1.5	33.7±0.3	34.2±0.3	33.8±0.6	0.1	0.112	0.113	0.881	0.883
<i>Micromonas polaris</i>	RCC2258	EXP2	Light-limited	YG-beads	1	3	1.8	33.8±0.8	33.6±0.2	33.1±0.1	-0.7	0.673	0.689	0.208	0.270
<i>Micromonas polaris</i>	RCC2306	EXP2	Dark-replete	YG-beads	1	3	2.1	4.6±0.2	4.9±0.2	4.6±0.2	0.0	0.113	0.117	0.741	0.742
<i>Micromonas polaris</i>	RCC2306	EXP2	Dark-limited	YG-beads	1	3	1.9	4.4±0.2	3.9±0.4	4.7±0.3	0.3	0.172	0.190	0.279	0.290
<i>Micromonas polaris</i>	RCC2306	EXP2	Light-replete	YG-beads	1	3	1.6	27.8±1.1	28.8±0.4	29.2±0.3	1.4	0.213	0.253	0.099	0.151
<i>Micromonas polaris</i>	RCC2306	EXP2	Light-replete-AntiB	YG-beads	1	3	1.6	27.8±0.3	29.8±2.5	28.7±0.5	0.9	0.235	0.294	0.068	0.086
<i>Micromonas polaris</i>	RCC2306	EXP2	Light-limited	YG-beads	1	3	1.6	28.0±0.2	28.0±0.1	27.9±0.1	-0.1	0.782	0.785	0.551	0.557
<i>Micromonas polaris</i>	RCC4298	EXP3	Light-replete	FLBs	1	3	1.8	24.5±2.3		24.6±2.7	0.0			0.984	0.984
<i>Micromonas polaris</i>	RCC4298	EXP3	Light-replete	YG-beads	1	3	1.6	35.6±2.2		34.9±2.0	-0.7			0.709	0.709
<i>Micromonas polaris</i>	RCC2306	EXP5	Dark-replete	YG-beads	1	2	1.1	4.4±0.3							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.9	4.6±0.4							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.7	4.6±0.0							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.1	0.4±0.1							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.0	0.1±0.0							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	4.0	6.3±0.5							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.6	0.8±0.1							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.1	0.2±0.2							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	1.3	4.4±0.2							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-limited	YG-beads	1	2	2.1	4.4±0.1							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	2	0.5	5.6±0.2							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	2	0.1	0.5±0.2							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	2	0.0	0.1±0.0							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	2	4.8	4.8±1.5							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	2	0.5	0.5±0.5							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	2	0.1	0.2±0.2							
<i>Micromonas polaris</i>	RCC4298	EXP5	Dark-replete	YG-beads	1	3	1.8	7.0±0.1							
<i>Micromonas polaris</i>	RCC4298	EXP5	Dark-limited	YG-beads	1	3	1.9	6.4±0.2							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	3	1.7	24.7±0.4							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete-AntiB	YG-beads	1	3	1.9	23.8±0.7							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-limited	YG-beads	1	3	1.8	22.5±0.2							

Table S3. Comparison of feeding on three different YG-bead sizes (diameter 0.5, 1, and 2 μm) for *M. polaris* (EXP7). The percent of cells with prey (mean \pm sd) was measured independently for each bead size and is indicated for each time point (T_0 and T_{40} , where the subscript correspond to minutes).

Species	strain	Treatment	Prey	Replication	Prey/cell ratio	T_0	T_{40}	$T_{40}-T_0$
<i>Micromonas polaris</i>	RCC2306	Light-limited	YG-beads 0.5 μm	2	1.0	4.09 \pm 0.32	4.08 \pm 0.07	0.0
<i>Micromonas polaris</i>	RCC2306	Light-limited	YG-beads 1.0 μm	2	0.9	3.27 \pm 0.14	2.30 \pm 0.10	-1.0
<i>Micromonas polaris</i>	RCC2306	Light-limited	YG-beads 2.0 μm	2	0.7	3.54 \pm 0.03	2.74 \pm 0.05	-0.8

Table S4. Comparison of Lugol's iodine and glutaraldehyde fixation, and live (no fixation) measurements of the percent of *M. polaris* cells with YG-beads (EXP6). The percent of cells with prey (mean \pm sd) is indicated for each time point after the addition of prey (T_0 and T_{40} , where the subscript corresponds to minutes).

Species	strain	Treatment	Prey	Fixation	Replication	Prey/cell ratio	T_0	T_{40}	$T_{40}-T_0$
<i>Micromonas polaris</i>	RCC2306	Light-replete	YG-beads	No (Live)	2	1.6	7.57 \pm 0.60	7.20 \pm 0.28	-0.4
<i>Micromonas polaris</i>	RCC2306	Light-replete	YG-beads	Lugol's iodine	2	1.4	5.85 \pm 0.39	5.58 \pm 0.18	-0.3
<i>Micromonas polaris</i>	RCC2306	Light-replete	YG-beads	Glutaraldehyde	2	1.5	6.97 \pm 0.39	6.62 \pm 0.01	-0.3

Table S5. Lysosensor experiment (EXP9). Last column shows the mean \pm sd Lysosensor green.

Species	strain	treatment	Unstained or Stained	Replication	Green fluorescence
<i>Ochromonas triangulata</i>	RCC21	Light-limited	Unstained	2	64.0 \pm 1.1
<i>Ochromonas triangulata</i>	RCC21	Light-limited	Stained	2	221.5 \pm 40.5
<i>Micromonas polaris</i>	RCC2306	Light-limited	Unstained	2	56.7 \pm 2.4
<i>Micromonas polaris</i>	RCC2306	Light-limited	Stained	2	67.8 \pm 1.9

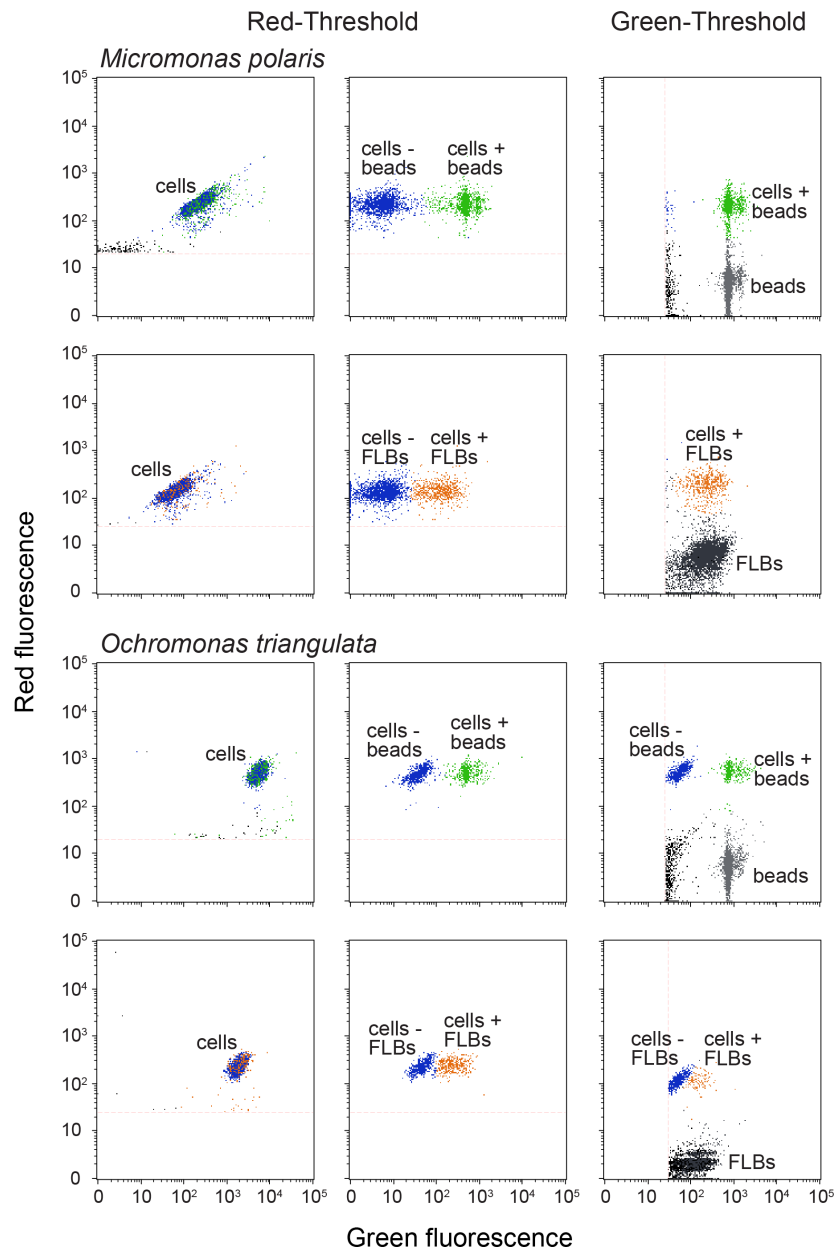


Figure S1. Examples of flow cytograms for *M. polaris* and the positive control *O. trianguata*. Flow cytometry was used to determine the percent of cells with prey (YG-beads and FLBs) in fixed samples using a protocol modified from Sherr & Sherr (1993). Data collection was performed with threshold on red (695 ± 50 nm band pass filter) or green fluorescence (525 ± 30 nm band pass filter). Cells that displayed red autofluorescence from chlorophyll as well as green fluorescence were considered to be containing prey (cells with YG-beads in green, cells with FLBs in orange and cells without prey in blue). In addition, to confirm the total concentration of prey added to each experimental flask, the same sample was also run with the threshold on green fluorescence (YG-beads and FLBs in grey and black respectively).

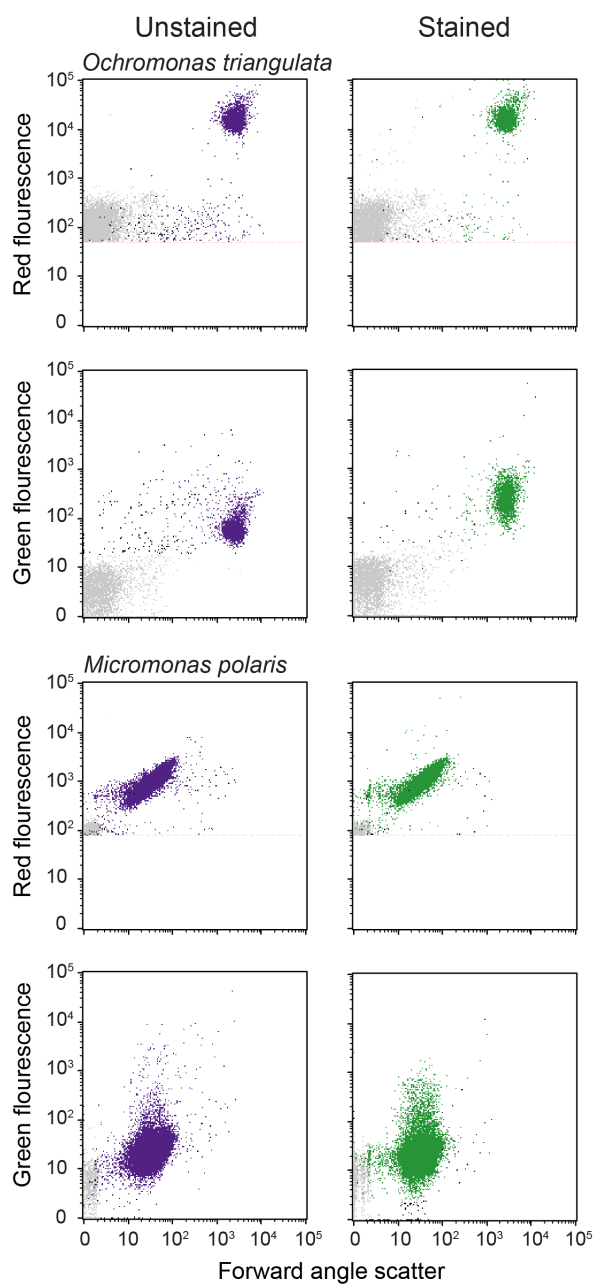


Figure S2. Flow cytograms of *O. triangulata* and *M. polaris* before (purple) and after (green) staining with Lysosensor. Red fluorescence corresponds to chlorophyll fluorescence, while green fluorescence corresponds to autofluorescence before staining or to Lysosensor fluorescence after staining. Green fluorescence clearly increases after Lysosensor staining for *O. triangulata* and not for *M. polaris*.

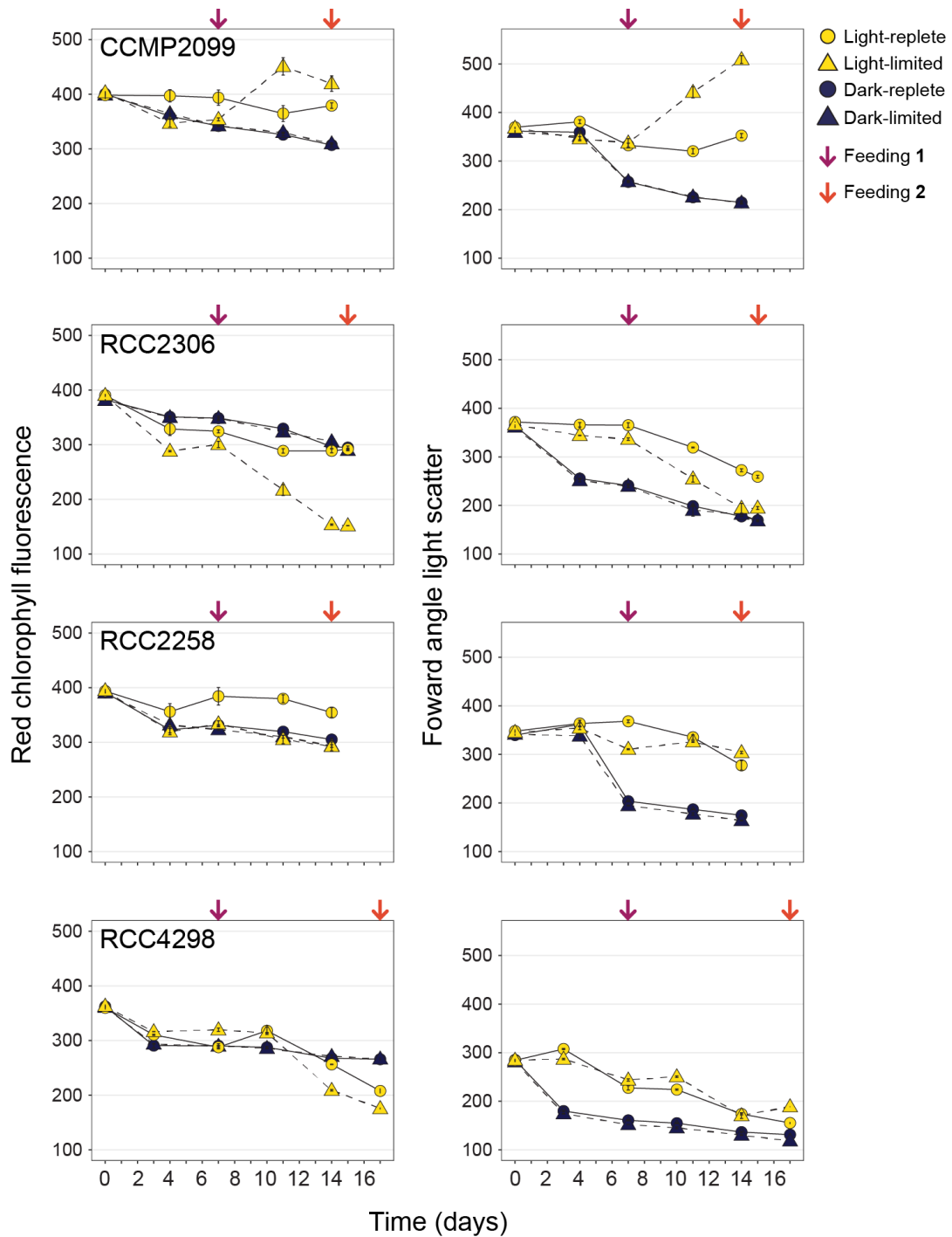


Figure S3. Change in forward scatter and red chlorophyll fluorescence measured by flow cytometry during the experiments reported in Figure 1 (*M. polaris*-EXP1).

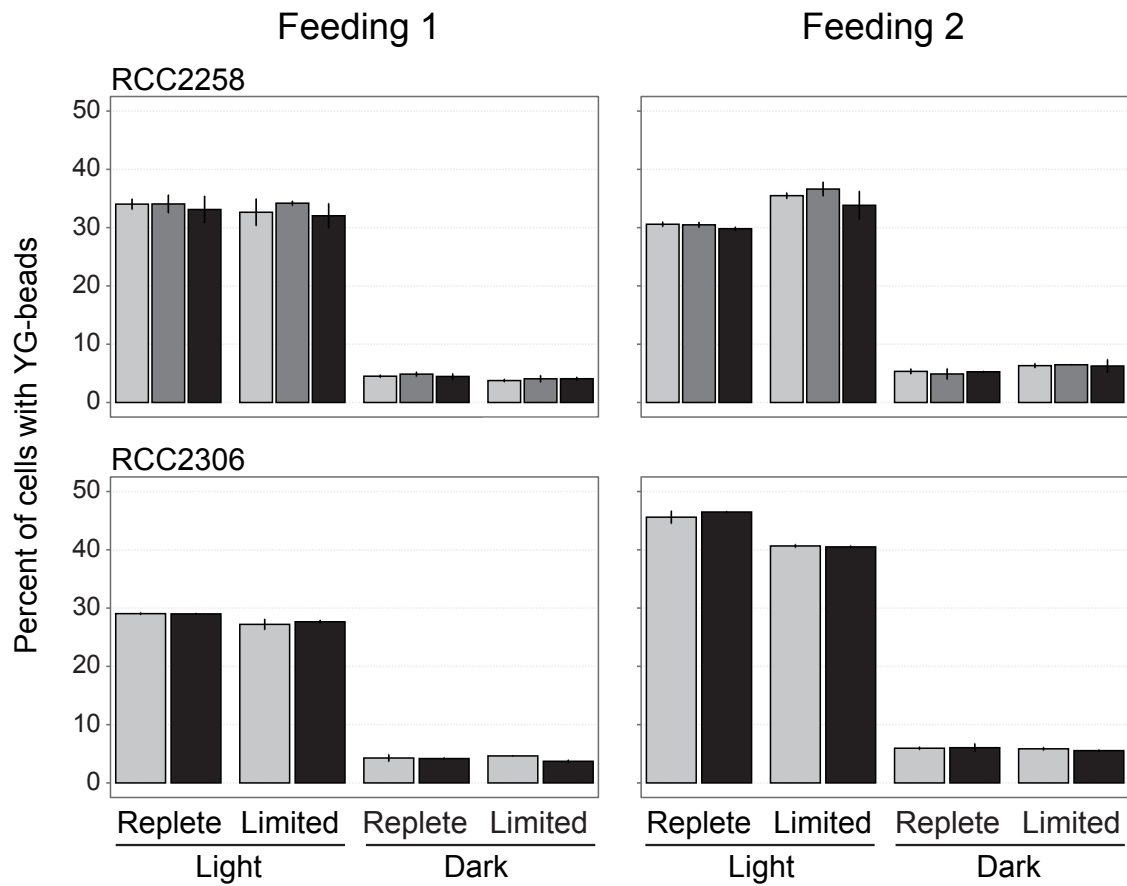


Figure S4. Percent of *M. polaris* cells with YG-beads (*M. polaris*-EXP1) for strains RCC2258 and RCC2306 and different treatments. Two feeding experiments were performed. The color of the bars represent the time point (in minutes) after the addition of YG-beads (0 minutes; light grey, 20 minutes; dark grey, 40 minutes; black). Error bars correspond to standard deviation.

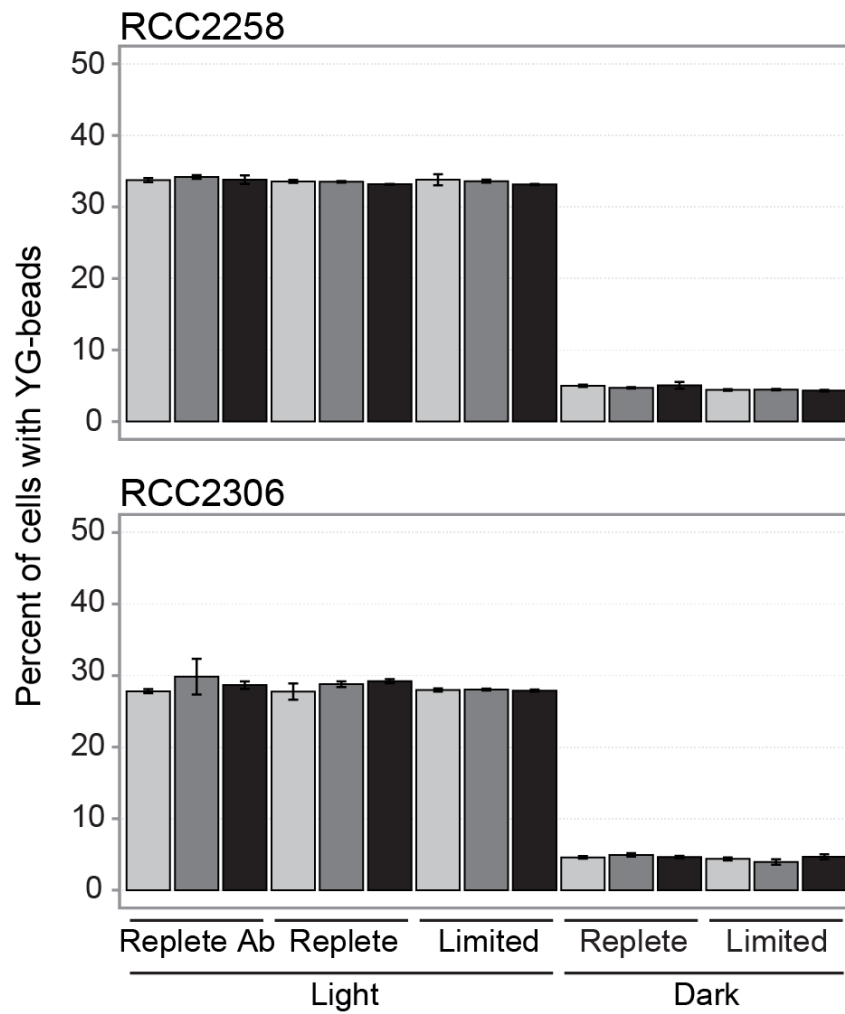


Figure S5. Percent of *M. polaris* cells with YG-beads (*M. polaris*-EXP2) for each strain and treatment. The color of the bars represents the time point (in minutes) after the addition of YG-beads (0 minutes; light grey, 20 minutes; dark grey, 40 minutes; black). Replete Ab correspond to nutrient replete conditions with antibiotics.

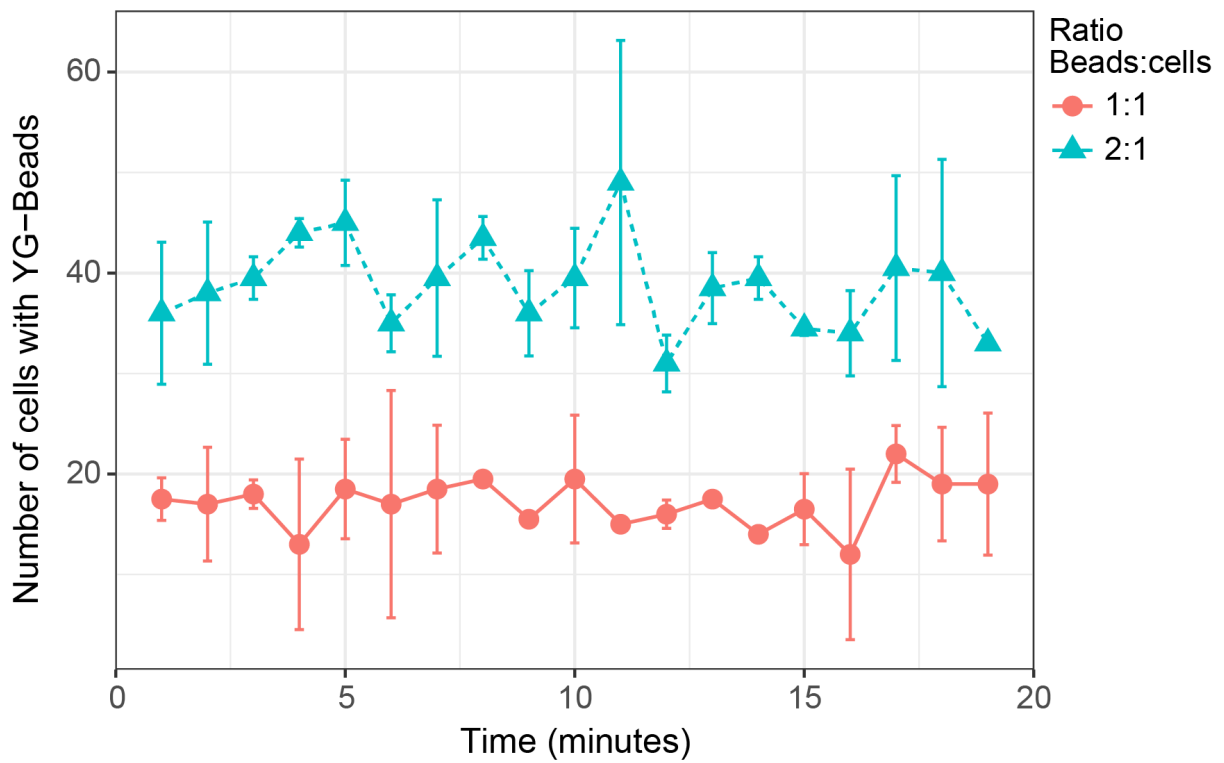


Figure S6. Changes with time in the number of *M. polaris* (strain RCC2306) cells with YG-beads measured by continuously running a live sample for 20 minutes immediately after the addition of YG-beads. Two ratios of beads to cells were tested, 1:1 and 2:1, each in duplicate.

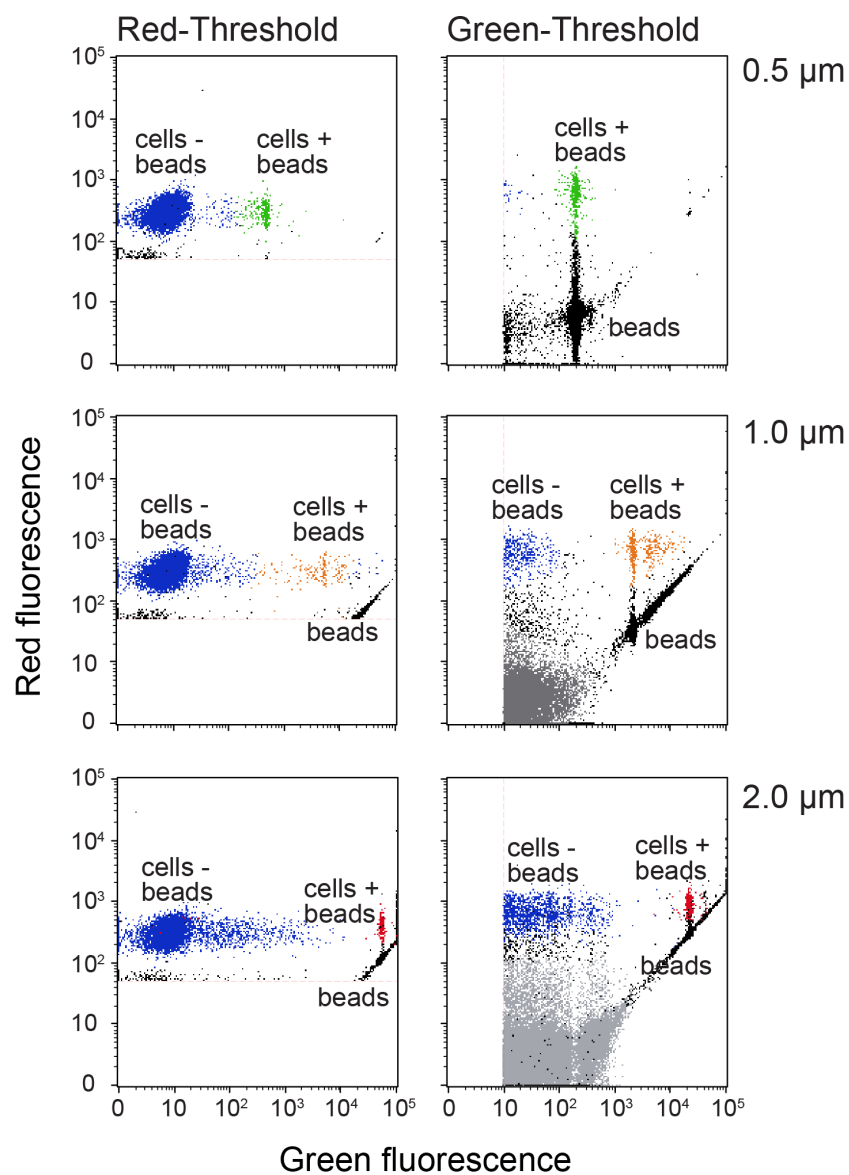


Figure S7. Flow cytograms for *M. polaris* cells incubated with YG-beads of three different sizes: 0.5 (green), 1.0 (orange) and 2.0 (red) μm . See legend of Figure S1 for details.

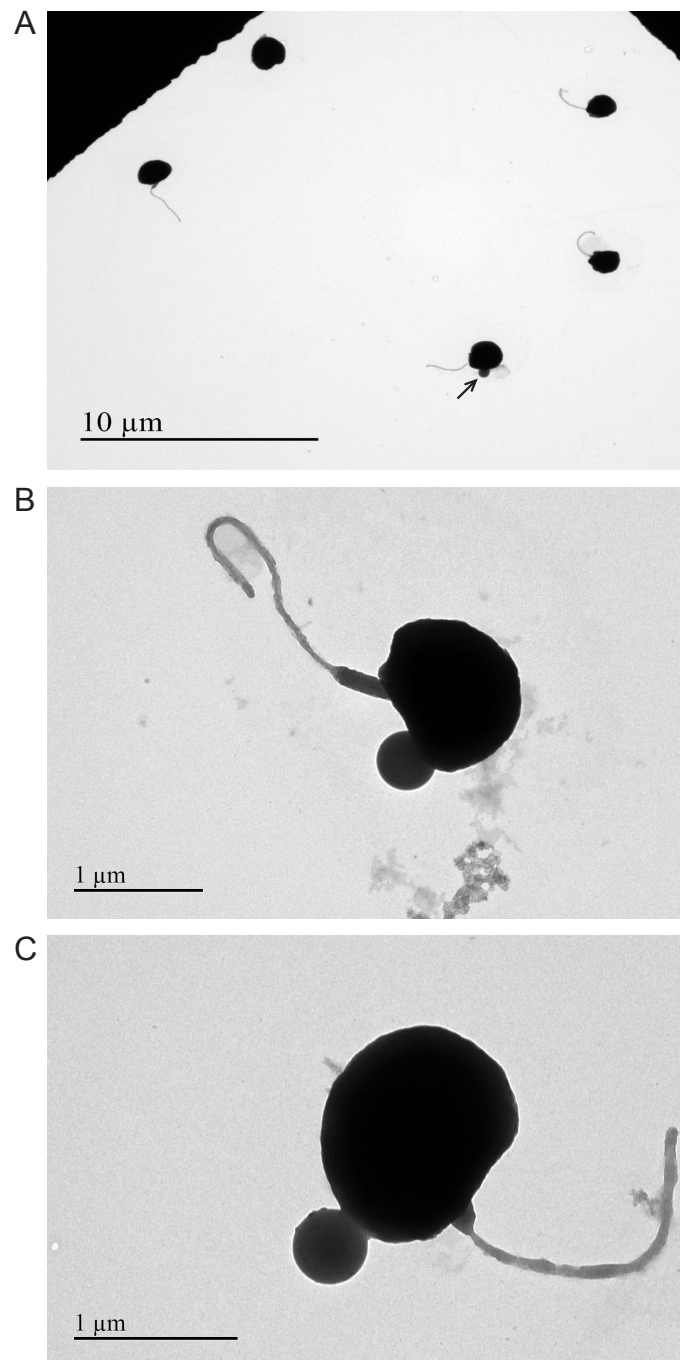


Figure S8. Transmission electron microscopy images of *M. polaris* (strain RCC2306) with YG-beads (0.5 μm) after negative staining. A. Arrow indicates a *M. polaris* cell with a YG-bead. B and C. Close up views of *M. polaris* cells with attached YG-bead.