

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

Valeria Jimenez¹, John A. Burns², Florence Le Gall¹, Fabrice Not^{1,4}, Daniel Vaulot^{1,3,5*}

¹ Sorbonne Université, CNRS, UMR7144, Ecology of Marine Plankton team, Station Biologique de Roscoff, 29680 Roscoff, France

² Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA

³ Asian School of the Environment, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

⁴ ORCID: 0000-0002-9342-195X

⁵ ORCID: 0000-0002-0717-5685

* Corresponding author: vaulot@gmail.com

Journal of Phycology

Date: December 11, 2020

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. Polaris* and *O. triangulata* strains. The percent of cells with prey (mean \pm 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 *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 \pm 0.1	9.3 \pm 0.3	12.3 \pm 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 \pm 0.1	7.6 \pm 0.0	9.9 \pm 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 \pm 0.8		9.4 \pm 2.2	6.2			0.066	0.130
<i>Ochromonas triangulata</i>	RCC21	EXP2	Light-limited	YG-beads	1	2	2.3	3.8 \pm 0.2		15.7 \pm 0.5	11.9			0.001	0.006
<i>Ochromonas triangulata</i>	RCC21	EXP3	Light-replete	FLBs	1	3	1.6	11.4 \pm 2.1		38.3 \pm 8.3	26.9			0.006	0.025
<i>Ochromonas triangulata</i>	RCC21	EXP3	Light-replete	YG-Beads	1	3	3.2	17.2 \pm 3.0		31.2 \pm 6.7	14.0			0.029	0.050
<i>Ochromonas triangulata</i>	RCC21	EXP4a	Light-replete	FLBs	1	2	2.1	4.6 \pm 0.1		25.1 \pm 0.1	20.5			0.000	0.000
<i>Ochromonas triangulata</i>	RCC21	EXP4b	Light-replete	FLBs	1	2	2.8	7.2 \pm 0.8		33.4 \pm 1.4	26.2			0.002	0.006
<i>Micromonas polaris</i>	CCMP2099	EXP1	Dark-replete	YG-beads	1	3	2.3	4.9 \pm 0.6	4.1 \pm 0.3	4.7 \pm 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 \pm 0.2	3.5 \pm 0.6	3.8 \pm 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 \pm 6.1	31.3 \pm 1.3	33.3 \pm 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 \pm 0.6	30.8 \pm 0.3	33.4 \pm 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 \pm 1.1	10.5 \pm 0.6	9.8 \pm 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 \pm 1.9	9.8 \pm 0.3	10.8 \pm 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 \pm 1.7	44.9 \pm 1.9	40.4 \pm 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 \pm 0.5	44.1 \pm 0.9	41.6 \pm 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 \pm 0.2	4.9 \pm 0.4	4.5 \pm 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 \pm 0.2	4.1 \pm 0.6	4.1 \pm 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 \pm 0.9	34.1 \pm 1.6	33.1 \pm 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 \pm 2.3	34.2 \pm 0.4	32.0 \pm 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 \pm 0.5	4.9 \pm 0.9	5.3 \pm 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 \pm 0.4	6.5 \pm 0.0	6.3 \pm 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 \pm 0.4	30.5 \pm 0.5	29.8 \pm 0.3	-0.8	0.795	0.795	0.065	0.069
<i>Micromonas polaris</i>	RCC2258	EXP1	Light-limited	YG-beads	2	3	1.4	35.5 \pm 0.5	36.6 \pm 1.2	33.8 \pm 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 \pm 0.6	4.2 \pm 0.2	4.2 \pm 0.2	-0.1			0.853	0.865
<i>Micromonas polaris</i>	RCC2306	EXP1	Dark-limited	YG-beads	1	2	2.5	4.6 \pm 0.0		3.7 \pm 0.2	-0.9			0.035	0.115
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-replete	YG-beads	1	2	1.7	29.0 \pm 0.2		29.0 \pm 0.1	-0.0			0.832	0.833
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-limited	YG-beads	1	2	1.7	27.2 \pm 0.9		27.7 \pm 0.3	0.4			0.579	0.615
<i>Micromonas polaris</i>	RCC2306	EXP1	Dark-replete	YG-beads	2	2	2.9	6.0 \pm 0.3		6.0 \pm 0.7	0.1			0.883	0.891
<i>Micromonas polaris</i>	RCC2306	EXP1	Dark-limited	YG-beads	2	2	2.9	5.9 \pm 0.3		5.5 \pm 0.2	-0.3			0.296	0.323
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-replete	YG-beads	2	2	0.5	45.6 \pm 1.1		46.5 \pm 0.1	0.9			0.377	0.458
<i>Micromonas polaris</i>	RCC2306	EXP1	Light-limited	YG-beads	2	2	0.8	40.7 \pm 0.3		40.5 \pm 0.2	-0.2			0.593	0.596
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-replete	YG-beads	1	2	2.6	7.8 \pm 0.5		7.7 \pm 0.5	-0.2			0.740	0.740
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-limited	YG-beads	1	2	2.3	7.1 \pm 0.2		6.8 \pm 0.1	-0.3			0.147	0.150
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-replete	YG-beads	1	2	1.9	41.4 \pm 0.8		40.7 \pm 0.6	-0.7			0.429	0.433
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-limited	YG-beads	1	2	2.0	30.6 \pm 0.3		30.5 \pm 0.1	-0.1			0.598	0.618
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-replete	YG-beads	2	2	0.5	3.0 \pm 0.1		3.0 \pm 0.6	-0.0			0.928	0.935
<i>Micromonas polaris</i>	RCC4298	EXP1	Dark-limited	YG-beads	2	2	0.5	2.0 \pm 0.2		2.3 \pm 0.2	0.3			0.293	0.302
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-replete	YG-beads	2	2	0.2	20.4 \pm 1.2		19.5 \pm 0.6	-0.9			0.452	0.482
<i>Micromonas polaris</i>	RCC4298	EXP1	Light-limited	YG-beads	2	2	0.2	16.1 \pm 1.2		14.9 \pm 0.7	-1.2			0.340	0.365
<i>Micromonas polaris</i>	RCC2258	EXP2	Dark-replete	YG-beads	1	3	2.5	5.0 \pm 0.1	4.7 \pm 0.1	5.1 \pm 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 \pm 0.1	4.5 \pm 0.1	4.3 \pm 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 \pm 0.2	33.5 \pm 0.1	33.2 \pm 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 \pm 0.3	34.2 \pm 0.3	33.8 \pm 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 \pm 0.8	33.6 \pm 0.2	33.1 \pm 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 \pm 0.2	4.9 \pm 0.2	4.6 \pm 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 \pm 0.2	3.9 \pm 0.4	4.7 \pm 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 \pm 1.1	28.8 \pm 0.4	29.2 \pm 0.3	1.4	0.213	0.213	0.253	0.099
<i>Micromonas polaris</i>	RCC2306	EXP2	Light-replete-AntiB	YG-beads	1	3	1.6	27.8 \pm 0.3	29.8 \pm 2.5	28.7 \pm 0.5	0.9	0.235	0.234	0.068	0.086
<i>Micromonas polaris</i>	RCC4298	EXP3	Light-replete	FLBs	1	3	1.8	24.5 \pm 2.3		24.6 \pm 2.7	0.0			0.984	0.984
<i>Micromonas polaris</i>	RCC4298	EXP3	Light-replete	YG-beads	1	3	1.6	35.6 \pm 2.2		34.9 \pm 2.0	-0.7			0.709	0.709
<i>Micromonas polaris</i>	RCC2306	EXP5	Dark-replete	YG-beads	1	2	1.1	4.4 \pm 0.3							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.9	4.6 \pm 0.4							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.7	4.6 \pm 0.0							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.1	0.4 \pm 0.1							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.0	0.1 \pm 0.0							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	4.0	6.3 \pm 0.5							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.6	0.8 \pm 0.1							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	0.1	0.2 \pm 0.2							
<i>Micromonas polaris</i>	RCC2306	EXP5	Light-replete	YG-beads	1	2	1.3	4.4 \pm 0.2							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	3	1.8	7.0 \pm 0.1							
<i>Micromonas polaris</i>	RCC4298	EXP5	Dark-limited	YG-beads	1	3	1.9	6.4 \pm 0.2							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete	YG-beads	1	3	1.7	24.7 \pm 0.4							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-replete-AntiB	YG-beads	1	3	1.9	23.8 \pm 0.7							
<i>Micromonas polaris</i>	RCC4298	EXP5	Light-limited	YG-beads	1	3	1.8	22.5 \pm 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 of Lysosensor green fluorescence.

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

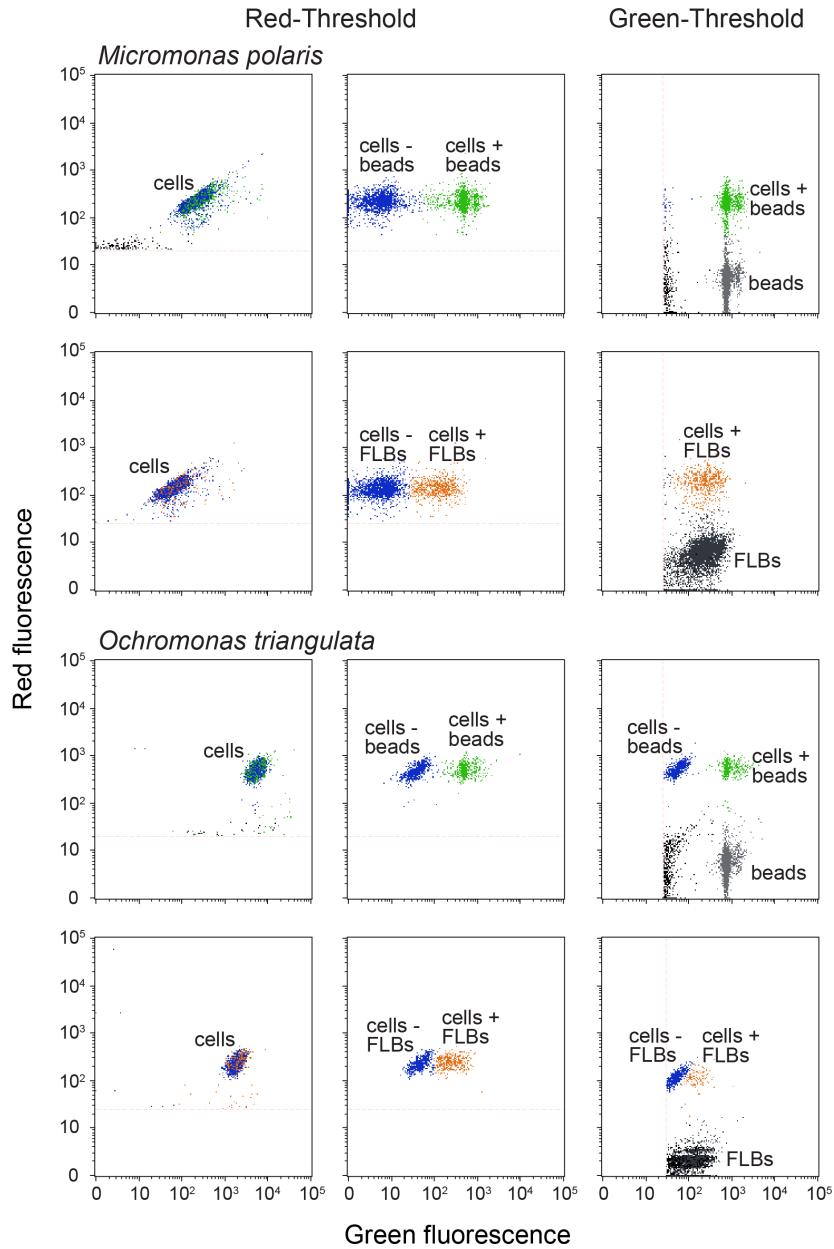


Figure S1. Examples of flow cytograms for *M. polaris* and the positive control *O. triangulata*. Flow cytometry was used to determine the percent of cells with prey (YG-beads and FLBs) in fixed samples using a protocol modified from Sherr & Sherr (1993). Data collection was performed with threshold 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).

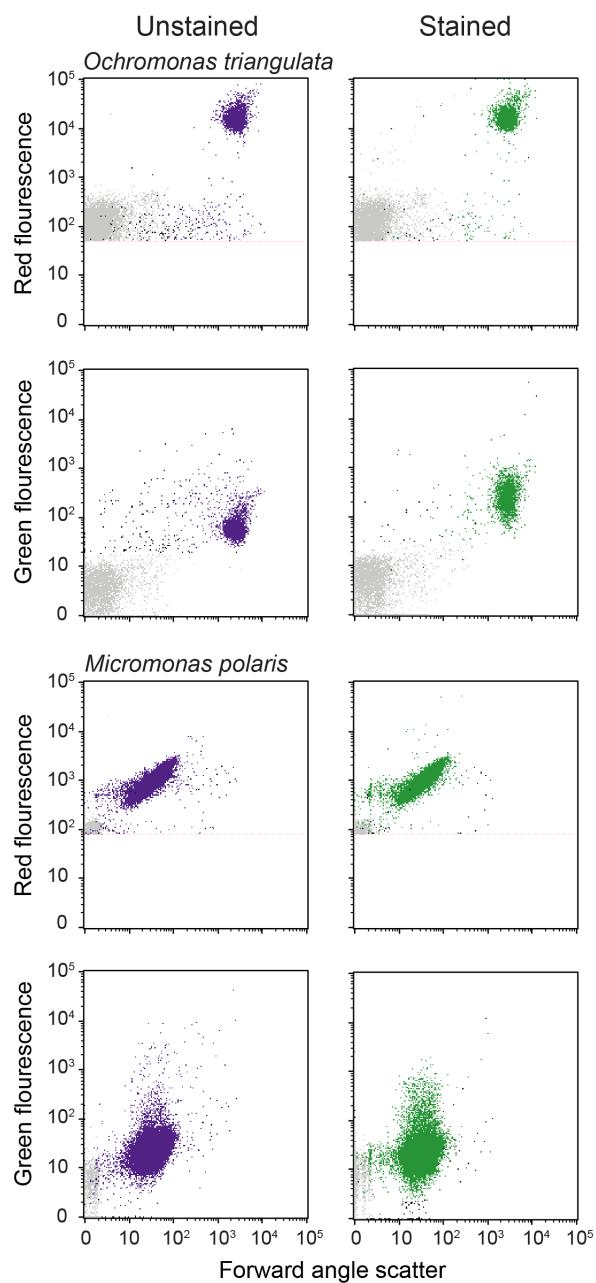


Figure S2. Flow cytograms of *O. triangulata* and *M. polaris* before (purple) and after (green) staining with Lysosensor. Red fluorescence corresponds to chlorophyll fluorescence, while green fluorescence corresponds to autofluorescence before staining or to Lysosensor fluorescence after staining. Green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

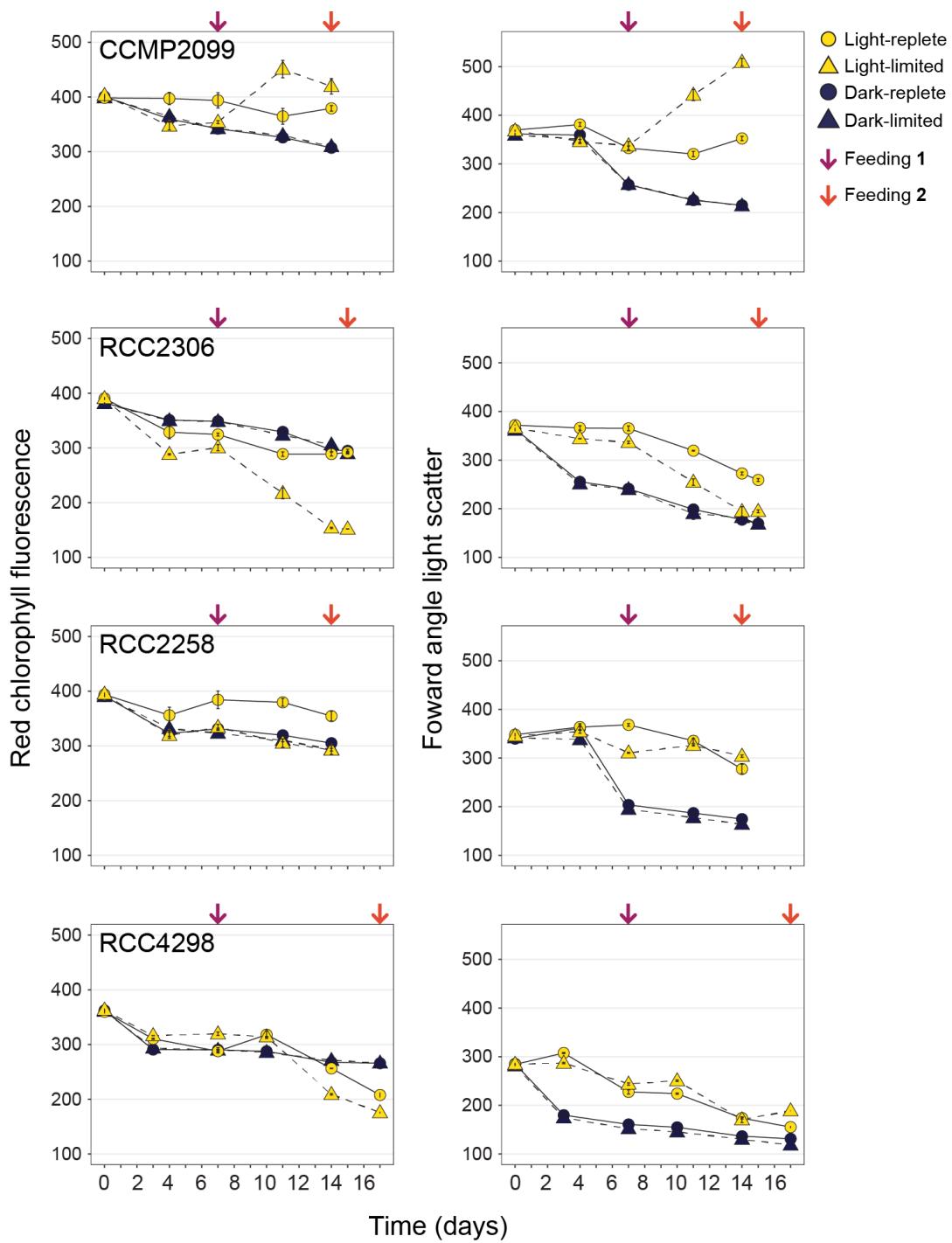


Figure S3. Change in forward scatter and red chlorophyll fluorescence measured by flow cytometry during the experiments reported in Figure 1 (*M. polaris*-EXP1).

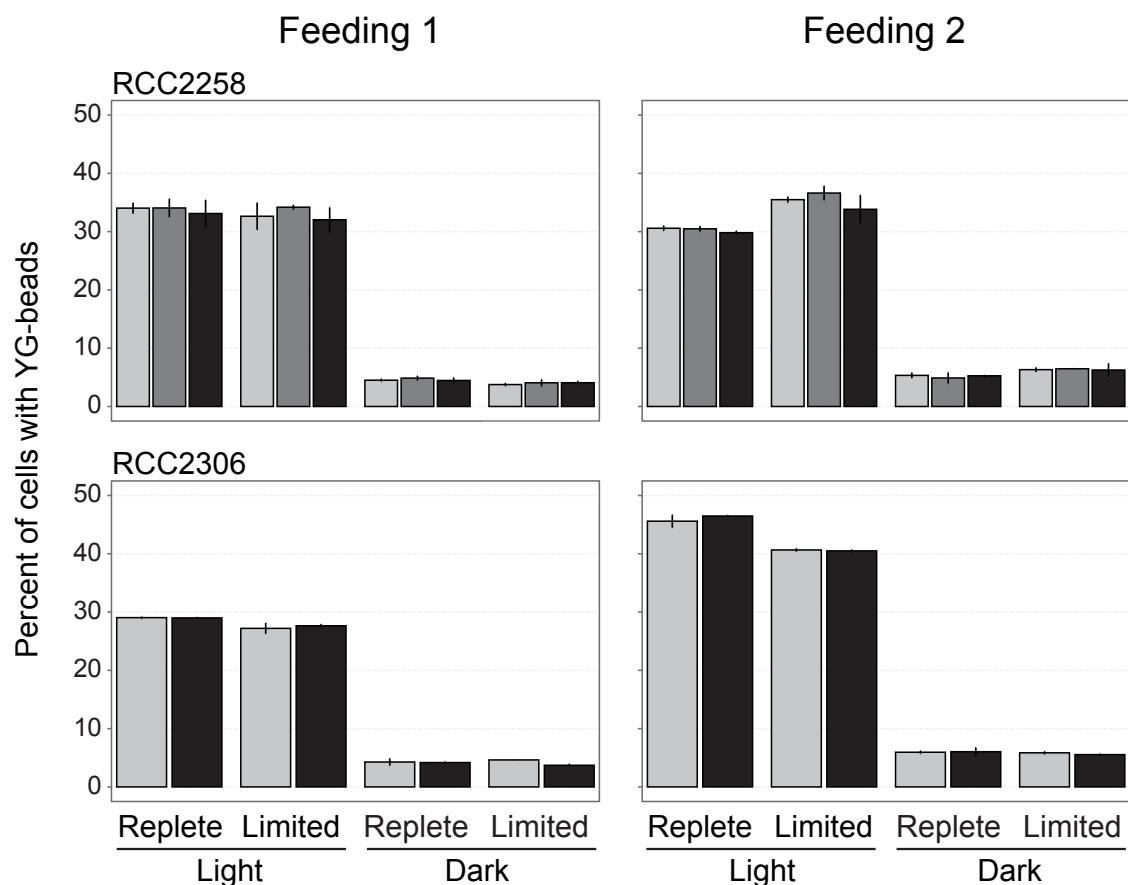


Figure S4. Percent of *M. polaris* cells with YG-beads (*M. polaris*-EXP1) for strains RCC2258 and RCC2306 and different treatments. Two feeding experiments were performed. The color of the bars represent the time point (in minutes) after the addition of YG-beads (0 minutes; light grey, 20 minutes; dark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

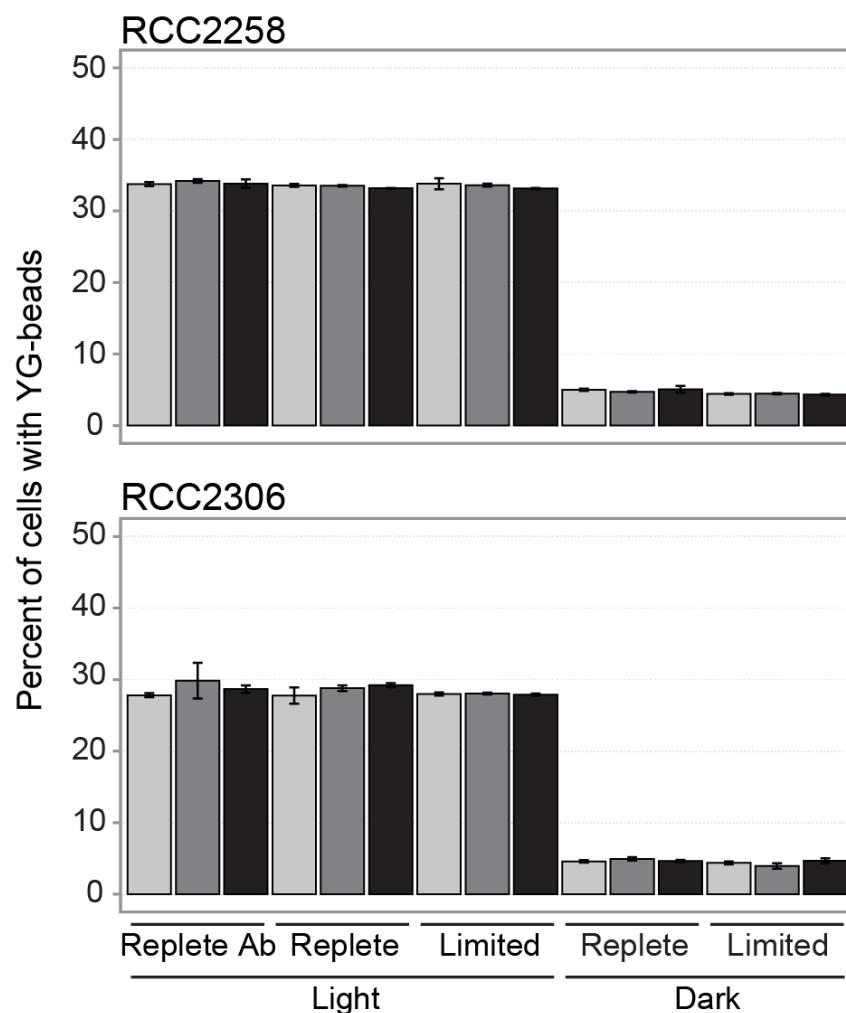


Figure S5. Percent of *M. polaris* cells with YG-beads (*M. polaris*-EXP2) for each strain and treatment. The color of the bars represents the time point (in minutes) after the addition of YG-beads (0 minutes; light grey, 20 minutes; dark grey, 40 minutes; black). Replete Ab correspond to nutrient replete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

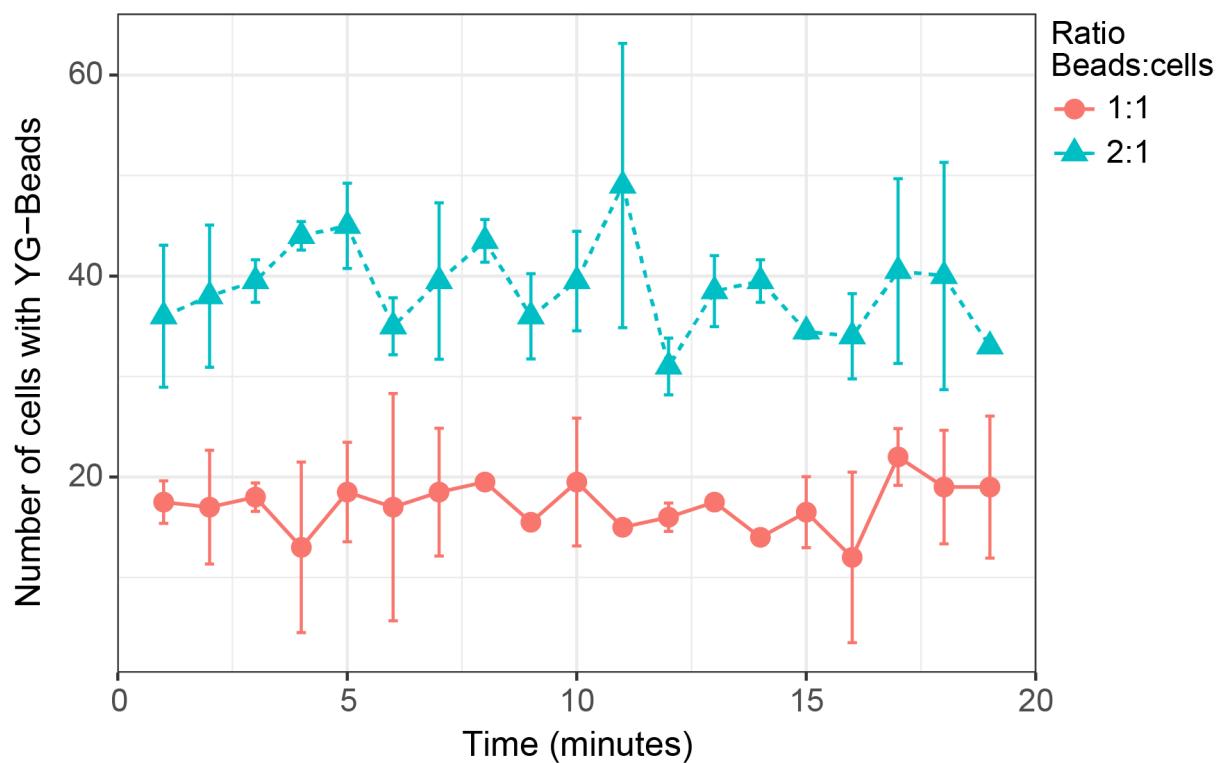


Figure S6. Changes with time in the number of *M. polaris* (strain RCC2306) cells with YG-beads measured by continuously running a live sample for 20 minutes immediately after the addition of YG-beads. Two ratios of beads to cells were tested, 1:1 and 2:1, each in duplicate.

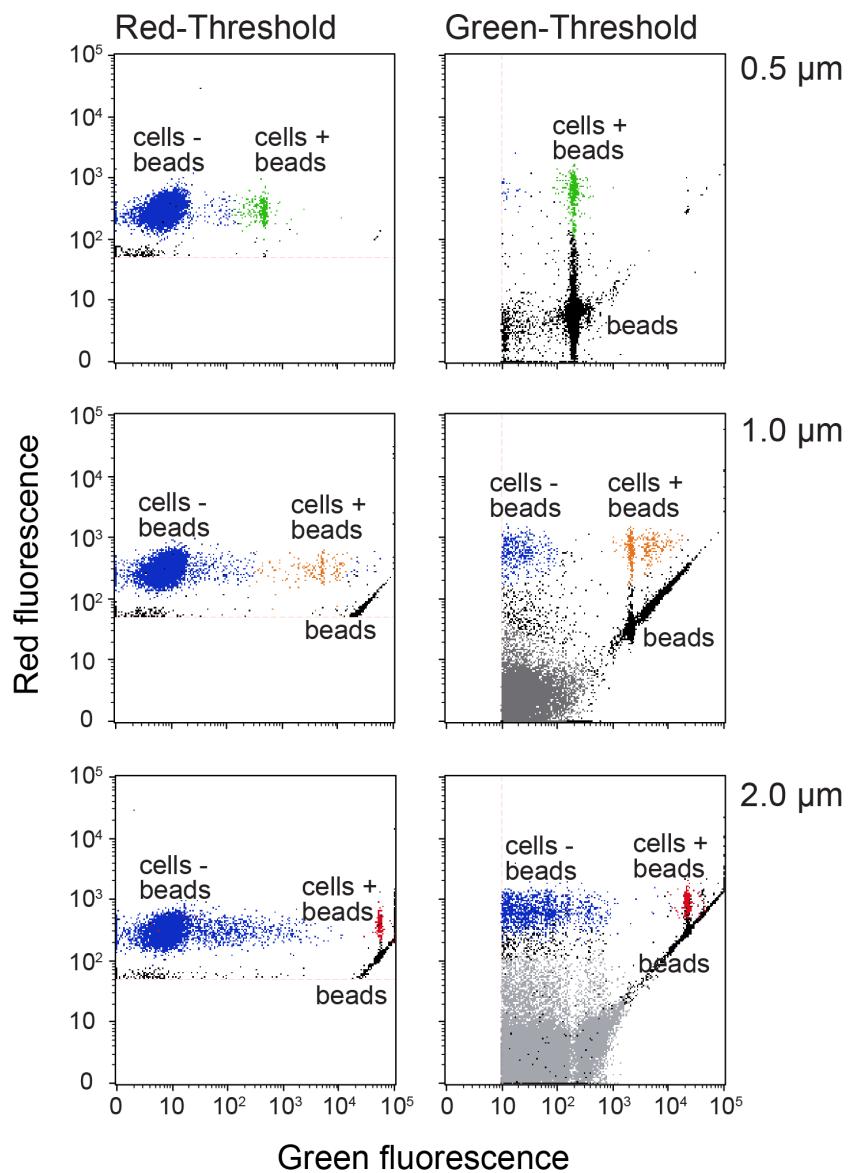


Figure S7. Flow cytograms for *M. polaris* cells incubated with YG-beads of three different sizes: 0.5 (green), 1.0 (orange) and 2.0 (red) μm . See legend of Figure S1 for details.

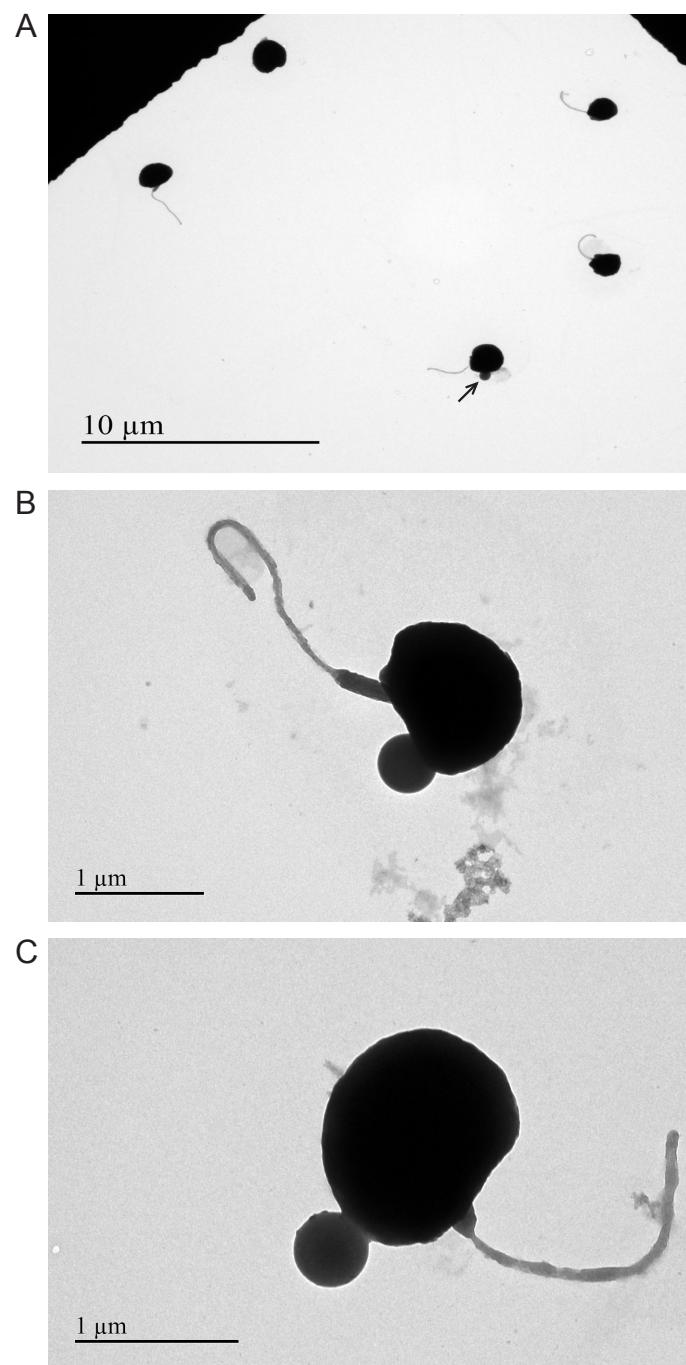


Figure S8. Transmission electron microscopy images of *M. polaris* (strain RCC2306) with YG-beads ($0.5 \mu\text{m}$) after negative staining. **A.** Arrow indicates a *M. polaris* cell with a YG-bead. **B** and **C.** Close up views of *M. polaris* cells with attached YG-bead.