Supporting Information

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SI Text

Bacterioplankton cells were pulse–chase–labeled with two amino acids, [^{35}S]methionine and [^{3}H]leucine (*Materials and Methods*). This permitted examination of the digestion of prey biomass by protist predators, because the two isotopes are retained to different extents in the processes of digestion and assimilation. Whereas ^{3}H radioactivity of bacterioplankton cells was always higher than ^{35}S radioactivity, ^{3}H and ^{35}S radioactivities of protists were more similar (Fig. S2). However, prey digestion by protists was not uniform: Small plastidic (Plast-S) cells retained more ^{3}H tracer relative to ^{35}S tracer compared with large plastidic (Plast-L) or aplastidic (Aplast) cells (*t* test, $P < 0.001$), whereas no difference was detected in ^{35}S:^{3}H between the latter two types of cells. Hence, all three protist types retained more ^{35}S-labeled moieties of methionine-labeled bacterioplankton biomass than ^{3}H-labeled moieties of leucine-labeled bacterioplankton biomass (*t* test, $P < 0.001$) (Figs. S2–S4). Consequently, the ^{3}H-based assessments of rates of bacterivory by protists were generally lower and less robust than ^{35}S-based assessments, and therefore the latter were used to compare bacterivory by protists in different oceanic regions (Figs. 2 and 3).

To independently gauge ^{35}S-based rates of bacterivory, we have used phosphate-uptake rates, measured in the Northern subtropical gyre (1). Assuming that aplastidic protists acquire phosphorus from prey only, the uptake rates of aplastidic and plastidic protists were compared. Phosphate-derived rates of cell bacterivory were a little higher than the rates measured in this study, but they were in the same range, between 0.5 and 8 bacterioplankton cells·h^{-1}. The difference could be caused by differential retention of the isotopic tracers: [^{33}P]Phosphate could be more efficiently retained by protist cells than [^{35}S]methionine. Differential assimilation and retention of [^{3}H]leucine and [^{35}S]methionine was observed in the present study (see above) as well as in earlier studies (2–4).

To assess flow-sorting recovery, the tracer radioactivity of an average bacterioplankton cell was multiplied by the concentration of bacterioplankton in the sample. The resulting total bacterioplankton population radioactivity was compared with the tracer radioactivity of particulate material collected from the same sample directly onto 0.2-μm pore size filters. The two datasets for both ^{35}S and ^{3}H tracers correlated with a close to 1:1 relationship (Fig. S8), validating the high accuracy of flow cytometric counting and sorting.

To assess cell survival during relatively long incubations, bacterioplankton and protist concentrations were compared after 3-h and 9-h incubations. The data shown came from the 2009 cruise. No significant change in cell concentration was observed (Fig. S9).

1. Hartmann M, et al. (2011) Comparison of phosphate uptake rates by the smallest plastidic and aplastidic protists in the North Atlantic subtropical gyre. *FEMS Microbiol Ecol* 78:327–335.
2. Zubkov MV, Sleigh MA (1995) Ingestion and assimilation by marine protists fed on bacteria labeled with radioactive thymidine and leucine estimated without separating predator and prey. *Microb Ecol* 30:157–170.
3. Zubkov MV, Sleigh MA (2005) Assimilation efficiency of *Vibrio* bacterial protein biomass by the flagellate *Pteridomonas*: Assessment using flow cytometric sorting. *FEMS Microbiol Ecol* 54:281–286.
4. Zubkov MV, Tarran GA (2008) High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. *Nature* 455:224–226.

Table S1. Mean size of flow-sorted small and large plastidic and aplastidic protists in different oceanic regions and overall averages

	Plast-S		Plast-L		Aplast	
	Mean (μm)	SD	Mean (μm)	SD	Mean (μm)	SD
NG	2.1	0.4	3.3	1.0	2.9	1.1
EQ	2.0	0.4	2.8	0.7	3.0	1.1
SG	2.0	0.3	2.6	0.7	2.6	0.9
ST	1.9	0.3	3.2	0.9	2.7	0.7
Mean Atlantic	2.0	0.1	3.1	0.3	2.9	0.3

Fig. S1. Characteristic flow cytometric signatures of SYBR Green I-DNA stained bacterioplankton (*A* and *B*) and smallest planktonic protists (*C*–*F*). The groups were differentiated according to light-scattering properties (90° or side light scatter), relative concentration of SYBR Green I stain per particle (green fluorescence; FL1, 530 ± 30 nm), and chlorophyll content (red fluorescence; FL3, >650 nm). (*A*) A density plot showing all bacterioplankton cells (Bpl). (*B*) A density plot showing the actual flow-sorted bacterioplankton group. (*C*) A density plot showing populations of aplastidic and larger plastidic protists and smaller plastidic protists, the latter adjacent to a fraction of larger (i.e., higher-90° light-scattering) bacterioplankton (Bpl-L) including a fraction of *Synechococcus* cyanobacteria (*Syn*) and debris. (*D*) A density plot showing the separation between larger bacterioplankton cells, aplastidic protists, and large and small plastidic protists. The plastidic protists possess extra red fluorescence due to autofluorescence of chlorophyll *a* inside their plastids. (*E*) A density plot showing the two flow-sorted groups of protists: aplastidic protists and small plastidic protists. (*F*) A density plot showing the flow-sorted group of large plastidic protists. Red arrows indicate groups of identified populations of cells or debris particles, and black arrows indicate internal reference 1.0- and 0.5-μm beads.

Fig. S1

Fig. S2. A comparison of ^{35}S and ^{3}H cellular radioactivity of bacterioplankton (*A*) and aplastidic (*B*), large plastidic (*C*), and small plastidic (*D*) protists flow-sorted after 3 h and 9 h of pulse–chase experiments in each of four oceanic regions: Northern subtropical gyre (NG), equatorial waters (EQ), Southern subtropical gyre (SG) in 2008, 2009, and 2010 (dark and light incubations), as well as Southern temperate waters (ST) combining 2008, 2009, and 2010. Dashed lines indicate the unity line. Error bars show single SDs of mean values.

Fig. S2

Fig. S3. A comparison of ^{35}S (*Left*) and ^{3}H (*Right*) dynamics in bacterioplankton (*A*) and aplastidic (*B*), large plastidic (*C*), and small plastidic (*D*) protist cells flow-sorted at four time points during the chase period of the pulse–chase experiments. The experiments were carried out in Southern temperate waters in 2008 (*A* and *B*) and 2009 (*C* and *D*) and in the Southern gyre in 2009 (*E* and *F*). Dashed lines indicate linear regressions. Error bars show single SDs of mean values.

Fig. S3

Fig. S4. A comparison of ^{35}S (*Left*) and ^{3}H (*Right*) dynamics in bacterioplankton (*A* and *B*) and small plastidic (*C* and *D*), large plastidic (*E* and *F*), and aplastidic (*G* and *H*) protist cells flow-sorted at 3 h and 9 h of the pulse–chase (PC) and chase–pulse (CP) experiments. The experiments were carried out at three stations (33°S, 14°S, and 23°N) in 2009 (*C* and *D*). At 23°N the cells were sorted only at 9 h of the chase–pulse experiment. Error bars show single SDs of mean values. The majority of the chase–pulse measurements were insignificantly different from the background, and the differences between the 3-h and 9-h measurements were insignificant ($P > 0.05$) for all chase–pulse experiments. In contrast, in the pulse–chase experiments, many 9-h measurements for protists were significantly higher than the 3-h measurements, whereas the 3-h measurements for bacterioplankton (*A* and *B*) were often higher than the 9-h measurements because of tracer metabolism during the chase phase.

Fig. S4

Fig. S5. Latitudinal changes in bacterioplankton (*A*) and aplastidic (*B*), small plastidic (*C*), and large plastidic (*D*) protist concentrations in samples used for determining bacterivory rates in 2008, 2009, and 2010. Error bars indicate single SDs of measurements. Vertical dotted lines indicate province boundaries.

Fig. S5

Fig. S6. Contour plots of vertical distribution of *Prochlorococcus* cyanobacteria along the transatlantic transect in 2008 (*A*), 2009 (*B*), and 2010 (*C*). Small circles indicate sampled depths.

Fig. S6

Fig. S7. Characteristic time course of combined [^{3}H]leucine and [^{35}S]methionine tracer radioactivity in the total particulate material in pulse–chase experiments done in the NG, EQ, and SG regions in 2009 and in the ST region in 2009 and 2010.

Fig. S7

Fig. S8. Comparison of radiotracer uptake by the whole microbial community (particulate) and flow-sorted average bacterioplankton cell multiplied by bacterioplankton concentration. Error bars indicate single SDs of triplicate measurements. Dotted lines are unity lines.

Fig. S8

Fig. S9. Comparison of bacterioplankton and protist concentrations at the beginning (3 h) and end (9 h) of 2009 bacterivory experiments. Error bars indicate single SDs of replicated measurements. Dotted lines indicate unity lines.

Fig. S9