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Mixotrophic basis of Atlantic oligotrophic ecosystems

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sorted microbial cells; G.A.T. did flow cytometric analyses of microbial samples; M.H., A.P.M., D.J.S., P.H.B. and M.V.Z. wrote the manuscript.

Abstract

Oligotrophic subtropical gyres are the largest oceanic ecosystems covering >40% of the Earth's surface. Unicellular cyanobacteria and the smallest algae (plastidic protists) dominate CO_2 fixation in these ecosystems competing for dissolved inorganic nutrients. Here we present direct evidence from the surface mixed layer of the subtropical gyres and adjacent equatorial and temperate regions of the Atlantic Ocean, collected on three Atlantic Meridional Transect cruises on consecutive years, that bacterioplankton are fed on by plastidic and aplastidic protists at comparable rates. Rates of bacterivory were similar in the light and dark. Furthermore, because of their higher abundance, it is the plastidic protists, rather than the aplastidic forms, that control bacterivory in these waters. These findings change our basic understanding of food web function in the open ocean, since plastidic protists should now be considered as the main bacterivores as well as the main CO_2 fixers in the oligotrophic gyres.

Keywords: Oligotrophic gyres; Bacterivory; Protist predation; Pulse-chase labelling

Text

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Introduction

Oligotrophic ecosystems of subtropical oceanic gyres are the most extensive ecosystems on Earth. These ecosystems cover approximately 40% of the planet's surface, with their area currently expanding (1). *Prochlorococcus* cyanobacteria and the SAR11 group of alpha-proteobacteria are the most numerous microbes in these ecosystems (2, 3), whilst the smallest plastidic protists, comprising various taxonomic groups including uncultured members of the Prymnesiophyceae and Chrysophyceae (4-8), are the most numerous among the eukaryotes, dominating over their aplastidic counterparts (9). The large area of these oligotrophic gyres means that they play a key role in global biogeochemical cycles. However, current knowledge about the functioning of these microbe-controlled systems is relatively limited owing to the difficulty of studying microbes in a photic layer typified by nanomolar concentrations of inorganic macro-nutrients.

According to our current understanding of oligotrophic ecosystem functioning, the roles of different microbial populations are tightly defined. In the established paradigm (10) for these systems, phytoplankton such as cyanobacteria and plastidic protists harvest light, fix CO_2 and take up inorganic nutrients. They are the primary producers of organic matter that fuel the entire system, allowing heterotrophic bacterioplankton, dominated by the SAR11 group, to thrive. Populations of both cyanobacteria and heterotrophic bacterioplankton

68 are controlled by viruses and aplastidic protist predators. Organic matter and
69 inorganic nutrients, released by these control processes, in addition to cell death
70 and bacterioplankton remineralisation of dissolved organic matter, sustain
71 heterotrophic bacterioplankton and phytoplankton. However, some more recent
72 observations are at variance with this paradigm.

73 It is generally accepted that prokaryotes are more efficient than protists in
74 acquiring nutrients at low concentration because of their higher cell surface to
75 volume ratio (11). Indeed, in the North Atlantic subtropical gyre bacterioplankton
76 dominate phosphate uptake and outcompete protists for this nutrient (12, 13).
77 However, despite their low phosphate uptake plastidic protists are major
78 contributors to CO_2 fixation (5, 14). Consequently, the C:P ratio, calculated using
79 CO_2 and phosphate uptake rates by plastidic protists is unrealistically high,
80 suggesting that osmotrophic uptake of phosphate cannot satisfy protist
81 requirements for growth (12). Therefore, in order to sustain themselves in
82 oligotrophic ecosystems, plastidic protists must somehow be able to compensate
83 for a lack of inorganic nutrients. We hypothesise that they do this by mixotrophy:
84 they gain energy from sunlight and simultaneously prey on bacterioplankton to
85 acquire inorganic, and perhaps some essential organic, nutrients, e.g. amino
86 acids and vitamins.

87 Mixotrophy in natural populations of large (>3μm) plastidic protists has
88 been previously documented microscopically in coastal oligotrophic and
89 upwelling regions as well as in the open equatorial Pacific Ocean and the
90 Mediterranean Sea (15-17). There is also qualitative molecular evidence from the

subtropical North Pacific of the presence of mixotrophs among picocyanobacterial predators (18). Furthermore, the quantitative dominance of bacterivory by small plastidic protists (<3μm) over aplastidic protists has been reported for the temperate North Atlantic Ocean in summer (19). The latter paper also outlined preliminary evidence of bacterivory by plastidic protists in the mesotrophic subtropical northeast Atlantic Ocean. However, the ecological extent of mixotrophy in the vast ecosystems of the oligotrophic open ocean remains unknown.

Here, we show that plastidic protists prey on bacterioplankton in the surface mixed layer of both oligotrophic subtropical gyres and adjoining low latitude pelagic regions of the Atlantic Ocean (40^{o}N to 40^{o}S). Owing to their high abundance, plastidic protists prevail over aplastidic protists in bacterivory. This new evidence suggests that mixotrophy is crucial to sustain the functioning of oligotrophic marine ecosystems.

Results

Protist bacterivory was assessed on three Atlantic Meridional Transect (AMT) research cruises in October-November 2008, 2009 and 2010 encompassing subtropical oligotrophic gyres of the Northern and Southern hemisphere and the Equatorial convergence area (Fig. 1). Temperate waters adjoining the Southern gyre were also examined. The results of an earlier study conducted in 2007 in North Atlantic temperate waters (19) are included for comparison.

Protist feeding on bacterioplankton (Bpl) was determined using a dual-labelling pulse-chase method (20) and flow cytometric sorting of labelled prey and predator cells (19, 21). Three populations of the smallest planktonic protists were examined (supporting information (SI) Fig. S1): plastidic protists (i.e. chloroplast containing) smaller, approximately 2 μm (Plast-S) and larger, approximately 3 μm (Plast-L) as well as aplastidic (without chloroplast) protists, approximately 3 μm (Aplast) (Table S1). The two size classes of plastidic protists were operationally differentiated by flow cytometry using cell 90^{o} light scatter, DNA content and autofluorescence (Fig. S1).

Bacterioplankton cells were labelled using a pulse-chase of two amino acids, ^{35}S-methionine and ^{3}H-leucine (see Methods), to examine digestion of prey biomass by protist predators (see SI). In the majority of experiments, in all regions studied, tracer content of protist cells increased with time during the chase phase in contrast to stable or slightly decreased tracer content of bacterioplankton cells (Figs S2-4). The increase demonstrates bacterivory by the three types of protist cells. More robust ^{35}S-based assessments (see SI) were used to compare protist bacterivory in different oceanic regions (Fig. 2). Because only two stations were examined on each cruise in the Southern temperate (ST) region in austral spring, the 2008, 2009 and 2010 measurements were combined to get a more representative average estimate of protist bacterivory. The rates of bacterivory compared favourably with independent estimates (see SI) derived from cell uptake rates of phosphate in surface waters of the Northern subtropical gyre (NG). Moreover, the influence of light and dark incubation on bacterivory

137 was studied on the cruise in 2010. No statistically significant light-induced
138 differences (t-test, $p>0.1$) in protist bacterivory were determined (Fig. S2, SG
139 2010).

140 The rates of cell bacterivory (i.e. the number of bacterioplankton cells
141 consumed per protist cell h^{-1}) by the Aplast protists were comparable in all
142 regions apart from the Northern temperate (NT) region in summer (Fig. 2). The
143 difference between the ST and the NT regions was probably seasonal.
144 Bacterivory by the Plast-L cells was the lowest in the Southern subtropical gyre
145 (SG) in 2008 and 2010 but it was comparable to bacterivory by Aplast cells in the
146 ST region. Bacterivory by the Plast-S cells was lowest in temperate waters and in
147 the SG in 2010. It was similar to bacterivory by the Plast-L cells in the NG in
148 2009 and in the SG in 2008, but lower in the SG in 2009 and 2010.

149 Rates of bacterivory in the SG varied inter-annually. Cell bacterivory by all
150 three types of protists was significantly higher in 2009 compared to 2008 and
151 2010, while the differences between 2008 and 2010 were insignificant (Fig. 2).
152 On the other hand the concentration/biomass of the Plast-S population and
153 concentration of bacterioplankton were comparable between the three years (Fig.
154 3a, Fig. S5), whereas the concentration/biomass of Aplast and the Plast-L
155 protists were higher in 2010. For comparison, *Synechococcus* and
156 *Prochlorococcus* concentrations were 70% and 50% higher in 2010 compared to
157 2008 (Figs 1b-d, Fig. S6). Bacterioplankton, *Synechococcus* and
158 *Prochlorococcus* concentrations in the surface mixed layer of the two gyres were
159 similar in 2009, while Plast-L and Aplast biomass was lower in the SG than in the

NG, while the opposite was true for the Plast-S protists (Fig. 1c, Figs S5-6). The biomass of Plast-L protists was highest in all regions followed by the biomass of Aplast and Plast-S protists (Figs 3a, b). The combined biomass of the two plastidic protist groups made up between 65% and 90% of the combined biomass of the smallest protists (Fig. 3b).

In contrast to cell bacterivory, population bacterivory (i.e. the total number of bacterioplankton consumed ml^{-1} h^{-1} by each protist population) in the NG and NT regions was not significantly different between the three protist populations (Fig. 3c). Population bacterivory was significantly higher in more productive temperate regions followed by the equatorial region (Fig. 3c), owing to higher protist concentrations (Fig. 3a, Fig. S5). In the equatorial waters (EQ), and particularly in the SG in 2009, bacterivory by the Plast-L population was the highest compared to other populations, comprising 50% of total protist bacterivory (Fig. 3d). A comparison between the sum of plastidic populations and the aplastidic population showed a significant difference in population bacterivory by plastidic and aplastidic protists (t-test, $p=0.01$). Cumulative bacterivory by plastidic protists accounted for 60 to 77% of total protist bacterivory across the Atlantic Ocean. Furthermore, inter-annual variability had a minor effect on the domination of bacterivory by plastidic protists.

Discussion

The uniformly higher population rates of bacterivory by plastidic protists compared to aplastidic protists in the surface mixed layer of the northern and

183 southern gyres and the equatorial region shows for the first time the large
184 contribution of phytoplankton to harvesting bacterioplankton in the low latitude
185 Atlantic Ocean. There are several important implications of this finding.

186 Firstly, it challenges the long-standing assumption of the total dependence
187 of phytoplankton on dissolved inorganic nutrients in oligotrophic oceanic waters.
188 Independent culture studies have shown that some marine and freshwater algae
189 can acquire scarce nutrients, such as phosphorus and iron, using bacterivory
190 (22, 23). There are also field reports from non-gyre regions that rates of
191 bacterivory by plastidic protists between 3-5 μm and larger than 5 μm negatively
192 correlate with the concentration of soluble reactive phosphorus or iron (16, 17). In
193 this study, rates of bacterivory by Plast-S cells were lower in surface waters of
194 the South Atlantic subtropical gyre compared to the North Atlantic subtropical
195 gyre, which is depleted in phosphate (13). This suggests that the mixotrophy of
196 Plast-S cells may be linked to phosphate depletion. However, differences linked
197 to the seasonality of studies in the gyres (boreal autumn, austral spring) as well
198 as inter-annual variability may also play a role here. In contrast, rates of
199 bacterivory by Plast-L protists were comparable along the whole transect in
200 2009. Rates of bacterivory by Plast-L cells similar to those found in 2009 have
201 also been measured in the temperate North Atlantic Ocean (19), further
202 supporting the lack of correlation between macro-nutrient availability and
203 bacterivory in Plast-L cells. The main temporal variability in Plast-L bacterivory
204 was inter-annual in the SG. Both biomass and rates of bacterivory by Plast-L and
205 Aplast populations are broadly comparable between the phosphate depleted

206 Northern and relatively phosphate replete Southern Atlantic oligotrophic gyres
207 (Fig. 3). This corroborates earlier observations of similar microbial abundance
208 and bacterioplankton activities in the two gyres (24) (Fig. 1, Fig. S5).

209 The second major implication of this work is related to the sustainability of
210 oligotrophic ecosystems, because of the metabolic “inefficiency” of mixotrophs.
211 Notionally, mixotrophs require energy investment in both photosynthetic and
212 phagotrophic cellular apparatus, and laboratory experiments suggest that they
213 may be most ecologically successful when nutrient resources are limited (25) but
214 light energy is plentiful, making surface waters of oligotrophic oceanic gyres their
215 ideal habitat. High basic metabolic requirements and hence a decreased
216 efficiency of nutrient retention by mixotrophic protist generalists compared to
217 phototrophic or phagotrophic specialists (25, 26) should enhance rates of nutrient
218 regeneration in the surface mixed layer of oligotrophic gyres. It has been claimed
219 (26, 27) that by being mixotrophs, algae could also escape nutrient competition
220 with bacterioplankton by reducing bacterioplankton concentrations to levels that
221 would reduce or even arrest growth of specialist phagotrophs, e.g. aplastidic
222 protists.

223 The third implication of our work is related to the cell metabolism of
224 mixotrophs. Because CO_2 fixation as well as predation and respiration are
225 concomitantly taking place in the same cells, mixotrophy could help to explain the
226 tightness of biogenic carbon budgets at the community level (28). Tight
227 intercellular coupling of production and respiration could contribute to the stability
228 of oligotrophic ecosystems in the absence of seasonal or episodic perturbations

229 (29) such as seasonally accumulated bio-available organic matter (30), or
230 allochthonous matter transported by advection (31) or deposited from the
231 atmosphere (32, 33) which enhance growth of opportunistic species and
232 ultimately change the composition of microbial communities.

233 The fourth implication concerns the ecological significance of the smallest
234 plastidic protists in oligotrophic ecosystems. Apart from being key CO_2 fixers (5,
235 14), plastidic protists control bacterioplankton abundance, acting as producers of
236 organic matter and predators at the same time. Such dual control and
237 interdependence of bacterioplankton and protists could help to explain the
238 constancy of low bacterioplankton concentrations in the oligotrophic ocean
239 compared to more productive regions (34, 35). The scarcity of bacterioplankton
240 prey in oligotrophic gyres in turn probably reduces both propagation of phage
241 infections and growth of specialised predators like aplastidic protists (Fig. S5).

242 In summary, this work shows the significance and ubiquity of mixotrophy
243 in the survival of the smallest pelagic protists in sunlit oligotrophic surface waters.
244 This deceptively “inefficient” life style should reduce nutrient export and maintain
245 faster nutrient turnover in the surface mixed layer, both of which are essential for
246 sustainable functioning of oligotrophic ecosystems. Consequently, future food
247 web models should consider including mixotrophs as a basic ecosystem element.
248
249

250 Materials and methods

251 **Sampling**

252 This study, comprising 68 experiments, was carried out in the Atlantic Ocean
253 during one Atlantic Meridional Transect cruise (AMT) on board the UK RRS
254 *James Clark Ross* in October-November 2008 and on two AMT cruises on board
255 the UK RRS *James Cook* in October-November 2009 and 2010 (Fig. 1).
256 Seawater samples were collected from a depth of 20 m before dawn with 20-litre
257 Niskin bottles mounted on a sampling rosette of a conductivity-temperature-depth
258 profiler (Sea-Bird Electronics, Washington, USA). In 2008 and 2009 experiments
259 were set up within 20 min of sample collection in the dark at ambient
260 temperature, controlled by a water bath within 0.5^{o}C. In 2010 experiments were
261 set up for dark and light measurements in a dark room using only a dim green
262 light (LEE filter 090, transmission of 20-30% of light at 500-550 nm). Light
263 incubation experiments were placed in a 6 L water tank illuminated by a warm
264 white light emitting diodes (LED) array (Photon Systems Instruments, Drasov,
265 Czech Republic). Parallel dark incubations were done in a similar water tank but
266 were isolated from light. Both tanks were plumbed into a refrigerated bath (Grant
267 Instruments, Cambridge, UK) to maintain temperature within 0.5^{o}C of *in situ*
268 temperature at the depth of sample collection. The LED array was adjusted to
269 keep light intensity at 300 µmol Q m^{-2} s^{-1} inside the incubation bottles.

270 **Cell counting**

271 Bacterioplankton and protist cell concentrations were determined by flow
272 cytometry (Fig. S1) using FACSort and FACSCalibur instruments (Becton

273 Dickinson, Oxford, UK). *Synechococcus* and *Prochlorococcus* cyanobacteria
274 were counted in unfixed samples (Figs 1b-d, Fig. S6). Concentrations of
275 *Prochlorococcus* in the surface mixed layer were likely underestimated owing to
276 low chlorophyll content of cells that led to red autofluorescence of cells lying
277 closer to the detection limit of the flow cytometers. The other samples were fixed
278 with paraformaldehyde (PFA) 1% (w/v) final concentration and stained with
279 SYBR Green I DNA dye (13, 36). A mixture of multifluorescent beads, diameter
280 0.5 µm and 1.0 µm (fluoresbrite microparticles, Polysciences, Germany), was
281 used as internal standard for fluorescence and flow rates (37). To compare
282 protist population biomass (Figs 3a, b), protist concentrations were multiplied by
283 the corresponding cell biomass values.
284 To estimate their cell sizes, the three groups of protists were flow sorted (Fig.
285 S1), sorted cells being deposited on polycarbonate membrane filters with 0.2 µm
286 pore diameter. Filters were mounted onto glass slides and stained excessively
287 with DAPI (final concentration 1 µg ml^{-1}) to reveal cell cytoplasm. Aplast cells
288 were sorted and sized from 4 experimental samples on the 2009 cruise and from
289 5 experimental samples on the 2010 cruise, which represented all regions
290 studied. Plast-L and Plast-S cells were sorted from 5 and 4 experimental
291 samples on the 2010 cruise. At least 200 cells were measured at 1000×
292 magnification of an epifluorescence microscope (Axioscope 2, Zeiss, Germany)
293 to estimate mean cell diameters. Mean cell diameters of each of the three protist
294 groups were statistically similar in analysed samples (Table S1). The overall
295 mean size of Plast-S cells of 2.0 ± 0.1 µm was significantly lower (t-test,

296 p=0.0002) than the overall mean for Plast-L cells of 3.1 ± 0.3 µm, while the
297 overall mean size of Aplast cells of 2.9 ± 0.3 µm was statistically similar to the
298 size of Plast-L cells. To estimate the biomass of the three protist groups their cell
299 biovolume was computed assuming that the cells are spheres with a diameter
300 equal to the mean cell size. Cell biovolume was converted into cell biomass
301 using a specific carbon content of 200 fg C μm^{-3}, taken as a mean value from
302 Christian and Karl (38).

303 **Determining rates of protist bacterivory using pulse-chase dual-labelling of**
304 **natural communities**

305 Before an experiment, glass bottles (250 ml, Schott, Fisher Scientific, UK) were
306 soaked in 10% hydrochloric acid and rinsed twice with 50 ml of sampled
307 seawater (taken from the same Niskin bottle as that for the subsequent
308 experiment). Seawater (250 ml) from the sample was subsequently added to
309 each washed glass bottle and spiked with L-[^{35}S] methionine (specific activity >37
310 TBq/mmol, Hartmann Analytic, Germany), final concentration 0.25 nM or 0.4 nM,
311 and L-[4,5-^{3}H] leucine (specific activity 1.48-2.22 TBq/mmol, Hartmann Analytic),
312 final concentration 0.5 nM. An increase in the amount of label (and thus the
313 sensitivity of the experiment) was necessary to compensate for the low
314 abundance of protists in the oligotrophic gyres. After 1.5 h incubation, non-
315 radioactive methionine and leucine were added to final concentrations of 0.25
316 µM, or 0.4 µM and 0.5 µM, respectively, to chase the radioactive amino acids
317 (19, 20). Samples were incubated for 1.5 h to stabilise pulses in bacterioplankton
318 cells before taking subsamples (120 ml, fixed with 1% (w/v) PFA at 3 h and 9 h)

for the measurement of protist tracer uptake rates. In parallel, the pulse-chase was monitored over 9 h by taking 1.6 ml subsamples every 15 min for 3h followed by sampling intervals of 1h until the end of the 9 h experiment. These samples were fixed with 1% (w/v) PFA and after 1h of fixation particulate material was collected onto 0.2 μm polycarbonate filters (Nuclepore, Whatman, UK) to measure the total sample radioactivity (Fig. S7).

In addition to the two point experiments (3 h vs. 9 h), time course experiments were carried out at several stations on two cruises (2008 and 2009) with samples fixed at four time points for subsequent population sorting to resolve protist feeding dynamics (Fig. S3).

To check for potential osmotrophic uptake of tracers by protists during the chase phase, additional so-called “chase-pulse” experiments (in parallel to “pulse-chase” experiments) were carried out at three stations on the 2009 cruise. In the “chase-pulse” experiments samples were initially spiked with unlabelled leucine and methionine molecules at concentrations of radiotracers used in the parallel pulse-chase experiment. After 1.5 h incubation unlabelled amino acids were added at concentrations to make up to the values used in the chase (see above) and in addition radiotracers were added at concentrations identical to the ones used in the pulse phase. Subsamples were fixed for flow sorting after 3 h and 9 h. The measured radioactivity in chase-pulse labelled and sorted cells was comparable to the background (Fig. S4), confirming the insignificant osmotrophic uptake of tracer molecules by sorted protists and bacterioplankton during the chase phase.

342 **Flow cytometric cell sorting**

343 PFA-fixed radioactively-labelled samples were stored at 4°C and sorted within 10
344 h. For each time point four different populations (total bacterioplankton (Bpl),
345 Plast-S, Plast-L and Aplast protists) were sorted (Fig. S1). For each population
346 four to eight replicates of different cell number were sorted (19).

347 Sorted bacterioplankton cells were collected onto 0.2 µm polycarbonate
348 filters. Sorted protist cells were collected onto 0.8 µm polycarbonate filters to
349 reduce the retention of potentially by-sorted Bpl cells. Filters were washed with
350 deionised water to remove contaminating tracer and placed into scintillation vials
351 to which 5 ml of scintillation cocktail (GoldStar, Meridian, UK) was added.
352 Subsequently, the vials were radioassayed for 0.5-2 h (depending on sample
353 radioactivity) using ultra-low-level liquid scintillation counters (1220 Quantalus,
354 Wallac, Finland).

355 **Data analyses**

356 Using quench curves, the ^{3}H label was deconvoluted from the ^{35}S label and the
357 amount of each label was computed as Bq cell^{-1} by dividing the cumulative ^{3}H or
358 ^{35}S radioactivity by the corresponding number of sorted cells. Cell radioactivities
359 at 3 h and 9 h were compared including dark and light incubations (Fig. S2).

360 Cell bacterivory was calculated according to the following formula:

361 $$\text{Bacterivory} = (Prt_{avg}T2 \times Bpl_{avg}T2^{-1} - Prt_{avg}T1 \times Bpl_{avg}T1^{-1}) \times (T2\text{-}T1)^{-1}$$

362 where $Prt_{avg}T2$ is the average activity of 4-8 replicates of one of the protist groups
363 at the second time point and $Prt_{avg}T1$, the same at the first time point; T1 and T2
364 are the first and the second time points of the experiment (e.g. 3 h and 9h,

365 respectively); $Bpl_{avg}T1$ and $Bpl_{avg}T2$ are the average activity of 4-8 replicates of
366 the bacterioplankton cells at T1 and T2, respectively. Because of the pulse-chase
367 experimental design, the activity of the Bpl was in most cases the same at T1
368 and T2 (Figs S2-4), a cumulative mean could be used.

369 To verify that the increase in label between the first and second time
370 points was statistically significant, t-tests ($p<0.05$) were carried out using
371 SigmaPlot 11.0 (Systat Software, USA) and Quattro-Pro X4 (Corel, Ottawa,
372 Canada) software. Errors were calculated according to standard error
373 propagation procedures. The majority of experiments (80%) showed a significant
374 difference in protist radioactivity between the two time points (Figs S2, S4) and
375 hence demonstrated bacterivorous activity of the Plast-S, Plast-L and Aplast
376 cells. We attribute non-significant bacterivory in some experiments to the
377 detection limit of our method owing to the low radioactivities measured. All
378 estimates of rates of cell bacterivory were included in calculations of average
379 regional rates (Fig. 2). The mean rates were all significantly higher than zero (t-
380 tests, $p<0.05$). To calculate regional bacterivory of the protist populations (Fig.
381 3c), bacterivory per cell was multiplied by the corresponding mean concentration
382 of protist cells ml^{-1}. T-tests were used to compare mean values; standard
383 deviations, derived from pooled variance, were used to show variability within
384 regions (Figs 2, 3).

385

386 ## Acknowledgements

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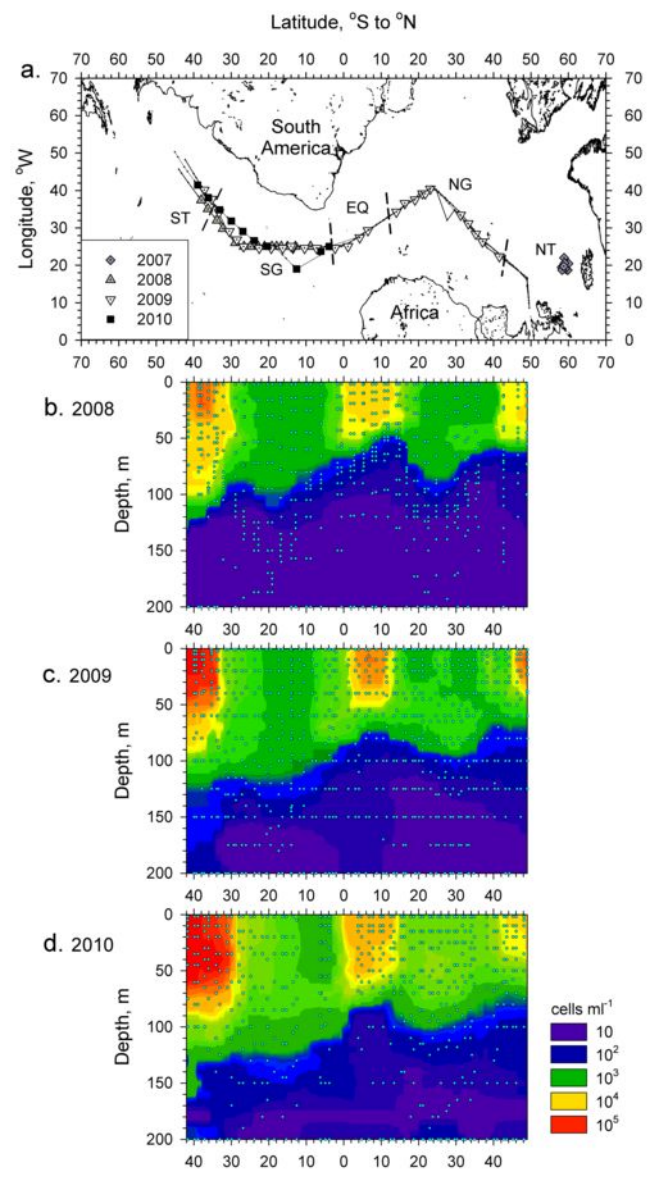
508 Figure legends

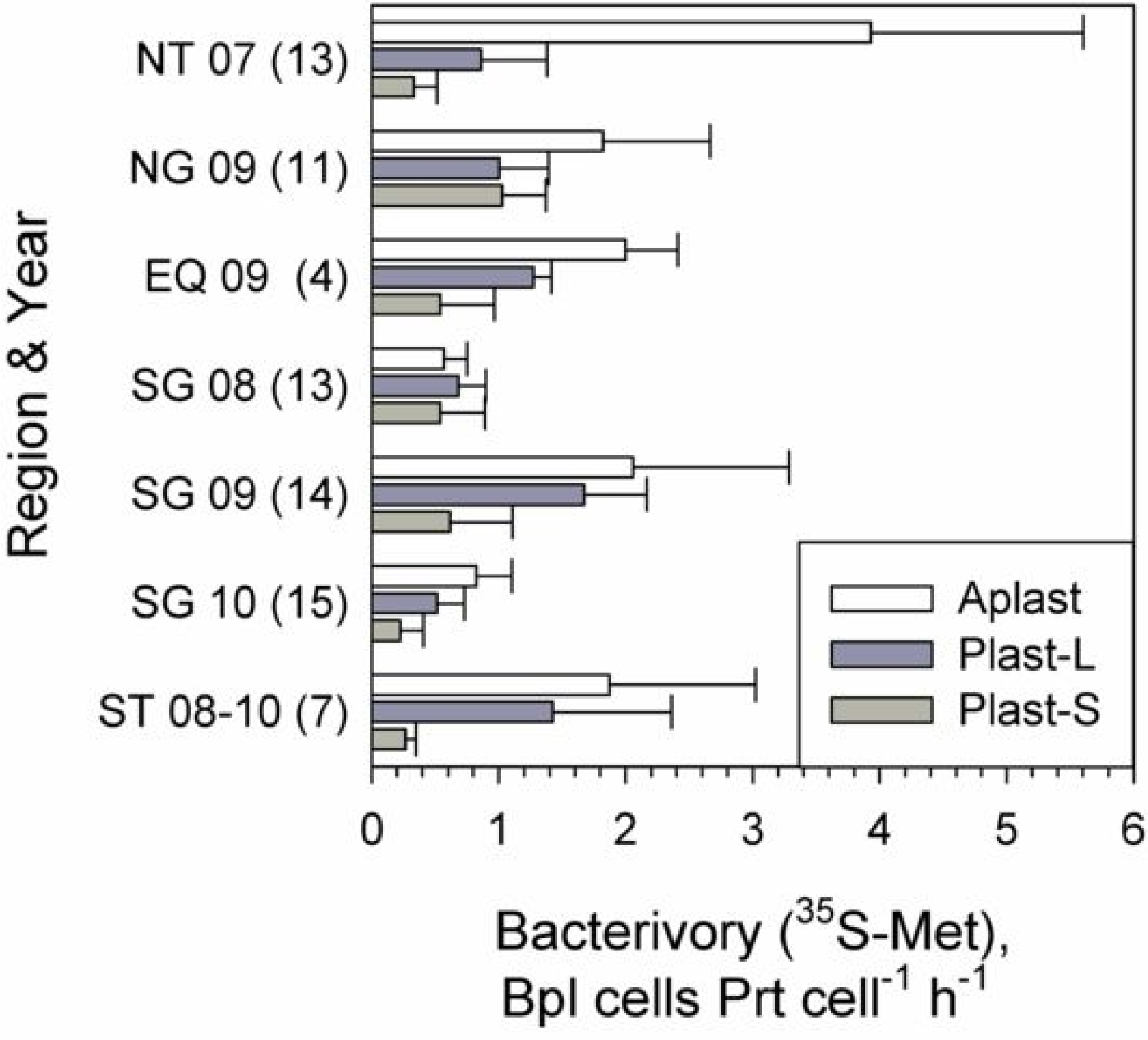
509 Fig. 1. A schematic map of the Atlantic Ocean showing the area sampled in the
510 2008, 2009 and 2010 cruises (a) with corresponding contour plots of vertical
511 distribution of *Synechococcus* cyanobacteria in 2008 (b), 2009 (c) and 2010 (d).
512 These distributions were used to identify the boundaries of the five oceanic
513 regions: Northern temperate waters (NT), Northern subtropical gyre (NG),
514 equatorial waters (EQ), Southern subtropical gyre (SG) and Southern temperate
515 waters (ST). Short dashed lines (a) indicate these boundaries. Solid lines with
516 dots (a) indicate the ship tracks and sampled stations, respectively. Circles (b, c)
517 indicate sampled depths. The stations sampled in 2007 in North Atlantic
518 temperate waters are also indicated.

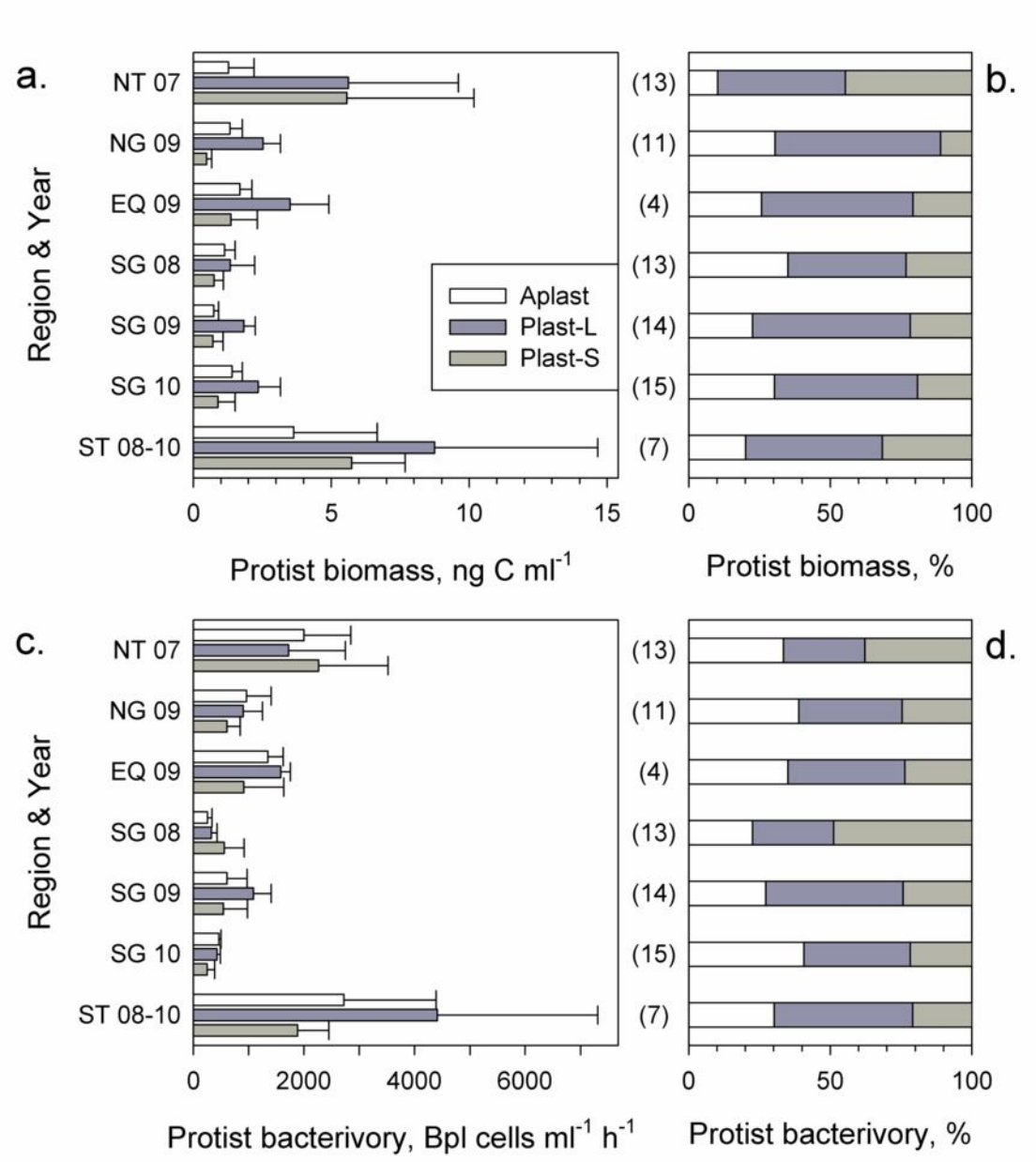
519 Fig. 2. A comparison of mean rates of cell bacterivory by the flow-sorted
520 aplastidic (Aplast), large plastidic (Plast-L) and small plastidic (Plast-S) protists in
521 the five regions (see Fig. 1 for details). The numbers next to the region
522 abbreviations indicate the year of sampling, and then the numbers in brackets
523 indicate the number of separate experiments performed in each region. The rates
524 were calculated using ^{35}S-methionine pulse-chase tracing. Error bars show single
525 standard deviations to indicate the variance of rates within regions. The results of
526 an earlier study conducted in 2007 in North Atlantic temperate waters (19) are
527 included for comparison.

528 Fig. 3. A comparison of mean absolute (a) and relative (b) population biomass
529 and mean absolute (c) and relative (d) population bacterivory of aplastidic
530 (Aplast), large plastidic (Plast-L) and small plastidic (Plast-S) protists in the five

regions (see Fig. 1 for details). The numbers next to the region abbreviations indicate the year of sampling. The numbers in brackets indicate the number of experiments done in each region. The rates were calculated using ^{35}S-methionine pulse-chase tracing. Error bars show single standard deviations to indicate the variance of biomass and rates within regions.


Latitude, °S to °N
a.
Longitude, °W
South America
Africa
ST
EQ
NG
NT
SG
2007
2008
2009
2010
b. 2008
c. 2009
d. 2010
Depth, m
cells ml-1
10
10^2
10^3
10^4
10^5


NT 07 (13)
NG 09 (11)
EQ 09 (4)
SG 08 (13)
SG 09 (14)
SG 10 (15)
ST 08-10 (7)
Region & Year
Aplast
Plast-L
Plast-S
0
1
2
3
4
5
6
Bacterivory (^{35}S-Met),
Bpl cells Prt cell^{-1} h^{-1}


a.
b.
c.
d.
Region & Year
NT 07
NG 09
EQ 09
SG 08
SG 09
SG 10
ST 08-10
(13)
(11)
(4)
(13)
(14)
(15)
(7)
Aplast
Plast-L
Plast-S
0
5
10
15
0
50
100
0
2000
4000
6000
Protist biomass, ng C ml-1
Protist biomass, %
Protist bacterivory, Bpl cells ml-1 h-1
Protist bacterivory, %