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Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre

Mikhail V. Zubkov [a,*], Glen A. Tarran [b], Bernhard M. Fuchs [c]

[a] *Southampton Oceanography Centre, Waterfront Campus, European Way, Southampton, Hants SO14 3ZH, UK*
[b] *Plymouth Marine Laboratory, Plymouth PL1 3DH, UK*
[c] *Max-Planck-Institute for Marine Microbiology, D-28359 Bremen, Germany*

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Abstract

Ambient concentrations and turnover rates of two amino acids, leucine and methionine, by total bacterioplankton and *Prochlorococcus* cyanobacteria were determined along a latitudinal transect across the Southern Atlantic gyre using a combined isotopic dilution and flow cytometric sorting technique. The ambient concentrations of methionine (0.2–0.65 nM) were about 2 times higher than the concentrations of leucine, while the turnover rates of the two amino acids were remarkably similar (0.1–0.7 nM d^{-1}). The concentrations of both amino acids did not vary significantly with depth between 3 and 150 m but their turnover rates were 1.5–2 times higher in the top 3–80 m. *Prochlorococcus* took up amino acids in situ at high rates. Using a representative ^{35}S-methionine precursor, about 25% of total bacterioplankton consumption of amino acids could be assigned to *Prochlorococcus* with low red fluorescence (Pro LRF) inhabiting the surface mixed layer down to 80 m and about 50% assigned to *Prochlorococcus* with high red fluorescence (Pro HRF) living below 100 m. In the same deep waters the cellular amino acid uptake of Pro LRF was less than 6% of that of the Pro HRF, indicating declining metabolic activity of the former. The mean cellular uptake rate of Pro HRF at depths below 120 m was 2.5 amol $cell^{-1}$ d^{-1}, 4 times higher than the rates of Pro LRF in the top 80 m. The difference could be partially explained by Pro HRF cellular biomass being twice that of Pro LRF. The biomass specific rates of *Prochlorococcus* were comparable or higher (particular of the Pro HRF) than that of other bacterioplankton. The reported findings could explain ecological success of mixotrophic *Prochlorococcus* cyanobacteria over both strictly autotrophic algae and heterotrophic bacteria in oligotrophic regions sustained by nutrient remineralisation.

Keywords: Marine bacterioplankton; Marine phytoplankton; Mixotrophy; Flow cytometric sorting; Isotopic tracer bioassay

1. Introduction

The ubiquity of phototrophic cyanobacteria of the genus *Prochlorococcus* [1] within a latitudinal band between the 40°S to 40°N to depths of 200 m makes it probably the most abundant photosynthetic organism in the ocean [2]. One of the reasons for *Prochlorococcus*' extraordinary ecological success is the occurrence of several different species or ecotypes along the vertical light gradient [3,4] adapted to different light regimes [5]. The diversity of laboratory cultures [6,7] as well as within natural populations [8] indicates the ecological importance of its genetic variability.

In subtropical oligotrophic gyres the vertical distribution of *Prochlorococcus* often exceeds the boundaries of the euphotic layer and these organisms seem to be able to sustain their populations over an irradiance range spanning three orders of magnitude. In these regions,

* Corresponding author. Tel.: +44-23-8059-6335; fax: +44-23-8059-6247.
E-mail address: mvz@soc.soton.ac.uk (M.V. Zubkov).

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where nutrients such as nitrogen are extremely scarce and nutrient regeneration within a microbial loop [9,10] predominates over nitrate influx, *Prochlorococcus* has a competitive advantage in nutrient uptake over eukaryotic algae because of its higher surface area to volume ratio [11]. Experimental studies of *Prochlorococcus* strains [12] as well as *Prochlorococcus* genomic analysis [13] demonstrated that they predominantly use reduced forms of inorganic nitrogen (ammonium) and could be unable to assimilate the oxidised forms (nitrate, nitrite).

Because it is generally accepted that *Prochlorococcus* is an obligate photoautotroph there have been a considerable number of studies concerning its photosynthetic activities as well as inorganic nutrient acquisition, while less attention has been paid to its potential heterotrophic activity. Recently, it was shown that natural *Prochlorococcus* populations could take up significant amounts of dissolved organic nitrogen in the form of amino acids [14]. The unexpectedly high heterotrophic activity reveals *Prochlorococcus* ecological success from a completely different angle. By tapping into another source of nitrogen in nutrient poor environments and by controlling a pool of labile organic nutrients and consequently exerting competitive pressure on other bacterioplankton populations, mixotrophy could give *Prochlorococcus* very considerable ecological advantages in oligotrophic oceanic conditions. However, studies to date considered the amino acid uptake by *Prochlorococcus* in relative values and only in the Arabian Sea.

The aim of the present study was to assess how important this process is to *Prochlorococcus* growth, to estimate the rate of amino acid uptake of *Prochlorococcus* in absolute units, taking into account the ambient amino acid concentrations. The second aim was to compare these rates in *Prochlorococcus* adapted to high and low irradiance, living at different depths. We hypothesised that in low light conditions these cyanobacteria could be more heterotrophic, possibly using photosynthesis energy to fuel their organic nutrient transport. To answer these questions a previously developed flow sorting technique was refined and augmented with a bioassay of ambient amino acid concentrations and turnover rates.

2. Methods

2.1. Sampling site

The experimental work was done during an oceanographic cruise on board the Royal Research Ship James Clark Ross (Cruise No. JR91) in the Atlantic Ocean in September–October 2003, using a meridional transect of eight stations from 6.6°S and 25°W to 33.8°S and 32.1°W (Fig. 1). Seawater was collected with a rosette of 20 l Niskin bottles mounted on a conductivity–temperature–density (CTD) profiler with additional fluorescence and oxygen detectors at two depths: one depth was chosen at 3–10 m and the other depth was chosen just above the deep chlorophyll maximum at 55–150 m. Bacterial and cyanobacterial abundance, microbial turnover rates of amino acids: methionine and leucine, and group flow sorting of *Prochlorococcus* and other bacterioplankton groups preloaded with ^{35}S-methionine were determined in each collected sample. The latter analyses included flow sorting of four to five main cytometric groups of bacterioplankton for radiotracer studies. In the present report we particularly concentrated on uptake rates of flow cytometrically identified *Prochlorococcus* cyanobacteria and the results of studies of other bacterial groups and *Prochlorococcus* phylogenetic characterisation will be reported later elsewhere.

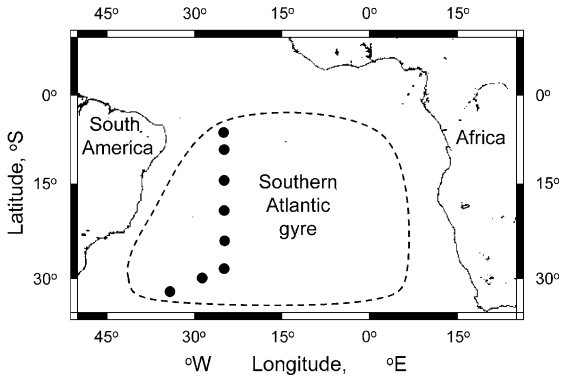

Fig. 1. Map of the studied area with station locations (circles). Boundaries of the Southern Atlantic gyre were approximated using satellite colour imagery.

2.2. Bioassay of amino acid concentration and microbial turnover rates using radioactively labelled precursors

The concentrations and turnover rates of amino acids were estimated using a concentration series bioassay [15]. The samples used for rate determinations were initially collected into acid washed 1 l thermos flasks using acid soaked silicone tubing and processed within 1 h after sampling. Two labelled amino acids, ^{3}H-leucine and ^{35}S-methionine, were used. The L-[^{35}S]methionine (specific activity 1000 Ci/mmol) was added at a standard concentration of 0.13 nM and diluted with non-labelled methionine using a dilution series of 0.2, 0.5, 1.0, 1.5 and 2.0 nM. The L-[4,5-^{3}H]leucine (specific activity 160 Ci/mmol) was added in a series of 0.1, 0.2, 0.4, 0.6 and 0.8 nM final concentration. The radioactively labelled chemicals were purchased from Amersham Biosciences, UK.

Triplicate samples (1.6 ml) for each amino acid addition were incubated in 2 ml capped screw top sterile polypropylene microcentrifuge tubes in the dark at in situ temperatures. One sample was fixed at 10, 20 and 30 min, respectively, by adding 20% paraformaldehyde

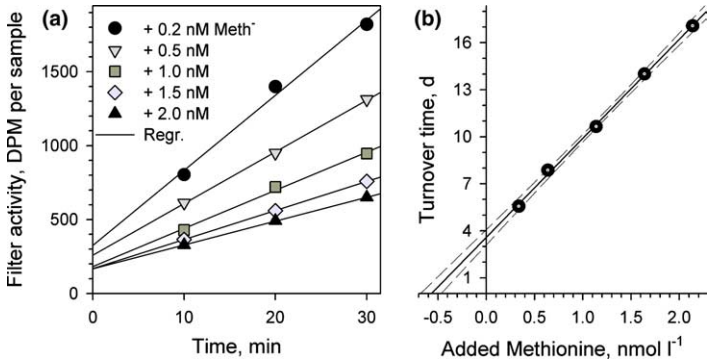

Fig. 2. A bioassay estimation of ambient methionine (Meth) concentration and turnover rate in a sample collected at 145 m; i.e. microbial uptake of Meth in a concentration dilution series, in which ^{35}S-Meth at 0.13 nM was diluted with different amounts of non-labelled Meth^{-}. (a) Time series at different Meth concentrations with linear regression lines (Regr.). (b) The resulting relationship between added Meth concentration and dissolved Meth pool turnover time, the *y*-axis intercept of the regression line ($r^2 = 0.998$, $P < 0.0001$) is an estimate of turnover time at an ambient Meth concentration which is the *x*-axis intercept. Dashed lines indicate 95% confidence intervals.

(PFA) to 1% final concentration. The sample particulate material was harvested onto 0.2 μm pore size polycarbonate filters (Poretics Corporation, USA) and washed with deionised water. Radioactivity retained on filters was measured as disintegrations per minute (DPM) using a liquid scintillation counter (Tri-Carb 3100, Perkin–Elmer) onboard ship. The rate of precursor uptake was calculated as the slope of the linear regression of radioactivity against incubation time ($r^2 > 0.93$, $P < 0.0001$; see example in Fig. 2(a)) and used to compute an amino acid turnover time by dividing the amount of radioactivity added to a sample by the rate of its uptake per time unit, e.g. day. The resulting turnover times were plotted against a corresponding concentration of *added* amino acid and extrapolated using linear regression ($r^2 > 0.94$, $P < 0.0001$; see example in Fig. 2(b)). The regression line intercept of the *x*-axis gave an estimate of an ambient concentration of amino acid, whereas the regression line intercept of the *y*-axis gave an estimate of amino acid turnover time at the ambient concentration.

2.3. Flow cytometric sorting of radioactively labelled bacterioplankton groups

Amino acid uptake of *Prochlorococcus* cyanobacteria and other bacterioplankton groups was determined using ^{35}S-methionine [14,16], which was added at 0.84 ± 0.12 nM concentration. Five replicated 1.6 ml samples were incubated at in situ temperature in the dark, fixed with 1% PFA after 3 h, incubated at 2 °C for 24 h and stored frozen at −80 °C before being analysed ashore.

Prochlorococcus cyanobacteria in thawed samples were enumerated and flow sorted with a FACSort flow cytometer (Becton Dickinson, Oxford, UK) in unstained samples using their chlorophyll-specific autofluorescence [1] that gave good discrimination between low red fluorescence (Pro LRF) and high red fluorescence (Pro HRF) cells (Fig. 3(a)) in samples that came from near the deep chlorophyll maximum. Abundance of bacterioplankton was determined after staining with SYBR Green I DNA dye [17] (Fig. 3(b)–(c)). A yellow–green 0.5 μm bead standard (Fluoresbrite Microparticles, Polysciences, Warrington, USA) was used in all analyses.

The low chlorophyll content of the Pro LRF did not allow their clear separation from other bacterioplankton. In seven out of eight surface samples the Pro LRF population could not be completely resolved even in unstained samples, and consequently only the visible part of the population was flow sorted. In these cases we estimated a visible fraction of the population (0.3–0.8) to compute the total Pro LRF concentration. The staining was used to visualize other bacterioplankton groups (Fig. 3(b)–(c)) and the target cells were flow sorted using single-cell sort mode, sorting at a rate of 10–250 particles s^{-1}. *Prochlorococcus* cells in surface water samples were sorted from unstained samples and in deep water samples from both unstained and stained samples using their red autofluorescence (Fig. 3). Sorted cells were collected onto 0.2 μm pore size nylon filters (Nylaflow, Pall Life Science, USA), washed with deionised water and radioassayed. Three proportional numbers of the cells (from 1, 2 and 3×10^3 cells to 10, 20 and 30×10^3 cells, depending on sample concentration) were sorted, and the mean cellular methionine uptake was determined as the slope of the linear regression of radioactivity against the number of sorted bacteria (Fig. 4(a)). Radioactivity retained on filters was measured as counts per minute (CPM) using an ultra-low level liquid scintillation counter (1220 Quantulus, Wallac, Finland) and desintegrations per minute (DPM) were computed to correct for the radioactive decay.

Two types of control were employed to check reproducibility of gate sorting. Firstly, we compared the

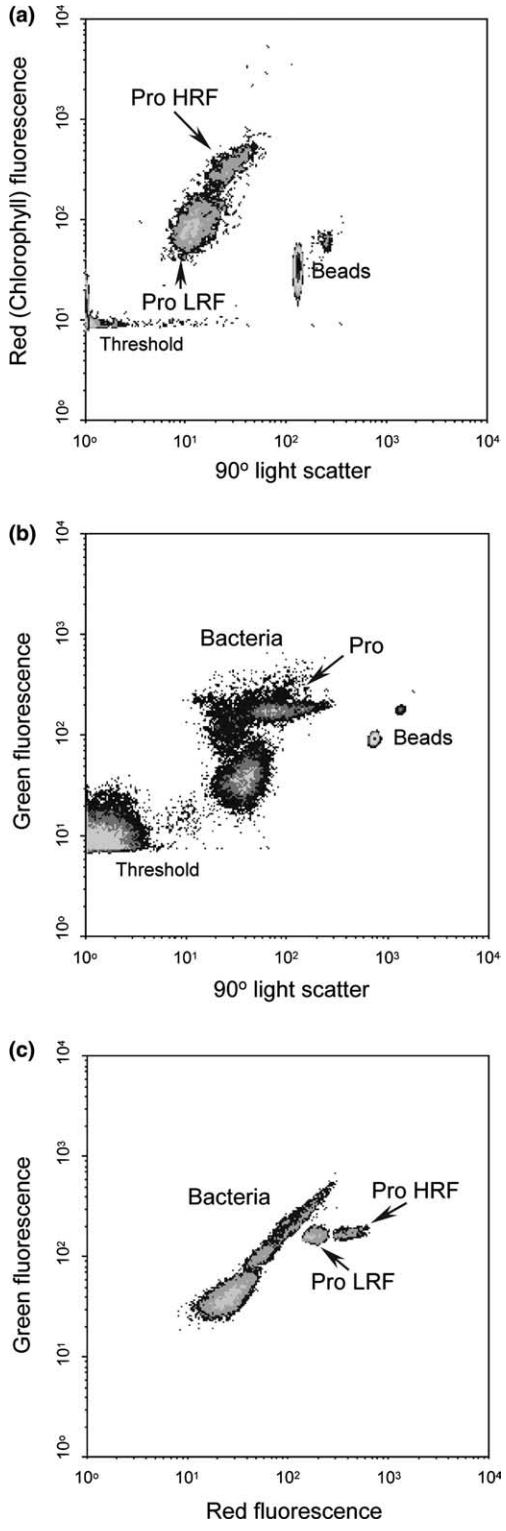

Fig. 3. Characteristic flow cytometric signature of high (HRF) and low red fluorescent (LRF) *Prochlorococcus* cyanobacteria in unstained (a) and SYBR Green I stained samples ((b) and (c)) collected from 135 m. Total bacterioplankton (bacteria) was visualised by plotting 90° light scatter of analysed particles versus their green DNA fluorescence (b). Then red fluorescence of bacterioplankton was plotted against their green fluorescence to reveal *Prochlorococcus* and to separate Pro HRF and Pro LRF (c). Arrows indicate the sorted cell populations.

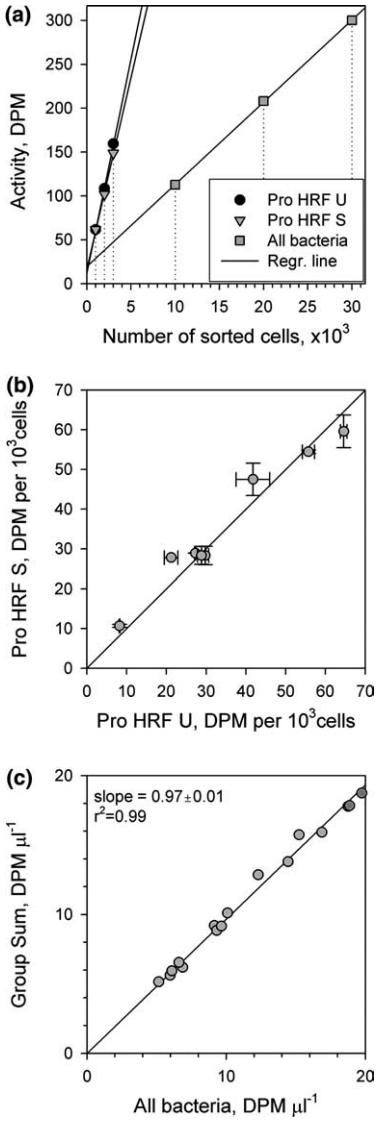

Fig. 4. An example of the flow sorting radioassay. (a) ^{35}S-Methionine uptake by flow cytometrically sorted general bacterial cells (all bacteria) and *Prochlorococcus* cells with high red fluorescence (Pro HRF), the latter were sorted from the replicated unstained (U) and SYBR Green I stained (S) samples (sorted cell number series). (b) Comparison of ^{35}S-Met uptake by unstained and stained *Prochlorococcus* HRF sorted from different deepwater samples. Error bars indicate a single standard error of triple sortings. Solid lines show linear regressions. (c) Comparison of methionine uptake of all bacterioplankton, i.e. determined uptake rate of a flow sorted mean bacterial cell multiplied by total bacterioplankton concentration, and a sum of the sorted group uptake rates, i.e. a sum of determined uptake rate of sorted groups multiplied by corresponding group concentrations.

cellular activities of *Prochlorococcus* sorted in duplicate in eight unstained and stained deepwater samples (Fig. 4(b)). The cellular activity determined correlated with a 1:1 relationship ($r^2 = 0.96$, $P < 0.0001$, $n = 8$). Secondly, we compared activities of all bacterioplankton communities and a sum of four to five bacterial group populations that comprised the communities. The population

activities were calculated by multiplying the mean cellular activities of a group by its cell abundance. The relationship between all the bacterioplankton and sum of group populations was also very close to 1:1 (Fig. 4(c)).

3. Results and discussion

3.1. Concentration and microbial turnover of amino acids

Microbial turnover of individual compounds is seldom determined [18]; partly because of methodological difficulties, particularly in separation of particulate and dissolved pools of a compound and partly because of a perception that individual compounds would be inadequate to explain the dynamics of a complex pool of dissolved organic matter.

There were two reasons why it was decided to estimate amino acid concentrations using bioassays. Firstly, it was important to know the ambient concentration of amino acids for conducting isotopic tracer studies on microorganisms. Secondly, a bioassay would avoid the uncertainties of analytical determinations associated with separation of dissolved and particulate pools, because bioassays are done with untreated live samples. The main disadvantage of a concentration series bioassay is the extrapolation step of an empirical relationship for estimating the ambient concentration and turnover. The regressions in the presented assays were statistically significant.

The concentrations and microbial turnover rates of the two dissolved amino acids, leucine and methionine, in the oligotrophic gyre varied within a threefold margin (Fig. 5 and Table 1). There was some increase of the turnover of leucine towards the southern end of the transected area (Fig. 5(c), two right outstanding points). The leucine concentration was about half of the methionine concentration, the methionine concentration was insignificantly higher at depth. The methionine pool was turning over 2.4 and 2 times slower than the leucine pool in the surface and deep waters, respectively and consequently the turnover rates of both amino acids were remarkably similar. If one assumes that leucine and methionine are representative dissolved free amino acids (DFAA) and multiply their average turnover rates by 20, the number of common amino acids, the estimated total turnover rate of DFAA in the Southern Atlantic gyre would be about 6 $\mathrm{nmol\,N\,l^{-1}\,d^{-1}}$ (0.25 $\mathrm{nmol\,N\,l^{-1}\,h^{-1}}$), that is at the low end of values reported for more productive oceanic and coastal areas [18].

One of the important practical outcomes of these estimates was that it would be very difficult to conduct successful flow sorting experiments using tracer (<0.01–0.05 nM) concentrations of radiolabelled methionine, i.e. to flow sort a sufficient number of cells by a low speed sorter for detectable measurement of incorporated radiolabel. We had to adopt a different approach by adding radiolabelled methionine at concentrations comparable to ambient but that would not alter the dynamics of the precursor uptake. The uptake of compounds by microorganisms is linearly proportional to the compound concentration when the latter concentration is close to ambient (Fig. 2(b)). Indeed this proportionality was confirmed in all bioassays used to estimate ambient compound concentrations and turnover rates, and it was expected that bacterioplankton would take up radiolabelled methionine similarly to natural concentrations. The long incubation time of 3 h was chosen to maximise the amount of radiolabel taken up

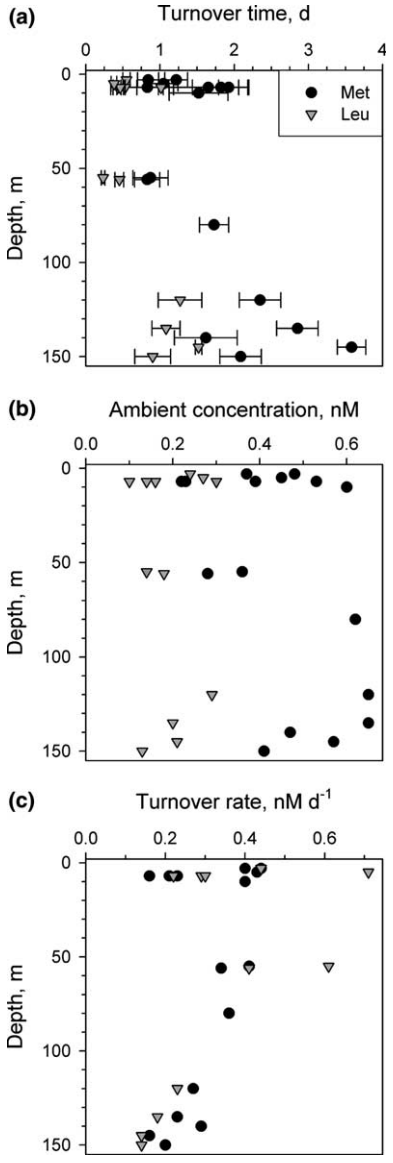

Fig. 5. Comparison of vertical distributions (a) of microbial turnover time of amino acids, methionine and leucine, (b) of ambient concentrations of methionine and leucine and (c) of their turnover rates by the whole microbial community. Error bars indicate single standard error of the bioassay measurements.

Table 1
Bioassay estimated concentrations and microbial dynamics of the dissolved methionine (Meth) and leucine (Leu) in the Southern Atlantic gyre

Depth range (m)	Concentration (nM), avg ± sd		Turnover time (d), avg ± sd		Turnover rate (nM d^{-1}), avg ± sd	
	Meth	Leu	Meth	Leu	Meth	Leu
3–80	0.40 ± 0.13	0.19 ± 0.07	1.30 ± 0.44	0.51 ± 0.23	0.32 ± 0.10	0.41 ± 0.17
120–150	0.54 ± 0.10	0.21 ± 0.07	2.50 ± 0.75	1.19 ± 0.26	0.22 ± 0.05	0.17 ± 0.04

by bacterioplankton cells, because the flow sorting was done two months after the cruise and ^{35}S isotope has a relatively short half-life of 87.5 days. If the sorting is done on board the incubation time could be easily decreased to 1–2 h. The estimated microbial turnover rates of methionine were assumed to correspond to the whole bacterioplankton community, i.e. an activity of an average flow sorted bacterial cell multiplied by the total concentration of bacterioplankton. Using this link we could estimate the actual uptake rate of ambient dissolved methionine by *Prochlorococcus* cells.

3.2. Determination of methionine uptake by high- and low-red fluorescence Prochlorococcus cells

Pro LRF was abundant in traversed waters down to 130 m with highest concentrations above 200×10^3 cells ml^{-1} at the surface (Fig. 6(a)) and Pro HRF was common at depths below 130 m, 50×10^3 cells ml^{-1}. In one case (depth 120 m) the Pro LRF and Pro HRF populations could not be clearly separated using their scatter properties and chlorophyll contents and we sampled them together and report these data as Pro total only. The clear switching of dominance between the two types of cells was usually below 130 m and Pro HRF were not observed at the edges of the gyre.

The original radioactivity counts of flow sorted *Prochlorococcus* (Fig. 6(b)) are presented primarily to show reproducibility of the measurements made. An outstanding feature of these data is a clearly higher uptake of ^{35}S-methionine by the Pro HRF compared to the Pro LRF. By combining the flow sorting data with estimated total microbial uptake of methionine, assuming that the latter integrates the activity of all bacterioplankton cells, we calculated the cellular specific uptake rate of the *Prochlorococcus* cells (Fig. 7(a)) and compared them with the corresponding uptake rates of a mean bacterial cell in the same sample (Fig. 7(b)). After the differences in estimated ambient methionine concentrations and turnover rates were taken into account the Pro HRF still had considerably, on average 4 times, higher cellular specific uptake rate than the Pro LRF. The difference was particularly striking in the same deepwater samples. The uptake rate of the Pro LRF was on average comparable to the uptake rate of a mean bacterial cell down to 80 m, while the uptake rate of the Pro HRF was 2.5–7 times higher than that of a mean bacterial cell. The data unequivocally demonstrated that the Pro HRF was taking methionine and very possibly other dissolved amino acids at a rate considerably higher than other bacterial cells.

Because the Pro HRF cells are usually bigger than the Pro LRF cells the biomass specific rates of the *Prochlorococcus* cells were calculated. The bead standard was used for estimating the relative values of 90° or side light scatter (SSC) of bacterial cells. Using the side scatter as a proxy of cell biomass [19] we could tentatively compare biomass specific uptakes. The computed biomass specific uptake of *Prochlorococcus* cells showed higher variability compared with the cell specific activity and the differences between the two types of cells became less pronounced (Fig. 7(c)) and more comparable with other

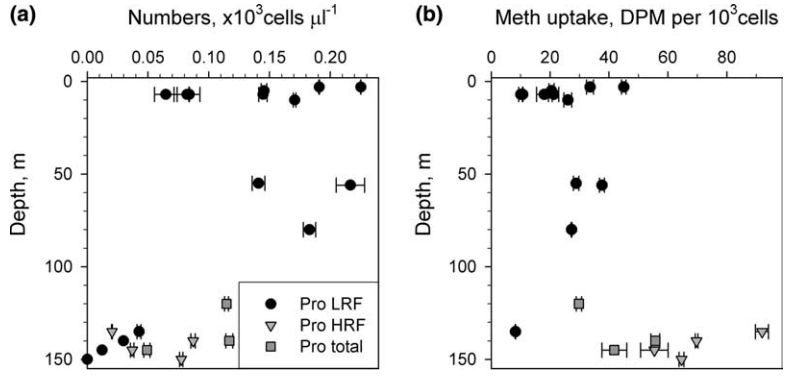

Fig. 6. Vertical distributions of *Prochlorococcus* LRF and HRF as well as the total population (Pro total) (a) abundance and (b) uncorrected flow sorting measurements of methionine uptake rates. Error bars indicate a single standard error of three measurements.

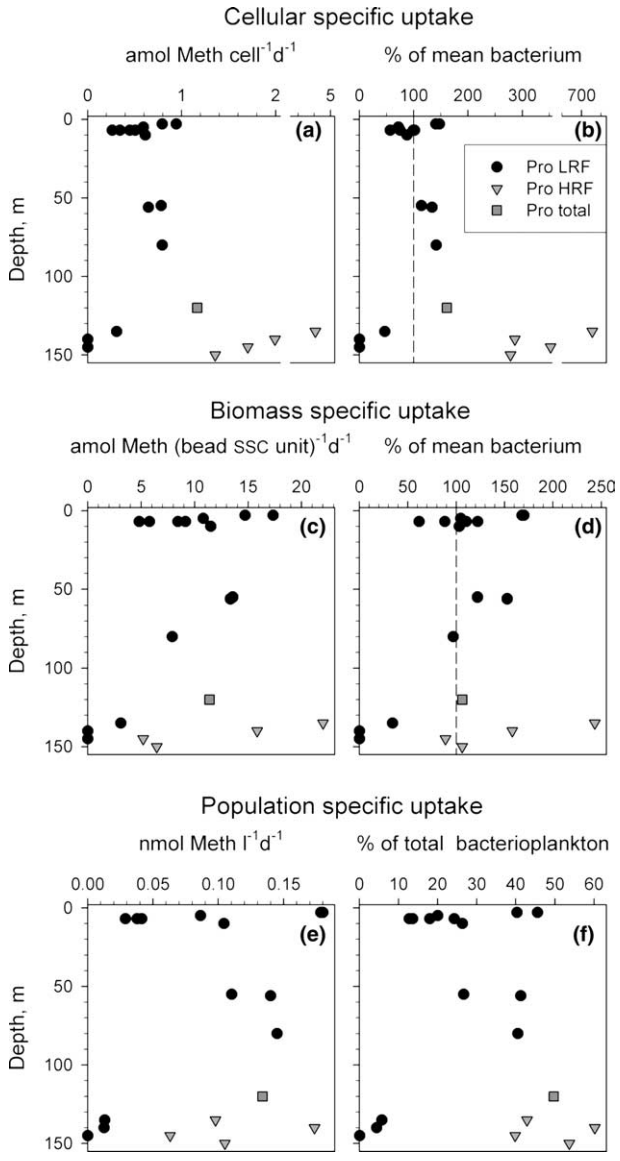

Fig. 7. Vertical distributions of *Prochlorococcus* LRF and HRF absolute (a, c and e) and relative to all bacterioplankton, including *Prochlorococcus* (b, d and f) cellular specific (a, b), biomass specific (c, d) and population specific (e, f) uptake rates of methionine in the Southern Atlantic gyre. Cellular biomass was estimated using cellular 90° light scatter or side scatter (SSC) in bead standard relative units. Dashed lines indicate 100% value, i.e. activity equal to an average bacterioplankton cell. In a case where separation between Pro LRF and HRF was unclear a value of combined *Prochlorococcus* (total) was shown.

bacterial cells (Fig. 7(d)). Therefore it seems likely that the high cellular uptake rate of Pro HRF could be at least partially explained by its large cell size.

The population specific uptake rates of the Pro LRF and Pro HRF were relatively similar (Fig. 7(e)), they were consuming about the same amount of methionine daily. The observed high variability in the surface waters could be explained by high variability of ambient methionine concentration (Fig. 5(b)). The Pro LRF population was contributing about 25% to the uptake of methionine by total bacterioplankton community (All Bact) and the contribution of the Pro HRF population was twice as high, about 50% (Fig. 7(f)).

If methionine dynamics are representative of dynamics of other amino acids as a previous study suggested [14] and the agreement between methionine and leucine dynamics further supports (Fig. 5(c)), one could speculate that the Pro LRF population was consuming about a quarter of the total dissolved amino acid pool in the surface mixed layer. Its uptake drastically decreased at depth, most likely indicating general reduction of metabolic activity in the low light conditions. The Pro HRF was very successful in amino acid uptake at depths generally below 100 m, where it seemed to dominate bacterioplankton. ProHRF controlled the amino acid pool by consuming half of it whilst numerically representing only 15% of all bacterioplankton. Therefore, the amino acid pool seems to be an important source of nutrients for the Pro HRF.

The present results from the Atlantic Ocean confirm the previous demonstration of the role of *Prochlorococcus* in the consumption of organic nitrogen compounds in the oligotrophic Indian Ocean [14] suggesting that partial heterotrophy of *Prochlorococcus* is a general feature. It seems that in oligotrophic waters *Prochlorococcus* directly and successfully competes with other bacterioplankton populations, probably using photosynthesis to fuel an energy demanding transport system for efficient acquisition of organic nitrogen compounds at ultra low (<1 nM) ambient concentrations. The methionine uptake by Pro LRF in the dark was usually somewhat lower than under light (our unpublished data from the same cruise). By consuming amino acids *Prochlorococcus* deprived other bacteria of an important source of organic nitrogen, forcing the latter to use inorganic nitrogen at a higher energetic cost in the organic nutrient limited oligotrophic environment. To evaluate how important dissolved amino acids could be to *Prochlorococcus* diet we estimated how much the uptake of methionine could contribute to their biomass and production (Table 2). Additionally the biomass-specific activity was calculated by dividing the cellular activity by mean cellular biomass SSC proxy. The Pro LRF uptake of methionine was similar down to depths of Pro HRF dominance (135 m) where they formed mixed communities, however, below 135 m Pro LRF concentrations dropped sharply and their methionine uptake rate also dropped (Fig. 7(c) and (d)). The cellular Pro HRF methionine uptake was 4 times higher than the Pro LRF one. As mentioned above, this was only partly because of the large cell size of the Pro HRF. The amount of methionine taken up by a mean *Prochlorococcus* cell daily was estimated to contribute about 0.44% and 0.76% towards cellular biomass of Pro LRF and Pro HRF respectively (Table 2).

Table 2
Estimated cell size, biomass and methionine uptake by the LRF *Prochlorococcus* in the mixed layer (LRF, 3–80 m) and HRF type in deep waters (135–150 m)

Prochlorococcus type	Diameter[a] (μm)	Biomass (fg protein cell^{-1})	Methionine uptake	
			amol cell^{-1} d^{-1}	% cell protein d^{-1}
Pro LRF	0.58 ± 0.05 (14)	21	0.61 ± 0.22 (11)	0.44
Pro HRF	0.77 ± 0.08 (4)	48	2.5 ± 1.6 (4)	0.76

[a] Cellular diameter data was taken from our previous study of *Prochlorococcus* in the same region [22]. Diameters were estimated using a size fractionation method. A factor of 0.2 pg C μm^{-3} was used to convert cell volume to cell carbon content, and carbon and protein cellular contents were assumed to be equal [23].

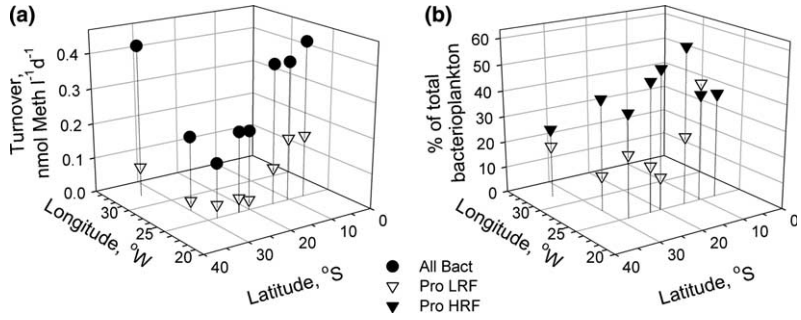

Fig. 8. Spatial distribution of absolute methionine (Meth) rates of total bacterioplankton (All Bact) and Pro LRF population in surface waters (a) and percentages of Pro LRF and Pro HRF contributions to total turnover of Meth by bacterioplankton in surface and deep (below 120 m) waters, respectively (b).

Therefore a single dissolved amino acid at an ambient concentration below 0.5 nM seems to be a measurable and relatively important source of organic nitrogen for *Prochlorococcus* cells at all depths. If we speculate that the other amino acids are present at similar concentrations and could be taken at comparable rates, as leucine (Table 1) and tyrosine data (our unpublished data from the same cruise) suggest, the combined contribution of about 20 amino acids to *Prochlorococcus* diet could be 10–15% of biomass. Considering that only a fraction of *Prochlorococcus* cells divide every day, such a contribution would have even more importance. It could be from 12% and 20% for Pro LRF and Pro HRF, respectively, using the higher estimates of *Prochlorococcus* division rates 0.55 d^{-1} [20], to up to 45% and 70% respectively using the lower estimates of *Prochlorococcus* division rates 0.15 d^{-1} [21].

Thus the present study has confirmed that there is no classical clear distinction between auto/photo- and hetero-trophic microorganisms in the oligotrophic ocean and photosynthetic *Prochlorococcus* cyanobacteria can take up key nutrients such as nitrogen heterotrophically in the form of amino acids at rates comparable or even exceeding the rates of other bacterioplankton populations (Fig. 8(a)). Although the absolute rates of amino acid turnover by bacterioplankton were lower in the middle of the gyre the contribution of *Prochlorococcus* population was relatively similar, somewhat higher values were measured in the northern area close to the equator. The contribution of amino acids to *Prochlorococcus* diet seems to be higher in deep waters (Fig. 8(b)). The uptake of dissolved amino acids could satisfy from 12%–20% to 45%–70% of nutrient requirements for *Prochlorococcus* LRF and HRF growth, respectively, depending on growth rate. *Prochlorococcus* HRF living at depths below 120 m has on average four times higher cellular specific amino acids uptake rate than *Prochlorococcus* LRF living in the surface mixed layer. This difference could be partially explained by the fact that deep *Prochlorococcus* cells are twice the biomass of the surface *Prochlorococcus*. In mixed populations, when surface LRF *Prochlorococcus* was found at depths below 120 m its cellular amino acid uptake rate was 6% or less that of the HRF *Prochlorococcus*, suggesting that the former was in an environment outside its optimal growth range. To answer the question as to whether the observed difference could be attributed to physiological adaptation of the same ecotypes or to changes in the *Prochlorococcus* ecotype composition [3,4] the phylogentic characterisation of the dominant *Prochlorococcus* ecotypes at different depths will be presented in a separate study.

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