

## Photoheterotrophy of bacterioplankton is ubiquitous in the oligotrophic ocean

Claire Evans<sup>a</sup>, Paola R. Gómez-Pereira<sup>a,1</sup>, Adrian P. Martin<sup>a</sup>, David J. Scanlan<sup>b</sup>, Mikhail V. Zubkov<sup>a\*</sup>

<sup>a</sup>National Oceanography Centre, Ocean Biogeochemistry and Ecosystems Research Group, European Way, Southampton, UK

<sup>b</sup>School of Life Sciences, University of Warwick, Coventry, UK

<sup>1</sup>Present address: Phillips Research, 101 Cambridge Science Park, Milton Road, Cambridge, UK

\*Communicating author email: [mvz@noc.ac.uk](mailto:mvz@noc.ac.uk) Tel: +44 (0)2380 596335 Fax: +44 (0)2380 596247

### Abstract

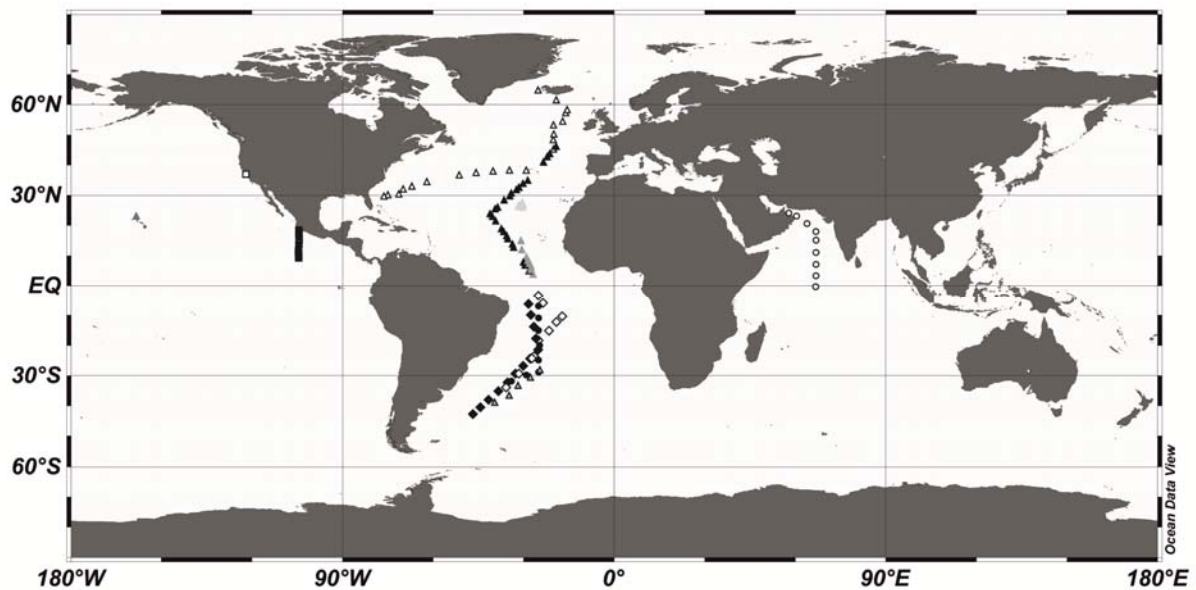
Accurate measurements in the Southern Hemisphere were required to test a hypothesis of the ubiquity of photoheterotrophy in the oligotrophic ocean. We present experimental results of light-enhanced uptake of methionine, leucine and ATP by bacterioplankton during two large-scale transects of the South Atlantic. Light increased the uptake of substrates by both dominant bacterioplankton groups: *Prochlorococcus* and SAR11, as well as for the bulk microbial community. Our consistent experimental evidence strongly indicates that photoheterotrophy is characteristic of dominant bacterioplankton populations in the global oligotrophic ocean.

**Keywords:** bacterioplankton, photoheterotrophy, oligotrophic, light, South Atlantic

## 1. Introduction

The widespread ability of marine bacteria to utilize light was first indicated by the observation of bacteriochlorophyll *a*-containing microorganisms in the Pacific (Kolber et al., 2000) and the discovery of the proteorhodopsin gene in samples from Monterey Bay (Beja et al., 2000). The proteorhodopsin pigment was found to function as a light-driven proton pump capable of generating chemiosmotic membrane potential that can be directed towards processes such as ATP synthesis, motility or active transport (Spudich et al., 2000). Soon after, the cyanobacteria *Prochlorococcus*, which harvests light using a chlorophyll-based antenna complex (Chisholm et al., 1988), were found to be able to import organic substrates (Zubkov et al., 2003). Prokaryotes possessing the means to harvest light, such as the proteorhodopsin containing SAR11 alphaproteobacteria and *Prochlorococcus*, dominate oceanic prokaryotic communities (Chisholm et al., 1988; Morris et al., 2002).

At present, a consensus is lacking as to the prevalence of photoheterotrophy in marine systems. However, it appears to be influenced by ecosystem productivity, since in coastal waters light can inhibit substrate uptake (Alonso-Saez et al., 2006; Ruiz-González et al., 2012), whereas photoheterotrophy has been consistently reported in studies of oligotrophic stratified waters (Church et al., 2004; 2006; Michelou et al., 2007; Mary et al., 2008; Gómez-Pereira et al., 2013). Given that these oligotrophic waters harbour the most extensive ecosystems on Earth, it is important to establish whether photoheterotrophy is a general feature of the bacterioplankton which dominate these regions, in order to determine the full significance of light to biomass accumulation and biogeochemical functioning. To date, this endeavour is hampered by the scarcity of data available from the Southern hemisphere (Mary et al., 2008) as the majority of measurements have been made in the north Pacific and Atlantic (Church et al., 2004; 2006; Michelou et al., 2007; Mary et al., 2008; Gómez-Pereira et al., 2013) (Figure 1). The northern hemisphere has a larger continental land mass resulting in greater mineral aerosol deposition which increases the availability of trace metals, such as Fe and Pb, in the north relative to the south (Duce and Tindale 1991; Henderson and Maier-Reimer 2002). Hence the nutrient regimes of the oligotrophic waters of the northern and southern hemispheres are distinct (Moore et al 2013) which could potentially cause differences in the role and significance of photoheterotrophic nutrient acquisition between these biogeochemically distinct hemispheres. Photoheterotrophy is typically assessed by the quantification of light mediated organic nutrient uptake by either bulk bacterioplankton (Church et al., 2004; 2006) or on flow cytometrically sorted bacterial groups (Michelou et al., 2007; Mary et al., 2008; Gómez-Pereira et al., 2013). The rate at which a bacterial cell takes up different organic molecules is



**Figure 1** Overview of the sites for which evidence of photoheterotrophy has been shown or photoheterotrophy has been recorded in the form of light-enhanced bacterial substrates uptake (not comprehensive). (□, Beja et al., 2000) detection of bacterial Rhodopsin, (■, Kolber et al., 2000) bacterial photosynthesis detected, (●, Zubkov et al., 2004; ○, Zubkov et al., 2006a) amino acid uptake by photosynthetic bacterioplankton, (▲, Church et al., 2004; 2006) bulk light-enhanced leucine uptake, (△, Michelou et al., 2007) bulk and cell-specific light-enhanced leucine uptake, (▲,▲, Mary et al., 2008) SAR11 and *Prochlorococcus* specific light-enhanced amino acid uptake, (▲,▲, Gómez-Pereira et al., 2013; ◇,◆, this study).

related to its specific metabolic requirements. For instance amino acids are taken at a greater rate than nucleotides because cells synthesise more protein than nucleic acids (Gómez-Pereira et al., 2013). Similarly, leucine is taken up at a higher rate than methionine because of a higher proportion of the former in bacterial protein (Mary et al., 2008). Organic substrate uptake is also likely driven by nutrient requirements, for example ATP may help satisfy cellular phosphorus requirements (Alonso-Saez and Gasol, 2007), whereas, methionine may supply sulphur (Mary et al., 2008). Hence, when assessing photoheterotrophy by light-mediated substrate uptake a range of different substrates should be used.

We sought to assess the ubiquity of bacterioplankton photoheterotrophy in the oligotrophic ocean and identify the organism responsible. This was achieved by redressing the imbalance between the more extensive measurements in the northern hemisphere and the scarcity of measurements in the southern hemisphere (Figure 1). To do this, the effect of light on the uptake of ATP and the amino acids leucine and methionine by the bacterioplankton was tested during two cruises to the South Atlantic. Here we show that photoheterotrophy was consistently detected in the bulk bacterioplankton, as well as the two dominant groups *Prochlorococcus* and SAR11. These measurements indicate that photoheterotrophy is a

widespread biological process in the south Atlantic subtropical gyre, which when combined with the existing data, supports the conclusion that photoheterotrophy is ubiquitous in the oligotrophic ocean.

## **2. Materials and methods**

### **2.1 Study site and sampling procedure**

The study was conducted on board the Royal Research Ships the *James Cook* (cruise no. JC53) and the *James Clark Ross* (cruise no. JR300) from October to November 2010 and 2013, respectively (Figure 1). Seawater samples were collected from 20 m as a representative depth for the surface mixed layer using a sampling rosette of 20 L Niskin bottles mounted on a conductivity-temperature-depth (CTD) profiler. A depth of 20 m was selected as it represented the shallowest depth at which the majority of biologically harmful higher energy wavelengths of light are attenuated. Stations sampled were located throughout the Southern Atlantic Gyre (SAG) with three in the Southern Temperate Waters (STW). During JC53 a preliminary study of the ambient concentration and turnover times of methionine and ATP, and the influence of light on the uptake of these organic substrates by total bacterioplankton, was conducted. On JR300 leucine and ATP were used to assess light-stimulated uptake by specific bacterioplankton groups in addition to the total bacterioplankton.

### **2.2 Bacterioplankton**

Bacterioplankton were enumerated by flow cytometry (FACSort, Becton Dickinson, UK) from samples fixed with paraformaldehyde (PFA, 1% final concentration) and stained with the DNA-specific dye SYBR Green I (ref. Marie et al., 1997). An internal standard of 0.5 and 1.0  $\mu\text{m}$  beads (Fluoresbrite microparticles, Polysciences), the concentration of which was determined by syringe pump flow cytometry (Zubkov and Burkill, 2006b), was added to each of the samples. Bacterioplankton groups were distinguished according to their DNA content and scatter properties. SAR11 alphaproteobacteria and *Prochlorococcus* cyanobacteria were defined in accordance with previous molecular identification of flow cytometrically sorted bacterioplankton cells (Mary et al., 2006; Gómez-Pereira et al., 2013).

### **2.3 Ambient concentration and turnover rates of organic substrates**

The ambient concentrations and turnover rates of leucine, methionine and ATP in the waters were determined using the isotopic dilution time series bioassay (Wright and Hobbie, 1966; Zubkov et al., 2004). L-[4,5-<sup>3</sup>H]-leucine (specific activity 140 Ci mmol<sup>-1</sup>) was added into 2 mL polypropylene crystal clear microcentrifuge tubes (Starlab, Milton Keynes) to achieve final concentrations ranging from 0.1 to 1 nM in the 1.6 mL seawater samples. Immediately after collection, seawater was mixed with the labelled substrate (marking the start of the experiment) and a sample from each concentration was fixed with PFA (1% final concentration) at 10, 20, 30 and 40 min. Particulate matter in the samples was collected by

filtration onto 0.2 µm pore-size polycarbonate filters, which were then washed twice with 4 mL of deionised water. To determine the radioactivity of the retained particulate matter the filters were placed in scintillation vials, which were subsequently filled with scintillation cocktail. Vials were placed into a liquid scintillation counter (Tri-Carb, 3100TR, Perkin-Elmer, Beaconsfield, UK) and analysed. Substrate concentration, uptake rate and turnover time were calculated as previously described (Wright and Hobbie, 1966; Zubkov et al., 2005). Briefly, leucine uptake rates were calculated from regression analysis of the radioactivity incorporated into particulate material plotted against incubation time. This was used to derive a turnover time for each of the concentrations of leucine by dividing the amount of radioactivity added to the sample by its uptake rate per unit time. The calculated turnover time was then plotted for each of the leucine concentrations used and extrapolated using linear regression. Assuming constant rates of removal and regeneration, leucine uptake rate ( $V$ ) was estimated from the slope of the linear regression. The y intercept gave an estimate of amino acid turnover time at ambient concentration ( $t$ ) which can otherwise be expressed as the sum of the ambient concentrations ( $S$ ) plus the transport constant ( $K_t$ ). This can be expressed according to the equation:

$$S + K_t = V \times t$$

Where  $K_t$  is a measure of the uptake system's affinity for leucine, with a low value indicative of a high affinity. As bacterioplankton are well-adapted to living at ambient organic substrate concentrations we assume they are efficient at organic substrate uptake at ambient concentration and thus have a negligible  $K_t$  relative to ambient concentration. Therefore, it should be noted that our calculated ambient concentrations represent upper estimates.

The L-[<sup>35</sup>S]-methionine (specific activity >1000 Ci mmol<sup>-1</sup>) bioassay was completed in a similar manner, except that the labelled substrate was added at a standard concentration of 0.05 nM and diluted with unlabelled (cold) methionine, using a dilution series ranging from 0.05 to 1.0 nM. For the [ $\alpha$  <sup>33</sup>P]-ATP (specific activity 3000 Ci mmol<sup>-1</sup>) bioassay the labelled substrate was added at a standard concentration of 0.15 nM and also diluted with unlabelled (cold) ATP, using a dilution series ranging from 0.2 to 1.0 nM. Samples were fixed at 15, 30, 45, and 60 min.

#### **2.4 Bacterioplankton light and dark uptake**

All experimental setup and sample handling was completed under dim green light (<1 µmol photons m<sup>-2</sup> s<sup>-1</sup>, Gómez-Pereira et al., 2013). During the first cruise seawater samples were placed in 2 mL polypropylene crystal clear microcentrifuge tubes to which either 0.5 nM final concentration L-[<sup>35</sup>S]-methionine, or 0.1-0.4 nM final concentration [ $\alpha$  <sup>33</sup>P]-ATP was added. Each treatment was performed in four tubes which were placed into the light or dark simultaneously. The tubes were incubated in 6 L water-filled transparent tanks that were maintained at ambient seawater temperature by continual water recirculation through a

thermostatically controlled bath. For the dark incubations the water tank was sealed in two layers of black, plastic bags. For the light incubations two separate tanks were used, each of which was equipped with a warm white light emitting diode array (Photon Systems Instruments, Drasov, Czech Republic) adjusted to create two discrete light intensities. For the majority of experiments a setup receiving a standard light intensity of  $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  was paired with a setup receiving either a lower light intensity of 100, 110 or 250  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , or a higher light intensity of 750 or 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The tubes transmit 72% of the light at 400 nm increasing to 82% at 700 nm (Mary et al., 2008). Thus, the contents of the tubes would have received 350-410  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in the reference setups, 72-82  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in the low-light setups, and 720-820  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in the high-light setups. At each time point of a time series one of the tubes was removed and its content fixed with PFA. The fixed sample was filtered and analysed as described above, and the radioactivity taken up in the light and dark treatments was compared.

During the second cruise seawater samples were placed in 30 mL borosilicate glass bottles, to which either 0.4 nM final concentration L-[4,5-<sup>3</sup>H]-leucine or 0.1-0.4 nM final concentration [ $\alpha$  <sup>33</sup>P]-ATP was added. The bottles were incubated using the same tanks, except that only one light intensity was used (160  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at stations south to 27°S, and 250-300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  thereafter). A lower light intensity was purposefully selected for the first set of stations based on results obtained during the first cruise, which indicated that this level should be sufficient to stimulate light-enhanced uptake, whilst at the same time minimizing the risk of photoinhibition (Church et al., 2004). To examine isotope uptake experiments were sub-sampled every two hours over a time period of six hours, starting at either one or two hours post isotope addition. The subsamples were fixed, filtered and analysed as described above and the difference between radioactivity taken up in the light and dark treatments was compared. Additional subsamples were also taken from the incubations at the six hour time point to determine isotope uptake by specific microbial groups. This was achieved by radioassaying individual flow-sorted bacterioplankton populations.

After fixing and staining (as described in section 2.2), cells were sorted onto pre-washed 0.2  $\mu\text{m}$  polycarbonate filters at a rate of  $<300 \text{ particles s}^{-1}$  in single-cell sort mode for 1, 2, 3, and 4 min for each group, and analysed as described in section 2.3. At stations located within the SAG the sorted bacterioplankton groups were: average bacterioplankton, SAR11 and *Prochlorococcus* cells, whilst in the STW *Prochlorococcus* declined sharply and could not be sorted with confidence. For each group isotope uptake per cell was calculated by dividing the radioactivity taken up by the number of cells sorted, and then deriving an average from the four sorts conducted per group. For both the light and dark treatments an uptake rate in  $\text{nmol cell}^{-1} \text{ h}^{-1}$  was calculated (Gómez-Pereira et al., 2013). Briefly, the uptake rate per cell (c.p.m.  $\text{cell}^{-1}$ ) for either the light or dark treatment was divided by the total uptake (c.p.m.  $\text{l}^{-1}$ ) from that same treatment, and then multiplied by the microbial uptake rate in  $\text{nmol l}^{-1} \text{ h}^{-1}$

(as derived from the isotopic dilution assay described in section 2.3). Total uptake was then calculated from the filtered whole-water subsamples, as described above. To finally calculate leucine and ATP uptake rates in molecules per cell this rate was multiplied by Avogadro's number.

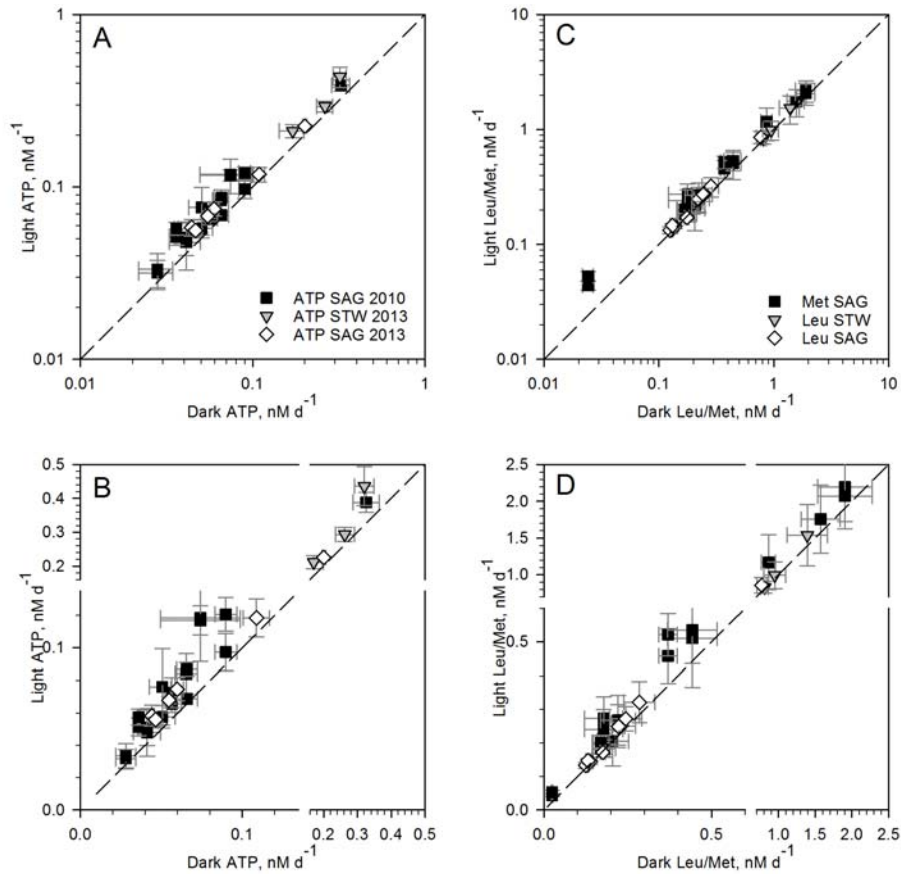
## 2.5 Statistics

To test whether the differences between light and dark treatments were significant, a paired t-test was applied to pooled data sets. Prior to this the data was checked for normality using the Shapiro-Wilk test, and non-normally distributed data was first log transformed. All statistical analyses were conducted in SigmaPlot 12.3.

## 3 Results

### 3.1 Effect of light on bacterioplankton organic nutrient uptake

Light significantly enhanced the microbial uptake of ATP and amino acids in the surface waters of the South Atlantic Ocean (Figure 2a, b, c and d). In the SAG total microbial  $^{33}\text{P}$ -ATP uptake in 2010 (JC53) was on average  $29 \pm 18\%$  higher in the light than in the dark. Three years later (JR300) the light-enhanced ATP uptake relative to uptake in the dark the dark recorded for the same region was approximately a third lower at  $19 \pm 7\%$  in the SAG and  $23 \pm 2\%$  in the STW. The lower values for the latter cruise are likely attributable to the range of light intensities employed for incubations (see section 3.2). During both cruises absolute uptakes rates of ATP in the SAG were comparable at  $0.10 \pm 0.07 \text{ nM d}^{-1}$  and  $0.07 \pm 0.07 \text{ nM d}^{-1}$  for 2010 and 2013 respectively, and in the STW were  $0.30 \pm 0.13 \text{ nM d}^{-1}$ . Light was found to enhance the amino acid methionine's uptake in the SAG by on average  $28 \pm 30\%$ . However, levels of light stimulated leucine uptake were lower at  $8 \pm 5\%$  and  $6 \pm 4\%$  in the SAG and in the STW respectively which was likely due to the on average lower light intensities employed. Absolute uptake rates in the light recorded for both amino acids in the SAG covered a similar range and were on average  $0.51 \pm 0.61 \text{ nM d}^{-1}$  for methionine and  $0.27 \pm 0.23 \text{ nM d}^{-1}$  for leucine. As for ATP, leucine uptake rates were higher in the STW at  $1.12 \pm 0.37 \text{ nM d}^{-1}$ .

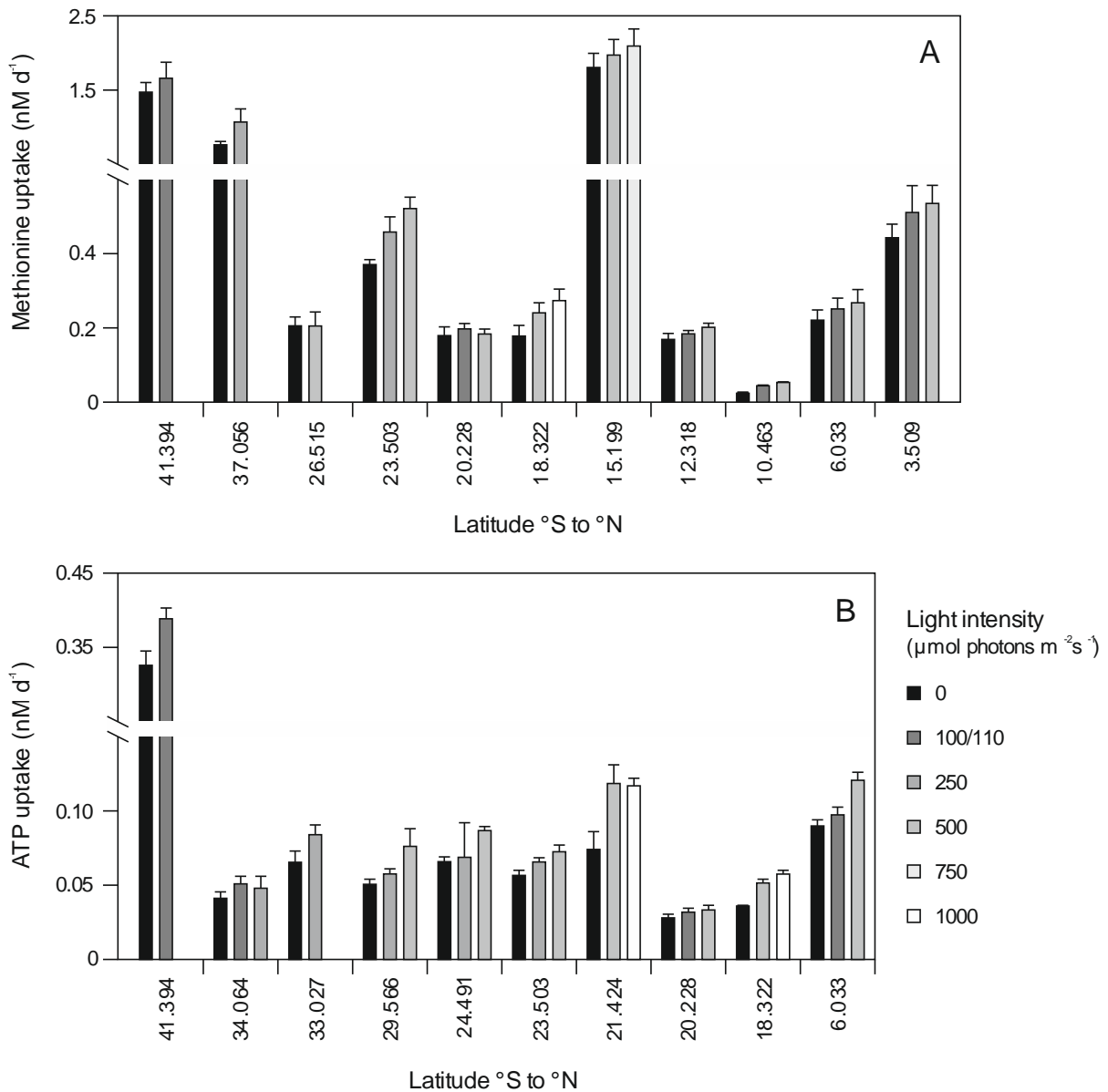


**Figure 2** Scatter plot comparison of the total bacterioplankton uptake of (A, B) ATP and (C, D) Leucine (Leu) and methionine (Met) in the light and the dark using (A, C) logarithmic and (B, D) linear scales, note the breaks. The dashed line indicates the unity line and error bars show se.

### 3.2 Effect of light intensity on bacterioplankton organic nutrient uptake

Incubation at a higher light intensity of 350-410  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  on cruise JC53 resulted in a greater light-stimulated microbial <sup>33</sup>P-ATP uptake of on average  $33 \pm 14\%$  in the waters of the South Atlantic, when compared to the same measurement at lower light intensity during cruise JR300 in the same region ( $20 \pm 6\%$  average increase at 160 to 300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) (Figure 2a and b). This relationship between light intensity and the level of light-stimulated ATP uptake is supported by the results of parallel incubations to determine the





**Figure 3** Uptake rates by total bacterioplankton of (A) ATP and (B) methionine (Met) in the dark and under varying light intensity. Light intensities are given in  $\mu\text{mol}$  per photon per  $\text{m}^2$  per  $\text{s}$ .

effect of light intensity on substrate uptake (Figure 3a). When compared to uptake in the dark, the degree of light-stimulated microbial ATP uptake was greatest in the populations incubated at the highest light intensity for six out of eight experiments (t-test P-value <0.05). This relationship was also found for light-stimulated uptake of the amino acid methionine, with all but one of the eight experiments showing proportionally greater light-enhanced uptake at the higher light intensity (Figure 3b; t-test P-value <0.05). On average methionine exhibited a much greater light-stimulated uptake ( $33 \pm 35\%$ ) in the South Atlantic surface waters when incubated at  $350\text{--}410 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  compared to leucine in the same region ( $8 \pm 5\%$ ) incubated at the lower light intensity of  $160\text{--}300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Figure 2b).

Despite the fact that incubation at higher light intensity generally resulted in greater uptake of organic nutrients, a direct relationship between light intensity and uptake rate could not be identified in the data. Specifically, even large increases in light intensity did not always increase uptake substantially, (for example ATP uptake at stations 21.424 °S and 18.322 °S; Figure 3a) potentially indicative of an irradiance threshold beyond which substrate uptake is not greatly increased.

### **3.3 ATP and leucine uptake by SAR11 and *Prochlorococcus***

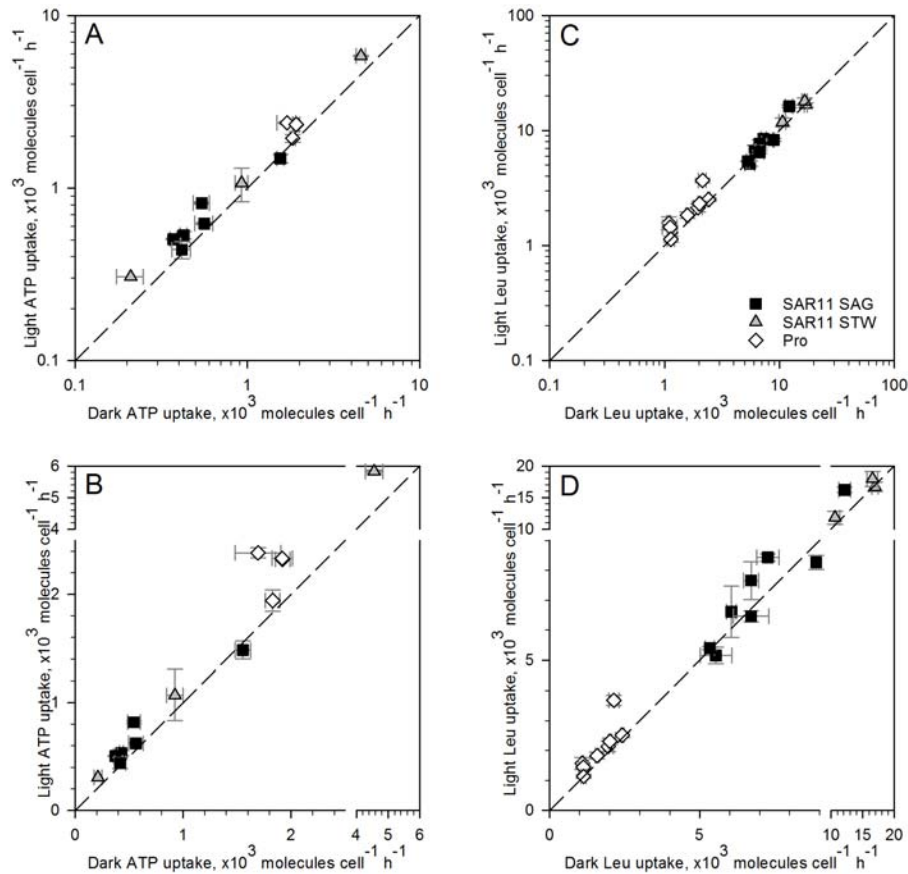
On average, uptake of ATP and amino acids by bulk bacterioplankton was higher in the light than in the dark (see section 3.1). However, it is unclear as to whether all the cells were responding in a similar way, or if only one or several groups behaved differently. To address this issue, individual subgroups of the bacterioplankton were flow-sorted.

The *Prochlorococcus* populations in the SAG took up similar amounts of both ATP and leucine at around 2000 and 2400 molecules cell<sup>-1</sup> h<sup>-1</sup>, respectively, in the presence of light (Figures 4a, b, c and d). Conversely, uptake rates of ATP and leucine by the SAR11 populations in the SAG differed by more than an order of magnitude, at on average 600 molecules cell<sup>-1</sup> h<sup>-1</sup> compared to 7500 molecules cell<sup>-1</sup> h<sup>-1</sup>, respectively. SAR11 uptake rates were on average greater in the STW compared to the SAG, being almost double for leucine, at on average 13000 molecules cell<sup>-1</sup> h<sup>-1</sup>, and approximately five times as much for ATP, at 3100 molecules cell<sup>-1</sup> h<sup>-1</sup>.

### **3.4 The effect of light on SAR11 and *Prochlorococcus***

For *Prochlorococcus*, light stimulation increased the uptake of leucine and ATP by a similar proportion at, on average, 24% and 21% respectively, whereas, for SAR11, light-stimulated uptake of leucine was (7%) below that of ATP (21%).

To assess whether any other major groups of bacterioplankton, aside from those sorted and described above, contributed to the light-stimulated uptakes of leucine and ATP, a budget was calculated. For each group its abundance relative to the total bacterioplankton abundance was multiplied by its average light-enhanced uptake. In the case of leucine in the surface water, *Prochlorococcus* accounted for more of the increase than SAR11 (60% versus 39%, of the total bacterioplankton light-stimulated uptake respectively), and taken together their contributions closed the budget for light-stimulated uptake by the total bacterioplankton. In the case of ATP, SAR11 made a greater overall contribution to the light-enhanced uptake compared with *Prochlorococcus* (49% versus 28%, of the total bacterioplankton light-stimulated uptake respectively) accounting for two thirds of light-stimulated uptake by the total bacterioplankton.



**Figure 4** Scatter plot comparison of (A, B) ATP and (C, D) Leu uptake in the light and dark by *Prochlorococcus* and SAR11 using (A, C) logarithmic and (B, D) linear scales, note the inclusion of breaks. The dashed line indicates the unity line and error bars show SE.

#### 4. Discussion

The consistently higher rates of substrate uptake by microbes in the light compared with the dark at stations in the South Atlantic indicate photoheterotrophy is common throughout this region. These findings overcome the previous barrier to establishing the global importance of photoheterotrophic photosynthetic processes as we provide the first major body of evidence from a region in the previously scarcely sampled Southern Hemisphere.

These findings contradict the conclusion made from a recent compilation of the data by Ruiz-González and colleagues that light may have a net inhibitory effect on marine microbial substrate uptake (Ruiz-González et al., 2013). Closer inspection of the compiled data, combined with the fact that photoheterotrophy is common in the oligotrophic stratified waters, suggest that this process may be dependent on ecosystem productivity (Gómez-Pereira et al., 2013). Pioneering studies of the North Pacific Gyre indicated light-stimulated uptake of the amino acid leucine in *Prochlorococcus*-dominated waters (Church et al., 2004; 2006). Later, a single-cell approach determined that *Prochlorococcus* were responsible for the majority of light-stimulated amino acid uptake in the North Atlantic, and it was

speculated that the unaccounted for remainder of the uptake could most likely be attributed to proteorhodopsin-containing bacteria (Michelou et al., 2007). Mary and colleagues (2007) were the first to provide actual light-stimulated uptake rates for both SAR11 and *Prochlorococcus* which they did using near-ambient concentrations of methionine and leucine in the Atlantic Ocean (Mary et al., 2008). Similar methods were recently employed in the North Atlantic Subtropical Gyre by Gómez-Pereira and colleagues (2012), who found that light stimulation increased the uptake rates of methionine and ATP by a third in these bacterioplankton groups. Here, light-enhanced uptake of organic substrates in both SAR11 and *Prochlorococcus* during two cruises traversing the south Atlantic, give confidence that photoheterotrophy can be confidently extrapolated to all oligotrophic water masses. Furthermore, reports of light enhanced organic nutrient uptake in temperate waters (Michelou et al., 2007; Mary et al., 2008; Gómez-Pereira et al., 2013; this study) suggests that while photoheterotrophic bacterioplankton groups are a ubiquitous feature of oligotrophic waters they are not confined to them.

The purpose of a photoheterotrophic nutritional strategy to cell metabolism has been debated in the literature in the context of contrasting experimental findings. Culture studies have indicated that light does not promote the growth of proteorhodopsin containing bacteria but rather may serve to support them during times of energy starvation (Steindler et al., 2011). However, a modelling approach has determined that whilst the energetic benefits of proteorhodopsin-based phototrophy may be slight the associated costs are low enough to ensure proteorhodopsin's persistence in marine bacterial genomes (Kirchman and Hanson, 2013). Indeed, the elimination of genes not vital for cell survival (Giovannoni et al., 2005) suggests that the acquisition of even a small proportion of the required organic molecules by photoheterotrophy must be energetically more beneficial than de novo synthesis (Zubkov, 2009). Thus photoheterotrophy supports the nutrition of bacterioplankton and likely confers an advantage under conditions where an abundance of sunlight can be harvested to power the acquisition of scarce organic nutrients.

The stratified oligotrophic oceanic regions represent a vast ecosystem intimately linked to the Earth's climate and biogeochemistry which are considered to be carbon sinks (del Giorgio and Duarte, 2002). By far the most biogeochemically active component of these systems are the abundant bacterioplankton constituents (Kirchman, 1997) which are dominated by the photoheterotrophs SAR11 and *Prochlorococcus* (Chisholm et al., 1988; Morris et al., 2002). The ubiquitous utilization of light, a resource abundant in the open ocean, in order to help satisfy their nutritional requirements, makes photoheterotrophy a key process to Earth system functioning. Furthermore, the ability to harvest light energy could decrease the requirement for respiration thereby reducing the production of carbon dioxide by bacterioplankton (Koblížek, 2011). Therefore, we recommend the inclusion of Photoheterotrophy alongside photosynthetic fixation of carbon in future endeavours to assess the biological significance of light.

The absolute uptake rates of methionine and leucine reported here are in a similar range to those reported for amino acids throughout the Atlantic Ocean (Mary et al., 2008; Hill et al., 2011), whereas uptake rates of ATP in the South Atlantic are lower than those found in the North Atlantic (Michelou et al., 2011; Gómez-Pereira et al., 2013). This difference likely results from the stronger phosphate limitation in the north resulting from iron input by Saharan dust (Moore et al., 2009) and/or ocean circulation (Straub et al., 2013). The dissolved organic phosphorus (DOP) pool has been identified as a possible alternative P source in the North Atlantic and its use in the upper water column was found to equal that of inorganic phosphorus (Björkman and Karl, 2003). Three quarters of the natural DOP pool consists of P-esters such as ATP (Kolowitz et al., 2001), and bacteria are able to utilise ATP to help satisfy their phosphorus requirement (Alonso-Saez and Gasol, 2007). Hence, the lower absolute uptake rates of ATP reported here are likely due to a lower dependence on DOP in the South Atlantic compared to the North Atlantic Ocean.

### **Conclusions**

Our consistent detection of light enhanced substrate uptake over two large-scale transects of a southern hemispheric ocean region combined with reports from the northern Hemisphere (Church et al., 2004; 2006; Michelou et al., 2007; Mary et al., 2008; Gómez-Pereira et al., 2013) could be considered as a critical mass of evidence from which we can confidently extrapolate light stimulated microbial substrate uptake to the global ocean. To date, the use of light to reduce carbon and generate oxygen has received vastly more scientific attention than photoheterotrophy. However, here we establish that photoheterotrophy is prevalent in the oligotrophic, stratified regions of the world's ocean, indicating it is widespread and should also be considered when appraising the biological and biogeochemical significance of light.

### **Acknowledgments**

We gratefully acknowledge the captains, officers, crew and all those onboard the Royal Research Ships *James Cook* and *James Clark Ross* for their support during these cruises. This study was supported by the UK Natural Environmental Research Council through Research grants NE/H007083/1. This is Atlantic Meridional Transect publication no. XX

## 5. References

- Alonso-Sáez, L. and J. M. Gasol. 2007. Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern Mediterranean coastal waters. *Applied and Environmental Microbiology* 73:3528-3535.
- Alonso-Saez, L., J. M. Gasol, T. Lefort, J. Hofer, and R. Sommaruga. 2006. Effect of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in northwestern Mediterranean coastal waters. *Applied and Environmental Microbiology* 72:5806-5813.
- Beja, O., L. Aravind, E. V. Koonin, M. T. Suzuki, A. Hadd, L. P. Nguyen, S. Jovanovich, C. M. Gates, R. A. Feldman, J. L. Spudich, E. N. Spudich, and E. F. DeLong. 2000. Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science* 289:1902-1906.
- Bjorkman, K. M. and D. M. Karl. 2003. Bioavailability of dissolved organic phosphorus in the euphotic zone at station ALOHA, North Pacific Subtropical Gyre. *Limnology and Oceanography* 48:1049-1057.
- Chisholm, S. W., R. J. Olson, E. R. Zettler, R. Goericke, J. B. Waterbury, and N. A. Welschmeyer. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* 334:340-343.
- Church, M. J., H. W. Ducklow, and D. A. Karl. 2004. Light dependence of [ $H^3$ ]leucine incorporation in the oligotrophic North Pacific ocean. *Applied and Environmental Microbiology* 70:4079-4087.
- Church, M. J., H. W. Ducklow, R. M. Letelier, and D. M. Karl. 2006. Temporal and vertical dynamics in picoplankton photoheterotrophic production in the subtropical North Pacific Ocean. *Aquatic Microbial Ecology* 45:41-53.
- del Giorgio, P. A. and C. M. Duarte. 2002. Respiration in the open ocean. *Nature* 420:379-384.
- Duce, R. A. and N. W. Tindale. 1991. Atmospheric transport of iron and its deposition in the ocean. *Limnology and Oceanography* 36:1715-1716.
- Falkowski, P. G. and J. A. Raven. 2007. *Aquatic Photosynthesis*. 2 edition. Princeton University Press, Princeton.
- Giovannoni, S. J., H. J. Tripp, S. Givan, M. Podar, K. L. Vergin, D. Baptista, L. Bibbs, J. Eads, T. H. Richardson, M. Noordewier, M. S. Rappe, J. M. Short, J. C. Carrington, and E. J. Mathur. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. *Science* 309:1242-1245.
- Gomez-Pereira, P. R., M. Hartmann, C. Grob, G. A. Tarran, A. P. Martin, B. M. Fuchs, D. J. Scanlan, and M. V. Zubkov. 2013. Comparable light stimulation of organic nutrient uptake by SAR11 and *Prochlorococcus* in the North Atlantic subtropical gyre. *Isme Journal* 7:603-614.
- Henderson, G.M., E. Maier-Reimer. 2002. Advection and removal of  $^{210}Pb$  and stable Pb isotopes in the oceans: a general circulation model study. *Geochimica et Cosmochimica Acta* 66:257-272.
- Hill, P. G., I. Mary, D. A. Purdie, and M. V. Zubkov. 2011. Similarity in microbial amino acid uptake in surface waters of the North and South Atlantic (sub-)tropical gyres. *Progress in Oceanography* 91:437-446.
- Kirchman, D. L. 1997. Carbon cycle - Microbial breathing lessons. *Nature* 385:121-122.

- Kirchman, D. L. and T. E. Hanson. 2013. Bioenergetics of photoheterotrophic bacteria in the oceans. *Environmental Microbiology Reports* 5:188-199.
- Koblížek, M. 2011. Role of photoheterotrophic bacteria in the marine carbon cycle. *Microbial Carbon Pump in the Ocean*. Science AAAS, Washington DC.
- Kolber, Z. S., C. L. Van Dover, R. A. Niederman, and P. G. Falkowski. 2000. Bacterial photosynthesis in surface waters of the open ocean. *Nature* 407:177-179.
- Kolowitz, L. C., E. D. Ingall, and R. Benner. 2001. Composition and cycling of marine organic phosphorus. *Limnology and Oceanography* 46:309-320.
- Mary, I., J. L. Heywood, B. M. Fuchs, R. Amann, G. A. Tarran, P. H. Burkill, and M. V. Zubkov. 2006. SAR11 dominance among metabolically active low nucleic acid bacterioplankton in the surface waters along an Atlantic meridional transect. *Aquatic Microbial Ecology* 45:107-113.
- Mary, I., G. A. Tarran, P. E. Warwick, M. J. Terry, D. J. Scanlan, P. H. Burkill, and M. V. Zubkov. 2008. Light enhanced amino acid uptake by dominant bacterioplankton groups in surface waters of the Atlantic Ocean. *FEMS Microbiol Ecol* 63:36-45.
- Michelou, V. K., M. T. Cottrell, and D. L. Kirchman. 2007. Light-stimulated bacterial production and amino acid assimilation by cyanobacteria and other microbes in the North Atlantic Ocean. *Applied and Environmental Microbiology* 73:5539-5546.
- Michelou, V. K., M. W. Lomas, and D. L. Kirchman. 2011. Phosphate and adenosine-5'-triphosphate uptake by cyanobacteria and heterotrophic bacteria in the Sargasso Sea. *Limnology and Oceanography* 56:323-332.
- Moore, C. M., M. M. Mills, E. P. Achterberg, R. J. Geider, J. LaRoche, M. I. Lucas, E. L. McDonagh, X. Pan, A. J. Poulton, M. J. A. Rijkenberg, D. J. Suggett, S. J. Ussher, and E. M. S. Woodward. 2009. Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. *Nature Geoscience* 2:867-871.
- Morris, R. M., M. S. Rappe, S. A. Connon, K. L. Vergin, W. A. Siebold, C. A. Carlson, and S. J. Giovannoni. 2002. SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* 420:806-810.
- Ruiz-Gonzalez, C., R. Simo, R. Sommaruga, and J. M. Gasol. 2013. Away from darkness: a review on the effects of solar radiation on heterotrophic bacterioplankton activity. *Frontiers in Microbiology* 4.
- Ruiz-Gonzalez, C., R. Simo, M. Vila-Costa, R. Sommaruga, and J. M. Gasol. 2012. Sunlight modulates the relative importance of heterotrophic bacteria and picophytoplankton in DMSP-sulphur uptake. *ISME Journal* 6:650-659.
- Spudich, J. L., C. S. Yang, K. H. Jung, and E. N. Spudich. 2000. Retinylidene proteins: Structures and functions from archaea to humans. *Annual Review of Cell and Developmental Biology* 16:365-+.
- Steindler, L., M. S. Schwalbach, D. P. Smith, F. Chan, and S. J. Giovannoni. 2011. Energy starved *Candidatus Pelagibacter ubique* substitutes light-mediated ATP production for endogenous carbon respiration. *Plos One* 6.
- Straub, M., D. M. Sigman, H. Ren, A. Martinez-Garcia, A. N. Meckler, M. P. Hain, and G. H. Haug. 2013. Changes in North Atlantic nitrogen fixation controlled by ocean circulation. *Nature* 501:200-203.
- Wright, R. T. and J. E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* 47:447-464.
- Zubkov, M. V. 2009. Photoheterotrophy in marine prokaryotes. *Journal of Plankton Research* 31:933-938.

- Zubkov, M. V. and P. H. Burkill. 2006b. Syringe pumped high speed flow cytometry of oceanic phytoplankton. *Cytometry A* 69:1010-1019.
- Zubkov, M. V., B. M. Fuchs, G. A. Tarran, P. H. Burkill, and R. Amann. 2003. High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Appl Environ Microbiol* 69:1299-1304.
- Zubkov, M. V., I. Mary, E. M. S. Woodward, P. E. Warwick, B. M. Fuchs, D. J. Scanlan, and P. H. Burkill. 2007. Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. *Environmental Microbiology* 9:2079-2089.
- Zubkov, M. V., G. A. Tarran, and P. H. Burkill. 2006a. Bacterioplankton of low and high DNA content in the suboxic waters of the Arabian Sea and the Gulf of Oman: abundance and amino acid uptake. *Aquatic Microbial Ecology* 43:23-32.
- Zubkov, M. V., G. A. Tarran, and B. M. Fuchs. 2004. Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre. *Fems Microbiology Ecology* 50:153-161.