The ISME Journal (2016) 10, 2582–2592
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www.nature.com/ismej

ORIGINAL ARTICLE

# Bacterioplankton niche partitioning in the use of phytoplankton-derived dissolved organic carbon: quantity is more important than quality

Hugo Sarmento[1,2], Cédric Morana[3] and Josep M Gasol[2]
[1]*Department of Hydrobiology, Federal University of São Carlos (UFSCar), São Carlos, Brazil;* [2]*Institut de Ciències del Mar-CSIC, Pg. Marítim de la Barceloneta, Barcelona, Spain and* [3]*Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven, Leuven, Belgium*

**Some prokaryotes are known to be specialized in the use of phytoplankton-derived dissolved organic carbon (DOCp) originated by exudation or cell lysis; however, direct quantification measurements are extremely rare. Several studies have described bacterial selectivity based on DOCp quality, but very few have focused on the quantity of DOCp, and the relative importance of each of these variables (for example, quantity versus quality) on prokaryote responses. We applied an adapted version of the MAR-FISH (microautoradiography coupled with catalyzed reporter deposition fluorescence *in situ* hybridization) protocol using radiolabelled exudates from axenic algal cultures to calculate a specialization index (*d'*) for large bacterioplankton phylogenetic groups using DOCp from different phytoplankton species and at different concentrations to elucidate to what extent the bacterial response to DOCp is driven by resource quantity (different DOCp concentrations) or by quality (DOCp from different phytoplankton species). All bacterial phylogenetic groups studied had lower *d'* at higher DOCp concentration, indicating more generalist behavior at higher resource availabilities. Indeed, at increasing resource concentrations, most bacterial groups incorporated DOCp indiscriminately, regardless of its origin (or quality). At low resource concentrations, only some specialists were able to actively incorporate the various types of organic matter effectively. The variability of bacterial responses to different treatments was systematically higher at varying concentrations than at varying DOCp types, suggesting that, at least for this range of concentrations (10–100 μM), DOCp quantity affects bacterial responses more than quality does. Therefore, resource quantity may be more relevant than resource quality in the bacterial responses to DOCp and affect how bacterioplankton use phytoplankton-derived carbon.**
*The ISME Journal* (2016) **10,** 2582–2592; doi:10.1038/ismej.2016.66; published online 29 April 2016

## Introduction

Oceanic dissolved organic carbon (DOC) is one of the largest organic carbon reservoirs on Earth, and is approximately equivalent to the atmospheric $CO_2$ stock (Hedges, 1992). Marine heterotrophic bacteria are the major regulators of this DOC pool, incorporating it into biomass (bacterial secondary production), respiring it into inorganic carbon or degrading the labile fractions of this DOC into more refractory compounds (Jiao *et al.,* 2010). Despite their importance in the global carbon cycle, DOC–microbe interactions, in particular bacterioplankton specialization in DOC utilization, are not yet fully understood (Azam and Malfatti, 2007; Kujawinski, 2011).

Correspondence: H Sarmento, Department of Hydrobiology, Federal University of São Carlos (UFSCar), Rodovia Washington Luis, km 235, Sao Carlos, São Paulo 13565-905, Brazil.
E-mail: hsarmento@ufscar.br
Received 12 October 2015; revised 18 March 2016; accepted 23 March 2016; published online 29 April 2016

Most of the DOC found in the sea surface has marine origin (Opsahl and Benner, 1997). Phytoplankton exudation or cell lysis (DOCp) represents a primary source of labile organic carbon-supplying bacterial metabolism (Baines and Pace, 1991). Different phytoplankton species release a vast range of different molecules (Repeta *et al.*, 2002; Romera-Castillo *et al.*, 2010; Sarmento *et al.*, 2013), and these organic compounds have different degrees of lability (Romera-Castillo *et al.*, 2011). In nature, DOCp varies in quality (lability) and quantity in space and time, yet information on whether which of these two factors (quantity or quality) is more relevant to bacterial activity is scarce or absent.

Prokaryotic specialization in the use of DOCp is a recurrent topic in microbial ecology literature; however, quantitative direct measurements are extremely difficult to achieve, and the few attempts required sophisticated methods such as nanoscale secondary-ion mass spectrometry combined with halogen *in situ* hybridization (Alonso *et al.*, 2012). Still, recent advances provided a new look at an old

topic: our understanding of DOC–bacteria interaction has moved from an extreme specialization view, where few specific bacterioplankton strains responded to a particular phytoplankton species (for example, González *et al.*, 2000; Schafer *et al.*, 2002; Pinhassi *et al.*, 2004; Grossart *et al.*, 2005; Rooney-Varga *et al.*, 2005; Sapp *et al.*, 2007a) to a more moderate (generalist) and complex view where many types of bacteria are involved in the decomposition of different types of DOCp with differences in their uptake rates (Nelson and Carlson, 2012; Sarmento and Gasol, 2012; Landa *et al.*, 2013; Paver *et al.*, 2013; Morana *et al.*, 2014).

Recent studies have demonstrated that different bacterial phylogenetic groups do not incorporate DOCp originated from different sources (that is, different phytoplankton species) in the same proportions (Nelson and Carlson, 2012; Sarmento and Gasol, 2012). Nelson and Carlson (2012) evaluated the performance of different bacterial clades growing on different DOC sources (namely, phytoplankton exudates and lysates) disregarding the particular DOC concentrations added. Other similar enrichment experiments have been performed with a fixed DOC concentration, varying only the quality (phytoplankton species origin) of the amendment (Sarmento and Gasol, 2012). However, the 'resource allocation hypothesis' (Koch, 1985) predicts that microbial decomposition rates should be concentration-dependent, and that substrate decomposition might not be linear and decrease progressively once the concentration of a certain substrate falls below a certain threshold. Independent studies on soil (German *et al.,* 2011) and aquatic microbial communities (Attermeyer *et al.*, 2014) have found indications that the 'resource allocation hypothesis' was accurate for some substrates but not for others, depending on the ubiquity or rarity of the specific microbial enzymes involved in those substrate's decomposition. The fact is that very few studies have evaluated the performance of specific bacterial phylogenetic groups at varying DOC sources (testing for quality) and quantities simultaneously (but see Eiler *et al.*, 2003; Alonso and Pernthaler, 2006).

Various studies have used the network approach to illustrate the interactions among microbes, yet the interactions are usually defined by co-existence in a water sample or by co-variation throughout time series (for example, Barberan *et al.*, 2011; Eiler *et al.*, 2011; Steele *et al.*, 2011; Lima-Mendez *et al.*, 2015), yet rarely by a real quantification of the interaction strengths (ISs) in terms of carbon flow, for example. We previously developed a metric that captures the mutualistic interaction of several bacterioplankton phylogenetic groups and different phytoplankton species mediated by DOCp, applying an adapted version of the MAR-FISH (microautoradiography combined with fluorescent *in situ* hybridization) technique with radiolabeled exudates from axenic algal cultures (Sarmento and Gasol, 2012). This approach can be used to determine the specialization indices of various bacterioplankton phylogenetic groups in using DOCp from different phytoplankton species at different concentrations.

We set to evaluate the variation in the degree of specialization of each phylogenetic group as a function of resource availability, and to elucidate which factor is more determinant in the bacterial response to DOCp: either resource quantity (different DOCp concentrations) or quality (DOCp from different phytoplankton species, which we had showed before that was of different value).

## Materials and methods

The experimental procedures are explained in detail in Sarmento and Gasol (2012), where similar experiments were performed but using only one fixed DOCp concentration.

### *Phytoplankton cultures and preparation of radiolabeled exudates*

We prepared radiolabeled DOCp from three axenic cultures obtained from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP) and used it as a tracer in the MAR-FISH technique. The three axenic strains used were as follows: the prymnesiophyte *Isochrysis galbana* Parke (CCMP1323), the prasinophyte *Micromonas pusilla* (RW Butcher) I Manton and M Parke (CCMP1545) and the cyanobacteria *Synechococcus* sp. (CCMP1183).

The cultures were grown to exponential phase in 50-ml tissue-culture flasks in presence of 150 μCi of $NaH^{14}CO_3$, in F/2 culture medium elaborated with aged, filtered and autoclaved coastal Mediterranean seawater and incubated at 20 °C under an artificial radiation of 100 $\mu mol_{photon}\, m^{-2}\, s^{-1}$, in a 16:8 h light:dark cycle, until cell density increased about one order of magnitude. Aliquots for phytoplankton counts were taken at the beginning and end of the growing period of 3 days. Bacterial contamination was checked in the same samples using epifluorescence microscope observations under blue and ultraviolet wavelength excitation, following 4,6-diamidino-2-phenylindole (DAPI) staining (10 $\mu g\, ml^{-1}$, final concentration). Parallel non-radioactive cultures were run simultaneously in the exact same conditions to measure DOC concentration in each culture. DOC analysis was performed using standard methods as described in Romera-Castillo *et al.* (2010).

Labeled DOCp stocks were prepared as described in Sarmento and Gasol (2012); 40 ml of cultures were gently filtered onto previously flushed 0.2-μm Sterivex filters to isolate the DOCp from cells. The filtrates were acidified with 3 ml of 6 M HCl and left open in an orbital shaker overnight for dissipation of unassimilated $NaH^{14}CO_3$. The pH of the exudates was then adjusted to ~8 with

6 M NaOH, and a 3-ml subsample was radioassayed ($DO^{14}C$) in 15 ml of Ultima Gold liquid scintillation cocktail.

*Microautoradiography coupled with catalyzed reporter deposition fluorescence in situ hybridization*

MAR-FISH allows tracing the carbon transference through phytoplankton and its re-assimilation by heterotrophic prokaryotes, revealing how many and which kind of heterotrophic prokaryote incorporate each type of DOCp. Subsurface 150-µm prefiltered seawater samples (20 ml each) taken on 22 March 2010 from a NW Mediterranean coastal site (Blanes Bay Microbial Observatory, http://www.icm.csic.es/bio/projects/icmicrobis/bbmo/) were incubated in controlled *in situ* temperature in the dark with addition of the different radiolabeled compounds at three different concentrations: 10, 30 and 100 µM DOC final concentration. The DOC concentration on that date at the Blanes Bay Microbial Observatory was 64.9 µM. DOC in Blanes Bay accumulates during summer, from an annual minimum by early March (less than 60 µM) to the annual maximum by early September (higher than 100 µM). The average value for the 2008–2010 period was 79.1 µM, with maxima reaching 140 µM (Romera-Castillo *et al.*, 2013).

To determine the exact amounts of DOCp additions in each incubation flask, we varied the volume of exudate solution added that ranged from 200 µl to 4.5 ml. Dead control incubations were performed by fixing the sample with formaldehyde (1.8%) before ($>5$ min) the addition of the radiolabeled compounds. False positives obtained in these dead controls (always $<0.5\%$ of DAPI-stained cells) were subtracted.

A previous study tested the reproducibility of this technique and showed good agreement between incubation replicates (Sarmento and Gasol, 2012). In order to generate a complete matrix on our experimental design in a reasonable time and effort, we did not do replicated incubations, as processing MAR-FISH samples from end to end is a very time-consuming task.

After 5-h incubation, the samples were fixed overnight with formaldehyde (1.8%) at 4 ºC, gently filtered on 0.2-µm polycarbonate filters (EMD Millipore, Billerica, MA, USA, 25 mm diameter) and processed with the MAR-FISH protocol.

MAR-FISH was performed with the protocol initially described by Alonso and Pernthaler (2005), with some modifications detailed in Sarmento and Gasol (2012). After hybridization following the CARD-FISH (catalyzed reporter deposition fluorescence *in situ* hybridization) protocol (described hereafter), the filters were glued onto glass slides with an epoxy adhesive (UHU plus; UHU GmbH, Bühl, Germany). The slides were embedded in 46-°C-tempered photographic emulsion (KODAK NTB-2) containing 0.1% agarose (gel strength 1%, $>1$ kg cm$^{-2}$) in a dark room and were placed on an ice-cold metal bar for ~5 min to allow the emulsion to solidify. They were subsequently placed inside black boxes at 4 °C until development. We found that the optimal exposure time was 9 days (data not presented). For development, we submerged the exposed slides for 3 min in the developer (KODAK D19), 30 s rinsing with distilled water and 3 min in fixer (KODAK Tmax), followed by 5 min of washing with tap water. The slides were then dried in a dessicator overnight, stained with DAPI (1 µg ml$^{-1}$) and inspected in an Olympus BX61 epifluorescence microscope. CARD-FISH-positive cells (hybridized with the specific probe) appear in bright green under blue light excitation. MAR-FISH positives contain, additionally, dark silver grains accumulated above the bacterial cells on the photographic emulsion, resulting from radioactive decay of labeled DOCp, and are clearly visible under white light in the same microscope.

CARD-FISH was carried out following the protocol described previously (Pernthaler *et al.*, 2004). Several horseradish peroxidase probes were used to characterize the composition of the bacterial community in the original water samples, using the same procedure as described in Alonso-Sáez and Gasol (2007). The horseradish peroxidase-labeled probes used were as follows: EUB-338, EUB-II and EUB-III (target most Eubacteria; Amann *et al.*, 1990; Daims *et al.*, 1999); GAM42a (targets most Gammaproteobacteria; Manz *et al.*, 1992); ALF968 (targets most Alphaproteobacteria; Neef, 1997); CF319 (targets many groups belonging to the Bacteroidetes group; Manz *et al.*, 1996); ROS537 (targets members of the *Roseobacter–Sulfitobacter–Silicibacter* group; Eilers *et al.*, 2001); NOR5-730 (targets members of the NOR5 cluster; Eilers *et al.*, 2001); Alt1413 (targets *Alteromonas* and *Colwellia*; Eilers *et al.*, 2000b); and SAR11-441 R (targets the SAR11 cluster; Morris *et al.*, 2002); EUB antisense probe NON338 (Wallner, 1993) was used as a negative control. All probes were purchased from biomers.net (Ulm, Germany). Specific hybridization conditions were established by addition of formamide to the hybridization buffers (20% formamide for the NON338 probe, 45% formamide for the ALF968 and SAR11-441 R probes, 50% for NOR5-730, 60% for Alt1413 and 55% for the other probes).

Counterstaining of CARD-FISH preparations was performed with DAPI (1 µg ml$^{-1}$). Between 500 and 1000 DAPI-positive cells of each phylogenetic group were counted in a minimum of 10 fields, at least 50 MAR-FISH-positive cells.

*Calculating IS and network properties*

The MAR-FISH results can be presented in different ways, depending on which parameter is chosen to relate to the number of cells taking up the radiolabeled compounds. Hence, the number of MAR-positive cells belonging to a certain bacterial

phylogenetic group (MAR+$_g$) can be divided by the number of cells from that phylogenetic group (MAR +$_g$/CARD-FISH+$_g$), by MAR positives within Eubacteria-hybridized cells (MAR+$_g$/MAR+$_{Eub}$) or by the total bacterial abundance (MAR+$_g$/DAPI counts). Here, we have calculated the IS between a phytoplankton strain and the different heterotrophic prokaryote groups mediated by the use of DOCp in two different ways: (1) the number of MAR-FISH positives (cells actively taking up the radiolabeled compounds) as a proportion of the total bacterial community (MAR+$_g$/DAPI counts) and (2) as the number of MAR-FISH positives (cells actively taking up the radiolabeled compounds) as a proportion of the active community (MAR+$_g$/MAR+$_{Eub}$). Thus, in both cases, if all cells of a given bacterial group would use a particular DOCp, then that group and the corresponding algae would have a strong interaction, and both MAR+$_g$/MAR+$_{Eub}$ and MAR+$_g$/DAPI counts would approach 100%, whereas when no cells used the substrate, this interaction would be zero (no interaction). We also present the results as MAR+$_g$/CARD-FISH+$_g$, which reflects the number of active cells within each bacterial phylogenetic group. Although informative, this metric is not pertinent for IS calculation purposes.

Networks were built aggregating the results of the different treatments in one single network, and specialization indices (Blüthgen *et al.*, 2006; Blüthgen *et al.*, 2007) were calculated at the network level ($H_2$') and at the bacterial phylogenetic group level (*d*') using *H2fun* and *dfun* functions from *bipartite* package (Dormann *et al.*, 2008; Dormann *et al.*, 2009; Dormann, 2011) in Software R (http://www.r-project.org), after multiplying IS values by 1000 and rounding them to integers, as calculations of specialization indices require absence of decimals. Specialization indices $H_2$' and *d*' range from 0 for the most generalized to 1.0 for the most specialized case (Blüthgen *et al.*, 2006).

## Results

Bacterial community composition at the Blanes Bay Microbial Observatory on 22 March 2010 was dominated by members of the SAR11 clade (51.1%) and Bacteroidetes (22.7%). *Gammaproteobacteria* (5.5%) and *Roseobacter* (4.0%) had lower relative abundance. Other targeted groups, namely NOR5 and *Alteromonas*, represented less than 1% of the community in that particular sample, and were discarded from our further analyses. The probes targeting Eubacteria hybridized 88.2% of DAPI-stained cells, and no significant changes in the community structure were observed during the 5-h incubations (data not shown).

The results expressed as a percentage of active cells within each heterotrophic bacterial phylogenetic group (MAR+$_g$/CARD-FISH+$_g$) illustrate the variety of responses in processing the DOCp originated from different DOCp concentrations, independently of the phytoplankton species that originated that DOCp (Figure 1). The proportion of active cells belonging to Bacteroidetes or *Gammaproteobacteria* increased with increasing DOCp concentrations. On the contrary, within SAR11 and *Roseobacter* the percentage of active cells decreased with DOCp availability. Overall, the more active groups were *Roseobacter* (on average 38.7% of active cells) and *Gammaproteobacteria* (on average 30.2% of active cells), followed by Bacteroidetes (on average 23.3% of active cells) and SAR11 were the least active (only 10.2% of active cells, on average).

A bidimensional heat map of IS obtained aggregating the results of the different treatments, using the contribution of MAR-FISH-positive cells to the total community (MAR+$_g$/DAPI counts), illustrates the different responses of the various bacterial phylogenetic groups to different DOC sources at different concentrations (Figure 2). In this figure, rows represent different DOC types and columns represent different DOC concentrations. Within different DOCp sources, some differences were observed. IS increased notably with DOCp concentration in Bacteroidetes and *Gammaproteobacteria*, whereas *Roseobacter* and SAR11 presented the opposite trend (Figure 2).

To explore which metric better expresses the IS between phytoplankton and the bacterial phylogenetic groups, we tested the different ways of presenting MAR-FISH results (Table 1): the number of MAR-positive cells belonging to a certain bacterial phylogenetic group (MAR+$_g$) divided by the sum of the active cells from the different groups (MAR+$_g$/MAR+$_{Eub}$) or total bacterial abundance (MAR+$_g$/DAPI counts). The coefficients of variation and specialization indices for results expressed both as MAR+$_g$/MAR+$_{Eub}$ and MAR+$_g$/DAPI counts (Tables 2 and 3) show differences in absolute values, but similar trends for both IS metrics (Figure 3).

The average coefficients of variation of the bacterial responses to the different treatments were systematically higher at varying concentrations (in Table 2, 'same DOCp type at different concentration') than at varying DOCp sources (in Table 2, 'same DOCp concentration (μM) from different sources'). Moreover, higher coefficients of variation were always observed at lower DOCp concentrations (10 μM), except for SAR11 in the results expressed as MAR+$_g$/DAPI counts that followed the opposite trend (Table 2).

The construction of interaction networks allowed calculating specialization indices for each bacterial phylogenetic group (*d*') and for the entire network ($H_2$'). These indices reflect how much the distribution of ISs deviates from that occurring by chance. Higher values indicate higher degree of specialization and lower values indicate higher generalism. The specialization index values obtained were relatively low (Table 3). Still, a comparative analysis between

bacterial phylogenetic groups and between networks allows the identification of trends in the relative degrees of specialism. All bacterial phylogenetic groups had higher *d*' at lower DOCp concentration (10 µM), regardless of the DOCp source. Members of *Gammaproteobacteria* were more specialized when exposed to *Micromonas* exudates; SAR11 were more specialized in the *Synechococcus* treatment; and Bacteroidetes were more specialized using *Isochrysis*-derived DOCp. *Roseobacter* was the only group that showed some divergence between the two different IS metrics. Using $MAR+_g$/DAPI counts, *Roseobacter* were more specialized with *Micromonas* exudates, whereas using $MAR+_g/MAR+_{Eub}$ the *Isochrysis* treatment had higher *d*' (Table 3).

Comparing the results of the different bacterial groups in the use of algal-derived DOC merging all treatments in a single network, Bacteroidetes had higher *d*' (were more specialists), and *Gammaproteobacteria* were the most generalist group ('altogether' in Table 3). Concerning the specialization indices for the entire networks ($H_2$'), lower DOCp concentration (10 µM) had higher $H_2$' values, and *Isochrysis* was the algae that induced higher specialization within the interaction network (Table 3).

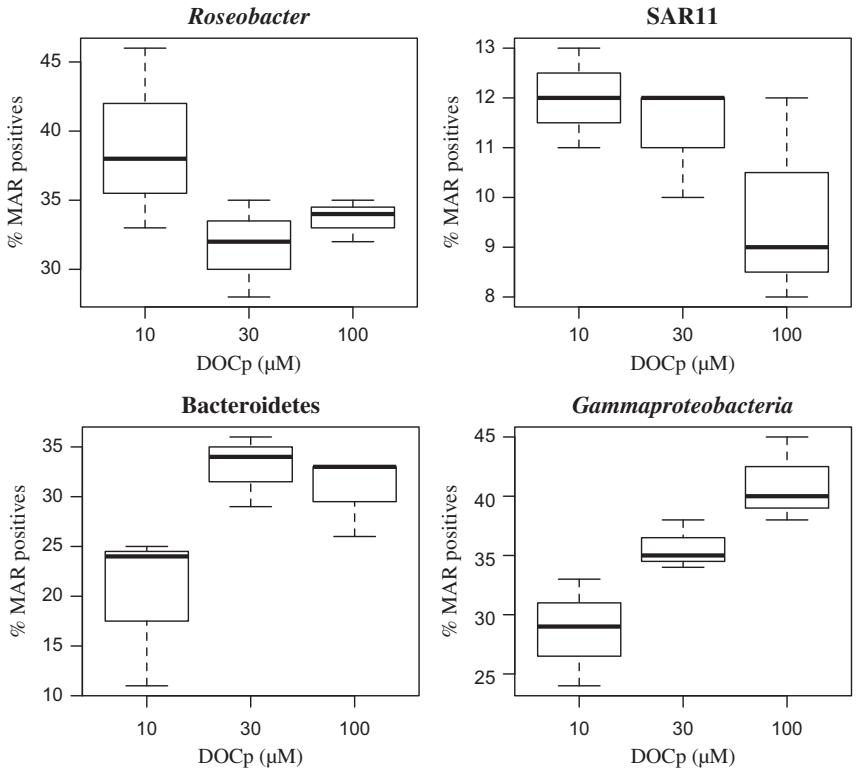


**Figure 1** Box plots presenting the percentage of MAR-FISH-positive (cells actively taking up the radiolabeled compounds) within each heterotrophic bacterial phylogenetic group ($MAR+_g$/CARD-FISH$+_g$, see text), for the different DOCp concentrations (grouping the results by the three different algal treatments). The central full line indicates the median value, the boxes indicate the lower and upper quartiles, and vertical lines indicate the 10th and 90th percentiles. Note the different scales on the *y* axes.

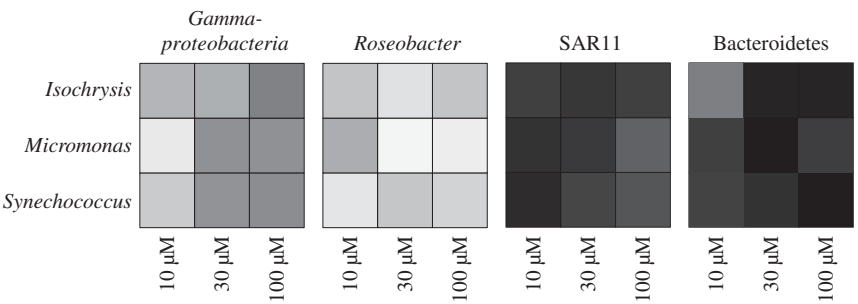


**Figure 2** Bidimensional heat map of the IS between marine heterotrophic bacteria phylogenetic groups and different DOC sources at different concentrations. Darker tones of gray indicate stronger IS ('$MAR+_g$/DAPI counts' data from Table 1 and Figure 2). *Roseobacter* and SAR11 are both subgroups of *Alphaproteobacteria*.

**Table 1** MAR-FISH results

| *DOC type* | *DOC conc. (µM)* | *Alphaproteobacteria* | | *Gammaproteobacteria* | *Bacteroidetes* |
|---|---|---|---|---|---|
| | | *Roseobacter* | *SAR11* | | |
| *MAR+$_g$/DAPI counts (%)* | | | | | |
| *Isochrysis* | 10 | 1.5 | 5.7 | 1.8 | 2.5 |
| | 30 | 1.3 | 6.1 | 1.9 | 7.8 |
| | 100 | 1.4 | 5.9 | 2.5 | 7.5 |
| *Micromonas* | 10 | 1.8 | 6.3 | 1.3 | 5.7 |
| | 30 | 1.1 | 5.9 | 2.1 | 8.1 |
| | 100 | 1.3 | 4.3 | 2.1 | 5.8 |
| *Synechococcus* | 10 | 1.3 | 6.9 | 1.6 | 5.5 |
| | 30 | 1.4 | 5.0 | 1.9 | 6.7 |
| | 100 | 1.4 | 4.3 | 2.2 | 7.4 |
| *MAR+$_g$/MAR+$_{Eub}$ (%)* | | | | | |
| *Isochrysis* | 10 | 13.2 | 49.7 | 15.7 | 21.5 |
| | 30 | 7.6 | 35.6 | 11.1 | 45.7 |
| | 100 | 8.1 | 34.2 | 14.3 | 43.4 |
| *Micromonas* | 10 | 12.2 | 41.5 | 8.8 | 37.5 |
| | 30 | 6.6 | 34.5 | 12.1 | 46.8 |
| | 100 | 9.7 | 31.7 | 15.6 | 43.1 |
| *Synechococcus* | 10 | 8.8 | 44.8 | 10.4 | 36.0 |
| | 30 | 9.4 | 33.5 | 12.9 | 44.2 |
| | 100 | 8.8 | 28.4 | 14.2 | 48.5 |

Abbreviations: Conc., concentration; DAPI, 4,6-diamidino-2-phenylindole; DOC, dissolved organic carbon; MAR-FISH, microautoradiography combined with fluorescent *in situ* hybridization.
Table results presented (1) as the number of MAR-FISH positives as a proportion of the total bacterial community (MAR+$_g$/DAPI counts) and (2) number of MAR-FISH positives as a proportion of the interacting community (MAR+$_g$/MAR+$_{Eub}$)*; Roseobacter* and SAR11 are both subgroups of *Alphaproteobacteria.*

**Table 2** Coefficients of variation of the bacterial responses to different treatments

| | *Alphaproteobacteria* | | *Gammaproteobacteria* | *Bacteroidetes* |
|---|---|---|---|---|
| | *Roseobacter* | *SAR11* | | |
| *MAR+$_g$/DAPI counts (%)* | | | | |
| Same DOCp type at different conc. | | | | |
| *Isochrysis* | 0.08 | 0.03 | 0.18 | 0.51 |
| *Micromonas* | 0.26 | 0.20 | 0.24 | 0.21 |
| *Synechococcus* | 0.03 | 0.24 | 0.15 | 0.15 |
| Average | 0.12 | 0.16 | 0.19 | 0.29 |
| Same DOCp conc. (µM) from different sources | | | | |
| 10 | 0.16 | 0.09 | 0.15 | 0.39 |
| 30 | 0.11 | 0.10 | 0.05 | 0.10 |
| 100 | 0.04 | 0.19 | 0.09 | 0.14 |
| Average | 0.10 | 0.13 | 0.10 | 0.21 |
| *MAR+$_g$/MAR+$_{Eub}$ (%)* | | | | |
| Same DOCp type at different conc. | | | | |
| *Isochrysis* | 0.32 | 0.22 | 0.17 | 0.36 |
| *Micromonas* | 0.30 | 0.14 | 0.28 | 0.11 |
| *Synechococcus* | 0.04 | 0.24 | 0.15 | 0.15 |
| Average | 0.22 | 0.20 | 0.20 | 0.21 |
| Same DOCp conc. (µM) from different sources | | | | |
| 10 | 0.20 | 0.09 | 0.31 | 0.28 |
| 30 | 0.18 | 0.03 | 0.08 | 0.03 |
| 100 | 0.09 | 0.09 | 0.05 | 0.07 |
| Average | 0.16 | 0.07 | 0.14 | 0.13 |

Abbreviations: Conc., concentration; DAPI, 4,6-diamidino-2-phenylindole; DOCp, phytoplankton-derived dissolved organic carbon; IS, interaction strength; MAR, microautoradiography.
Coefficient use two different IS metrics (MAR+$_g$/DAPI counts and MAR+$_g$/MAR+$_{Eub}$, see text for details); *Roseobacter* and SAR11 are both subgroups of *Alphaproteobacteria.*
Note that these are not true replicates.

**Table 3** Specialization indices

| *Subnetwork* | d' ($\times 10^{-2}$) | | | | $H_2'$ ($\times 10^{-2}$) Entire network |
|---|---|---|---|---|---|
| | *Alphaproteobacteria* | | *Gammaproteobacteria* | *Bacteroidetes* | |
| | *Roseobacter* | *SAR11* | | | |
| *MAR+$_g$/DAPI counts (%)* | | | | | |
| *Isochrysis* | 1.23 | 1.39 | 0.52 | 4.02 | 2.46 |
| *Micromonas* | 1.37 | 0.62 | 1.15 | 0.51 | 1.21 |
| *Synechococcus* | 0.02 | 1.78 | 0.39 | 0.89 | 1.36 |
| 10 µM | 0.67 | 0.32 | 1.31 | 2.13 | 1.69 |
| 30 µM | 0.41 | 0.03 | 0.09 | 0.03 | 0.15 |
| 100 µM | 0.11 | 0.26 | 0.04 | 0.19 | 0.23 |
| Altogether | 0.88 | 1.33 | 0.73 | 1.94 | 1.42 |
| *MAR+$_g$/MAR+$_{Eub}$ (%)* | | | | | |
| *Isochrysis* | 1.39 | 1.62 | 0.52 | 4.82 | 3.50 |
| *Micromonas* | 1.27 | 0.64 | 1.22 | 0.49 | 1.29 |
| *Synechococcus* | 0.02 | 1.77 | 0.39 | 0.89 | 1.37 |
| 10 µM | 0.66 | 0.34 | 1.41 | 2.41 | 1.92 |
| 30 µM | 0.42 | 0.03 | 0.10 | 0.03 | 0.17 |
| 100 µM | 0.11 | 0.25 | 0.04 | 0.19 | 0.24 |
| Altogether | 0.92 | 1.48 | 0.77 | 2.33 | 1.64 |

Abbreviations: DAPI, 4,6-diamidino-2-phenylindole; IS, interaction strength; MAR, microautoradiography.
Specialization indices use two different IS metrics (MAR+$_g$/DAPI counts and MAR+$_g$/MAR+$_{Eub}$, see text for details), calculated for each bacterial phylogenetic group (*d*') and for the entire interaction networks ($H_2$') as proposed by Blüthgen *et al.* (2007).
'Altogether' refers to the results merging all treatments in a single network.

## Discussion

Resource availability and nutritional value determine competition and niche partitioning among coexisting species in a community. Optimal foraging models for consumer-prey interactions predict higher specialization in highly productive systems, and more generalist diets in low-resource regimes (for example, Werner and Hall, 1974; Gende *et al.*, 2001). However, this applies only at the individual or population level. The picture might be different when scaling up to the community level, as indicated by our measures of DOCp incorporation by a bacterioplankton community. At the community level, specialization can be expressed as the mean specialization of the species present in that community (Devictor *et al.*, 2010). Our observations suggest that higher resource availability actually enhanced generalism in the interaction network among bacterial large phylogenetic groups (Table 3; Figure 4). This is somehow expected at a community level perspective: communities that can survive in low-resource environments, such as deserts, for example, are usually composed of specialized species. Here, we provide experimental evidence that increasing resources enhance generalism in bacterioplankton communities.

The two different metrics used to express IS differed in absolute numbers (Table 1), but suggested similar trends regarding specialization among bacterial groups (*d*') and entire networks ($H_2$'; Table 3). Actually, both metrics were highly correlated (Figure 3), indicating that it does not make much difference whether we consider the proportion of active cells over the whole community or only over the interacting community, at least for the DOCp treatments. We conclude that both metrics are tenable and, at least for DOCp, provide similar results.

Within each different DOCp source, some differences were also observed among treatments. Bacteroidetes and SAR11 had the stronger interactions. IS increased notably with DOCp concentration in Bacteroidetes and *Gammaproteobacteria*, whereas *Roseobacter* and SAR11 presented the opposite trend (Figure 2). Despite the fact that the probes used target groups from different taxonomical ranks, most of these groups present ecological coherence (Philippot *et al.*, 2010). Thus, our results provide valuable information concerning the auto-ecology of the most abundant bacterial large phylogenetic groups in the sea, sometimes difficult to discern in the huge amount of genomic data (for example, Lauro *et al.*, 2009). For example, our observations reinforce previous reports that SAR11 can grow in culture only with low substrate concentration (Rappé *et al.*, 2002).

Bacteroidetes had higher *d*' values, enhanced by *Isochrysis* DOCp, which was the alga that induced higher specialization within the interaction network (Table 3). Previous studies comparing bacterial communities associated to algal cultures in non-axenic conditions had already pointed out that *Isochrysis* had very specific bacterial associated taxa, namely isolates from the genera *Flexibacter* belonging to *the Cytophaga–Flavobacterium–Bacteroides*

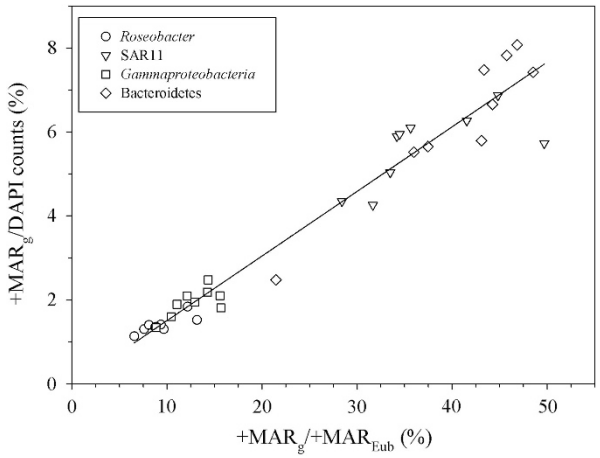


**Figure 3** Type II linear regression between two different IS metrics: %+MAR+$_g$/DAPI counts = % +MAR+$_g$/MAR+$_{Eub}$ × 0.154 − 0.036 ($n = 36$, $r = 0.98$; $P < 0.0001$).

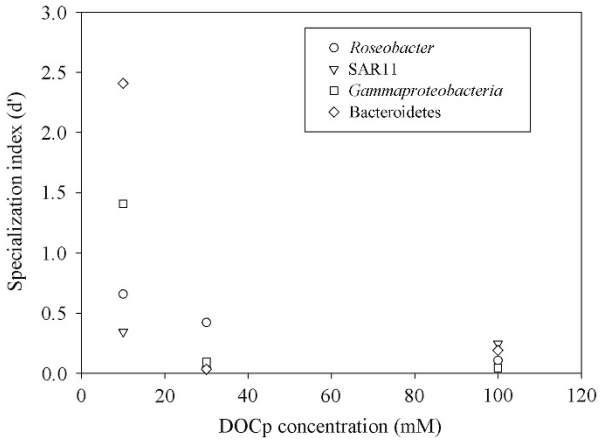


**Figure 4** Specialization index *d*' (Blüthgen *et al*., 2007) of the different bacterial phylogenetic groups as a function of DOCp concentration. *Roseobacter* and SAR11 are both subgroups of *Alphaproteobacteria*.

phylum (Schwenk *et al*., 2014). It is not uncommon to observe members of Bacteroidetes associated with phytoplankton (for example Schafer *et al*., 2002; Pinhassi *et al*., 2004; Grossart *et al*., 2005; Sapp *et al*., 2007b), and our results reinforce the relevance of this group in incorporating organic carbon derived from some phytoplankton species.

In contrast, *Gammaproteobacteria* were the most generalist group. *Gammaproteobacteria* have also many times been identified associated with particles, namely phytoplankton cells (for example, Sapp *et al*., 2007a, b), but also in enrichment experiments or disturbances (that is, bottle effects, Eilers *et al*., 2000a; Pinhassi and Berman, 2003). As we observed the same generalistic behavior in a previous study using the same MAR-FISH technique with different algal strains (Sarmento and Gasol, 2012), we deduce that this bacterial group can respond to enrichment and confinement, independently of the type or quantity of DOCp added.

All bacterial phylogenetic groups had higher *d*' at lower DOCp concentration (Figure 4; Table 3). Lower DOCp concentration (10 μM) had also higher $H_2$' values. In other words, the IS distribution of bacterial phylogenetic groups toward the different DOCps was more uneven when there was low DOCp concentration. With an increasing resource availability, IS distribution among DOCp sources became more even, indicating a more generalist network where various bacterial groups incorporated resources indiscriminately, regardless of the source (or quality). At low resource concentration, only few specialists were able to actively incorporate specific types of organic carbon effectively. A single-cell genome analysis (Swan *et al*., 2013) comparing the existing cultures with natural bacterioplankton from different oligotrophic surface oceans revealed extreme specialization and the prevalence of genome streamlining in low resource conditions, which supports our observations of inverse relationship between specialization and resource availability. The cost of genome streamlining in free-living bacteria would result in a reduction in physiological flexibility, leading to specialization in resource utilization (Swan *et al*., 2013). Conversely, large-genome copiotrophs would have higher genetic potential (more genes coding for a higher number of enzymes), allowing handling a wider range of molecules, which makes them more generalists (Lauro *et al*., 2009); however, they need a higher amount of energy to start the synthesis of such a complex enzymatic machinery.

The coefficients of variation of the bacterial responses to different treatments were systematically higher at varying concentrations ('same DOCp type at different concentrations') than at varying DOCp sources ('same DOCp concentration from different sources'), suggesting that, at least for these ranges of concentrations, DOCp quantity affects bacterial responses more than quality does. These results highlight the importance of DOC quantity in evaluating bacterial responses to DOC additions, as suggested by previous studies (Eiler *et al*., 2003; Alonso and Pernthaler, 2006; Attermeyer *et al*., 2014).

It has already been demonstrated that the organic matter excreted by different phytoplankton species do not stimulate all major phylogenetic groups of bacteria in the same way (Sarmento and Gasol, 2012). Indeed, it suggests that the diverse bacterial groups could coexist in seawater by utilizing different resources (that is, different types of organic molecules). Our results here indicate that the concentration of available substrate for bacteria could affect even more the strength of their interactions with phytoplankton species through DOCp. Moreover, it appears that different bacterial groups harbor diverse concentration-dependent patterns of DOCp uptake (Figure 1): some groups were more active with increasing DOCp concentrations (Bacteroidetes, *Gammaproteobacteria*), whereas some others followed an opposite pattern (SAR11,

*Roseobacter*). This observation provides evidence for niche partitioning in the use of DOCp by bacterioplankton. Temporal fluctuations in biotic and abiotic factors, as well as microscale processes, provide a variety of substrate quantity and quality, creating numerous heterogeneous habitats that maintain the high diversity of heterotrophic bacteria in the sea (Stocker, 2012).

Overall, we found that increasing resources enhance generalism in bacterioplankton communities, and that resource quantity may be more relevant than quality concerning bacterial responses to DOCp, at least at a large phylogenetic group level, and this should be taken into account when designing experiments and interpreting results of bacterioplankton use of phytoplankton-derived carbon.

## Conflict of Interest

The authors declare no conflict of interest.

## Acknowledgements

We are thankful to Professor Dr Nico Blüthgen and Dr Adriano Caliman for enriching discussions that inspired this work. Work of HS was supported by the Spanish MCyI (Juan de la Cierva Fellowship JCI-2008-2727), by CNPq (Project: 474759/2013-0) and FAPESP (Process: 2014-/14139-3). JMG was supported by Spanish MCyI grants DOREMI (CTM2012-34294) and HOTMIX (CTM2011-30010/MAR) and by Grup Consolidat de Recerca 2014SGR/1179 (Generalitat de Catalunya).

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