SUPPLEMENTARY INFORMATION

Differential recruitment of opportunistic taxa leads to contrasting abilities in carbon processing by bathypelagic and surface microbial communities

Marta Sebastián[1,2], Irene Forn[1], Adrià Auladell[1], Markel Gómez-Letona[2], M. Montserrat Sala[1], Josep M. Gasol[1], Celia Marrasé[1]

[1] Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CSIC, 08003 Barcelona, Catalunya, Spain

[2] Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, ULPGC, 35214, Gran Canaria, Spain.

This file contains supplementary methods and 7 supplementary figures. Three additional supplementary tables are supplied as separate files.

Supplementary Methods

Catalyzed Reported Deposition Fluorescence in situ hybridization (CARDFISH)

CARDFISH was performed to quantify the contribution of the main bacterioplankton groups to the community at the final day of sampling (day 9) following the Pernthaler *et al.*, (2002) protocol as detailed in Sebastián and Gasol, (2013).

Filters were hybridized with one of the following horseradish peroxidase (HRP)-labelled probes: EUB338 I-II and –III (targets most Bacteria, Daims *et al.*, 1999), GAM42a together with its unlabelled competitor probe (targets most Gammaproteobacteria, Manz *et al.*, 1992), and Alf968 (targets most Alphaproteobacteria, Neef 1997). Specific hybridization conditions were established by addition of formamide to the hybridization buffers (45% formamide for the Alf968 probe, and 55% for the other probes). Hybridization was performed overnight at 35°C (Pernthaler et al., 2002). For amplification, we used tyramide labelled with Alexa 488. The abundances of the different phylogenetic groups in relation to total prokaryotic counts were calculated by counterstaining with 4',6-diamidino-2-phenylindole (DAPI, final concentration 1 μg mL^{-1}). Since 16S rRNA gene archaeal sequences were negligible at the end of the experiment, we consider that cells that could not hybridize with the general EUB338 I-II and –III lacked enough ribosomes to be detected, and therefore were either dormant or dead.

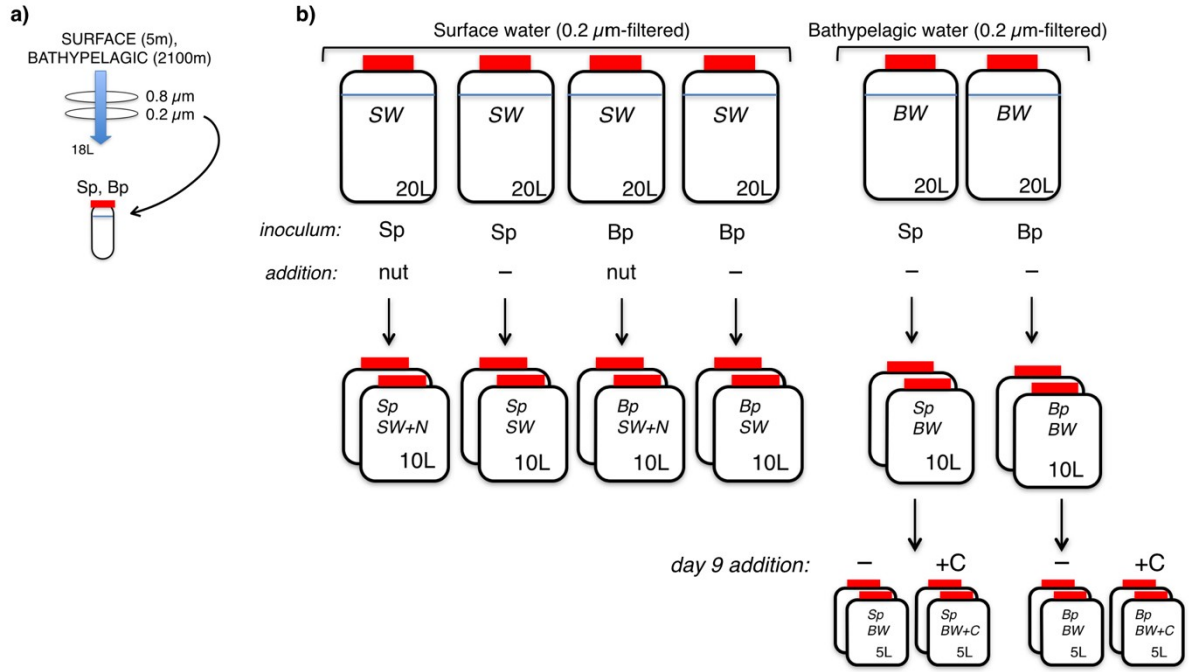

Figure S1. Experimental setup. a) collection of prokaryotic biomass from the surface (5 m) and bathypelagic (2,100 m): prefiltration through 0.8 µm was carried out to remove predators, and prokaryotic cells were collected in 0.2 µm filters. Cells collected from the surface and bathypelagic were resuspended in 0.9 L of their corresponding 0.2 µm filtered seawater by vortexing and prokaryotic abundance was quantified through flow cytometry, so that the starting inoculum was the same in all treatments (5x104 cells mL^{-1}). b) Four 20-L carboys containing 0.2 µm filtered surface seawater ('SW') and two 20-L carboys containing 0.2 µm filtered bathypelagic seawater ('BW') were prepared and inoculated with either surface (Sp) or bathypelagic (Bp) prokaryotic cells. Inorganic nutrients were then added to one of the SW carboys containing Sp and to another containing Bp to yield the SW+N treatments. Then, the 20 L of volume were divided into two replicates in 10-L containers. On day 9 of the experiment, the remaining water volume in the BW treatment was divided in half into two containers and carbon (mixed sources, including glucose, acetate, terrestrial humic acids and amino acids, see Table S1 for details) added to one of the containers, yielding the BW+C treatment.

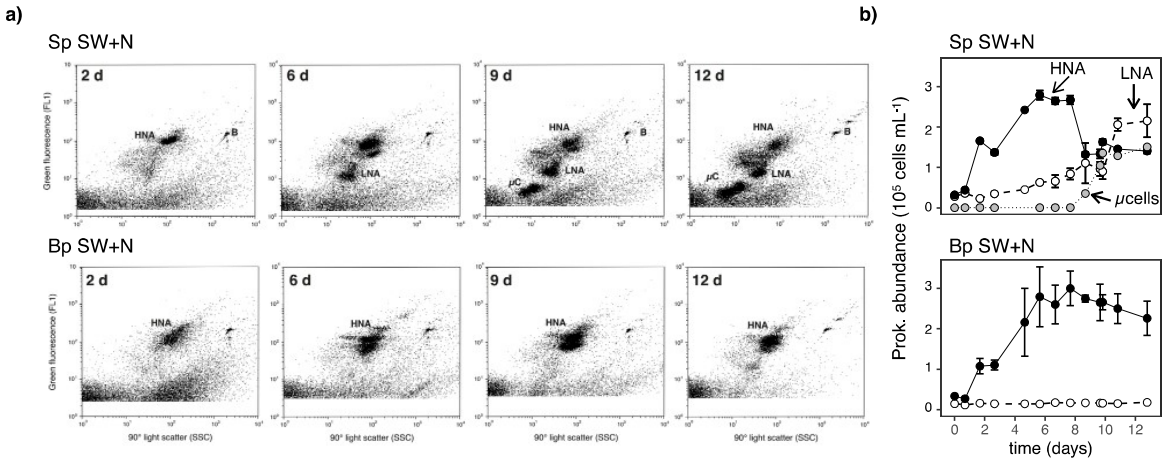

Figure S2. Dynamics of surface prokaryotes (Sp, upper panel) and bathypelagic prokaryotes (Bp, lower panel) in the surface water with nutrients treatment: a) cytograms, b) total cell abundance. Note that in the Sp SW+N treatment, low nucleic acid (LNA) prokaryotes increased in abundance after day 2, and after 8 days a fraction of the cells got miniaturized, resulting in the appearance of a population of tiny cells (μcells). In contrast, high nucleic acid prokaryotes (HNA) dominated the Bp assemblage until the end of the experiment. B stands for 1 μm Polysciences yellow-green fluorescent beads.

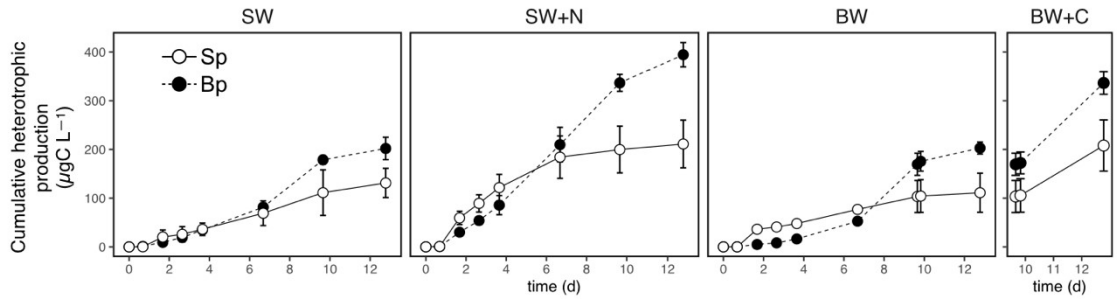

Figure S3. Cumulative heterotrophic prokaryotic production of surface prokaryotes (Sp open circles) and bathypelagic prokaryotes (Bp, filled circles) inoculated in surface waters (SW, left panel), surface waters with nutrients (SW+N, second panel), bathypelagic waters (BW, third panel) and bathypelagic waters after carbon addition on day 9 (see main text for details). Each data point represents the average of two replicates and error bars represent the range of values.

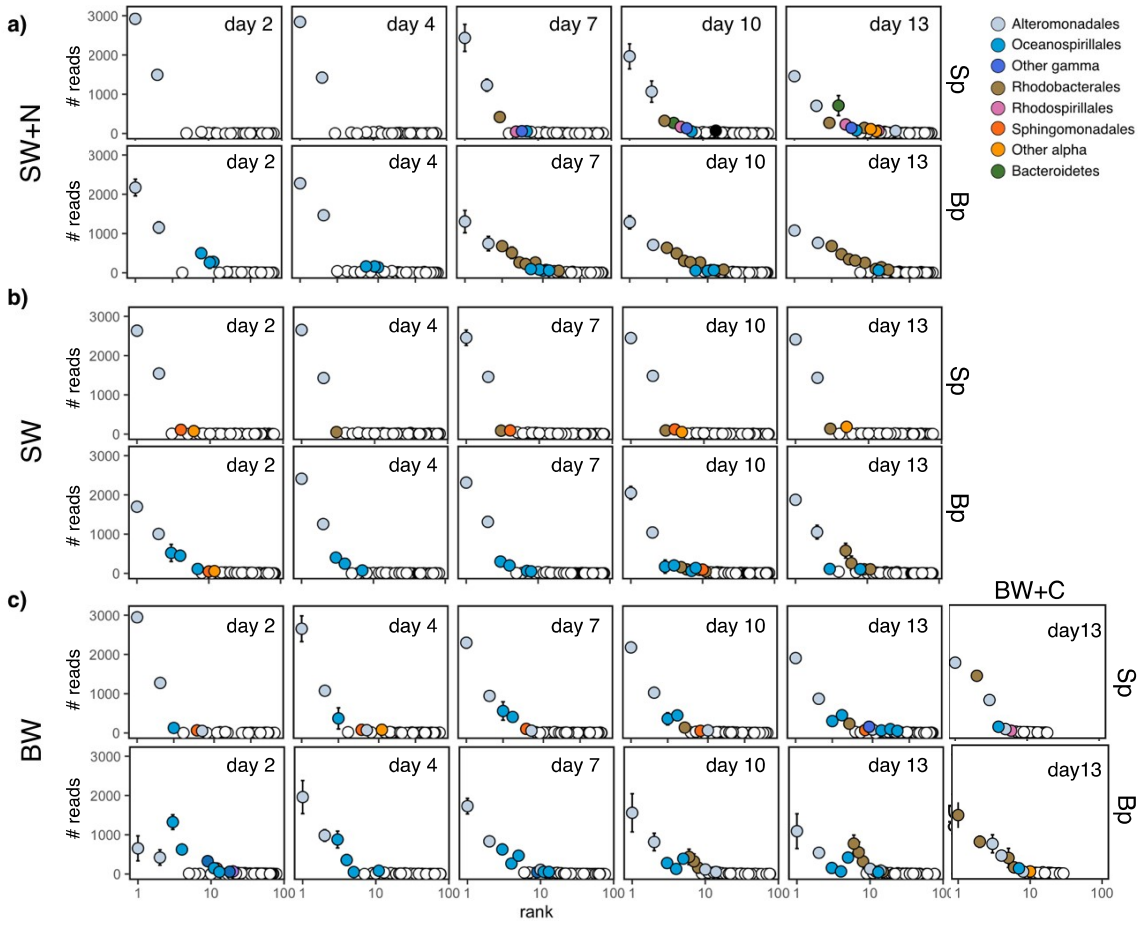

Figure S4. Temporal variation of the rank abundance distribution of amplicon sequence variants ordered by total abundance within each treatment (e.g. Sp in SW+N, Bp in SW+N, etc….). Sp: surface prokaryotes, Bb: bathypelagic prokaryotes, SW: surface waters, SW+N: surface waters with added nutrients, BW: bathypelagic waters, BW+C: bathypelagic waters with added carbon (see main text for details). Only those ASVs representing more than 1% of the reads are coloured.

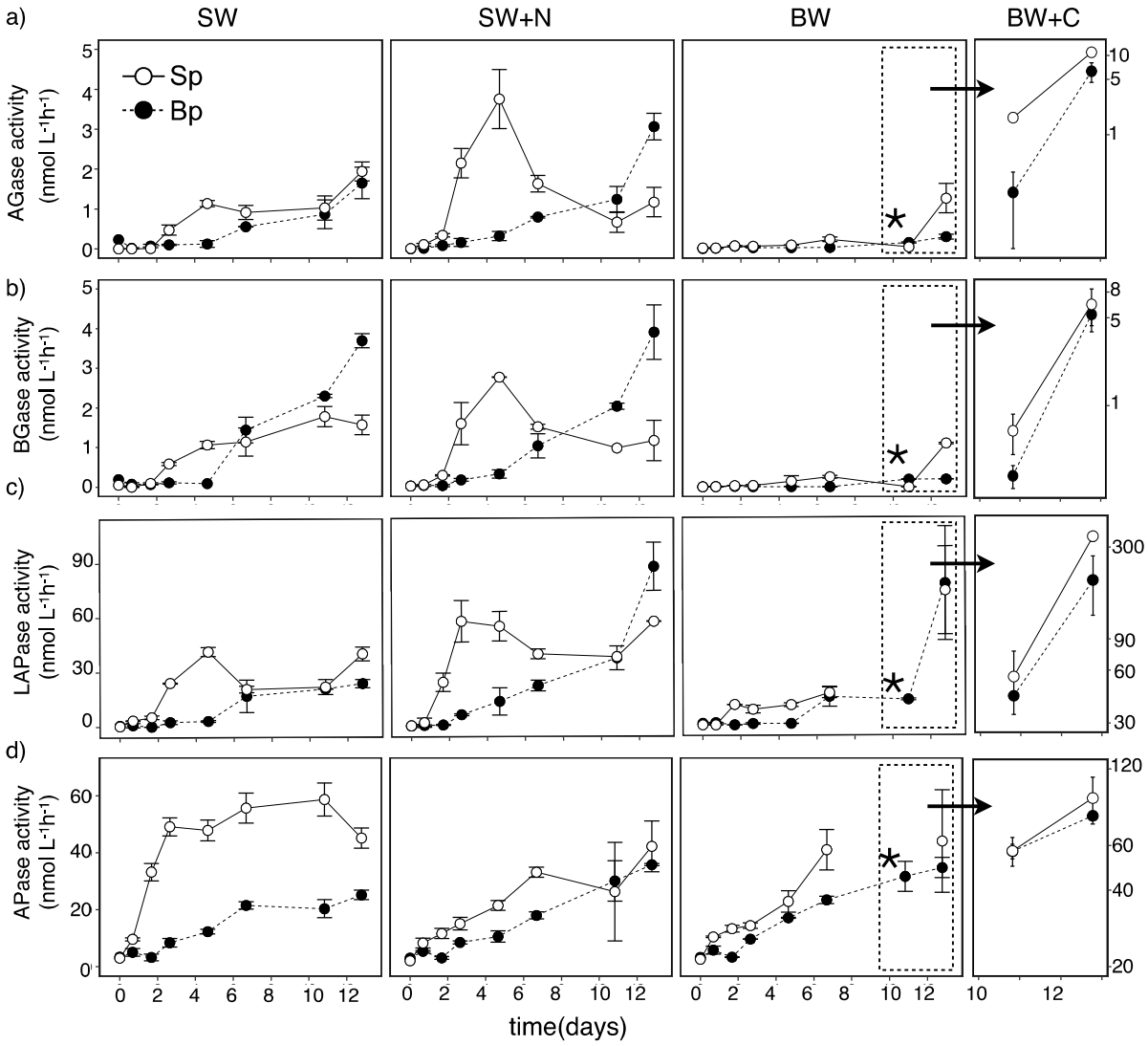

Figure S5. Temporal dynamics of different enzymatic activities of surface prokaryotes (Sp open circles) and bathypelagic prokaryotes (Bp, filled circles) inoculated in surface waters(SW, left panel), surface waters with nutrients (SW+N, second panel), and bathypelagic waters (BW, third panel). The asterisk represents the moment when labile carbon was added to a subsample of the bathypelagic waters treatment (see methods). The small panel on the right represents how this carbon-enriched BW treatment evolved (BW+C). Note the change in Y-axis scale for this plot. Each data point represents the average of two replicates and error bars represent the standard deviation.

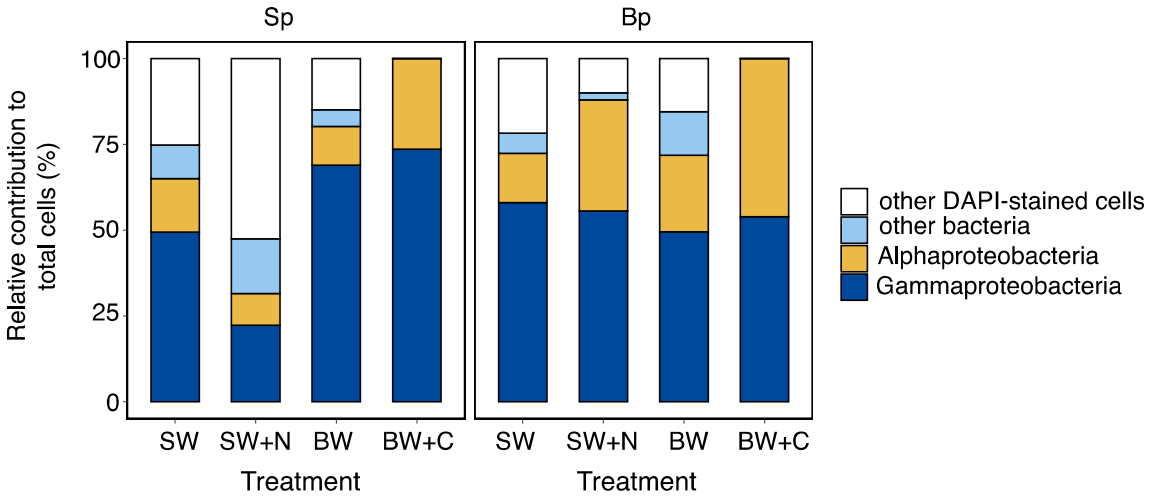

Figure S6. Relative contribution of CARDFISH identified groups to total cells at the last time point of the experiment (day 13). Note that the surface prokaryotes in the SW+N treatment have a high proportion of cells that could not be hybridized with the CARDFISH probe for Eubacteria. Given that the contribution of archaea was negligible at the end of the experiment (Figure 2), our results suggest that there was a large percentage of cells with not enough ribosomes to be detected, i.e. either dormant or dead. Data represent the average of two replicates.

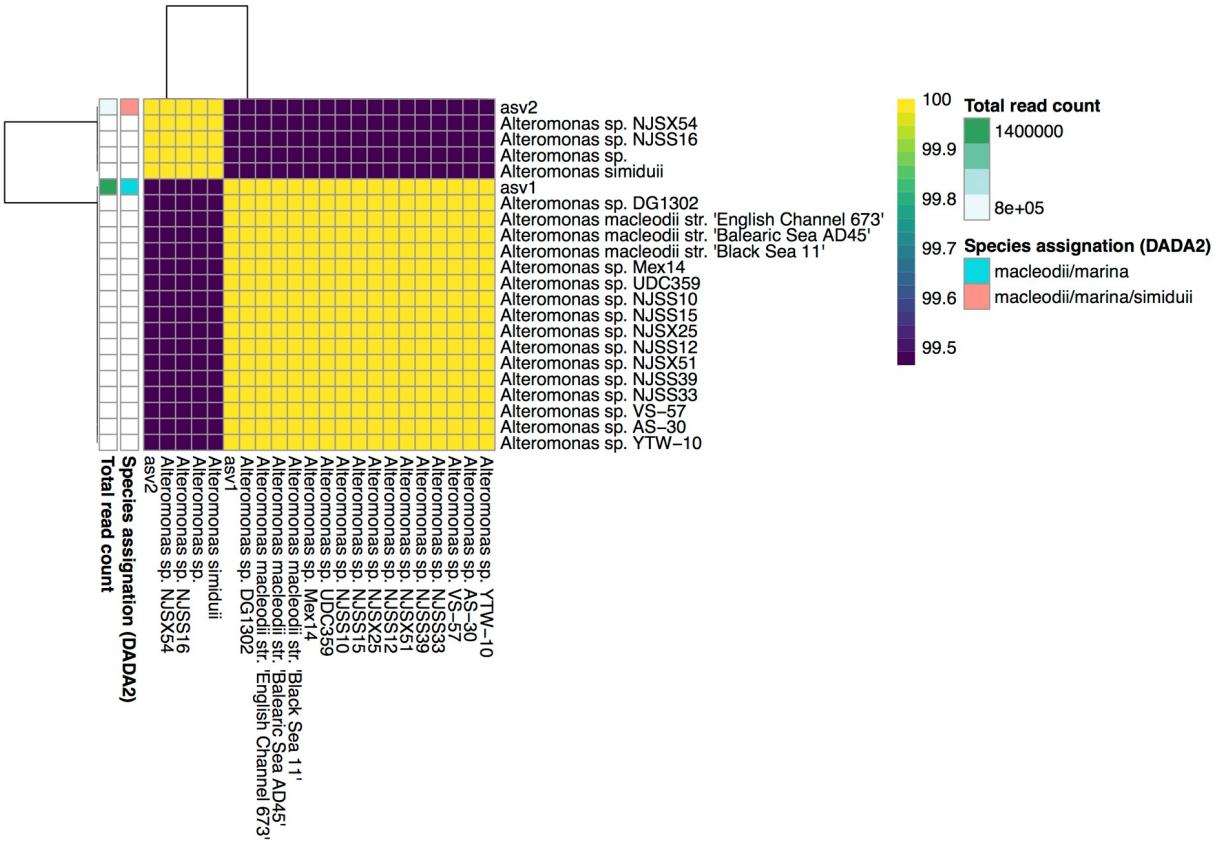

Figure S7. Heatmap showing the alignment of the two main ASV of *Alteromonas* (asv1 and asv2) that bloomed in both the surface and bathypelagic communities of all the water treatments with other *Alteromonas* species. Boxes shaded in yellow indicate 100% nucleotide identity between species. We can see that the V4 region (the one amplified in this study) does not provide enough resolution to distinguish between several species of *Alteromonas*.

REFERENCES

Daims, H., Brühl, A., Amann, R., Schleifer, K. H., & Wagner, M. (1999). The domain-specific probe EUB338 is insufficient for the detection of all bacteria: Development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology*. https://doi.org/10.1016/S0723-2020(99)80053-8

Manz, W., Amann, R., Ludwig, W., Wagner, M., & Schleifer, K.-H. H. (1992). Phylogenetic Oligodeoxynucleotide Probes for the Major Subclasses of Proteobacteria: Problems and Solutions. *Systematic and Applied Microbiology*, *15*(4), 593–600. https://doi.org/10.1016/S0723-2020(11)80121-9

Pernthaler, A., Pernthaler, J., & Amann, R. (2002). Fluorescence In Situ Hybridization and Catalyzed Reporter Deposition for the Identification of Marine Bacteria. *Applied and Environmental Microbiology*, *68*(6), 3094–3101. https://doi.org/10.1128/AEM.68.6.3094

Sebastián, M., & Gasol, J. M. (2013). Heterogeneity in the nutrient limitation of different bacterioplankton groups in the Eastern Mediterranean Sea. *The ISME Journal*, *7*(8), 1665–1668. https://doi.org/10.1038/ismej.2013.42