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Testing the temperature-size rule in marine microorganisms: effect of experimental warming on the size of major bacterioplankton groups as determined by CARD-FISH

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Summary

Knowledge of the cell sizes of different groups of marine bacteria and their controlling factors are of special importance to understand their contribution to oceanic biomass. Observations of organisms individual size shrinkage associated with rising temperatures are widespread in the ocean, but little is known about this effect on the smallest sized organisms, as marine bacteria. In order to understand the response of specific groups of bacterioplankton to temperature in terms of changes in biovolume, we performed 12 monthly incubation experiments at different temperature treatments. The single-cell method Catalyzed Reporter Deposition Fluorescent In Situ Hybridization (CARD-FISH) was used with broad and more specific phylogenetic bacterial groups (Gammaproteobacteria, Bacteroidetes and Rhodobacteraceae and SAR11 within *Alphaproteobacteria* group). Our results indicate a high temporal variability in the biovolume of these groups, and significant differences were also found when comparing their biovolume in different temperature treatments. This effect was especially marked in the case of *Rhodobacteraceae*, which is one of the most important bacterial groups in coastal oceans, but was also observed occasionally in other dominant marine bacterioplankton groups such as SAR11. In this work we demonstrate that the predicted inverse relationship between temperature and individual size also applies for marine microbes.

Introduction

Bacterioplankton represent the largest living biomass in the ocean, and play key roles in different biogeochemical processes. Individual biovolume is an essential characteristic conditioning their total biomass and hence, their contribution to the carbon cycle. However, the size range of different bacterial groups is not well understood (Lebaron and Joux, 1994) and average biovolume estimates for some dominant groups of marine bacteria, such as the widespread SAR11 clade, remain uncertain (Malmstrom et al. 2004). The increasing ocean global warming may also have an effect on bacterial sizes, by decreasing the mean biovolume of different phylogenetic groups according to the temperature-size rule, but there are no evidences confirming this effect yet. In this work we studied the variation of mean biovolumes of marine bacterioplankton over a seasonal cycle and in response to changes in temperature, finding evidence that in a warmer ocean the mean biovolume of some bacterial groups may decrease considerably.

Materials and Methods

To study the effect of temperature on bacterial biovolumes, 12 monthly incubation experiments were carried out under controlled temperature conditions. Seawater samples were collected near the coast of Gijón/Xixón (Spain) and filtered by 0.8 μm pore-size filters in order to isolate the bacterial communities from grazers. The water was incubated under 3 temperature treatments (*in situ*, -3 ºC and +3 ºC), with *in*

situ values varying between 12.7 ºC and 21.2 ºC. Incubations were maintained until bacterial abundances reached their maxima (5-7 days). During the incubations, seawater samples were filtered daily through 0.2 μm pore-size polycarbonate filters (Millipore) and the single-cell method Catalyzed Reporter Deposition Fluorescent In Situ Hybridization (CARD-FISH; Pernthaler et al. 2002) was used to determine changes in cell-size of different phylogenetic groups of bacteria (*Gammaproteobacteria*, *Bacteroidetes* and *Rhodobacteraceae* and SAR11 specific groups of *Alphaproteobacteria*). Samples were counterstained with 4′-6-diamidino-2-phenylindole (DAPI) and examined with an epifluorescence microscope (Leica DM 5500B). To calculate the bacterial biovolume an image analysis system based in R programming language in-house script was used.

Results and Discussion

The biovolume of distinct phylogenetic groups of marine bacteria measured in the western Cantabrian Sea during 2012 showed a high variability. The SAR11 clade was the most abundant group present in the samples, and had the lowest biovolume per cell (mean ± SD of 0.03 ± 0.006 μm^3). The highest biovolumes for this group were found during May (0.045 μm^3). The second most abundant group present in the samples, *Rhodobacteraceae*, had a mean biovolume of 0.067 ±0 .008μm^3, with maximum values in September (0.078 μm^3). The effect of increasing temperature in the incubation experiments was marked in the *Rhodobacteraceae* group, with a decrease in their biovolume for most of the months. This effect could also be observed for some months in the SAR11 clade, even though the differences in biovolume were lower.

The mean sizes found in this work are similar to others described in previous studies (Straza et al. 2009), including the small size of SAR11 cells (Rappé et al. 2002). The large variations in biovolume found indicates a high diversity in size in different phylogenetic groups along the year, leading to different contributions to total bacteria biomass. Drastic changes in bacterial biovolumes over seasons, even within days, have been described before (Carlson et al. 1996). However, here we demonstrated experimentally for the first time a decreasing biovolume with temperature for some groups, particularly *Rhodobacteraceae*, indicating that bacteria cell-size may be susceptible to changes in temperature and hence, to global ocean warming.

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