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Exploring the temperature-driven size reduction of marine bacteria over an annual cycle

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Summary:

Marine heterotrophic bacteria play key roles in carbon and nutrient cycling and as unicells and ectotherms are directly affected by temperature. One of the predicted effects of global warming is size reduction, with the subsequent consequences on biomass and turnover rates in the future ocean. To study this effect, we carried out monthly experiments during a complete annual cycle, incubating surface water (from 12.7 to 21.2 ºC) at three different temperature treatments (*in situ*, -3 ºC, +3 ºC). We followed the growth, standing stocks and biovolume changes of two widespread physiological groups detected by flow cytometry: high nucleic acid (HNA) and low nucleic acid (LNA) bacteria. The annual cycle showed that these groups have opposite seasonal trends, where smaller but more abundant abundant bacteria correspond to HNA cells in the colder spring and to LNA cells in the warmer summer. Surprisingly, the effect of temperature treatment on size was noticeable only upon the typically more active HNA bacteria, that showed a decrease in mean biovolume in 75% of experiments. The final effect on biomass was not significant in both cases, indicating that abundance has a greater effect than size for bacteria.

Introduction:

Marine heterotrophic planktonic bacteria are cosmopolitan, widespread and abundant organisms playing key roles in biogeochemical processes, including nutrient turnover and carbon cycling (Azam 1998). Our comprehension of oceanic carbon pools and fluxes requires understanding the regulation of bacterial biomass, dependent on both abundance and size, which have been proven to vary with temperature in different organisms (Gillooly *et al.* 2001). In the context of global warming, ocean temperature increases are expected to lead to a decrease in organismal size (Kingsolver and Huey 2008) with a possible effect on their standing stocks (Anderson-Texeira *et al.* 2012). Nonetheless, planktonic communities are complex and general ecological rules (Atkinson *et al.* 2003; Brown *et al.* 2004) may be obscured by differences in environmental conditions and trophic interactions, likely to affect the expected temperature response in natural assemblages. This study was conducted to further understand the effect of temperature on the size and biomass of heterotrophic marine bacteria, by following the bacterial community during a whole annual cycle in a hydrographically variable environment.

Material and Methods:

Surface seawater, both whole and pre-filtered by 0.8 µm to avoid grazers was collected every month during 2012 in the Bay of Biscay at 20 miles offshore of the city of Gijón/Xixón on board of RV José de Rioja. In the laboratory water was placed in triplicates in incubators set at 3 temperatures (*in situ*, + 3ºC and - 3ºC) maintaining the photoperiod of the sampling day. Sampling from bottles was aimed

towards following the growth curve of planktonic bacteria and took place twice a day for flow cytometry determinations of bacterial abundance and size and once for variables such as chlorophyll and nutrients (NO_3, NO_2, PO_4), analyzed as in Calvo-Díaz and Morán (2006). Flow cytometric analysis were conducted on a Bencton-Dickinson FACSCalibur equipped with a 488 nm laser and bacteria were separated in two groups (HNA or High Nucleic Acid and LNA or Low Nucleic Acid) in Green fluorescence (FL1) vs. Side Scatter (SSC) and Red fluorescence (FL3) vs. Green fluorescence (FL1) scatter plots. Conversion from SSC to cell biovolume was done following Calvo-Díaz and Morán (2009). All statistical analyses were performed using R open source free statistical software (version 3.1.1, http://www.R-project.org/).

Results and Discussion:

The study site corresponds to a typical temperate area, with a marked seasonality with water mixing in winter and stratification in summer. In situ conditions followed this seasonal pattern (SST: 12.7 – 21.2 ºC; total chlorophyll: 0.14 - 1.8 µg $l^{-1;}$ nitrate: 0.35 – 4.71 µmol l^{-1}, nitrite: 0.22 – 0.43 µmol l^{-1}; phospate: 0.11 – 0.49 µmol l^{-1}), with values within the usual range for this area (Calvo-Díaz *et al.* 2006, Bode *et al.* 2011). Total bacterial biomass was mostly driven by abundance and displayed a bimodal pattern, with HNA cells (3.17 – 13.13 µg C l^{-1}) being responsible for the major peak during the spring bloom, and LNA cells (1.4 - 8.9 µg C l^{-1}) being the mayor contributor to the secondary peak in summer. Bacterial volumes followed patterns opposite to biomass, with HNA cells being larger (range 0.046 - 0.071 μm^3) and LNA cells smaller (ranges 0.03 - 0.054 μm^3) during summer. Previous analyses showed that LNA cells had increased in abundance and decreased in size over a 10 year period, likely related to ocean temperature increases (Morán *et al.* submitted). Surprisingly, decrease of size with temperature in the incubations was rarely found for LNA cells, but took place in 75% of experiments for HNA cells, which followed a seasonal pattern with marked temperature-associated decreases from March to July. Moreover, the magnitude of the decrease of HNA cells was inversely correlated to temperature dependence (calculated as activation energy following Brown *et al* 2004, r^2=0.53, p<0.01), indicating strong effect of temperature during these months. In both cases (LNA and HNA cells), the final effect of size reduction was negligible for biomass, which again was driven by abundance and did not show any clear trend with increasing temperatures.

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