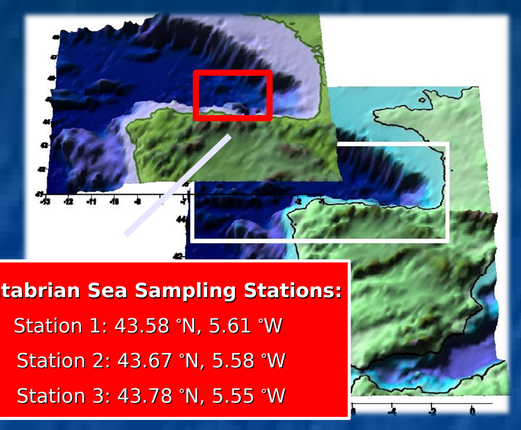
Cytometric Diversity of Marine Bacterioplankton: A 10 Years Interannual study In the Southern Bay of Biscay

F. C. García[1], L. Alonso-Sáez[1], X. A. G. Morán[1], A. López-Urrutia[1]

[1]Instituto Español de Oceanografía. Centro Oceanográfico de Gijón. Avda Príncipe de Asturias 70 bis, 33212 (Gijón)
e_mail: paqui.garcia@gi.ieo.es

INTRODUCTION

The application of molecular methods to marine ecology in the last decades has completely changed our view of the patterns of diversity and distribution of microorganisms in the ocean (Giovannoni et al. 1990, Zinger et al. 2012). However, these methods are expensive and time-consuming when applied on a large number of samples. Flow-cytometry, on the other hand, allows an efficient and rapid processing of a large number of samples. In this sense, the use of single-cell measurements by flow-cytometry for diversity purposes would be a great advance. In marine ecosystems, this concept has been introduced by Li 1997 as 'cytometric diversity'.



Cantabrian Sea Sampling Stations:
Station 1: 43.58 °N, 5.61 °W
Station 2: 43.67 °N, 5.58 °W
Station 3: 43.78 °N, 5.55 °W

OBJECTIVES: In this study we evaluated the power of cytometric diversity to detect changes in the composition of bacterioplankton communities:

1) By comparing changes in bacterial composition of 3.5 years surface samples obtained by cytometric diversity and molecular approaches.

2) Analysing the cytometric diversity patterns of a set of 10-years monthly bacterioplankton flow-cytometry samples for 3 coastal stations.

MATERIALS AND METHODS

Flow-Cytometry Analyses

Samples were collected monthly and were analysed and processed following the methodology of García et al. (2014). Side scatter (SSC), green fluorescence (FL1) and red fluorescence (FL3) were used as cytometric parameters to separate bacterioplankton groups.

To estimate cytometric diversity, we adapted the methodology used by Li (1997) for the study of bacterioplankton.

Genetic diversity estimates

Samples for 454 pyrosequencing were sampled monthly from the surface water at station 2. Bacterial sequences were amplified following the methodology described by Herlemann et al 2011. The purified amplicons were subject using a 454 FLX+ system. The raw reads were quality trimmed and denoised using the Mothur Platform (Schloss et al. 2009). Quimeras were removed using UQuime. Cyanobacteria and chloroplasts were removed and the sequencing depth of all samples was downsized to 4174 reads before calculating Shannon diversity estimates. OTUs were constructed at 99% similarity level.

RESULTS AND DISCUSSION

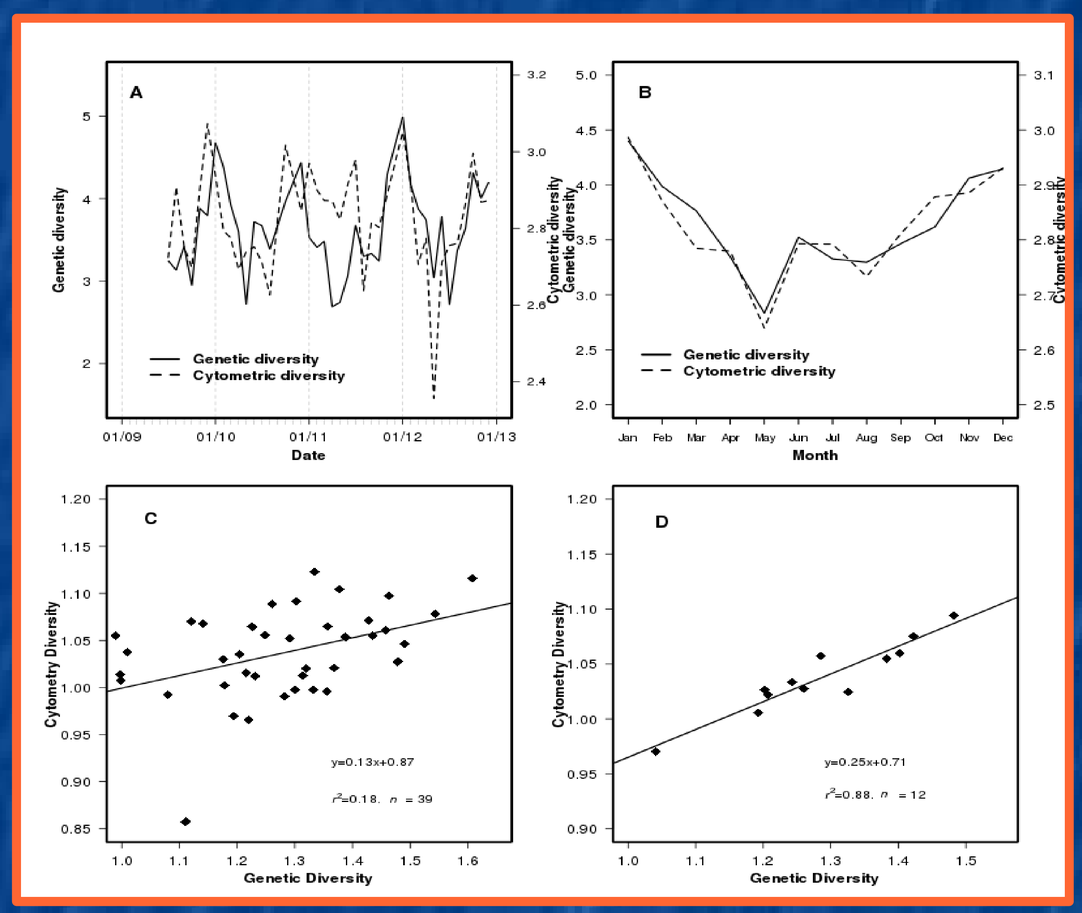


Figure 1: Comparison between surface genetic and cytometric Shannon diversities

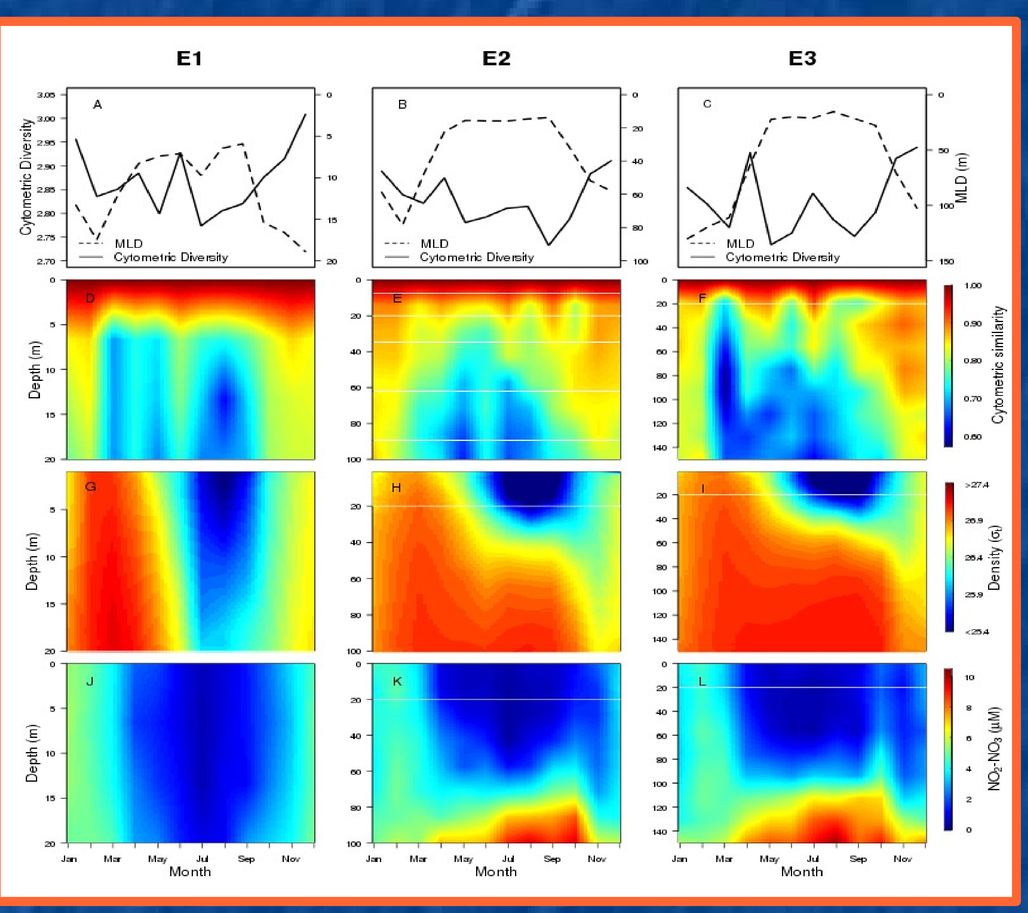


Figure 2: Seasonal distribution of cytometric diversity, Mixed-Layer depth (MLD) and nutrients concentration along the vertical profile at three coastal stations.

A high correlation was found between genetic and cytometric diversity at the level of samples similarity (Mantel statistic, r = 0.59, p <0.001). Furthermore, the seasonal tendency (i.e. monthly averages) between both methodologies was highly correlated (Figure 1 D). These results revealed that cytometric diversity could reliably detect changes in bacterioplankton community composition.

A cyclical diversity pattern was found with maximum Shannon diversity values in early winter and minimum values in spring/ early summer months. When we tested the effect of different environmental variables in the seasonal pattern found in the Cantabrian Sea, we found that day length, MLD and inorganic nutrients concentration are high correlated with the cytometric composition.

Further analyses of the similarity along the vertical profile (Figure 2) revealed that the MLD is the main factor driving the seasonal changes in the composition of bacterioplankton communities. We hypothesize that the increase in bacterial diversity in early winter could occur due to a vertical reshuffling of species during mixing period of the water column.

In summary: Our study points out the potential of flow-cytometry to assess heterotrophic bacterioplankton community structure and dynamics in aquatic systems and the MLD as the factor responsible of the reshuffling of species observed in the Cantabrian Sea.

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Acknowledgements

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