Monitoring the diversity of photosynthetic picoplankton in marine waters - (PICODIV)

Vaulot D.[1], Scanlan D.J.[2], Medlin L.K.[3], Pedrós-Alió C. [4], Throndsen J.[5] and the PICODIV participants

[1] Station Biologique de Roscoff, France (vaulot@sb-roscoff.fr); [2]University of Warwick, UK; [3]Alfred Wegener Institute for Polar Research, Bremerhaven, Germany; [4]Institut de Ciències del Mar, Barcelona, Spain; [5]University of Oslo, Norway.

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Objectives

Picoplankton (defined operationally as cells that pass through a 3 micron filter) dominate the photosynthetic biomass in many marine ecosystems, not only in the very oligotrophic regions of the world oceans, such as the Eastern Mediterranean Sea, but also in mesotrophic areas. However, picophytoplankton are clearly not exclusively restricted to pelagic environments. In many coastal regions, they are present throughout the year and constitute a 'background' population, onto which episodic phenomena, such as the spring bloom develops. In some environments, such as coastal lagoons, picoplankton can be a major component of biomass and productivity for most of the year. In addition, some bloom-forming picoplankters, such as *Aureococcus* spp., are toxic. However, fewer than 50 species of picophytoplankton have been described. A clear proof of our poor knowledge of picophytoplankton diversity is revealed by the discovery of three novel algal classes in the last ten years described from picophytoplanktonic taxa.

Because so little is known about the taxonomy and systematics of picophytoplankton we have very little data to estimate the levels of its biodiversity under natural conditions and how picophytoplankton are affected by environmental variability linked to either anthropogenic influence or to larger scale phenomena, such as those linked to climate change or global warming.

The major objective of the PICODIV project was to develop, test and validate methods based on molecular biology techniques that allow for routine and extensive assessment of picophytoplankton diversity (species composition and relative contribution of taxa to total community) in the marine environment.

Our strategy to meet this objective was encapsulated in the following four steps:

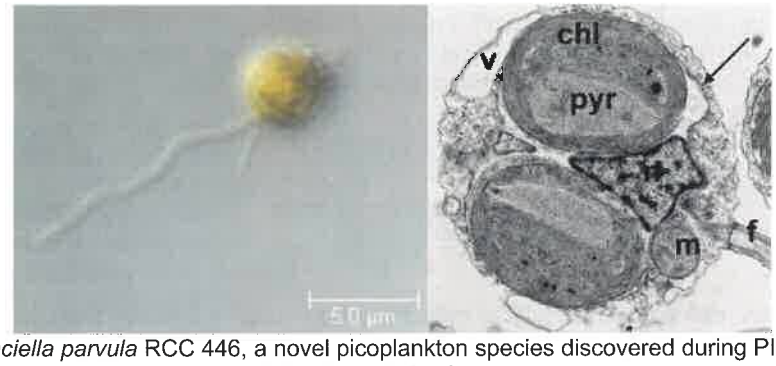
(1) Obtain SSU rDNA sequences for as many as possible picophytoplankton taxa from both cultures and natural samples. Novel taxa will be assessed using a combination of methods including in particular pigment analysis and electron microscopy. (2) Using this sequence database, develop hierarchical probes recognizing each taxonomic group having picophytoplanktonic representatives (3) Develop fast and efficient techniques to quantify the fraction of the pico-phytoplankton recognized by the probes in natural samples. (4) Test and validate these probes on time series of picophytoplankton biodiversity in three coastal ecosystems.

Results

Cultures of picoplankton.

Obtaining cultures of novel picoplanktonic species is a key step to assess picoplankton diversity. More than 250 cultures were established from three coastal sites (Roscoff, Helgoland, Blanes) and from open ocean cruises. These cultures have been characterized

by microscopy, pigment analysis and rDNA sequencing. We performed a detailed analysis of the phenotype (pigments, morphology) and genotype (18S rDNA phylogeny) of key groups of the picoplankton based on representative strains isolated during the project. This work led to the reorganization of the taxonomy of important groups such as the cyanobacteria and Prasinophyceae as well as to the description of novel species (e.g. *Florenciella parvula*) or the correct phylogenetic assignment of previously described species (e.g. *Telonema*).



Florenciella parvula RCC 446, a novel picoplankton species discovered during PICODIV: Light and electron microscopy

Clone libraries of marine picoplankton.

Construction of SSU rDNA clone libraries representative of the *in situ* picophytoplankton diversity, and subsequent sequencing of novel gene sequences, is critical for understanding and describing the total diversity present in contrasting marine aquatic environments. This is particularly so for identification of novel picophytoplankton groups for which cultured counterparts do not exist. The sequence data obtained provide the basis for the design of new phylogenetic probes, which can be used to retrieve their morphology..

In the course of the project we have obtained over 1,300 rDNA sequences from 46 clone libraries constructed both from coastal an open ocean samples. We analyzed the sequence data in order to both determine the spatial and seasonal variation of taxonomic groups at each coastal site and perform a detailed phylogenetic reconstruction for some of the groups, especially those for which no culture representatives are available (stramenopiles, alveolates). Two novel groups were discovered, one of which constitutes probably new algal class.

Molecular probes for marine picoplankton.

We have designed and tested the specificity of probes against some key taxonomic groups from the picoplankton. We then used probes to monitor the abundance in coastal waters of different groups among picoplankton (*Synechococcus*, Prasinophyceae, Prymnesiophyceae...) with detection techniques developed during the project, in particular fluorescent *in situ* hybridization coupled with the tyramide signal amplification (FISH-TSA) and DNA micro-arrays.

Potential exploitation by end users

The collection of cultures that have been isolated during the project should be a major resource both from a fundamental point of view because these strains are likely to represent key organisms in coastal waters and from the applied point of view because these strains could be screened in the future for interesting compounds. From the methodological point of view, the DGGE approach, that we have developed and applied, could prove very useful to assess and monitor the diversity of phytoplankton in oceanic waters. Finally the set of probes we have designed and tested as well the probing techniques (FISH-TSA, DNA micro-arrays) that have been developed should be very useful for routine monitoring of some key groups, such as the *Synechococcus* cyanobacteria and the Prasinophyceae, e.g. in the context of long term surveys to detect ecosystem response to global change or to pollution.