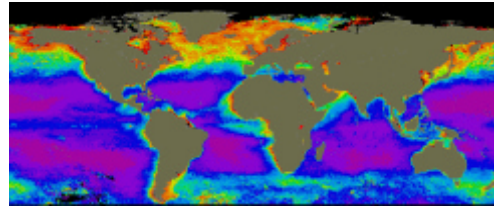


# PICODIV

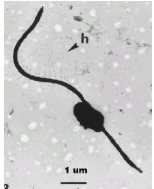
## Monitoring the Diversity of Photosynthetic Picoplankton in Marine Waters

Framework Program 5 - Contract EVK3-CT-1999-00021

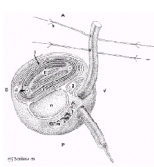
PICODIV is a program devoted to investigate the diversity of picoplankton in coastal and pelagic European waters using a combination of techniques including algal cultivation, HPLC pigment analysis, electron microscopy and the latest developments in molecular techniques.



*Bolidomonas mediterranea*



*Bolidomonas pacifica*



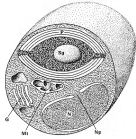
*Pseudoscurfeldia marina*



*Pelagomonas calceolata*



*Ostreococcus tauri*



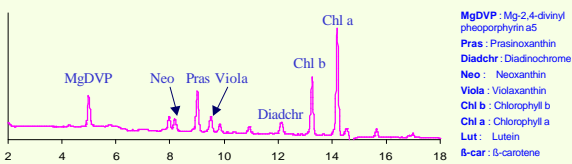
**Step 1 - Isolate picoplankton cultures and characterize new species**

Picoplankton cultures are isolated after filtration through a 3 micron filter. A combination of techniques are used to characterize them:

- Flow cytometry.** Basic information on cell size and abundance.
- Pigment analysis (HPLC).** Indicates algal class
- Electron microscopy.** Number and type of flagella. Scales.
- SSU rRNA gene sequence.** Phylogenetic position.

Cultures that do not belong to any known species are further characterized and described.

### HPLC pigment analysis of a Prasinophyte culture

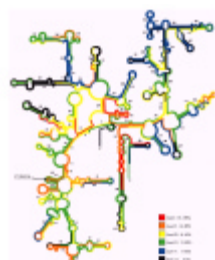


### Step 2 - Characterize directly natural picoplankton populations by RNA gene sequencing

A large fraction of picoplankton remains uncultured. In order to assess directly the nature and diversity of natural populations, one has to rely on molecular biology techniques. One of the most elegant approaches is to sequence directly the ribosomal RNA gene, which is used extensively for taxonomical purposes. By comparing the sequence from natural populations to the large number of eukaryotic sequences already stored in databanks (GenBank), one can assess their identity.

This approach applied to samples from the Pacific Ocean (right) revealed that the marine picoplankton contains a very large number of novel taxa, some of which are highly divergent compared to known groups (Moon et al. submitted).

### The SSU rRNA molecule



Small subunit of ribosomal RNA (from Fuchs et al. 1998)

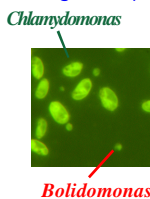


### Step 3 - Develop probes based on RNA gene sequence

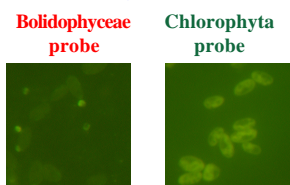
Based on the SSU rDNA sequences obtained both from cultures and natural samples, it is possible to design oligonucleotide **probes** (i.e. short DNA sequences about 20 bp long) that recognize specific taxonomic groups (e.g. a class such as the Prasinophyte, or a genus such as *Pelagococcus*, or a species such as *Micromonas pusilla*).

These probes can be labelled with a fluorescent molecule and used to detect cells by microscopy. Within PICODIV, we are developing a new highly efficient method called FISH-TSA (see pictures below). In the future, it should be possible to couple such probes with the extremely powerful DNA chip technologies that are currently emerging.

### Mixing two species



Using probes to distinguish them



### Step 4 - Monitor picoplankton populations at three coastal sites

Using probes targeted against all major picoplankton groups, as well as more classical techniques such as HPLC or flow cytometry, we will monitor during one year the abundance of picoplankton populations at three European coastal sites as well as on samples collected during selected cruises.



### Partners

- Daniel Vaillot, **Station Biologique de Roscoff**, CNRS et UPMC, France (coordinator)
- Dave Scanlan, **University of Warwick**, UK
- Linda Medlin, **Alfred Wegener Institute for Polar Research** Bremerhaven, Germany
- Carlos Pedrós-Alió, **Institut de Ciències del Mar**, Barcelona, Spain
- Jahn Thronsdén, **University of Oslo**, Section of Marine Botany, Norway



More informations: [www.sb-roscoff.fr/Phyto/PICODIV](http://www.sb-roscoff.fr/Phyto/PICODIV)  
Daniel Vaillot, Station Biologique, 29680 Roscoff, FRANCE, email: vaillot @sb-roscoff.fr