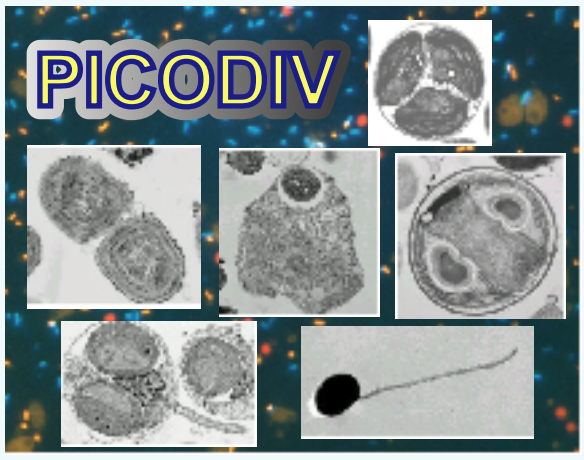

Diversity of Coastal Eukaryotic Picoplankton

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Abstract

Despite its key ecological role, the diversity of eukaryotic marine picoplankton (< 3 μm), has been little studied. Recent studies, based on the sequencing and analysis of the small sub-unit ribosomal RNA gene (rDNA), have revealed the presence in the picoplanktonic fraction of many new eukaryotic groups, not represented in cultures (Moon-van der Staay et al. 2001). Within the European program PICODIV and the regional program PICOManche we have analyzed picoplankton diversity in several coastal systems (North Sea, English Channel, Mediterranean Sea). We focus here on results obtained in the English Channel, off Roscoff (Britanny).

First, the 18S rRNA gene was directly amplified from coastal and estuarine samples taken at different periods of the year and cloned to construct clone libraries. A number of clones were partially sequenced and their phylogenetic affiliation determined. As observed in ecosystems, the majority of sequences did not match known or cultivated organisms and were related to potentially heterotrophic groups, more specifically Alveolates and Stramenopiles. Moreover, two new phylogenetic groups have been discovered, one related to the protist *Telonema* and one related to the red algae. Overall, clone library composition did not vary much with the period of the year, except during the diatom bloom from late Spring-early Summer.

Second, we designed oligonucleotide probes recognizing major taxa observed in coastal waters (in particular Prasinophyceae) and found that a single photosynthetic genus, *Micromonas*, dominates quantitatively the picoeukaryote community most of the year.

This study demonstrates that, despite their high diversity, picoeukaryote communities can be dominated numerically by a small number of taxa, but it also pleads for a much more detailed analysis of the nature and functional role of heterotrophic picoeukaryote.

Introduction: what is picoplankton ?

Picoplankton is defined as cells that pass through a 3 μm filter. They dominate the biomass in many marine ecosystems especially in the very oligotrophic regions of the world oceans, such as the central Pacific gyre or the Eastern Mediterranean Sea. Picoplankton contains both prokaryotes (proteobacteria, cyanobacteria, archea...) and eukaryotes (protists), both autotrophs and hetererotrophs. Picoeucaryotes have received very little attention until recently. Their taxonomic diversity is very broad. To date fewer than 40 species of picoeukaryotes have been described. This number pales in comparison, for example, to the more than 4,000 marine phytoplankton species that have been described to date and to the over 100,000 that are believed to exist. Therefore, a vast number of taxa remain unknown and undescribed. In many coastal regions, photosynthetic picoeukaryotes are present throughout the year and constitute a 'background' population, onto which episodic phenomena such as the spring phytoplankton bloom develops. Some photosynthetic picoplankton species are also very relevant from the human point of view, since some bloom-forming picoplankters such as *Aureococcus* spp. are toxic. Heterotrophic picoeukaryotes include in particular grazers feeding on bacteria, but probably also perform other functions such as particulate matter degradation.

Methods

Sequences. Marine waters off Roscoff (Brittany, English Channel) were sampled on 8 different occasions in 2000 and 2001 (7 coastal samples at Astan and 1 estuarine one at Dourduff). The 18S ribosomal RNA gene (rDNA) was amplified by PCR using universal primers and cloned following Moon-van der Staay et al. (2001). Between 40 and 80 clones for each library were partially (500 bp) sequenced. The data were analyzed with the ARB software (http://www.arb-home.de/) to determine sequence affiliation and build phylogenetic trees.
Probes. Oligonucleotide probes (18 bp) targeting 18S rRNA were designed using the ARB software to recognize three genera of the Mamiellales order: *Micromonas* (MICRO01), *Bathycoccus* (BATHY01) and *Ostreococcus* (OSTREO01). The probes were thoroughly validated on cultures. To recognize the whole eukaryotic community, we used a combination of three published probes (EUK1209R + CHLO01 + NCHLO01). Probes were applied to natural samples obtained biweekly from the Astan site off Roscoff using the TSA FISH procedure (Not et al. 2002).
Pigments. Pigments in the picoplankton fraction of the Roscoff Astan samples were determined by HPLC.

I - Diversity of picoplankton 18S rDNA sequences in the English Channel

The analysis of 416 partial 18S rDNA sequences obtained from 7 coastal and 1 estuarine samples taken off Roscoff (English Channel) reveals a very wide diversity (Fig. 1) similar to what has been observed in recent studies in the equatorial Pacific, Mediterranean Sea, North Atlantic, and off Antarctica (Diez et al. 2001; Moon-van der Staay, 2001).

For phylogenetic groups containing mostly **photosynthetic organisms**, only four classes were observed: Prasinophyceae, Cryptophyceae, Prymnesiophyceae, and Dinophyceae. Most (but not all) sequences clustered with known genera or species. For example, within the Prasinophyceae (division Chlorophyta), the most represented class, almost all of the 81 sequences from Roscoff clustered with three genera: *Micromonas*, *Ostreococcus* and *Bathycoccus*. However, within these genera, some heterogeneity is observed and ecotypes probably occur, as in *Prochlorococcus*. In contrast, for groups assumed to be **heterotrophic**, most sequences did not match any known organism. In particular, we recovered many sequences belonging to the novel phylogenetic groups from the Stramenopiles and Alveolates discovered recently in other environments (Diez et al. 2001; Moon-van der Staay, 2001). In particular, among Stramenopiles (Fig. 1, right), we observed sequences belonging to most of the clades recently described by Massana et al. (2002), demonstrating the ubiquity of these uncultivated groups.

Finally, we brought to light two novel phylogenetic groups labelled Rosko I and II. One seems to be related to *Telonema*, a protist of uncertain affiliation, while the other is phylogenetically close to the red algae (K. Valentin, unpublished).

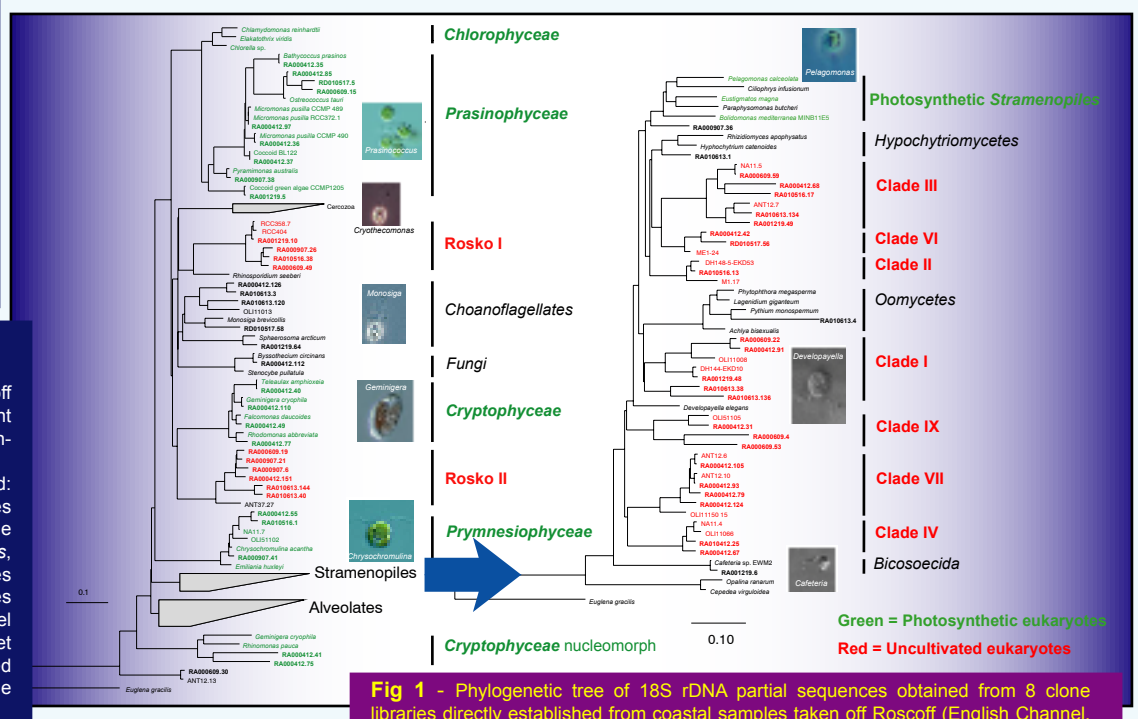

Fig 1 - Phylogenetic tree of 18S rDNA partial sequences obtained from 8 clone libraries directly established from coastal samples taken off Roscoff (English Channel, France) at different times of the year in 2000 and 2001. The Stramenopiles have been blown up at right (Romari and Vaulot, in prep). Images from Micro*scope web site.

II - Temporal and spatial patterns of picoplankton diversity in the English Channel

At higher taxonomic levels (i.e. that of the Division/Class), the overall composition of the 18S rDNA clone libraries remained fairly stable (Fig. 2, left). Three groups (Prasinophyceae, Stramenopiles and Alveolates II) were found in all samples, while other such as the Alveolates I and Choanoflagellates are more sporadic, but still observed at all seasons. The stability of the picoplankton community was also reflected in the pigment composition (Fig. 2, right) where the presence of prasinoxanthin (characteristics of Prasinophyceae) and alloxanthin (Cryptophyceae) confirmed the sequence data. Only two clone libraries differ markedly in their composition. The June 2001 sample corresponded to the diatom bloom and was characterized by the total disappearance of Cryptophyceae (despite the presence of alloxanthin) and of ciliates. In contrast, the estuarine library (Dourduff, May 01) was dominated by ciliates and displayed a lower diversity.

At a lower taxonomic level, however, 66% of the phylotypes (corresponding to sets of sequences with higher than 98% identity) occurred in a single sample, pointing out to a high number of species/ecotypes.

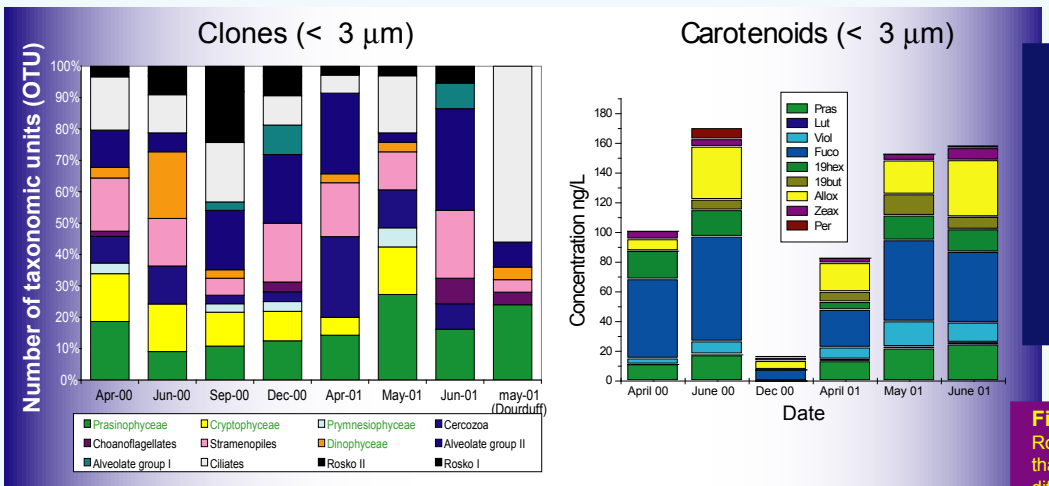

Fig 2 - **Left** : Seasonal variation of the relative importance of the different eukaryotic protist groups off Roscoff based on numbers of 18S rDNA operational taxonomic units (OTU), i.e. sequences with less than 99.5 identity (Romari and Vaulot, in prep.). **Right** : Seasonal variation of the abundance of the different photosynthetic pigments determined by HPLC in the same samples (Latasa, unpublished).

III - Population dynamics of specific picoplankton genera in the English Channel

As interesting as they may be, clone libraries only provide a qualitative image of the picoplankton community. However, based on the sequence obtained, it is possible to design oligonucleotide probes that recognize only specific taxonomic groups. Cells are then labeled with these probes using fluorescent in situ hybridization (FISH) with tyramide signal amplification (TSA), a procedure recently applied to picoplankton (Not et al. 2002). We designed such probes for three genera of Prasinophyceae (*Micromonas, Bathycoccus, Ostreococcus*) found repeatedly in our coastal clone libraries (see Fig. 1 and 2) and applied them to a seasonal time series off Roscoff (Fig. 3).

Very surprisingly a single genus (*Micromonas*) dominated the picoeukaryote community throughout the year representing up to 80 % of the cells. *Bathycoccus* made only sporadic but significant appearances while *Ostreococcus* was always found at negligible concentrations. This study is currently extended with probes targeting all major picoeukaryote groups.

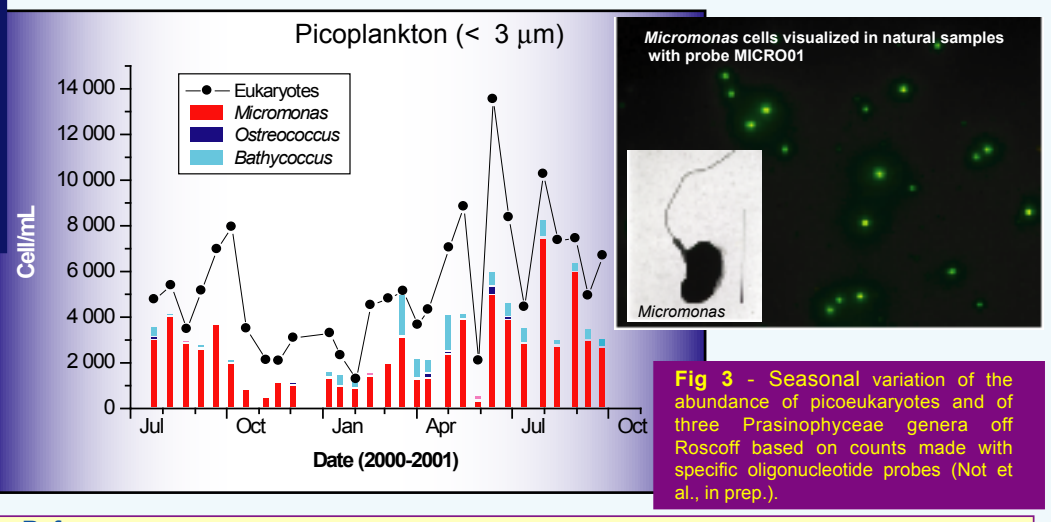

Fig 3 - Seasonal variation of the abundance of picoeukaryotes and of three Prasinophyceae genera off Roscoff based on counts made with specific oligonucleotide probes (Not et al., in prep.).

Perspectives

While, within photosynthetic picoeukaryotes, taxonomic diversity seems relatively low, although ecotypes probably occur, heterotrophic picoeukaryotes appear highly diversified and may play much more varied and complex roles than the sole predatory function, presently assigned to them. Thus, they may participate, on a par with heterotrophic bacteria, to the direct recycling of organic matter and may be involved in numerous parasitic and symbiotic relationships. They will deserve much more attention in the future.

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More informations: www.sb-roscoff.fr/Phyto/PICODIV
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