

TITLE : **PICODIV: MONITORING BIODIVERSITY OF
PICOPHYTOPLANKTON IN MARINE WATERS**

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PICODIV: EXPLORING THE DIVERSITY OF PICOPLANKTON

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INTRODUCTION

Picoplankton (defined operationally hereafter as cells that pass through a 3 µm filter) dominate the photosynthetic biomass in many marine ecosystems, not only in the very oligotrophic regions of the world oceans, such as the central Pacific gyre (Campbell et al. 1994) or the Eastern Mediterranean Sea, but also in mesotrophic areas, such as the high chlorophyll - low nutrient equatorial regions. However, picophytoplankton are clearly not exclusively restricted to oceanic environments. In many coastal regions, they are present throughout the year and constitute a 'background' population (Agawin et al. 1998), onto which episodic phenomena, such as the spring bloom develops. In some environments, such as coastal lagoons, picoplankton are a major component of biomass and productivity for most of the year. Picophytoplankton are also very relevant from the human point of view, because some bloom-forming picoplankters, such as *Aureococcus* spp. are toxic (Bricelj and Lonsdale 1997).

Photosynthetic picoplankton encompass both prokaryotic and eukaryotic species:

- Prokaryotes. Only two major genera are important for the picoplanktonic community in marine waters: *Synechococcus* and *Prochlorococcus*. Whereas *Prochlorococcus* dominates over *Synechococcus* in most oligotrophic regions, except at high latitudes, the reverse is true under meso- and eutrophic conditions (Partensky et al. 1999). With such wide ecological distributions, these two genera display a large genetic and phenotypic variability, that is just beginning to be assessed.
- Eukaryotes. In contrast to prokaryotes, the taxonomic diversity of picophytoplanktonic eukaryotes is much broader. In fact, nearly every algal division has picoplanktonic representatives (Figure 1). Still, a vast number of taxa undoubtedly remain unknown and undescribed.

To date fewer than 30 species of picophytoplankton have been described (see Table 1). This number pales in comparison 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. 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 (to put this into perspective, to ignore an algal class corresponds to ignoring the existence of mammals or birds among the vertebrates):

1991 class Pedinophyceae based on *Resultor mikron* 2 µm (Moestrup 1991)

1993 class Pelagophyceae based on *Pelagomonas calceolata* 2 µm (Andersen et al. 1993)

1999 class Bolidophyceae based on *Bolidomonas pacifica* 1.5 µm (Guillou et al. 1999)

Table 1: Chronology of taxonomic picoplankton knowledge.

Year	Name	Class	Size µm
1952	<i>Chromulina pusilla</i> Butcher (renamed <i>Micromonas</i> Manton & Parke)	Prasinophyceae	1-1.5
	<i>Nannochloris atomus</i> Butcher, <i>N. maculata</i> Butcher (renamed <i>Nannochloropsis</i> Hibberd)	Eustigmatophyceae	1.5-4
1955	<i>Nannochloris oculata</i> Droop (renamed <i>Nannochloropsis</i> Hibberd)	Eustigmatophyceae	1.5-4
1957	<i>Monallantus salina</i> Bourelly (renamed <i>Nannochloropsis</i> Hibberd)	Eustigmatophyceae	1.5-4
1967	<i>Hillea marina</i> Butcher	Cryptophyceae	2-2.5
1969	<i>Pedinomonas mikron</i> Throndsen (renamed <i>Resultor</i> Moestrup)	Prasinophyceae	1.5-2.5
	<i>Scourfieldia marina</i> Throndsen (renamed <i>Pseudoscourfieldia</i> Manton)	Prasinophyceae	2-3
1974	<i>Imantonia rotunda</i> Reynolds	Prymnesiophyceae	2-4
1977	<i>Pelagococcus subviridis</i> Norris	Pelagophyceae	2.5-5.5
1978	<i>Chlorella nana</i> Butcher	Chlorophyceae	1.8-2.6
1979	Discovery of oceanic picoplankton marine <i>Synechococcus</i> Naegeli	Cyanophyta	0.8-1.2
1982	<i>Nannochloropsis gaditana</i> Lubian <i>Nanochlorum eucaryotum</i> Wilhelm <i>et al.</i> (renamed <i>Nannochloris</i> Naumann)	Eustigmatophyceae Chlorophyceae	2.5-5
1987	<i>Triparma</i> Booth & Marchant spp, <i>Tetraparma pelagica</i> Booth	Chrysophyceae	2.2-4.7
1988	<i>Prochlorococcus marinus</i> Chisholm	Cyanophyta	0.5-0.7
	<i>Aureococcus anophagefferens</i> Hargraves & Sieburth	Pelagophyceae	2-4
1990	<i>Bathycoccus prasinus</i> Eikrem & Throndsen	Prasinophyceae	1.5-2.5
	<i>Pycnococcus provasolii</i> Guillard	Prasinophyceae	1- 4
1993	<i>Pelagomonas calceolata</i> Andersen & Saunders	Pelagophyceae	1.3-3
1995	<i>Ostreococcus tauri</i> Courties & Chrétiennot-Dinet	Prasinophyceae	0.8-1.1
1996	<i>Prasinoderma coloniale</i> Hasegawa & Chihara	Prasinophyceae	2.5-5.5
	<i>Nannochloropsis granulata</i> Karlson & Potter	Eustigmatophyceae	2- 4
1997	<i>Aureoumbra lagunensis</i> Stockwell <i>et al.</i>	Pelagophyceae	2.5-5
1999	<i>Bolidomonas pacifica</i> Guillou & Chrétiennot-Dinet <i>B. mediterranea</i> Guillou & Chrétiennot-Dinet	Bolidophyceae	1.5
2000	<i>Picophagus flagellatus</i> Guillou & Chrétiennot-Dinet	Chrysophyceae	2
	<i>Symbiomonas scintillans</i> Guillou & Chrétiennot-Dinet	Bicosocid	2

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 to understand how the picophytoplankton might be affected by environmental variability linked to either anthropogenic influence or to larger scale phenomena, such as those linked to climate change or global warming. However we have some indications that picophytoplankton species (and therefore picophytoplankton biodiversity) may react sharply to changes in marine systems:

- The prokaryote *Prochlorococcus* consists of at least two different genotypes/phenotypes, each one dominates under different environmental conditions: i.e., one is present under the high light/low nutrient conditions of the marine surface layer, and the other under the low light/higher nutrient conditions of the bottom of the euphotic zone. Thus *Prochlorococcus* is able to partition its niche genetically so that it is phenotypically adapted to its environment.

- The abundance of *Synechococcus* in the equatorial Pacific decreases during El Niño Southern Oscillation episodes.
- The potentially toxic brown picoplanktonic alga *Aureococcus* was unknown before 1985, but since then it has bloomed repeatedly in US coastal waters (Bricelj and Lonsdale 1997).

Our ignorance concerning picophytoplankton diversity is mostly explained by the fact that, because of their very small size, picophytoplankton cells most often lack any distinguishing features and are very difficult to identify by classical methods. In fact many have evolved to small "green or brown ball" morphotypes that mask a broad taxonomic diversity (Potter et al. 1997). Our present state of knowledge regarding picophytoplanktonic biodiversity is in fact analogous to that prevailing ten years ago for eubacteria and archaea. Until the early 1990's, the taxonomy and understanding of bacterial diversity was based primarily on species isolated into culture. No-one could have predicted the vast diversity of these organisms in nature (Giovannoni et al. 1990).

In order to remedy to this very poor state of knowledge concerning a group that, in many ecosystems, accounts for up to 60 to 80% of photosynthetic biomass and production, there is a very urgent need to develop efficient monitoring tools of picophytoplankton diversity. This problem is in fact very analogous to that encountered by microbiologists who cannot tell apart bacteria based on their shape or even on their metabolic requirements. The latter have relied more and more in recent years on molecular biology techniques to identify and detect bacteria in the environment

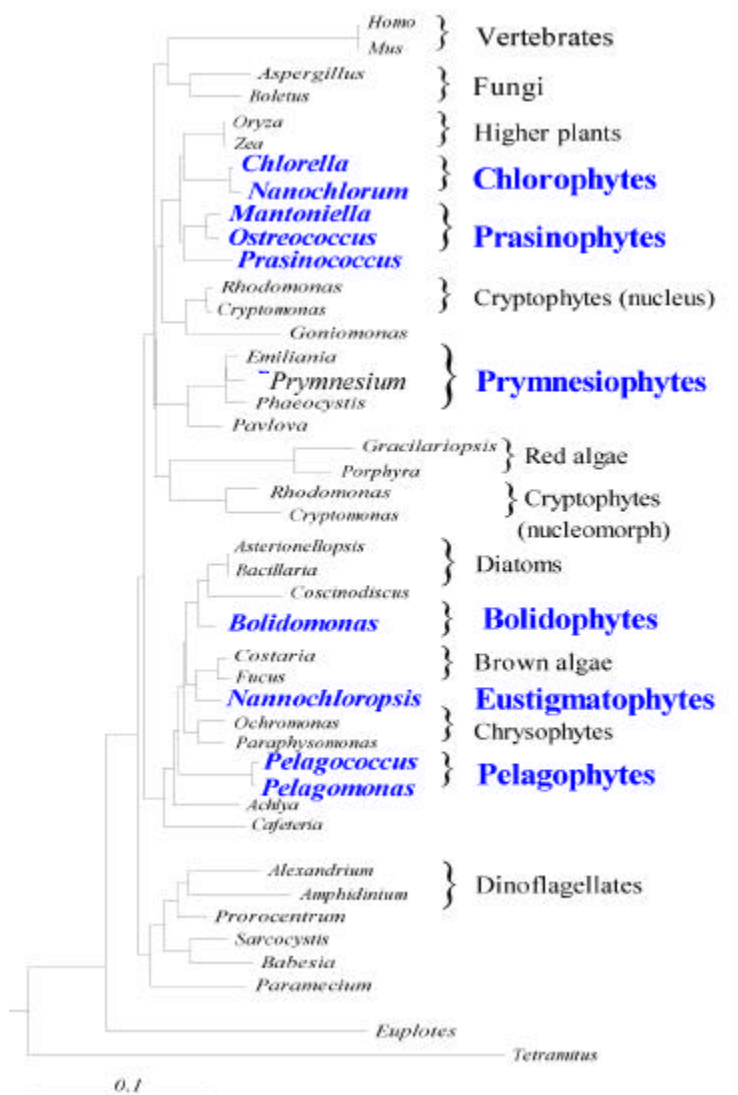


Figure 1: A tree showing the phylogenetic affinities of eukaryotic picophytoplankton species (in bold). Note that only one picoplanktonic Prymnesiophyte has been isolated yet (*I. rotunda*), but a number of picoplanktonic Prymnesiophyte sequences have been recovered from oceanic samples (Moon-van der Staay et al. 2000). Source: Guillou unpublished

(Giovannoni et al. 1990; Amann and Kuhl 1998). We plan during the course of this project to expand this very successful approach to picophytoplankton.

STRATEGY

Our strategy is 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 successive taxonomic groupings 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.

We will focus on the picophytoplankton from **coastal European waters** that has been much less studied in comparison to that of offshore oligotrophic waters. For this purpose we have selected three sites located in the following regions:

- English Channel (Roscoff)
- North Sea (Helgoland)
- Western Mediterranean Sea (Blanes Bay)

These sites have been carefully selected as offering a wide range of environmental conditions representative of EU coastal waters. Moreover, all have been extensively monitored in the past and abundant background information is available on environmental conditions as well as phytoplankton populations. One of them (Helgoland) has been designated as a flagship site for long-term and large-scale marine biodiversity research at a recent European meeting on biodiversity because its long-term sampling program stretches back at least 26 years.

Although these three sites will serve as focal points for our project, we are also taking advantage of oceanographic cruises planned outside this project to examine the diversity of picophytoplankton in other environments. In particular we have begun sample the following ecosystems:

- Mediterranean Sea (PROSOPE 99, MATER99, HIVERN00)
- Red Sea
- North Atlantic Ocean (PROSOPE 99)
- Celtic Sea (PROPHEZE D246)

First, for probe design we need to obtain SSU rDNA sequences covering the full taxonomic spectrum of picophytoplankton. For this purpose, we have adopted a two pronged approach:

a - We are obtaining sequences from **fully characterized laboratory strains**. We are in particular securing all picoplanktonic strains available from international culture collections, such as the CCMP (Center for Cultures of Marine Phytoplankton, Bigelow USA). However, we know that such collections only feature a limited number of picoplanktonic strains, because very little effort has been devoted to this size class to date. Therefore we need to embark on a very strong effort of strain isolation. For this purpose, we are establishing cultures of both prokaryotic and eukaryotic picophytoplankton from the environments listed above using methods that have already proved very successful for this purpose (prefiltration of natural samples, monitoring of cultures by flow cytometry). Once established, the cultures are screened by a variety of techniques (flow cytometry, electron microscopy, pigment analysis, molecular methods) to assess their taxonomic position. Those that obviously contain novel taxa are further purified by dilution or plating and more fully studied (electron microscopy sections), sequenced and described formally.

b - As we know that a large number of planktonic organisms still escape culture due to the lack of optimum culture conditions, we are also using the molecular approach that has been so successful

for bacteria i.e., **environmental ribosomal RNA gene cloning** and sequencing. These sequences are being obtained from the same environments from which we obtain cultures. It is highly likely that this will reveal novel groups that we can then target for culturing.

Second, using the sequence database obtained both from cultures and natural samples, we will use or design **hierarchical probes** for each taxonomic level containing picophytoplanktonic representatives (e.g., classes, such as the Pelagophyceae or species, such as *Micromonas pusilla*). These probes will be validated against cultured strains.

Third, we are developing **methods** to assess the fraction of the marine pico-phytoplankton recognized by a given probe. We focus on very recent techniques allowing quantitative and extensive probe measurements (fluorescent in situ hybridization, probe array, quantitative PCR).

Fourth, we will apply these methods in the second phase of the project to assess **picophytoplankton diversity** during a full year at our three coastal sites (English Channel, North Sea, Mediterranean Sea). At the same time, the composition and abundance of the picophytoplankton will be studied with more conventional techniques, such as electron microscopy or pigment analysis, and alternate molecular methods (DGGE). These data will permit a validation of the data obtained by the molecular probe approach. We will interpret then the biodiversity patterns as a function of the other environmental parameters of the site sampled. We will determine in particular whether there is a succession of groups and species (as is the case for the larger nano and micro-phytoplankton) or whether a small group of ubiquitous species are always present and merely change their abundance (but not their diversity) in response to environmental changes.

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