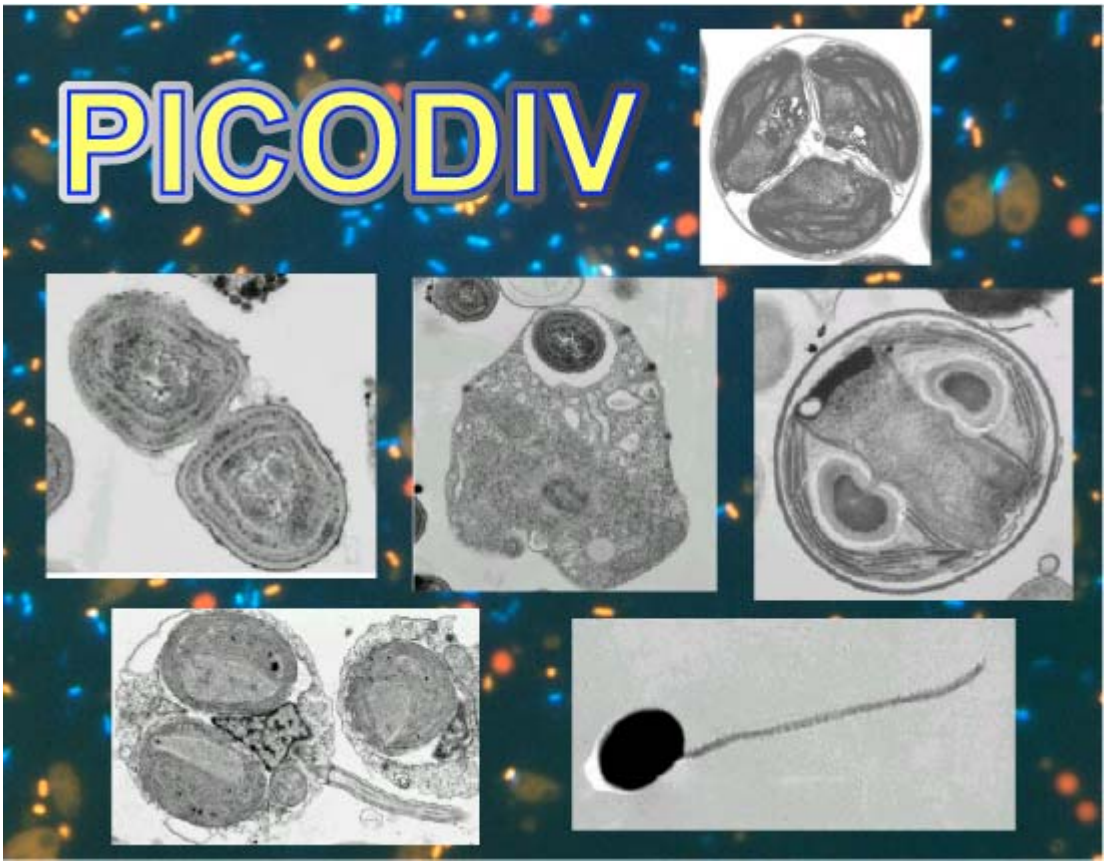

Monitoring the diversity of photosynthetic picoplankton in marine waters

Contract EVK3-CT-1999-00021

Report for period April 1, 2002 - September 30, 2002 (30 months)

Sections included: 1

Coordinator: Daniel Vaulot

Home page: www.sb-roscoff.fr/Phyto/PICODIV

Participants information:

N°	Institution/Organisation	Street name and number	Post Code	Town/City	Country Code	Title	Family Name	First Name	Telephone N°	Fax N°	E-Mail
1	**SBR** CNRS, Station Biologique	BP 74	29682	Roscoff	France		Vaulot	Daniel	+33 2 98 29 23 34	+33 2 98 29 23 24	vaulot@sb-roscoff.fr
2	**UW** University of Warwick	Gibbet Hill Road	CV4 7AL	Coventry	UK		Scanlan	Dave	+44-24 76 528363	+44-24 76 523701	dscanlan@bio.warwick.ac.uk
3	**AWI** Alfred Wegener Institute for Polar Research	Am Handelshafen 12	D-27570	Bremerhaven	Germany		Medlin	Linda	+49-471-4831-1443	+49-471-4831-1425	lmedlin@AWI-Bremerhaven.de
4	**ICM** Institut de Ciències del Mar, CMIMA, CSIC	Passeig Marítim de la Barceloneta 37-49	08003	Barcelona	Spain		Pedrós-Alió	Carles	+34-93-2309597	+34-93-2309555	cpedros@icm.csic.es
5	**UO** University of Oslo	P.O. Box 1069, Blindern	N-0316	Oslo	Norway		Throndsen	Jahn	+47 22 85 45 26	+47 22 85 44 38	jahn.throndsen@bio.uio.no

1. SECTION 1: MANAGEMENT AND RESOURCE USAGE SUMMARY (6 MONTHS: APRIL 2002-SEPT 2002)

This management report describes briefly the major actions undertaken **during the last six months of the project** (Please refer to previous reports for earlier activities). A very large amount of information and data is available from the PICODIV web site (www.sb-roscoff.fr/Phyto/PICODIV).

1.1. Objectives during the 6-month reporting period

The major objective of the **reporting period (Months 24-30)** was to initiate more detailed studies on selected cultures obtained from coastal sites and cruises and to start describing novel taxa (**Wkp 1**), to synthesize the information obtained from clone library sequence data (**Wkp 2**), to finalize probe design and testing (**Wkp 3**), to continue the development of quantitative PCR and DNA chips (**Wkp 4**) and to start processing samples obtained monthly at all coastal sites (**Wkp 5**).

Key common actions during that period have been:

- Holding an **open scientific workshop** on **molecular probes** (3 days) in April 2002 in Bremerhaven. This workshop (ASCMAP) was combined with a 4 day workshop on flow cytometry and the use of neural artificial networks for data analysis (**Groben and Medlin, 2002**).
- Holding the **third PICODIV annual meeting** (3 days) in July 2002 in Roscoff
- Participating in a **cruise of opportunity** in **Arctic waters** in August-September 2002 organized by the UO group.
- Update of the web site and centralized databases: www.sb-roscoff.fr/Phyto/PICODIV UserName=PICODIV Password=bolidol.

1.2. Scientific/Technical progress made in different work packages during the 6-month reporting period according to the planned time schedule:

1.2.1. Workpackage 1. Cultures

During the first 24 months of the PICODIV project, most of our efforts have been devoted to isolate and purify unialgal cultures from picoplankton from the different coastal sites and cruises (see **Table 1**). These cultures have been deposited to the Roscoff Culture Collection (RCC) which now holds 578 entries. These cultures have been characterized by the following set of methods (**Table 2**).

- Flow cytometry (Roscoff)
- HPLC or spectrofluorometry pigment analysis (Barcelona, Roscoff)
- Electron microscopy whole mount (Oslo)
- PCR-RFLP of SSU rDNA using *Hae* III restriction enzyme (Roscoff)
- Fingerprinting by SSCP (Bremerhaven) or DGGE (Barcelona)
- Sequencing of SSCP products (Bremerhaven)
- Sequencing of SSU rDNA (Warwick, Roscoff)

Although our isolation effort still continues, in particular for samples collected during the Arctic cruise conducted in the summer of 2002 (see below), we have now selected a number of strains for more detailed studies, leading in particular to the description of novel species or to the revision of existing taxonomy (**Table 3**).

- ***Synechococcus* cyanobacteria** (Warwick, Oslo). This is clearly the key group of photosynthetic bacteria in coastal waters. A review of their ecology as assessed by molecular approaches has been published during the reporting period (**Scanlan and West 2002**). Despite their importance, their diversity has been very little studied prior to the project. Based on 16S rDNA sequences of cultures, in particular those isolated in the framework of the project, we have been able to define 10 clades, three of which had not been described before. This analysis has then been used to design clade-specific probes (see Wpk 3). A paper presenting these results has been drafted (Fuller et al.). Further analysis of representative strains of these different clades is on-going, in particular *ntcA* gene analysis and transmission electron microscopy (TEM).
- **Prasinophyceae** (Barcelona, Roscoff, Oslo, Warwick). As the PICODIV project progressed, we found out that Prasinophyceae ("primitive" green algae) constitute clearly the major photosynthetic picoeukaryote group. We are currently drafting a paper describing the extent of its diversity based on 18S rDNA sequences and electron microscopy. Several new taxa will also be introduced. Because of the ecological importance of Prasinophyceae, we have also launched a number of studies on specific aspect of this group.
    - **Plastid genes.** In contrast to nuclear 18S rDNA genes, there are only few strains for which plastid 16S rDNA genes are available. However, these genes are often recovered in prokaryotic clone and metagenomic libraries and the unavailability of sequence from described species renders difficult the identification of genes from natural samples. Therefore we initiated collaborations to sequence 16S rDNA (Warwick and Bremerhaven) and *psbA* (Oded Beja, Israel) plastid genes from a number of RCC strains.

- ***Ostreococcus* genome diversity.** In the framework of PICODIV, we have isolated, from a wide variety of environments, a large number of strains of *Ostreococcus*, the smallest photosynthetic eukaryote known to date. Preliminary analysis by the team of Hervé Moreau in Banyuls has revealed that these strains differ in the number and size of their chromosomes. We have decided to pursue in this study which is of major interest in the context of the on-going *Ostreococcus* genome sequencing.
- **Acclimation of *Ostreococcus* strain to light and temperature.** The wide diversity of *Ostreococcus* strains raise the question whether this genus contains "ecotypes", similarly to what is observed for *Prochlorococcus*. In this context we have decided to compare growth and pigment content of the different *Ostreococcus* strains, in relation to light and temperature.

- **Dictyochophyceae** (Oslo, Roscoff, Barcelona). This group of algae was not considered to harbor picoplankton species. However, we have isolated several very small representatives. A paper is currently being drafted describing the new species *Florenciella ultra*.
- ***Telonema*.** This genus, although known for long, could never be clearly ascribed to any major protist group. During PICODIV, we have isolated several strains of *Telonema subtile*. A paper is drafted that assigns it at the base of the Stramenopiles and the Alveolates, making it a very interesting organism from a phylogenetic point of view.

Table 1: Cultures available as of September 30, 2002

Cruise or Site	Pre-cultures established	Unialgal cultures deposited to RCC
Arctic region: Norwegian and Barents Seas	37	
North Sea: Helgoland	1056	21
English Channel: Roscoff	77	61
Mediterranean Sea: Blanes	177	83
Mediterranean Sea/Atlantic PROSOPE cruise	160	81
Red Sea	274	31
China Sea: Vietnam	8	16
Pacific Ocean: Bali	4	2
Other unknown cultures to be characterized from Roscoff collection (RCC)		30

Table 2: Cultures deposited at RCC characterized as of September 30, 2002

Analysis performed	Unialgal cultures
Flow cytometry	309
Pigments	89
Electron microscopy	29
RFLP of rDNA	74
eukaryotic nuclear and cyanobacteria rDNA sequencing (500 bp)	135[a]
eukaryotic nuclear and cyanobacteria rDNA sequencing (full sequences)	48
eukaryotic plastid rDNA sequencing (partial sequences)	15

[a] For many RCC, more than one partial sequence was obtained: total number of sequences determined = 255

Table 3: Some of the eukaryotic cultures selected for further studies and taxonomic description as of September 30, 2002

RCC	Class	Genus	Species	Strain	Aim
137	Prasinophyceae	*Prasinoderma*		CCMP1220	Prasinophyceae phylogeny
136	Prasinophyceae	*Prasinococcus*	*capsulatus*	CCMP1407	Prasinophyceae phylogeny
261	Prasinophyceae	*Pseudoscourfeldia*	*marina*	TAK9801	Prasinophyceae phylogeny
244	Prasinophyceae	*Pycnococcus*	*provasolii*	MIN 018-75m B	Prasinophyceae phylogeny
15	Prasinophyceae	coccoid		CCMP1205	Prasinophyceae phylogeny
297	Prasinophyceae	coccoid		Açores 3	Prasinophyceae phylogeny
143	Prasinophyceae	*Ostreococcus*	*sp*	EUM13B	Genome size; Growth vs Light
141	Prasinophyceae	*Ostreococcus*	*sp*	EUM16BBL	Genome size; Growth vs Light
356	Prasinophyceae	*Ostreococcus*	*sp*	RA000412-9	Genome size; Growth vs Light
344	Prasinophyceae	*Ostreococcus*	*sp*	PROSOPE_3	Genome size; Growth vs Light
343	Prasinophyceae	*Ostreococcus*	*sp*	PROSOPE_5	Genome size; Growth vs Light
371	Prasinophyceae	*Ostreococcus*	*sp*	PROSOPE_44	Genome size; Growth vs Light
393	Prasinophyceae	*Ostreococcus*	*sp*	PROSOPE_122-4	Genome size; Growth vs Light
434	Prasinophyceae	*Micromonas ??*		BL_122	Novel species ??
391	Prasinophyceae	*Mamiella*	*sp*	PROSOPE_1-10	Novel species
287	Prasinophyceae	coccoid		NOUM15	Novel species
446	Dictyochophyceae ?	*Florenciella*	*ultra*	RA000412-1-1	Novel species
358	Incertae sedis	*Telonema*	*subtile*		Taxonomy analysis
404	Incertae sedis	*Telonema*	*subtile*	RA000412-2	Taxonomy analysis
341	Pelagophyceae	*Pelagomonas*		PROSOPE_63	Taxonomy analysis

1.2.2. Workpackage 2. Environmental clone libraries

We have completed construction of libraries from the **coastal sites** and **cruises** (**Table 4**). On all existing clone libraries, RFLP analysis and partial sequencing of clones has been performed. During the reporting period, we have intensified our effort to obtain full length sequences for selected clones corresponding either to key photosynthetic groups (*Synechococcus*, Prasinophyceae) or to groups with no cultivated representatives (environmental group close to red algae, Stramenopiles, Alveolates). Two alternative strategies of qualitative analysis of the natural picoplankton diversity have also been developed in the frame of PICODIV: DGGE (in Barcelona and Roscoff) and SSCP (in Bremerhaven). Both DDGE and SSCP will be applied to the coastal time series samples (**Wpk 5**).

A preliminary and synthetic analysis of the data obtained in Roscoff has been published during the reporting period (**Vaulot et al. 2002**). Several papers describing the extent of picoplankton diversity based on 18S rDNA clone libraries are currently in draft form. These papers emphasize the very high diversity of coastal picoplankton, similar in extent to that of oceanic plankton

Table 4: Clone libraries and sequences of rRNA available for PICODIV on September 30, 2002. Numbers in italic have not yet been deposited to the PICODIV database

Cruise or Site	**Samples obtained**	**Library established**		**Partial sequences**		**Full sequences**
		Prok	Euk	Cyanos/ Plastids[a]	Euk (nuclear)	Euk (nuclear)
English Channel: Roscoff	X	2	8	*5 (58)*	426	23
North Sea: Helgoland	X	3	6	*8 (56)*	251	2
Mediterranean Sea: Blanes	X	2	4	*26 (38)*	171	12
Pacific Ocean OLIPAC	X	0	10	0	59	65
Mediterranean Sea PROSOPE	X	2	0	20 (75)	0	9
Orkney Islands	X	0	1	0	45	0
Mediterranean Sea, Alborán. MATER	X	0	1	0	28	0
Antarctica IronEx 2000 experiment	X	0	3	0	*96*	0
Antarctica. DOVETAIL	X	0	2	0	35	0
North Atlantic Ocean. ACSOE-NAE	X	0	2	0	13	0
Total		**9**	**37**	**50**	**1124**	**111**

[a] The number between parenthesis corresponds to the total number of bacteria sequences determined. Among these only a fraction corresponded to cyanobacteria or plastid sequences.

1.2.3. Workpackage 3. Probe design

Most of the probe design and testing is now finished (**Table 5**, **Table 6**, **Table 7**). Our protist and cyanobacteria probe data base is now publicly accessible through a web interface (http://www.sb-roscoff.fr/Phyto/Databases/RNA_probes.php). During the reporting period, a paper on the novel stramenopile probes has been published (**Massana et al. 2002**) and two papers, respectively on *Synechococcus* and Prasinophyceae probes, have been drafted. We have also validated the following probes with **FISH**:

- NCHLO2: General Non-Chlorophyta probe (SBR)
- CRYPT13: Cryptophyta (SBR)
- ENV01, 02, 03: Novel group close to red algae (SBR)

We are currently designing FISH probes against the ubiquitous uncultivated Alveolates that should be ready for use by the end of the project.

During the reporting period, probes for **DNA arrays** have been extensively tested (AWI, see below). We have also tested primer sets for **quantitative PCR** (**Table 8**). As mentioned below (Wpk 4), we have encountered unexpected problems with both specificity and efficiency of the primers we designed. This implies some delay in using this technique for coastal sample analysis.

Finally, we have selected subsets of probes that will be used either in FISH or DNA array format to analyze the monthly samples collected at the three coastal sites (**Table 7**). More probes will be used in DNA array format than in FISH, since the former approach is best suited for high throughput.

Table 5: Probes available or under development for prokaryotic picoplankton using FISH on September 30, 2002

Probe name	**Target level**	**Target class**	**Target group**	**Under development in PICODIV**
PRO 645 HLI	Genus	Cyanobacteria	*Prochlorococcus* high light clade I (PCC 9511)	+
PRO 645 HLII	Genus	Cyanobacteria	*Prochlorococcus* high light clade II (Takapoto)	+

Probe name	Target level	Target class	Target group	Under development in PICODIV
PRO 645 LL	Genus	Cyanobacteria	*Prochlorococcus* low light (NATL1)	+
PRO 1445 NATL	Genus	Cyanobacteria	*Prochlorococcus* low light (NATL1)	+
PRO 181 HLI	Genus	Cyanobacteria	*Prochlorococcus* high light clade I (PCC 9511)	+
PRO 181 LL	Genus	Cyanobacteria	*Prochlorococcus* low light + *Synechococcus*	+
PRO 405	Genus	Cyanobacteria	*Prochlorococcus* high light + NATL1	+
SYN 405	Genus	Cyanobacteria	*Prochlorococcus* low light + *Synechococcus*	+

Table 6: Probes available or under development for prokaryotic picoplankton using dot-blot hybridisation on September 30, 2002

Probe	Target Level	Target Class	Strains detected	Under development in PICODIV
S1 (SYN1006RS)	Genus	Cyanobacteria	SynRS9910 cluster (clade II)	+
S2 (SYN1000)	Genus	Cyanobacteria	SynRS9901 & SynRS9916 (clade IX)	+
S3 (SYN635)	Genus	Cyanobacteria	UC SynNAC1-5 cluster (clade IV)	+
S4 (SYN262)	Genus	Cyanobacteria	SynWH8103 cluster (clade III)	+
S6 (SYN620)	Genus	Cyanobacteria	SynRS9918 cluster & SynWH8101 (clade VIII)	+
S7 (SYN1006)	Genus	Cyanobacteria	SynCC9311 cluster (clade I)	+
S9 (SYN1280)	Genus	Cyanobacteria	SynWH7803 / SynWH7805 / SynRS9920 cluster (clades V, VI, and VII)	+
S10 (SYN1007)	Genus	Cyanobacteria	SynMinos 11 cluster (clade X)	+
S11 (SYN1258)	Genus	Cyanobacteria	All marine *Synechococcus* except RS9918 / WH8101 cluster & RS9901 cluster; no *Prochlorococcus* except MIT9303 (clades I-VII, X)	+
P1 (S1PRO634R)	Genus	Cyanobacteria	HLI *Prochlorococcus*	+
P2 (S2PRO634R)	Genus	Cyanobacteria	HLII *Prochlorococcus*	+
P3 (DPRO634R)	Genus	Cyanobacteria	LL *Prochlorococcus*	+
P4 (MIT1023R)	Genus	Cyanobacteria	ProMIT9303	+
P5 (PRO444R)	Genus	Cyanobacteria	All *Prochlorococcus* except MIT9303 & MIT9313	+
P6 (SARG634R)	Genus	Cyanobacteria	ProSS120	+

Table 7: Probes available for eukaryotic picoplankton as of September 30, 2002

Target	Auto/ Hétéro	Probe name	Designed for PICODIV	FISH		DNA array	
				Tested	Applied	Tested	Applied
Eukaryota	A/H	Euk1209R+CHLO01+ NCHLO01		x	**x**	x	**x**
	A/H	Euk328					**x**
	A/H	EUk1528					**x**
Chlorophyta	A	CHLO01				x	**x**
	A	CHLO02		x	**x**	x	**x**
Mamiellales	A	PRAS04	x	x	**x**		**x**
Bathycoccus	A	BATHY01	x	x	subset		**x**
Micromonas	A	MICRO01	x	x	subset		**x**
Ostreococcus	A	OSTRE01	x	x	subset		**x**
Non-Chlorophyta	A/H	NCHLO02	x	x	**x**		
Stramenopiles	A/H	HETER01	x				**x**
Bolidophyceae	A	BOLI01		x	**x**	x	**x**
	A	BOLI02				x	**x**
Pelagophyceae	A	PELA01		x	**x**	x	**x**
	A	PELA02					**x**
Haptophyta	A	PRYM01				x	**x**
	A	PRYM02		x	**x**	x	**x**
Prymnesiophyceae	A	PRYM03	x			x	**x**
Cryptophyceae	A	CRYPTO07	x				**x**
	A	CRYPTOB	x				**x**
	A	CRYPT13	x	x	**x**		
Dinophyceae (some) + Apicomplexa (some)	A	DINO_B		x	**x**	x	**x**
Dinophyceae (some) + Aleveolates (some)	A/H	DINO E-12				x	**x**
Stramenopiles hetero groups	H	NS03	x	x	**x**		**x**
	H	NS04	x	x	**x**		**x**
	H	NS07	x	x	**x**		
Environmental group close to Rhodophyta	?	ENV01	x	x	subset		
	?	ENV02	x	x	subset		
	?	ENV03	x	x	subset		

Notes: Applied: Means that the probe will be applied to analyze monthly samples collected at each of the three coastal sites. Subset: Means that only a subset of the coastal samples will be analyzed.

Table 8: Primer pairs for quantitative PCR designed and tested for eukaryotic picoplankton on September 30, 2002

Specificity	Primer forward	Primer reverse	Specificity
Eukaryotes	Euk345f	Euk528r	Euk528r not specific (amplifies bacteria)
Eukaryotes	Euk528f	Euk765f	Yes
Chlorophyta	Euk528f	CHL02r	No (amplifies *Bolidomonas*)
Non-chlorophyta	Euk528f	NCHL02r	No (amplifies *Mamiellales*)
Mamiellales	Euk528f	PRAS04r	Yes
Ostreococcus all	Euk528f	OSTRE02r	Yes
Bathycoccus all	Euk528f	BATHY03r	Yes
Micromonas clade CCMP 490	Euk528f	MICRO02r	No (amplifies other *Micromonas* clades)
Micromonas clade CCMP 489	Euk528f	MICRO03r	No (amplifies other *Micromonas* clades)
Micromonas clade RCC 434 (BL122)	Euk528f	MICRO04r	No (amplifies other *Micromonas* clades)
Bolidophyceae	Euk528f	BOLI02r	No (amplifies *Pelagomonas* and *Rhodomonas*)
Pelagophyceae	Euk528f	PELA01r	Yes

1.2.4. Workpackage 4. Probe measurement

During the reporting period, one paper has been published (**Not et al. 2002**) on **TSA amplification**, a new method of fluorescent *in situ* hybridization (**FISH**) that increases the fluorescent signal and which has been applied to the small picoplankton. The Roscoff laboratory has finalized work to couple **TSA-FISH** with **flow cytometry** to speed up sample analysis and has tested it successfully on natural samples. A paper is almost ready to be submitted.

Major advances have been made on the **DNA array** approach by the Bremerhaven group. In particular the following steps have been carried out:

- Hybridization of arrays with DNA from picoplankton clones (Bolidophyceae, Prasinophyceae) has been successfully tested
- Successful hybridization of arrays with DNA from mixed cultures and clones (Prymnesiophyceae, Bolidophyceae, Prasinophyceae) suggest that DNA arrays can be used with complex natural samples. The relative signal intensities are proportional to the initial DNA concentrations.
- The poor signal intensity associated to certain probes appear to be linked to the position of the probe on the rDNA molecule. Probes located beyond position 1000 on the rDNA gene do not perform well. This problem can be solved by fragmenting the target DNA (this occurs for example with psoralen-biotin labeling).

The Roscoff group has continued development of **quantitative PCR.** We have tested general eukaryotic primers as well as primers specific to Prasinophyceae that are very common within the phytoplankton (**Table 8**). We have encountered two major problems. The first problem was the lack of specificity of some primers, despite the fact that the targeted region can be used without problem for FISH probing. Each primer pair must be very carefully tested and it is often necessary to redesign primers. The second problem came from the fact that some primers did not display 100% efficiency, which is an absolute requirement for the method to be quantitative. Again, when this occurs redesign is necessary. Preliminary tests on natural samples using Prasinophyceae probes are encouraging.

1.2.5. Workpackage 5. Picoplankton monitoring

Sample collection at the three coastal sites is now over. Samples have been exchanged between the different groups and during the reporting period, the following analyses have been initiated:

- HPLC pigment analysis (Barcelona)
- Flow cytometry (Barcelona and Roscoff)
- Microphytoplankton taxonomy and abundance (Barcelona, Roscoff, Helgoland)
- DGGE analysis (Barcelona)
- FISH analysis (Barcelona, Roscoff, Warwick)

The other measurements will be started very soon:

- DNA dot blot for prokaryotes (Warwick)
- DNA array (Bremerhaven)
- SSCP analysis (Bremerhaven)

During the reporting period three partners (Barcelona, Oslo, Roscoff) participated in a cruise of opportunity in Arctic waters aboard a Norwegian ship, following an invitation from W. Eikrem from the University of Oslo. This cruise covering a transect between Tromsoe (Norway) and Svalbard (Spitzberg) offered a unique occasion to sample picoplankton in cold marine waters where it has been little studied until now. Samples were taken to assess picoplankton diversity and abundance following the PICODIV approach. These samples will be analyzed with the following techniques:

- Cultures from shipboard enrichment
- Transmission electron microscopy

- Flow cytometry
- HPLC pigment measurements
- rDNA Clone libraries
- DGGE
- FISH

1.2.6. Gantt chart update

See at the end of this section.

1.2.7. Person-month used

Table 9: Person-months used per workpackage Months 24-30

	SBR	Coordination	UW	AWI	ICM	UO
Workpackage 1	2		1	6	4	3.5
Workpackage 2	6.5		2.5	2	1	
Workpackage 3			0.5	1		
Workpackage 4	2			3		
Workpackage 5	7.5		2		14	1.5
Coordination		1				
Total	18	1	6	12	19	5

1.3. Milestones and deliverables

Table 10: Milestones

Wpk	Objectives	Status	Planned date (last report)	Planned date revised
1	Sampling and pre-cultures from coastal sites and cruises begins	Done.	Sept 2000	
1	Sampling and pre-cultures from coastal sites achieved	Done.	Oct 2001	
1	All coastal pre-cultures screened	Done except for Arctic 2002 cruise.	April 2002	
1	All coastal cultures screened	See previous milestone	April 2002	
1	All coastal strains established	Done. Effort to purify mixed cultures will however continue until the end of the project.	Oct 2002	
1	All coastal and collection culture sequences obtained	Mostly done. Acquisition of full length sequence will continue until the end of the project.	Oct 2002	
1	Taxonomy of all coastal strains finalized and novel taxa described	Taxonomy of most strains established. We are preparing papers describing novel taxa discovered during the project.	April 2003	
2	Begin extraction of environmental DNA	Done	Sept 2000	
2	Begin Construction of SSU rDNA/rRNA clone libraries	Done	Sept 2000	
2	Clone libraries complete	Done except for Arctic 2002 cruise.	Dec 2001	
2	Environmental sequences available	Done for all coastal sites. Will continue for cruises of opportunity.	March 2002	
2	Full sequence analysis completed	In progress. Will continue as our attention shift to new taxonomic groups (e.g. alveolates)	Dec 2002	
3	Probe design begins based on sequences acquired during project	Done	April 2000	
3	Probes validated ready for use on coastal time series samples	Most probes designed within the PICODIV project are ready for application either with FISH or DNA arrays. We will however keep designing and validating probes for novel groups that are uncovered by clone library analysis (e.g. Alveolates)	Dec 2002	
4	Development of probe assessment methodologies begins	Done	April 2000	
4	Probe assessment methodologies ready	TSA-FISH technique coupled with microscopy and flow cytometry is operational. Good progress made on DNA arrays. Quantitative PCR still need more development time.	Dec 2002	Feb 2003
5	Sampling the coastal sites begins.	Done	April 2000	
5	Sampling the coastal sites achieved.	Done	Oct 2002	
5	All measurements of biodiversity achieved.	Some measurements such as those for pigments and FISH have already began. For some of the techniques still under	Feb 2003	

Wpk	Objectives	Status	Planned date (last report)	Planned date revised
		development (DNA chips), measurements will start latter than anticipated.		
5	Comparison and interpretation of biodiversity estimates achieved	Some comparisons (e.g. between FISH and pigments) are already underway	April 2003	

Table 11: Deliverables

Del.	Deliverable title	Delivery Date (planned)	Status
1	Picophytoplankton strains from coastal and oceanic regions	Oct 2002	Done (see **Table 1**).
2	SSU rDNA sequence from picophytoplankton strains	Oct 2002	Done for partial sequences (see **Table 2**). Full sequences are obtained for selected cultures.
3	Characterization and description of picophytoplankton strains	April 2003	Done. Flow cytometry, pigment and TEM data have been obtained for a large number of strains (see **Table 2**). Papers describing new taxa are being prepared.
4	Gene clone libraries from coastal and oceanic regions	Dec 2001	Eukaryotic clone libraries achieved for all coastal sites (see **Table 4**). About 45 clone libraries are available.
5	SSU rDNA sequences from coastal and oceanic regions	March 2002	More than 1220 partial and 99 full sequences are available (see **Table 4**).
6	Hierarchical probes	Sept 2002	Mostly done (see **Table 5**).
7	FISH methodology	April 2002	FISH-TSA detected by microscopy and flow cytometry is fully operational
8	Probe array methodology	Feb 2003	In progress
9	Quantitative PCR methodology	Feb 2003	In progress.
10	Estimation of picophytoplankton biodiversity with classical methods	Jan 2003	In progress
11	Molecular estimation of picophytoplankton biodiversity	Jan 2003	In progress
12	Validation of probe methodology for biodiversity	April 2003	In progress

1.4. Deviations from the work plan and/or time schedule and their impact to the project (if any please explain)

We have encountered some problems in the development of quantitative PCR (see above). This work has been halted at the end of July 2002, because the DEA student involved, B. Simonnet, did not obtain a PhD fellowship. Work should resume in early December 2002 with the arrival of new post doctoral fellow. This should not have a real impact on monthly sample measurements at coastal sites, because, to assess picoplankton abundance, we can rely on FISH and DNA array data for eukaryotes and on dot blot data for prokaryotes that are fully operational. Moreover, we will also apply to these samples additional diversity measurements not planned initially: DDGE and SSCP.

1.5. Co-ordination of the information between partners and communication activities (e.g. organised meetings, conference attendance, co-operation with other projects/networks, …).

1.5.1. Web site

The web site is the focal point for all activities within PICODIV (www.sb-roscoff.fr/Phyto/PICODIV). One part of the site is freely accessible, while the other part is restricted to the project participants (UserName=PICODIV Password=bolidol). It has been very frequently updated during the six month reporting period. It contains:

- List of all participants
- Abstract of project

- Administrative documents such as description of work, meeting minutes, periodic reports…
- List of publications resulting from the project with access to pdf reprints
- Updates from each research group at regular intervals
- Sampling and measurement protocols
- Details on sampling sites (illustrated by PowerPoint presentations)
- An overview of the work performed on cultures
- An overview of the work performed on clone libraries
- Links to project databases.
- An illustrated list of key picoplankton species
- A list of bibliographic references on picoplankton as well as on molecular techniques with in some cases access to on-line pdf reprints.
- An extensive summary of the international PICODIV workshops organized in Roscoff in June 2000, Barcelona in May 2001 and in Bremerhaven in April 2002 (ASCMAP meeting).
- An extensive summary of the internal PICODIV meetings organized in Roscoff in June 2000, Barcelona in May 2001, Bremerhaven in April 2002, Roscoff in July 2002 with links to PowerPoint files for each presentation.

1.5.2. Databases

Centralized databases have been updated during the 6-months reporting period:

- **Taxonomy of phytoplankton** (Access format). More than a hundred new species have been added and the database covers now both picoplankton and larger phytoplankton present at the coastal sites, in particular diatoms.
- **Cultures** (Access format). Twenty seven new cultures have been added to the database for a total of 426 entries. Moreover microscopy pictures and sequence data have been added for many existing entries.
- **rDNA sequences** (Access and ARB format). This database contains about 5 400 rDNA protist and cyanobacteria sequences.
- **rRNA probes** (Access format). The database contains now information on more than 150 probes and is now publicly accessible through the web (http://www.sb-roscoff.fr/Phyto/Databases/RNA_probes.php).
- **Coastal sites** (Access format). A prototype database has been built for the Roscoff site that will serve as a blueprint for the other sites. It contains all information about the samples taken in the frame of PICODIV (date, time, depth, volume and treatment for each sample), but also hydrological, flow cytometry and taxonomy data.

1.5.3. PICODIV meetings during the 6-month reporting period

Three PICODIV meeting have been held during the reporting period. Periodic updates on on-going work are provided on the web site.

- An **open scientific workshop** lasting 7 days devoted to " **Analysis of Single Cells in Marine Phytoplankton** " in Bremerhaven in April 2002 (see report in Protist: Groben and Medlin, 2002).
- A short 2 h **meeting** restricted to PICODIV partners in Bremerhaven in April 2002. This helped to organize the July meeting and addressed some immediate issue (coordination of time series measurements, planning the Arctic cruise).
- A three day **meeting** in Roscoff in July 2002. The first two days consisted of scientific talks summarizing progress made during year 2 of PICODIV and were opened to a few invited scientists working on topics very similar to those covered by PICODIV (e.g. A. Worden from the Scripps Institute of Oceanography, La Jolla, USA, who has constructed environmental 18S rDNA clone libraries). The last day was restricted to PICODIV partners and was devoted to planning the work for year 3.

1.5.4. Communication to the media and activities for the public during the 6-month reporting period

- Pedrós-Alió, C. Diario Ártico. The diary of an Arctic cruise. On-line magazine http://www.Thalassa-online.com
- Pedrós-Alió, C. Un bestiari del segle XXI: de les plantes microscòpiques a la flora intestinal. Bioconferència a la Universitat Pompeu Fabra – Societat Catalana de Biologia, Institut d'Estudis Catalans, Barcelona, 28 Maig 2002.
- Pedrós-Alió, C. Diversidad bacteriana y de genes. Su papel en el funcionamiento del ecosistema. Conferencia por invitación en el seminario sobre La sostenibilidad del planeta Tierra. La investigación en la Antártida. Palacio de la Magdalena, Santander, 26-27 abril 2002.
- Pedrós-Alió, C. Un bestiario del siglo XXI: de las plantas microscópicas a la flora intestinal. Conferencia en el Departamento de Química de la Universidad Católica del Norte, Antofagasta, Chile, marzo 2002.
- Roscoff group. Le picoplancton: des milliards de cellules dans l'océan. Workshop for school children. La Fête de la Science. 17-18 oct 2002.
- Valentin, K. Naturwissenschaft und Technik 'Winzlinge mit Potential'. Radio interview on Deutschlandfunk on April 19 2002 at 16.35.

1.6. Publications (cumulative list)[1]

1.6.1. Peer Reviewed Articles:

Papers published during the 6 month reporting period appear in ***bold***

Authors	Date	Title	Journal	Reference
Biegala I, Kennaway G, Alverca E, Lennon J-F, Vaulot D and Simon N.	2002	Identification of bacteria associated with dinoflagellates (*Alexandrium* spp.) using TSA-FISH (Tyramide signal amplification - fluorescent *in situ* hybridization) and confocal microscopy.	Journal of Phycology	38: 404-411
Daugbjerg N. and Guillou L	2001	Phylogenetic analyses of Bolidophyceae (Heterokontophyta) using rbcL gene sequences support their sister group relationship to diatoms	Phycologia	40: 153-161
Díez, B., Pedrós-Alió, C., Massana, R.	2001	Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by Small-Subunit rRNA gene cloning and sequencing	Appl. Environ. Microbiol.	67: 2932-2941
Díez, B., Pedrós-Alió, C., Marsh, T.L., Massana, R.	2001	Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques	Appl. Environ. Microbiol.	67: 2942-2951
Guillou L., Jacquet S., Chrétiennot-Dinet M-J, Vaulot D.	2001	Grazing impact of two picoplanktonic heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*	Aquatic Microbial Ecology	26: 201-207
Massana, R., L. Guillou, B. Díez & C. Pedrós-Alió.	**2002**	**Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean.**	**Appl. Environ. Microbiol.**	**68: 4554–4558.**
Medlin, L.K., Groben, R. and Valentin, K.	**2002**	**Molecular tools in the study of marine microbial diversity.**	**Encyclopedia of Life Support Systems**	**www.eolss.net**
Moon-van der Staay S.Y., De Wachter R., Vaulot D.	2001	Oceanic 18S rDNA sequences from picoplankton reveal new eukaryotic lineages	Nature	409: 607-610
Not F, Simon N, Biegala I, Vaulot D.	**2002**	**Application of fluorescent *in situ* hybridization coupled with tyramide signal amplification (FISH-TSA) to assess eukaryotic picoplankton composition.**	**Aquatic Microbial Ecology**	**28: 157-166**
Scanlan D.J. and West N.J.	**2002**	**Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*.**	**FEMS Microbiology Ecology**	**40: 1-12**
Vaulot D.	2001	Phytoplankton.	Encyclopedia of Life Sciences.	www.els.net
Vaulot D., Romari K., Not F.	**2002**	**Are autotrophs less diverse than heterotrophs in marine picoplankton?**	**Trends in Microbiology**	**10: 266-267**
West, N.J., Schonhuber, W.A., Fuller, N.J., Amann, R.I., Rippka, R., Post, A.F., Scanlan, D.J	2001	Closely related *Prochlorococcus* genotypes show remarkably different depth distributions in two oceanic regions as revealed by in situ hybridisation using 16S rRNA-targeted oligonucleotides	Microbiology - UK	147: 1731-1744

[1] Two copies of publications issued during reporting period should be annexed to the report, specific cases should be agreed by the Project Officer

1.6.2. Non refereed literature, communications at meetings:
*(events during the 6 month reporting period appear in **bold**)*

Authors / Editors	Date	Title	Event/Reference	Type[2]
Vaulot D. and the PICODIV participants	2003	The unsuspected diversity of marine eukaryotic picoplankton: results from the european PICODIV program.	Ocean Biogeochemistry and Ecosystem Analysis. International Open Science Conference. Paris 7-10 January 2003	Poster
Biegala I. C., Not F., Romari K., Marie D., Vaulot D., Simon N.	2002	Quantitative assessment of picoeukaryotes using taxon specific oligonucleotide probes in association with TSA-FISH (Tyramide Signal Amplification - Fluorescent In Situ Hybridization) and flow cytometry	8th Symposium on Aquatic Microbial Ecology. Taormina (Messina), Italy. October 25 - 30, 2002	Oral
Doussal D.	**2002**	**Dynamique saisonnière des peuplements de picoeucaryotes du système côtier de Roscoff.**	**Mémoire de fin d'études. Institut National Supérieur de Formation Agroalimentaire, Rennes. Defended Sept 2002**	Report
Fuller, N.J., Vaulot, D., and Scanlan, D.J.	**2002**	***In situ* community structure of marine photosynthetic picoeukaryotes**	**Marine and Freshwater Microbial Biodiversity & Challenger Meeting, Plymouth, UK. Sept. 9-13th 2002.**	Oral
Groben R. and L. K. Medlin	**2002**	**Meeting Report: EU Workshop "Analysis of Single Cells in the Marine Phytoplankton" (ASCMAP), Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, April 15 - 21, 2002**	**Protist 153: 193-195**	Report
Kerkmann, K., Groben, R. ,Valentin, K. and Medlin, L. Invited speaker,	**2002**	**Development of a DNA-microarray for the monitoring of phytoplankton diversity**	**Culture Collection of Algae: Increasing accessibility and exploring algal biodiversity. Goettingen Sept 2/6 2002**	Oral invited
Massana, R., L. Guillou, B. Díez and C. Pedrós-Alió	**2002**	**Diversity and distribution of marine picoeukaryotes.**	**ASLO 2002 Summer Meeting. Victoria, British Columbia (Canada), 2002.**	Oral invited
Medlin, L., Groben, R. , John , U., Lange, M., and Kerkmann K.	**2002**	**rRNA probes for identification and characterization of marine phytoplankton: their potential application for DNA microchips.**	**Culture Collection of Algae: Increasing accessibility and exploring algal biodiversity. Göttingen Sept 2/6 2002**	Oral invited
Medlin, L.	**2002**	**Probes and DNA chip technology: macro and micro views**	**HAB Probe Workshop, Galway Ireland, April 2002**	Oral invited
Pedrós-Alió, C.	**2002**	**Visualizing the invisible: microbial diversity in the oceans.**	**Keynote presentation in the 7th Symposium on Bacterial Genetics and Ecology (BAGECO-7), Bergen, Norway, 15-19 June 2002**	Oral invited
Simonnet B.	**2002**	**Détermination de l'abondance du picophytoplancton eucaryote par PCR quantitative**	**DEA Sciences de l'environnement marin. Université Aix-Marseille II. Defended July 2002**	Report

[2] Type: Abstract, Newsletter, Oral Presentation, Paper, Poster, Proceedings, Report, Thesis

Authors / Editors	Date	Title	Event/Reference	Type[2]
Valentin K, Mehl H, and Medlin L	**2002**	**Molecular analysis of the eukaryotic marine picoplankton at Helgoland (North Sea).**	**Deutsche Botanikertagung, Freiburg Sept. 2002-10-08**	Oral invited
Valentin, K.	**2002**	**Assessing picoplankton cultures using 18S fingerprinting and partial sequencing.**	**Deutsche Botanikertagung, Freiburg Sept. 2002-10-08**	Poster
Vaulot D. Le Gall F., Guillou L., Partensky F.	**2002**	**The Roscoff Culture Collection (RCC): a collection dedicated to marine picoplankton**	**Culture Collections of Algae: increasing accesibility and exploring algal biodiversity. Göttingen, Sept 2-6 2002**	Oral
Eikrem W. and J. Throndsen.	2002	PICODIV- monitoring the biodiversity of photosynthetic picoplancton	**Conference on Biodiversity Norwegian Council of Research**. Jan 2002.	Oral
Fuller, N.J., A. F. Post., D. J. Scanlan.	2002	Molecular characterisation of cyanobacterial Population dynamics in the Red Sea.	**UK Molecular Microbial Ecology Group Meeting**, Exeter March 2002.	Oral
Groben, R. and L.K. Medlin	2002	Hierarchical probes for measuring biodiversity	**ASCMAP Workshop** Bremerhaven, Germany, April 2002.	Oral invited
Hastings R., Orcutt, K., Howarth, R., Guillou, L., Fuller, N. J, and Post, A. F. Scanlan, D. J.	2002	Development of 16S rDNA oligonucleotide probes for use in identifying the niches occupied by specific *Synechococcus* and *Prochlorococcus* genotypes.	**ASCMAP Workshop** Bremerhaven, Germany, April 2002.	Poster
Kerkmann, K. and L.K. Medlin	2002	Probes and DNA chip technology: macro and micro views	**ASCMAP Workshop** Bremerhaven, Germany, April 2002.	Oral invited
Medlin, LK. and R. Groben	2002	Probes for Algal Classes	**ASCMAP Workshop** Bremerhaven, Germany, April 2002.	Oral invited
Scanlan, D.J., Fuller, N.J., Orcutt, K. and Post, A.F.	2002	Genetic analyses of photosynthetic picoplankton communities: a spatial and temporal study	**British Phycological Society** 50th Anniversary Meeting, London, UK Jan 2002	Oral invited
Scanlan, D.J., Fuller, N.J., West, N.J. Orcutt, K., Hastings, R. and Post, A.F.	2002	Development of clade-specific 16S rDNA oligonucleotides for use in assessing the spatial and temporal dynamics of community structure in the photosynthetic picoplankton genera *Synechococcus* and *Prochlorococcus*.	**ASCMAP Workshop** Bremerhaven, Germany, April 2002	Oral invited
Beatriz Díez Moreno	2001	Diversidad del picoplancton eucariótico marino mediante métodos moleculares.	**Ph D** Departament de Genètica i Microbiologia, **Universitat Autònoma de Barcelona**.	Thesis
de Wit E.	2001	RFLP based detection of eukaryotic picoplankton within the Roscoff Culture Collection.	Masters thesis. **University of Gröningen.** Netherlands. July 2001	Report
Fuller N.J. N.J. West, R. Howarth, A.F. Post, D.J. Scanlan	2001	A genetic analysis of the spatial and temporal dynamics of a photosynthetic picoplankton community.	**ASLO meeting 2001** Albuquerque, New Mexico, USA	Oral
Garczarek, L., Dufresne A. and Partensky F.	2001	Use of a highly variable gene, *pcb*, for the study of the diversity of natural *Prochlorococcus* populations.	**ASLO meeting 2001**, Albuquerque, New Mexico, USA	Poster

Authors / Editors	Date	Title	Event/Reference	Type[2]
Groben, R., . John, U., Lange, M. and Medlin, L.K,	2001	rRNA probes for identification and characterization of marine phytoplankton: their potenial application for DNA microchips	**American Phycological Society**, Estes Park, Col. June 2001	Oral
Groben, R., and Medlin, L.K,	2001	In-situ Hybridisation of Phytoplankton using Fluorescently -Labelled rRNA probes	**7th Conference on Methods and Application of Fluorescence,** Amsterdam, Nl. Sept 2001	Poster
Groben, R., John, U., Lange, M. and Medlin, L.K,	2001	rRNA probes for identification and characterization of marine phytoplankton: their potenial application for DNA microchips	**Society for Molecular Evolution,** Athens, Ga. July 2001	Oral
Groben, R., John, U., Lange, M. and Medlin, L.K,	2001	rRNA probes for identification and characterization of marine phytoplankton: their potenial application for DNA microchips	**International Phycology meeting**, Thessaloniki, Greece, Aug 2001	Oral
Guillou L.	2001	Picoplanktonic heterokont diversity in culture and natural samples assessed by molecular tools.	**VII th International Phycological Congress,** Thessaloniki, Greece (invited speaker).	Oral
Massana, R., Schauer, M., Díez, B., Pedrós-Alió, C	2001	Seasonal succession of bacterial and picoeukaryal assemblages in a coastal environment	**9th International Symposium on Microbial Ecology**, Amsterdam, 26-31 August	Poster
Medlin, L.K.	2001	Application of rRNA probes for measuring biodiversity.	**ESF Cyanofix Symposium**, Bologna, Italy	Oral invited
Medlin, L.K.	2001	Application of rRNA probes for measuring biodiversity.	Invited Seminar Speaker, University of Camerino, Camerino, Italy	Oral invited
Medlin, L.K	2001	PICODIV: An EU project to estimate the diversity of Eukaryotic Picoplankton in Coastal Waters	**American Phycological Society**, Estes Park, Col. June 2001	Oral
Medlin, L.K.and Valentin, K,	2001	Unexpected biodiversity in the marine picoplankton in the North Sea -a molecular analysis	**International Phycology meeting**, Thessaloniki, Greece, Aug 2001	Poster
Medlin, LK.	2001	Application of rRNA probes for measuring biodiversity.	**British Phycological Society** meeting in Liverpool UK. Jan 2001	Oral
Medlin, LK.	2001	Application of rRNA probes for measuring biodiversity.	**Woods Hole Oceanographic Institution** Seminar Series, May 2001	Oral
Muhling, N.J. Fuller D.J. Scanlan, A.F. Post, D. Vaulot, F. Partensky, W.H. Wilson and N.H. Mann.	2001	Marine viruses and cyanobacterial community structure.	**Molecular Biology of Cyanobacteria** meeting Asilomar, July 2001, California, USA	Oral
Not F, Simon N, Biegala IC, Vaulot D.	2001	Application of Fluorescent *In Situ* Hybridisation coupled with Tyramide Signal Amplification (FISH-TSA) to assess eukaryotic	**VII th International Phycological Congress,** Thessaloniki, Greece, Aug 2001	Poster

Authors / Editors	Date	Title	Event/Reference	Type[2]
		picoplankton composition		
Pedrós-Alió, C.	2001	Los microorganismos marinos vistos desde el espacio.	**Simposio Internacional Nuevas Fronteras en Ecología Microbiana**. Fundación Ramón Areces, Barcelona 11-13 diciembre	Oral
Pedrós-Alió, C.	2001	Diversity of microorganisms in polar regions.	**ESF/LESC Exploratory Workshop on The Polar Regions and Global Biodiversity Change**. Cambridge, United Kingdom, 21-22 May.	Oral
Pedrós-Alió, C.	2001	Diversity of microorganisms	**Masters Program on Analysis of Aquatic Ecosystems,** Universidad Internacional de Andalucía, Baeza, Spain, 1-5 October	Oral invited
Pedrós-Alió, C.	2001	La diversitat dels microorganismes	**Invisible Neighbours. Graduate course** at the Universitat Catalana d'Estiu, 23 August	Oral
Pedrós-Alió, C., B. Díez and R. Massana	2001	Diversity of Picoplankton	**18th Congress of the Spanish Society for Microbiology**, Alicante, Spain, 16-20 september	Oral invited
Pedrós-Alió, C., B. Díez, L. Guillou, M. Latasa and R. Massana	2001	Diversity of eukaryotic picoplankton in the oceans	**9th International Symposium on Microbial Ecology**, Amsterdam, 26-31 August	Oral
Ristori S.	2001	Analyse du phytoplancton côtier dans les eaux de Roscoff	Mémoire de stage 2e année, **Ecole des Métiers de l'Environnement, Rennes**	Report
Scanlan, D.J..	2001	Molecular approaches to assessing population structure and nutrient status of picocyanobacterial populations *in situ*.	**The Interuniversity Institute 33rd Anniversary Conference**, Eilat Israel, October 2001.	Oral invited
Scanlan, D.J..	2001	Cyanobacterial ecology, niche adaptation and genomics and introducing the most abundant photosynthetic organism on Earth.	**Microbiology Today** 28: 128-130.	News-letter
Valentin K, Mehl H and Medlin L	2001	Biodiversität des marinen Pikoplanktons vor Helgoland	**Deutsche Phycologentagung,** Helgoland Feb. 2001	Oral
Valentin K, Romari K, Not F.	2001	Uncovering picoplankton biodiversity by sequencing of environmental rRNA genes	**Qiagen News** 5: 16-17	News-letter
Vaulot D.	2001	Oceanic Picoplankton: from Ecology to Genomics	**Spring Lecture of the Catalan Society of Biology**	Oral invited
Vaulot D.	2001	The European program PICODIV: First results.	**PROMOLEC Final Meeting**. Institut Pasteur. Paris	Oral
Vaulot D.	2001	Diversity of eukaryotic picoplankton using molecular approaches.	**Biocomplexity/Food Web Symposium**, Kyoto, Japan, 2-3 Nov 2001	Oral invited
Díez, B., Massana, R., Pedrós-Alió, C.	2000	Genetic diversity of eukaryotic picoplankton in different oceanic regions by SSU ribosomal RNA gene cloning and sequencing.	**Aquatic Sciences Meeting, ASLO 2000**. Copenhagen, June 2000	Oral
Eikrem W. and J. Throndsen	2000	PICODIV- et project om de minste	**Annual meeting of the Norwegian Oceanographers**. Oct. 2000	Oral
Felman Héloïse	2000	Mise au point d'une technique de PCR-RFLP appliquée au criblage des cultures picoplanctoniques.	Mémoire de **DEA Océanologie Biologique**. Université Pierre et Marie Curie	Report

Authors / Editors	Date	Title	Event/Reference	Type[2]
Massana, R., Díez, B., Schauer, M., Pedrós-Alió, C	2000	Diversity of picoplankton assemblages in Mediterranean waters	**CIESM Workshop**. Zichron Yaakov, Israel, May 2000	Oral
Medlin, LK.	2000	Application of rRNA probes for measuring biodiversity	**Marine Research Station Network (MARS) meeting** in Venice. Oct 2000	Oral
Not Fabrice	2000	Etude de la diversité des picoeucaryotes en milieu côtier à l'aide de marqueurs phylogénétiques	Mémoire de **DEA Océanologie Biologique**. Université Pierre et Marie Curie	Report
Pedrós-Alió, C., Díez, B., Massana R.	2000	Diversity of the smallest eukaryotes in the oceans.	**7th EMMS** Noordwijkerhout, The Netherlands, Sept 2000	Oral
Scanlan, D. J. N. J. West, D. Erdner, N. Fuller, R. Howarth, R. Rippka, W. Schönhuber and R. Amann	2000	Niche adaptation in the marine cyanobacteria *Prochlorococcus* & *Synechococcus* : an assessment using phylogenetic and nutrient status molecular probes.	**Xth International Symposium on Photosynthetic Prokaryotes**, 26th-31st August 2000, Barcelona, Spain	Oral
Valentin K, Medlin L, Vaulot D, Scanlan D, Pedros-Alio C, Throndsen J.	2000	PICODIV: Monitoring the diversity of photosynthetic picoplankton in marine waters.	**Deutsche Botanikertagung in Jena** Sept 17-22, 2000	Oral
Vaulot D et al.	2000	PICODIV: Exploring the diversity of picoplankton.	**EurOcean 2000 meeting, Hamburg,** Aug 2000	Poster

1.6.3. Others: (Patents, CD ROM's, videos,…)

- PICODIV Web site: http://www.sb-roscoff.fr/Phyto/PICODIV/index.html

1.6.4. Planning of future publications: (type, date, contents, …)

Submitted

- Scanlan, D.J. Physiological diversity and niche adaptation in marine *Synechococcus*. Advances in Microbial Physiology
- Schauer, M., V. Balagué, C. Pedrós-Alió and R. Massana. Seasonal changes in bacterioplankton diversity in a coastal oligotrophic Mediterranean environment. Aquat. Microb. Ecol
- Zeidner G, Preston CM, Delong EF, Massana R, Post AF, Scanlan DJ, Béjà O. Molecular diversity among marine picophytoplankton as revealed by *psbA* analyses. Environmental Microbiology (in press)
- Massana. R. and K. Jürgens. Composition and population dynamics of planktonic bacteria and bacterivorous flagellates in seawater chemostat cultures. Aquat. Microb. Ecol.

In preparation

- Biegala I., Not F., Marie D, Vaulot D. , Simon N. Analysis of picoplankton labeled with TSA-FISH probes by flow cytometry
- Díez, B., C. Pedrós-Alió, T. Marsh, M. Estrada and R. Massana. Distribution of picoeukaryotic assemblages in Antarctic waters.
- Díez, B., R. Massana, M. Latasa and C. Pedrós-Alió. A spatio-temporal comparison of picoeukaryotes in the Alborán Sea (SW Mediterranean) by denaturing gradient gel electrophoresis and HPLC pigments analysis
- Eikrem W., Le Gall F., Romari K., Shalchian-Tabrizi K., Throndsen J. and D. Vaulot. The systematic position of *Telonema subtile* based on ultrastructure and 18S r DNA analysis.
- Eikrem W., Le Gall F., Romari K., Latasa M. and D. Vaulot. The pigments, ultrastructure and phylogenetic position of *Florenciella ultra* sp. nov. (Heterokontophyta), the first pico-sized member of the class Dictyochophyceae.
- Fuller, N.J., Marie, D., Partensky, F., Vaulot, D., Post, A. F., & Scanlan D.J. Clade-specific 16SrDNA oligonucleotides reveal the dominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. Appl. Environ. Microbiol.

- Fuller N.J., Post, A.F., and Scanlan D.J., Spatial and temporal dynamics of *Prochlorococcus* and *Synechococcus* community structure in the Gulf of Aqaba, Red Sea using molecular techniques. Environmental Microbiology
- Guillou, L., K. Romari, F. Le Gall, R. Massana, W. Eikrem, Chrétiennot-Dinet M.J., C. Pedrós-Alió, D. Vaulot. Diversity of picoplanktonic Prasinophytes.
- Marie D., Simon N. and Vaulot D. Counting algal cultures with flow cytometry. Invited Chapter. Handbook of Algal Culture (RA Andersen ed.).
- Medlin, L.K and Groben, R. An improved protocol for FISH hybridization for algae., invited chapter in Molecular Microbial Ecology
- Not F., Simon N., Latasa M., Vaulot D. Seasonal variability of picoplanktonic Prasinophyceae in coastal waters. Limnology Oceanography
- Romari, K., Vaulot D. Picoplankton diversity at a coastal site from 18S rDNA clone library analysis. Limnology & Oceanography
- Shalchian-Tabrizi K., Romari K., Eikrem W., Thomsen H.A., Throndsen J., Klaveness D. and D. Vaulot. *Telonema subtile* a probable common ancestor of the major eukaryotic lineage with tubular cristae. Nature
- Valentin K, Mehl H, Medlin L: Seasonal variability of picoplankton communities in Helgoland, North Sea – a molecular approach. Journal of Phycology
- Valentin K, Medlin L: Assessing picoalgal cultures using SSCP and partial 18S sequencing – an approach to identify picoplankton species in culture that lack morphological characters. European Journal of Phycology.
- Vaulot D. and Le Gall F. The Roscoff Culture Collection, a resource devoted to picoplankton. Nowa Hedwigia

Gantt Chart Update

Legend: Black bars correspond to initial plans, whereas gray bars represent deviations from that plan for tasks that will start earlier or finish later than anticipated.

Wpk	Task	Year 1												Year 2												Year 3											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
		avr-00	mai-00	juin-00	juil-00	août-00	sept-00	oct-00	nov-00	déc-00	janv-01	févr-01	mars-01	avr-01	mai-01	juin-01	juil-01	août-01	sept-01	oct-01	nov-01	déc-01	janv-02	févr-02	mars-02	avr-02	mai-02	juin-02	juil-02	août-02	sept-02	oct-02	nov-02	déc-02	janv-03	févr-03	mars-03
1	**Cultures**																																				
1.1	Obtain samples and start cultures																																				
1.2	Screen precultures																																				
1.3	Establish cultures																																				
1.4	Screen cultures																																				
1.5	Establish strains																																				
1.6	Characterize strains																																				
1.7	Sequence SSU rDNA																																				
1.8	Taxonomic assignment of strains																																				
2	**Sequences**																																				
2.1	Obtain samples																																				
2.2	Establish clone libraries																																				
2.3	Screen clone libraries																																				
2.4	Sequence SSU rDNA																																				
2.5	Taxonomic assignment of sequences																																				
3	**Probe design**																																				
3.1	Design probes																																				
3.2	Validate probes																																				
4	**Probing methods**																																				
4.1	FISH																																				
4.2	Probe arrays																																				
4.3	Quantitative PCR																																				
5	**Coastal time series**																																				
5.1	Get samples from coastal sites																																				
5.2	Measure diversity with probes																																				
5.3	Measure diversity with other techniques																																				
5.4	Compare methods																																				