Roscoff Biomarks Sampling
April 2010
Station Biologique de Roscoff, Roscoff, France

People
Roscoff: Sarah Romac, Frédéric Mahé, Johnn Decelle, Colomban de Vargas, Noan Le Bescot, Lucie Bittner, Christophe Boutte, Daniel Vaulot, Ian Probert – Crew members : Gilles Maron and François…
Marseille: Aurelie Chambouvet
Kaiserlautern: Micah Dunthorn
Oslo : Wenche Eikrem
Naples : Raffaele Siano
Geneva : Jan Pavlowski
Kerala Production : Pierre Augier, Pauline Plante
Genoscope: Roland Heilig

Roscoff sampling site location
Sampling occurred in the vicinity of the Station Biologique long term series site (SOMLIT-ASTAN : http://www.sb-roscoff.fr/Somlit/cartes.html
GPS position: 48°46'184N, 3°57'803W
Depth : 60 m
Nature of the sediment : heterogeneous sediment with pebbles (<15% of pebbles) and rocks

1. Realized schedule plan and samples taken

• *Monday 19 April*

In the lab : Discuss sampling, prepare material, label samples
On board : Prepare filtration ramps (Sarah, Lucie), bring material (Nathalie, Christophe, Colomban, Frédéric, Noan, Yohan)

• *Tuesday 20 April*

ONBOARD

8.00 - Leave from Roscoff old harbour to SOMLIT-ASTAN sampling site with the Neomysis (see figure for cruise track)
9:30 - CTD vertical profile: Temperature, salinity, fluorescence, PAR.
9:45 – 11:00 : sampling
Sample surface water with 30L Niskin bottles

- **5 Niskin bottles for genetic material, filtered onto 20 µm**

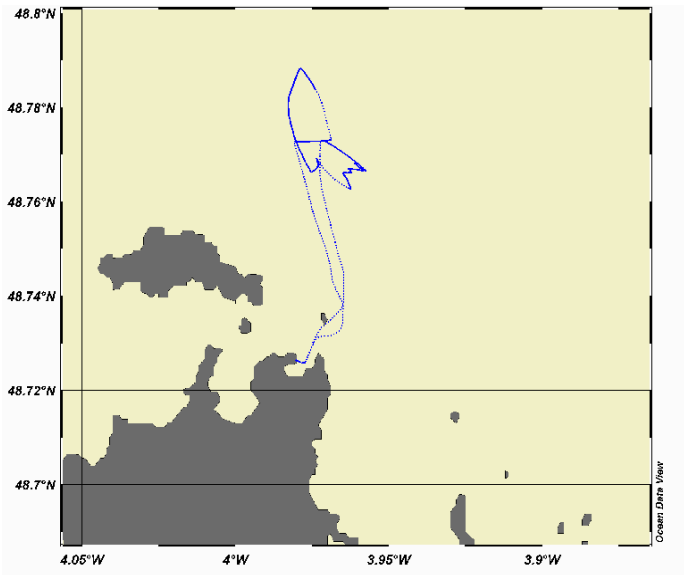


Figure 1 : Track of cruise on the 20th of April 2010

served to fill 2X 35 L carboys (for metagenomics), 4 X 20L coarboys (for PCR), 2 X 20L carboys (metagenomics). These samples were processed on board by Sarah and Lucie

- **2 Niskin bottles filtered for microscopy and pigments** : 10L of unfiltered SW collected for Microscopy, 10 L of water filtered onto 20 µm collected for Microscopy, unfiltered water collected for pollutants, nutrients and FCM, 2000 µm and 20 µm filtered water collected for pigments
- **2 Niskin bottles filtered onto 20 µm for Giruses**

Work onboard

- Sarah and Lucie process the Metagenomics, extracellular DNA
- Aurélie processes the PCR genomics samples
- Raffaele and Johan fix the samples for Microscopy (formol, glutaraldehyde and lugol) and liquid FISH
- Aurélie collects and fixes water for FISH
- Aurélie filters the water for Giruses
- Nathalie and Christophe collect water for flow cytometry, nutrients and pollutants, as well as for pigments. Fixation of samples for FCM.

11:00 – Lunch

11:30 – Deployment of Bongo nets (20 µm). Two tows each for 10 min. Net samples are prefiltered through 2000 µm sieve. Numerous *Pleurobrachia* (Ctenophora) are observed in the sieve. Samples are diluted in 4 liters of filtered sea water (filtrate from Sarah and Lucie's samples).

- Raffaele and Johan process the net samples for microscopy (formol, glutaraldehyde and lugol fixations)

11:45 – Deployment of 200 µm net (Johan)

12:15 : CTD

12:30 : Water sampled for microscopy and live imaging (3 Niskin bottles). The water (some unfiltered and some filtered onto 20 µm) is kept in the dark.

13.15. Arrive to the Roscoff harbor.

14:00- 17:00 ON BOARD IN HARBOR :

- Christophe filters the water for pigments
- Sarah and Lucie finish the filtering of the replicates

IN LAB 14:00

Work done on the water that was brought back in the lab

- Raffaele and Wenche work on the microscopy samples (fixing-concentration)
- Nathalie fixes the samples for the HTM and FISH-FCM
- Aurélie processes the FISH, Liquid FISH and DAPI samples
- Ian makes pictures of live samples
- Ian isolates some cultures, prepares some cultures.
- Johan, Margaux and Ian use the flowcam to get live samples analysed
- Micah has a look at samples for ciliates
- Christophe gives log sheets and labels RA_task_fraction_replicate

20:00 End of working day

• *Wenesday 21 february*

ON BOARD : Colomban, Noan, Sarah, Rolland

Work on tests for Genoscope. Filters are prepared

9:00 : Start of test of water ageing before filtration + filtration speed (all flash frozen) – [0.8-5µ] and [5-20µ] fractions from Niskin bottle. Around 15-20L of water filtered by Sarah & Roland.
11:00 : Start of test GPSS versus Net 5µm [5-20µ] fraction. Colomban
12:45 : Bongo Net 20µm out of the water, water prefiltered at 180µm
13:00 : Start of tests RNA later versus Flash-Freeze and Water ageing – [20-180µ] fraction. Sarah, Johan, Roland.

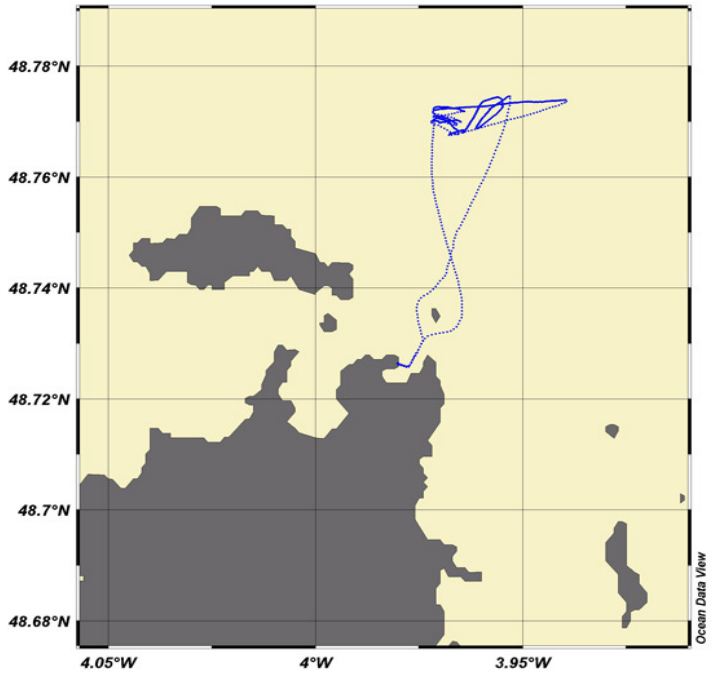


Figure 2 : track of the cruise on the 21rst of April 2010

IN LAB :

- Wenche and Raffaele prepare microscopy samples for SEM and TEM (concentration of cells from SW collected on board, fixations, etc..)
- Sarah, Lucie, Frédéric : cleaning of material
- Aurélie filters the samples for FISH (both eukaryotes and prokaryotes) and DAPI
- Labelling of samples

• *Thursday 22 April*

IN LAB :

- Wenche and Raffaele prepare microscopy samples for SEM and TEM.
- Sarah, Lucie, Frédéric, Aurélie: cleaning of material
- Labelling of samples, filling log sheets
- Aurélie filters the water for giruses

• *Friday 23 April*

- Debriefing, presentation of hydrological results and microscopy live samples observations.

• *Monday 26 April*

ON BOARD : Sarah, Jan and Noan with Gilles, François and Noël sample the benthos with a Reinek corer. 20 tries are made and only 4 samples are kept (the corer did not close properly for some samples because of the presence of either rocks of pebbles or large particules). Samples were taken at 60 m depth.

2. Meteorogical condition

Tuesday 20 th April : Sunny, rather cold day, no wind, swells and "clapot". The boat is moving but sampling is quite easy. The weather has been rather quiet the days before.

Wednesday 21th April : rather cloudy day with wind

Monday 26th April : sunny day with no wind

3. Samples collected and proccessed

Here we provide an overview on how the samples were collected and processed (see also details in the sampling part and in the related protocols)

- Molecular Plankton samples (29 in total) : all samples were processed on board.
Metagenomics: 45 L were filtered on 142 mm PC filters using the IP pump (x samples including all fractions). Different volumes of the net sample were filtered onto 47mm 12 µm PC filters (9 samples for [20-2000] fraction).
PCR genomics: around 10 L were filtered on 47 mm PC filters using the LS pump (x samples including all fractions)
Extracellular DNA (6 samples):
Molecular: 400 ml of CTAB were added to 20 L of 0.2-µm filtered seawater, incubated for 5 hours at RT and filtered on 142 mm 0.2 µm PC filter
Quantification: 20 ml of CTAB were added to 1 L of 0.2-µm filtered seawater, incubated for 5 hours at RT and filtered on 47 mm 0.2 µm PC filter
Total DNA pool: 40 ml of 0.2-µm filtered seawater

- Chemistry : all samples were taken onboard and kept in the fridge or freezer onboard.
Polutants : 6 L of total seawater (6 samples) taken in Perrier bottles : kept at 4°C on board
Nutrients: 100 ml of total seawater (4 samples) : kept at -20°C onboard.
Pigments: different volumes were filtered onto GF/F filters (8 samples including all fractions)

- **Microscopy (82 samples in total)**
Whole seawater. Quantitative fixation (3 fixatives [lugol, formol, lugolglutaraldehyde]) and coccolithophorids (unfixed samples filtered on stubs)
<20µm seawater. Fixation with the 3 fixatives. An extra lugol sample for ciliates. Coccolithophorids (unfixed samples filtered on stubs)
<20µm seawater concentrated on 3 µm (fraction 3-20 µm). SEM-osmium, TEMgrids and cultures. Live observations (pictures)
<3µm seawater. Fixation with the 3 fixatives.
Net samples filtered through 2000 µm (fraction 20-2000 µm). Fixation with the 3 fixatives and an extra fixation for ciliates with lugol. SEM-osmium. Cultures. Live observations (pictures)

- **Flow cytometry (3 samples in total)** : fixed onboard and frozen onboard.

- FISH samples (27 samples in total). Water collected onboard on the 20th and fixed in the lab on the 20th, afternoon.
FISH liquid: Formaldehyde fixed samples, sedimented for one hour, and part of the volume replaced with PBS + Ethanol
FISH HTM: Unfiltered seawater fixed with formalin and pluronic
FISH FCM: Unfiltered seawater fixed with formalin and pluronic
FISH on filters: Formaldehyde fixed samples (24h fixation) filtered through 0.2, 0.6, 3 and 12 µm PC filters
- Epifluporescence preparations (8 samples in total). Seawater prefiltered through 2000 µm and filtered onto 0.2 (5 ml), 0.6 (20 ml) and 2 µm (80 ml) black PC filters in the lab. Unfiltered seawater fixed with PFA + Glut

- Benthos (first cm of the cores) (11 samples in total)
First core:
Half total RNA/DNA, flash frozen
Half total RB for microscopy

Second core
Half total RNA/DNA, flash frozen
Half total RB for microscopy

Third core
Half total RNA/DNA, flash frozen
Half prefiltered between 63 and 500µ, flash frozen

Fourth core
Prefiltered between 63 and 500µ, flash frozen

Fifth core
Half total RNA/DNA, flash frozen
Half total RB for microscopy

One pool sample had been kept for live observations.

4. Culturing efforts

- 2 dilution series were prepared by Wenche. They are in the 15 degrees room (one <20 microns and one unfiltered)
- 2 raw cultures were prepared by Wenche : they were placed on top of the dilution series.
- Cultures were also prepared by Ian (for details, ask Ian).

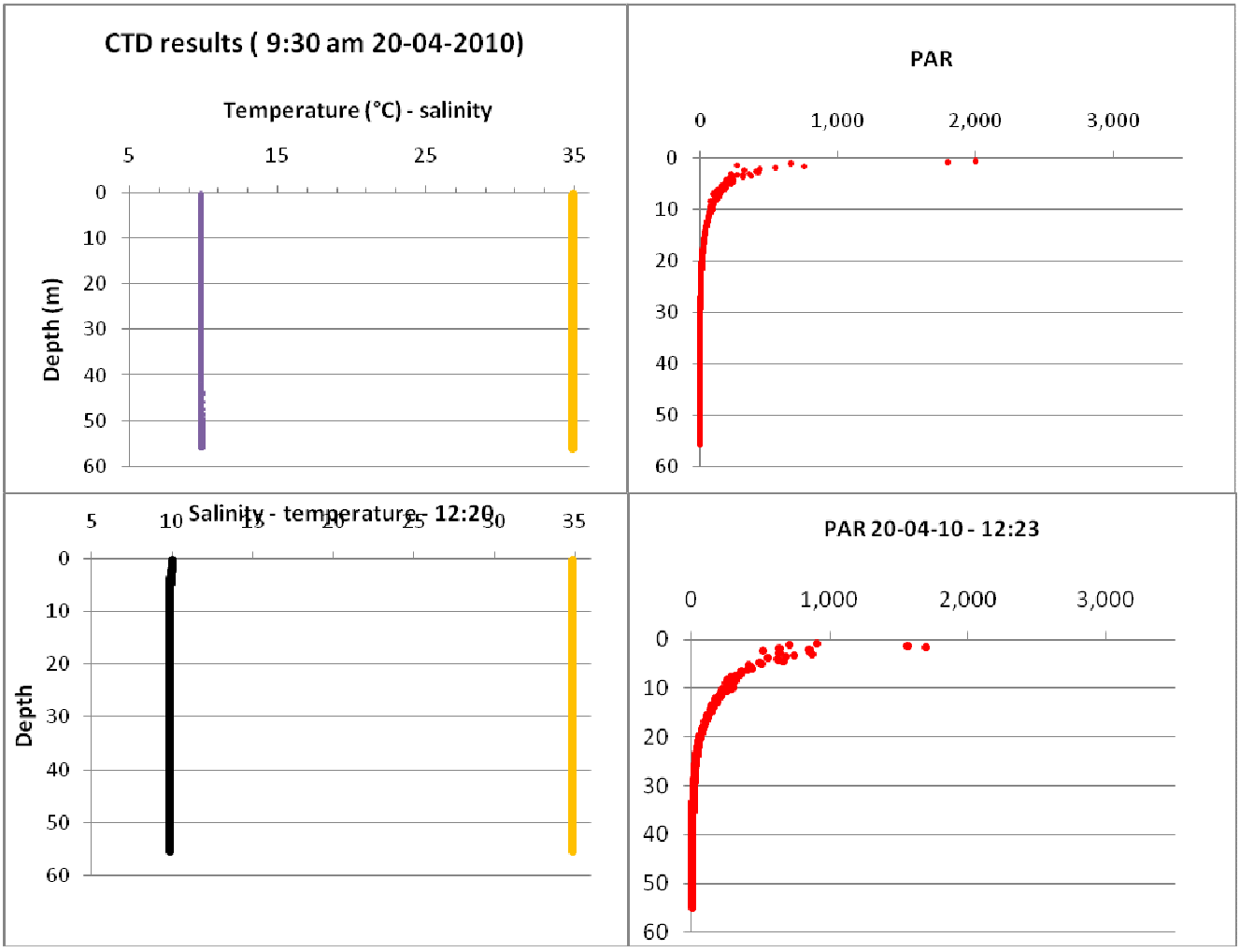
5 . Preliminary physico-chemical characterization

- *Temperature and salinity profiles, fluorescence*

Temperature and salinity was constant along the vertical profile at 9h15 at the beginning of the sampling day (9,87°C, salinity at 34,89) and at 12h15 after most samples were taken (9,87 to 10.03°C and salinity at 34.89) on the 20th of April. A problem was reported for the fluorimeter. We did not get any measurements for fluorescence but the profile was probably constant over the water column (given the hydrological conditions)

• *Light profile*

Light decreased from surface to depth exponentially. Units are in µE m^{-2} s^{-1}.



6. Preliminary results of microscopy

Observation of the net sample from the 20th of April (bongo net)

Live samples (net samples from the 20th of April) were observed by light microscopy both the day of sampling (20th of April, Ian's observations) and the day after sampling (Nathalie, Raffaele).

- The sample contained Copepods, metazoans eggs, sponges spicules
- Benthic foraminifers (according to Colomban) and tintinnids were observed in the samples.
- Debris were also observed in the net samples, with feces and as well as fragments of brown and red macroalgae were observed in the nets.
- The phytoplankton size fraction ~20-2000 µm was characterized by diatom species. Rhizosolenia species (R. delicatula for example, that are abundant during the spring bloom are present in the sample. Other diatoms, e.g. *Chaetoceros* spp., *Thalassiosira, Ditylum brightwellii* are also abundant. Dinoflagellates were also abundant (*Scriptiella*

was particularly abundant). Silicoflagellates were also identified in the sample, with *Dictyocha*. Benthic taxa (many pinnate diatoms such as *Gyrosigma* or *Pleurosigma*, Naviculaseae, and dinoflagellate cysts) were also observed.

- In the small fraction (~3-20 µm), some minute diatoms as well as unidentified dinoflagellat*es (Gymnodinioids*, *Prorocentrum??) and coccoid green balls were obs*erved. No coccolithophore was observed *in the samp*le (cf Ian's results of live observations)

After first microscopy analyses a total of p*hytoplank*ton taxa have been identified (diatoms, dino*flagellat*es, *coccolithop*hores and 8 taxa belonging to other groups).

See Annex I at the end of the report for the complete list of observed species.

7. Flow cytometry results

Peter von Dassow provided flow cytometry results from samples collected on the 20th of April. Analyses were performed live on the 21st (see figure below).
The cell populations detected include : *Synechococcus*, photosynthetic pico- and nano-eukaryotes (probably several cell populations) among which probably nanoplanktonic cryptophytes.

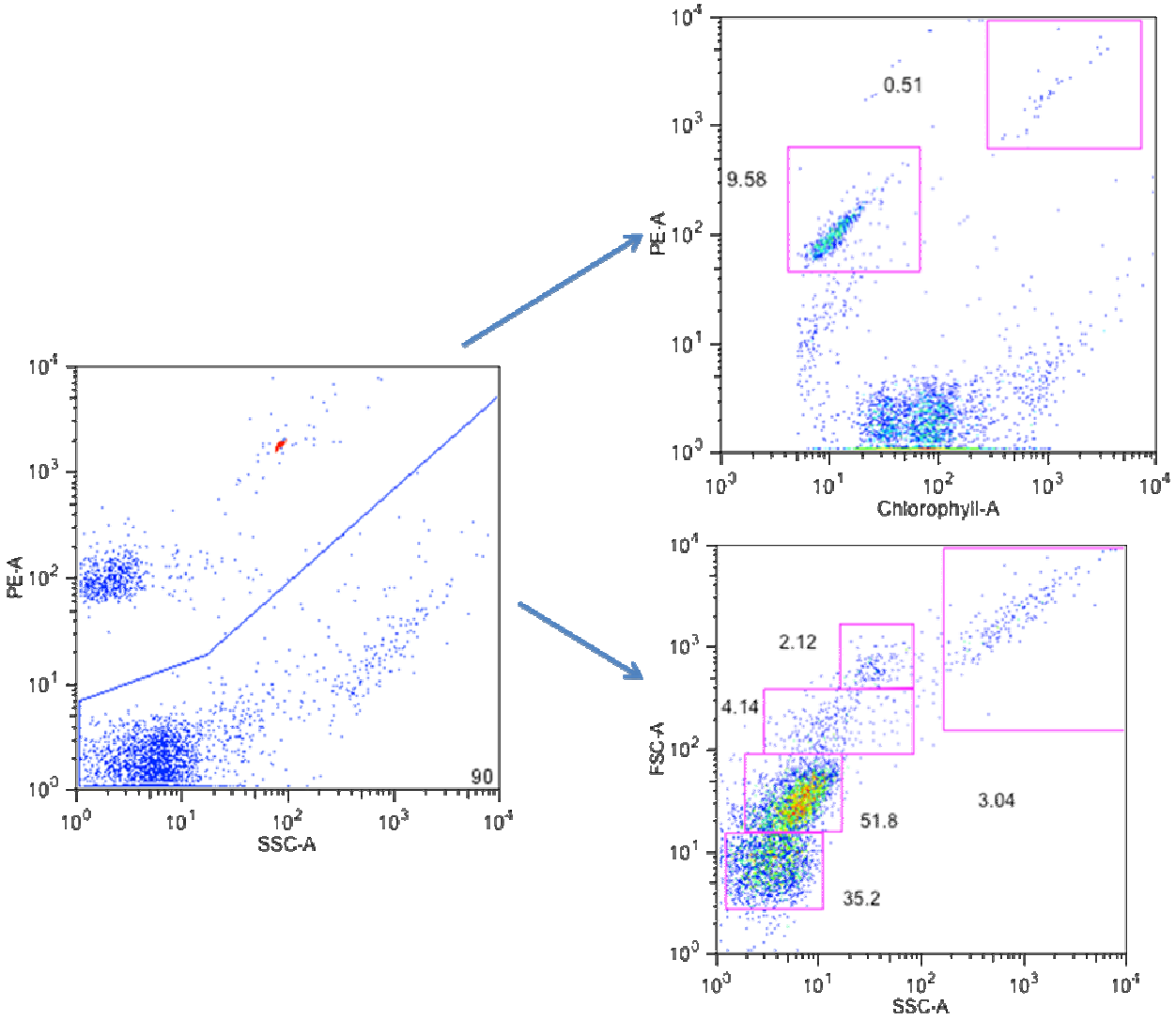
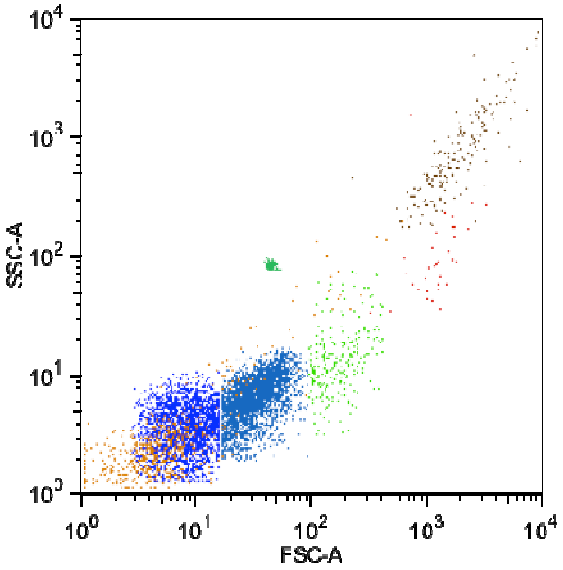


Figure 3 : Cytograms provided by Peter on live samples.



	concentration (cells/ml)	% of chl+ cells
Total number of photosynthetic cells (red fluorescing)	**9595**	
cyano	**919**	**9.58%**
picoeuk#1	**3042**	**31.70%**
picoeuk#2	**4478**	**46.67%**
picoeuk#3	**358**	**3.73%**
small nanoeuks	**183**	**1.91%**

Figure 4 : cytogram provided by Peter on live samples

non-PE larger nanoeuks	**263**	**2.74%**
possible cryptophytes	**49**	**0.51%**

8. FLOWCAM analysis

Live samples were processed with the FLOWCAM by Johan, Ian and Margaux. Files have been stored.

9. Localization of samples :

- Samples for genetic studies and molecular biology are kept in Roscoff (labeled and stored by Sarah and Lucie)
- FISH sample, DAPI, Liquid FISH, FISH HTM, FISH FCM, cytometry samples, nutrient samples as well as pigment samples: labeled, stored and kept by Sarah, Aurélie, Lucie.
- Microscopy samples (with fixatives, in glass bottles): labeled and stored at 4°C in Roscoff by Raffaele. We need to move them to another place.
- EM samples (grids, stubs for SEM and inclusions) have been labeled by Wenche (TEM samples) or Raffaele and Nathalie (Stubs). They are kept in Roscoff but will be sent probably to Oslo (at least those for TEM prepared by Wenche).
- Culture samples : they are kept in the culture room at 15°C in Roscoff.
- Benthic samples?

9. Naming of samples

137 samples have been collected: 18 for chemical analyses, 8 for Girus investigation, 82 for miscropics analyses, 29 for the molecular (Samples for tests in collaboration with genoscope are not included). Samples have been named non consecutely from RA048 to RA392. Samples list and details are provided in the xls file named "Biomarks Roscoff sampling list" and will be implemented in the BioMarKs databse. These files are available onto the FTP website

10. Samples destiny

This information is provided in the database along with the samples lists.

ANNEX - I. List of phytoplankton species (and other stuff) identified in the BONGO net from the 20th of April by light micrscopy.

	Estimation of abundance (+++ = very abundant, ++ = abundant)
DIATOMS	
Rhizosolenia pungens	
Rhizosolenia spp.	++
Chaetoceros spp.	+++
Thalassiosira rotula	+++
Coscinodiscus sp.	
Dytilum brightwellii	++
Cylindrotheca closterium	++

Thalassionema nitzschioides	
Guinardia delicatula	
Pseudo-Nitzschia sp.	
Pennate diatoms (Naviculaceae - benthic)	+++
Corethron criophilum	
Paralia sulcata	+++
Gyrosigma/Pleurosigma	++
Grammatophora sp.	
Helicotheca tamesis	
Odontella sp.	
Minidiscus?	
Small diatoms on sand grains	++
Odontella sp.	

DINOFLAGELLATES

Prorocentrum micans	
Prorocentrum gracile	
Scripsiella sp.	+++
Pyrocystis lunula	
Ceratium fusus	
Ceratium furca	
Ceratium longipes	
Ceratium furca	
Nanoplanktonic dinoflagellates	

DICTYOCHOPHYCEAE

Dictyocha sp.

OTHER PHYTOPLANKTON TAXA

Nanoplanktonic green balls

CILIATES

Ciliates (Tintinids + others)

Metazoans (Eggs, larvae)
Macroalgae debris
Copepod feces
Sponges spicules
Foraminifers (2 different forms)

Note:

- The sieve on which the net sample was filtered was full of the Ctenophore *Pleurobrachia.*

- On a sample from the 22nd of April, in addition to the species listed above, C. tenuissimus, as well as a cell of *Phalachroma* sp., *Actinoptycus* and *Leptocylindrus* was identified by Raffaele on a sample from the 22nd of April, same site)

11. Data availability

Data concerning protocoles used, sampling list, photos and video of the sampling will be available onto the Biomarks FTP server.