Cruise report from BIOMARKS sampling cruise in the Oslofjord (OF1) with R/V Trygve Braarud 22-23 September 2009

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October 2009

Contents

Background

BioMarKs integrates 8 EU research institutes and 30 EU experts in eukaryotic microbial taxonomy and evolution, marine biology and ecology, genomics and molecular biology, bioinformatics, as well as marine economy and policy, to assess the taxonomic depth, environmental significance, human health and economical implications of the least explored biodiversity compartment in the biosphere: the unicellular eukaryotes or protists.

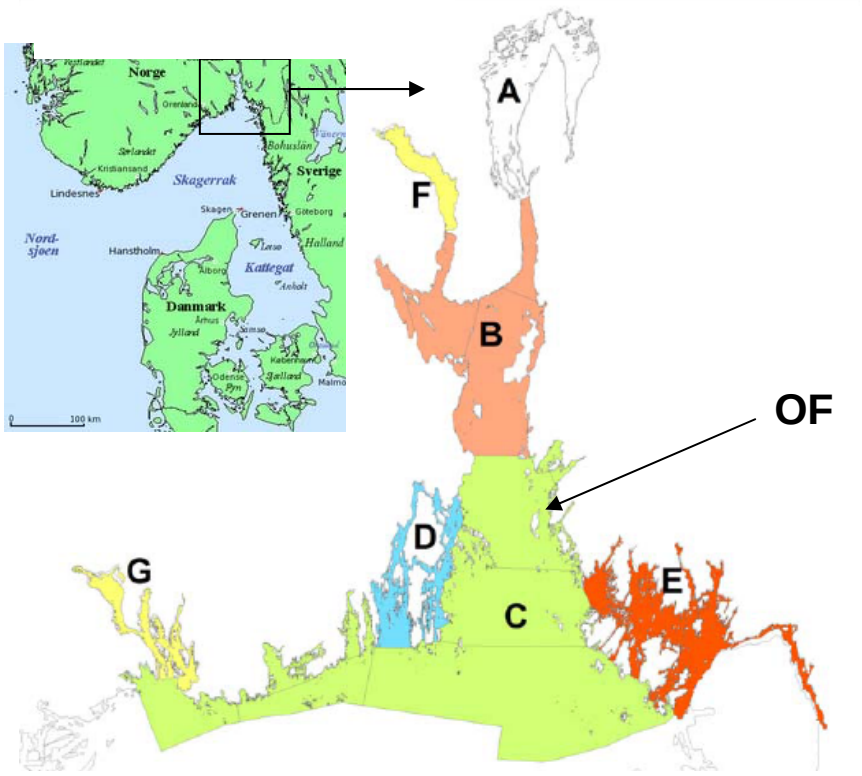
Project and cruise objectives

The scientific aims of the project BioMArKs (Biodiversity of marine eukaryotes) are to reassess coastal marine protist biodiversity using massive rDNA sequencing integrated into a network of taxonomic expertise and comprehensive phenotypic and environmental metadata. A suite of physical, chemical, and biological metadata from the same samples will allow statistical analyses of the ecological forces shaping marine protist biodiversity. Microscopy analyses, as well as downstream PCR-based and FISH molecular analyses of archive DNA, RNA, and cellular material (from the same samples) will allow anchoring of the genetic data into high quality phenotypic, phylogenetic, and ecological quantitative frameworks. Outer Oslofjord is one of nine locations and the first that will be sampled during the project.

The aim with the cruise was to obtain material from the plankton and soft bottom benthos from a north European coastal station for pyrosequencing, microscopy and a range of metadata, as well as to test and refine the sampling methods.

Study area

The sampling of plankton took place 22. and of benthos 23. September, 2009 on board the UiO R/V Trygve Braarud, leaving the harbour of Engelsviken, Østfold County at 9 and 8 a.m and returning at 7 and 1 p.m, respectively. Due to strong wind both days (up to 15 m/s) and high waves the sampling was undertaken at stations north east of Rauøy, an area somewhat protected from the south-easterly winds. The station for the plankton sampling was at position 59.253735N, 10.710908E (arrow). Benthos sampling took place at two stations a) at 59.254604N, 10.711379E (103 m depth) and b) 59.263081N, 10.724254E (24 m depth).



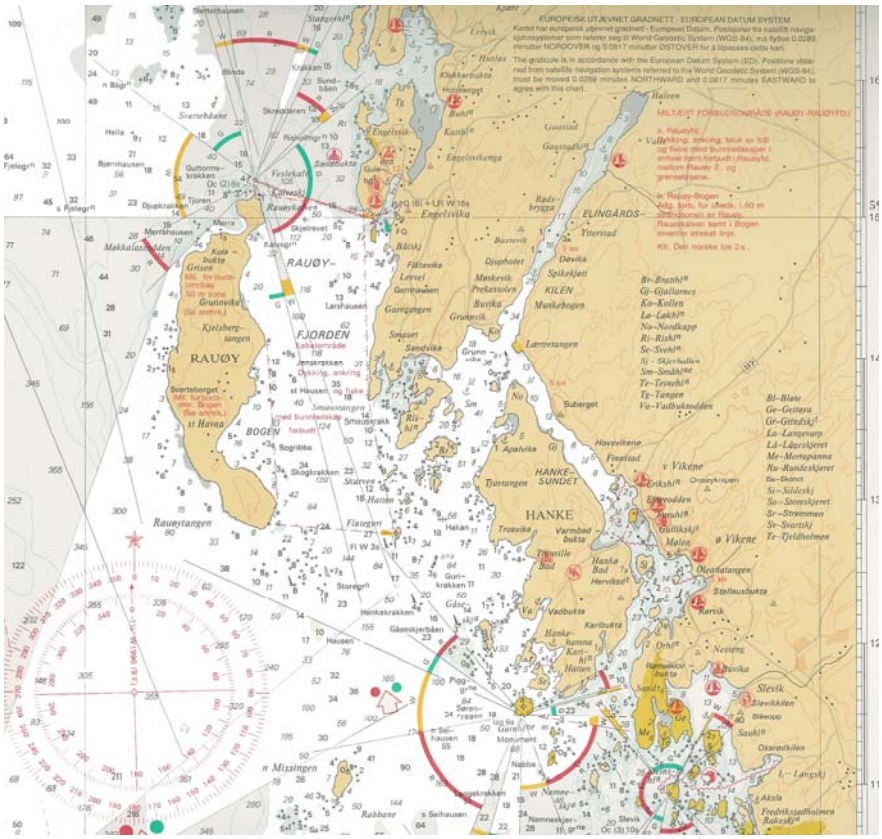


Fig. 1. Map of sampling station in Outer Oslofjord, Norway.

Participants

Partner	Name	Sampling day in September 2009
CNRS, Roscoff	Colomban de Vargas	22, 23
	Raffaele Siano	22
	Sarah Romac	22
CSIC, Barcelona	Ramon Massana	22, 23
Univ. Exeter	Meredith Jones	22
Univ. Kaiserslautern	Micah Dunthorn	22, 23
Univ. Oslo	Bente Edvardsen	22, 23
	Wenche Eikrem	22
	Rita Amundsen	22, 23
	Elianne Egge	22
	Thor Klevjer	22
	Vladyslava Hostyeva	22
	Jonas Hove	23
Switzerland	Jan Pawlowski	23

Sampling plan

	Samples/Tasks Plankton	Responsible persons
1	Physical parameters (T, S, fluorescens, irradiance, Secchi depth)	Thor & Bente
2	Collecting water, obtain <20 um size fraction	Mery & Mica
3	Chemical parameters (nutrients, pigments, chl a, pesticides)	Rita & Bente
4	Flow cytometry, FISH	Raffaele
5	FISH, Dapi	Ramon
6	SEM- coccolithophores	Wenche
7	Microscopy (LM and EM fixed with Lugols, GLA, formalin)	Raffaele& Wenche
8	Live material - cultures	Wenche
9	Syraco	Colomban
10	DNA and RNA (3 size fractions)	Sarah & Colomban
11	Extracellular DNA	Sarah & Colomban
12	Net haul >20 um microscopy (small net)	Vlada
13	Net haul >20 um DNA/RNA (large net)	Colomban
14	Sampling Haptodiv	Elianne
15	Sampling MIDTAL	Vlada
	Samples/Tasks Benthos	**Responsible person**
16	Microscopy, DNA/RNA, live material from sediment	Jan, Jonas
17	Ciliates from water and sediment samples	Mica
	Captain	Sindre Holm
	Engineer	Tor Egil
	Cruise leader	Bente

Methodology

1a. Hydrography (Thor 22.09)
Conductivity, temperature, depth and fluorescence were determined in 0-100 m depth by a CTD (FSI). Salinity and density by depth were obtained from these data.

1b. Irradiance and Secchi depth (Bente and others 23.09)
Photosynthetically available radiation (PAR) was measured with a cosinus underwater sensor and a light meter (Licore A-250) in each meter from 0-15 m depth. The values were normalised with simultaneous measurements from a similar deck sensor. Secchi depth was determined with a white 25 cm disk connected to a rope.

2. Water collection (Merry & Mica and others 22.09)
Water was collected with Niskin bottles attached to the CTD rosette. The first cast collected water from 1, 2, 4, 8, 12, 16, 20 and 40 m depths. The next casts collected approx. 30 L per cast (4 x 5 L and 8 x 1.2 L) from 1 m (subsurface) and from 20 m (deep chlorophyll maximum, DCM). The Niskin bottles were emptied by opening them in the bottom and pouring the content into 10L buckets. The content in the buckets was then poured into 60 L (2 per depth) carboys, either directly or through a plankton tissue with 20 µm mesh size stretched over a large (diameter approx. 30 cm) plastic funnel to obtain the <20 µm size fraction. The <3 µm size fraction was obtained by filtering the <20 µm size fraction through polycarbonate filters.

3a. Nutrients (Rita, Merry & Bente 22.09)
Water samples for nutrient analyses (N, P and Si) were collected from 1, 2, 4, 8, 12, 16, 20 and 40 m depth. Scintillation vials were rinsed with the sample three times and filled with 20 mL seawater, in 2 replicates. The flasks were immediately frozen at -20 °C on board. Additional water samples (125 mL) for nutrient analyses were collected from 1 and 20 m depths, in 2 replicates. Nutrient analysis will be performed at UiO.

3b. Chlorophyll *a* (Rita 22.09)
Water samples for fluorometric chlorophyll *a* determination were collected from 1, 2, 4, 8, 12, 16, 20 and 40 m depth and transferred into 1 L bottles. 200 (1 and 2 m) or 300 ml water, volume determined with a measure cylinder, was filtered and the seston collected on glass-fibre filters (Whatman 25 mm GF/F, 0.7 µm mesh size). The filters were transferred to cryo vials and frozen in liquid N_2. The samples are stored at -80^{o}C until analysis at UiO (Turners Design fluorometry). Two replicates.

3c. Pigments (Rita & Bente 22.09)
Water samples for HPLC pigment analysis were collected from 1 and 20 m depths and transferred to 1 L plastic bottles. One L, in two replicates, of the size fractions 'total-0.7 µm', '<0.7-20 µm' and '<0.7-3 µm' were filtered onto Whatman 25 mm GF/F, 0.7 µm mesh size. The < 3 fraction was obtained by filtering the <20 µm size fraction through a 47 mm < 3 µm pore size polycarbonate filter and collect the filtrate in a 1 L flask. The GF/F filters were then transferred into cryo vials and frozen in liquid N_2. Filters were sent on dry ice to Roscoff.

3d. Pesticides (Merry**)**
Water from 1 and 20 m was collected and poured into 750 mL new glass Perrier bottles, two replicates. The bottles were stored at + 4 °C until analysis at Veolia, France (?).

4. FISH and flow cytometry samples (Raffaele 22.09)
Samples were collected as described in Sample-list and protocols.

5a. FISH (Ramon Massana 22.09)
Samples from the 2 depths (1 and 20m) were fixed onboard with formaldehyde and kept at 4°C until processed in the lab the day after. Three fractions were collected:
A. Seawater prefiltered through 20 µm with a Nylon net (around 2 liters)
B. Seawater prefiltered by 20 µm and then through 3 µm in a PC (Polycarbonate) 47 mm filter (around 1 liter)
C. An aliquote of the plankton collected in the 20 µm net tow and prefiltered through 1000 µm.
Back in the lab, several filters for FISH were processed as follows:

Fraction A was filtered on 25 mm PC filters of 3 µm pore-size (200-400 ml in 4-5 replicates). **This corresponds to nanoplankton (3 to 20 µm).**

Fraction B was filtered on 25 mm PC filters of 0.8 µm pore-size (100 ml in 9-10 replicates). **This corresponds to picoplankton (0.8 to 3 µm). - excl proks**

Fraction B was filtered on 25 mm PC filters of 0.2 µm pore-size (5-10 ml in 5 replicates). **This corresponds to picoplankton (0.2 to 3 µm). - incl proks**

Fraction C was filtered on 47 mm PC filters of 12 µm pore-size (5 ml in 5 replicates). **This corresponds to microplankton (20 to 1000 µm).**

All filters were labelled in the side, and all replicates are kept together in the same Petri slide box. [In the DCM sample, the fraction 0.8-3 µm was lost and instead a fraction 0.8-20 µm was collected (see sampling list)]

5b. DAPI (Ramon Massana 22.09)
Samples (whole water) from the 2 depths (1 and 20m) were fixed onboard with glutaraldehyde and kept at 4°C until processed in the lab the day after. The following filters, stained with DAPI and mounted with oil, were prepared in 2 replicates:

5 ml on 0.2 µm pore-size black PC filters
10 ml on 0.6 µm pore-size black PC filters
20 ml on 0.6 µm pore-size black PC filters
50 ml on 2 µm pore-size black PC filters

6. Coccolithophorids for SEM (Wenche 22.09)
Samples were prepared as described in Sample-list and protocol, using a filtration rack for 10 13 mm filters (PC, 0.8 um pore size) and a Nalgene hand pump. Filters were sputtered with palladium, and examined in a Field emission scanning electron microscope at UiO.

7. Sampling for microscopy, fixed with Lugols, glutaraldehyde or formalin. (Raffaele, Wenche and others 22.09).
Samples were collected and preserved as described in Sample-list and protocol.

8. Cultures (Wenche 22.09)
Water from 1 and 20 m were brought cooled back to the lab where a dilution series was prepared in ES medium, 25 PSU. Each water sample was diluted 10, 100, 1000, 10^4, 10^5, 10^6 times with 5 replicates. The cultures are kept in a temperature controlled room at 15°C under white fluorescent light of approx. 50 µmol photons m^{-2} s^{-1} and a 12h:12h light:dark cycle.

9. Syraco (Colomban 22.09)

Samples were collected as described in Sample-list and protocols.

10a. DNA and RNA of 3 size fractions (Sarah & Colomban 22.09)
Samples were collected as described in Sample-list and protocols.

10b. DNA and RNA for Targeted genomics (Ramon Massana 22.09)
Seawater for 1 m depth was prefiltered through 20 µm with a Nylon net and kept in three plastic containers of 5 liters (the same was done later with sample from 20 m). Then seawater was filtered sequentially in a peristaltic pumping system through PC filters of 3 µm and 0.6 µm. Each filtration held 2.5 liters. So, 6 replicates were taken for the nanoplankton fraction (3-20 µm; each tube contained 2 filters) and the picoplankton fraction (0.6 to 3 µm). The filters were flash-frozen in liquid nitrogen and kept at -80°C.

11. Extracellular DNA (Sarah & Colomban 22.09)
Samples were collected as described in Sample-list and protocols.

12. Net haul >20 um microscopy - small net (Vlada 22.09)
A vertical net haul (20 µm mesh size plankton net) was collected from from 4-0 m depth. The plankton sample was divided in three parts into 100 ml medicine bottles: one sample was fixed with Lugol solution (1 %), another with formalin (2 %) and a third was kept alive cooled for subsequent examination on shore under the microscope.

13. Net haul >20 um DNA/RNA - large net (Colomban 22.09)
A horizontal net haul, at 0-2 m depth was collected during 5 min. The plankton was size fractioned through a 1000 µm sieve, and then through a 180 µm sieve.

14. DNA for haptophyte genomics (Haptodiv) (Elianne 22.09)
Water from 1 m and 20 m was collected with Niskin water bottles and filtered through a 45 µm nylon filter onto 2 l plastic bottles.

DNA - nano: 500 ml water was filtered through 3 µm pore-size polycarbonate filter, 47 mm diameter, with three replicate filters per depth. Filtrations were done using a Millipore manifold, filter holders and funnels, and a Millipore vacuum pump. The filters were placed in labeled 2 ml cryo tubes and frozen in liquid N_2.

DNA - nano+pico: As above, but cells collected on 0.6 µm pore-size filters.

DNA - nano+pico: Approx. 15 liters surface water (collected with bucket) were sieved through 180 µm nylon tissue, then run through a Millipore tripod with a 0.45 µm, 142 mm diameter Durapore filter by a Masterflex peristaltic pump. The filter was cut in four, and each piece was placed in a 5 ml cryo tube and frozen in liquid N_2.

15. Sampling MIDTAL (Vlada 22.09)
Microscopy: Water samples from 1 and 20 m were collected onto in total 6 medicine flasks and preserved with GLA (0.25%), Lugols (1%) and GLA+Lugols (0.25 and 1%) resp. for subsequent examination under the LM and in EM.

16 and 17. Benthic soft bottom sampling for DNA/RNA and microscopy.
A Gemini corer, taking 3 shots at each of two stations was used to collect material from the upper 2 cm of the sediment. Samples were collected as described in Sample-list and protocols.

Plate 1. Pictures showing the plankton sampling in the Oslofjord 22.09.2009.

Plate 2. Pictures showing the benthos sampling in the Oslofjord 23.09.2009.

16.

Preliminary results

Hydrography

The very strong stratification observed in august was now almost broken down and week pycnoclines were found deeper, at 0-15 m and in 18-25 m depth (Fig. 2). The salinity was 25 PSU in the surface and 35 PSU in the bottom water. The sea surface temperature was 15.5 °C and 8°C in the bottom water (100 m).

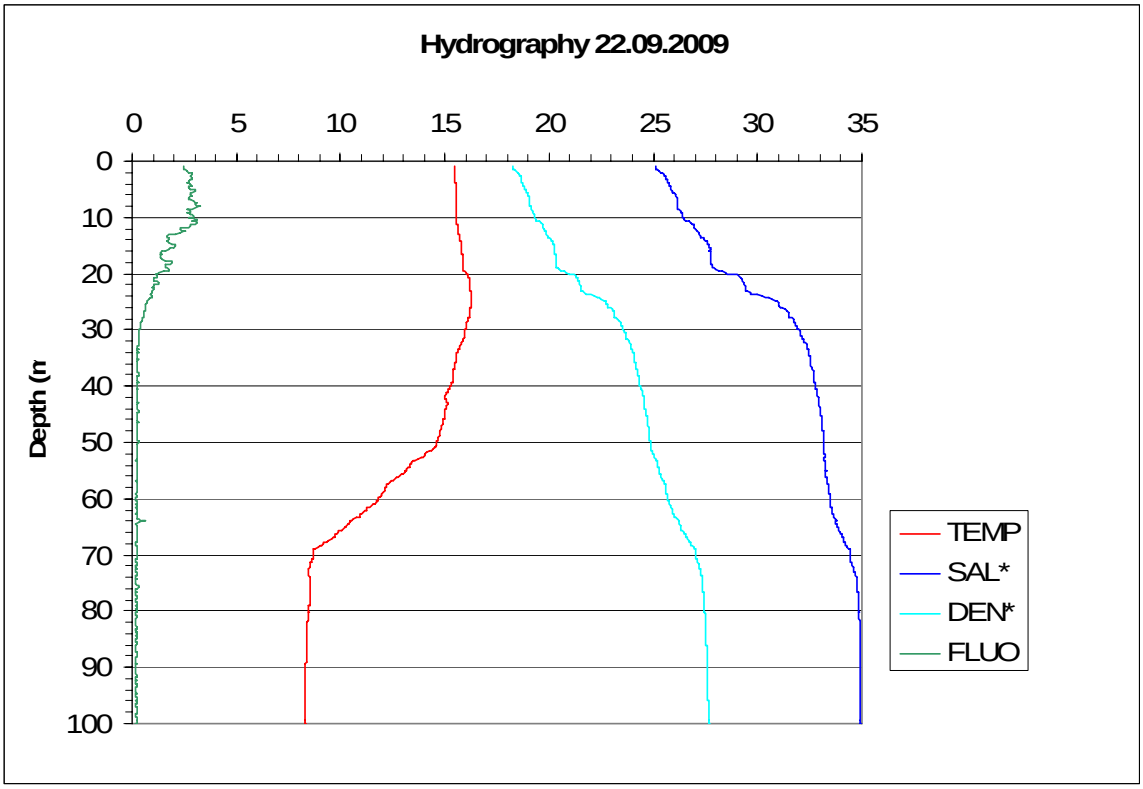


Fig. 2. Temperature, salinity, density and fluorescence with depth at station OF on 22.09.2009 as measured by a CTD.

Fluorescence

Fluorescence, a proxy for chlorophyll *a* concentration, showed highest levels in the upper 30 m, with a maximum in 8 m and minor deeper maxima in 11, 15 and 18 m (Fig. 3).

Irradiance and Secchi depth

Maximum photosynthetically available radiation (the irradiance between 350-700 nm available for photosynthesis) under water was measured at 0 m to 730 µmol photons m^{-2} s^{-1}, and 1% of the maximum level was found at 10 m depth (Fig. 4). The Secchi depth was found to be 4.2 m. The Secchi depth multiplied by a factor between 2 and 3 gives an estimate for 1% light depth. Here the factor was 2.4.

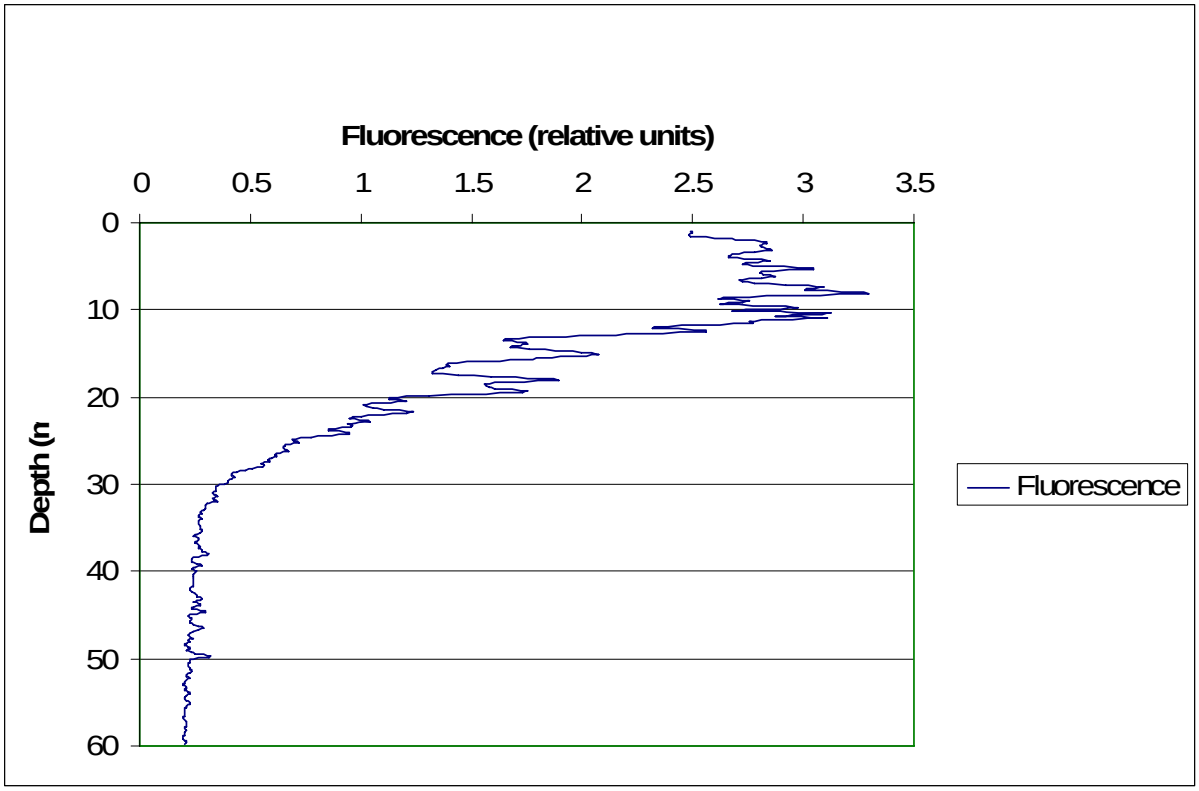


Fig. 3. Fluorescens, an estimate for chlorophyll *a* concentration and phytoplankton abundance, by depth at station OF on 22.09.2009.

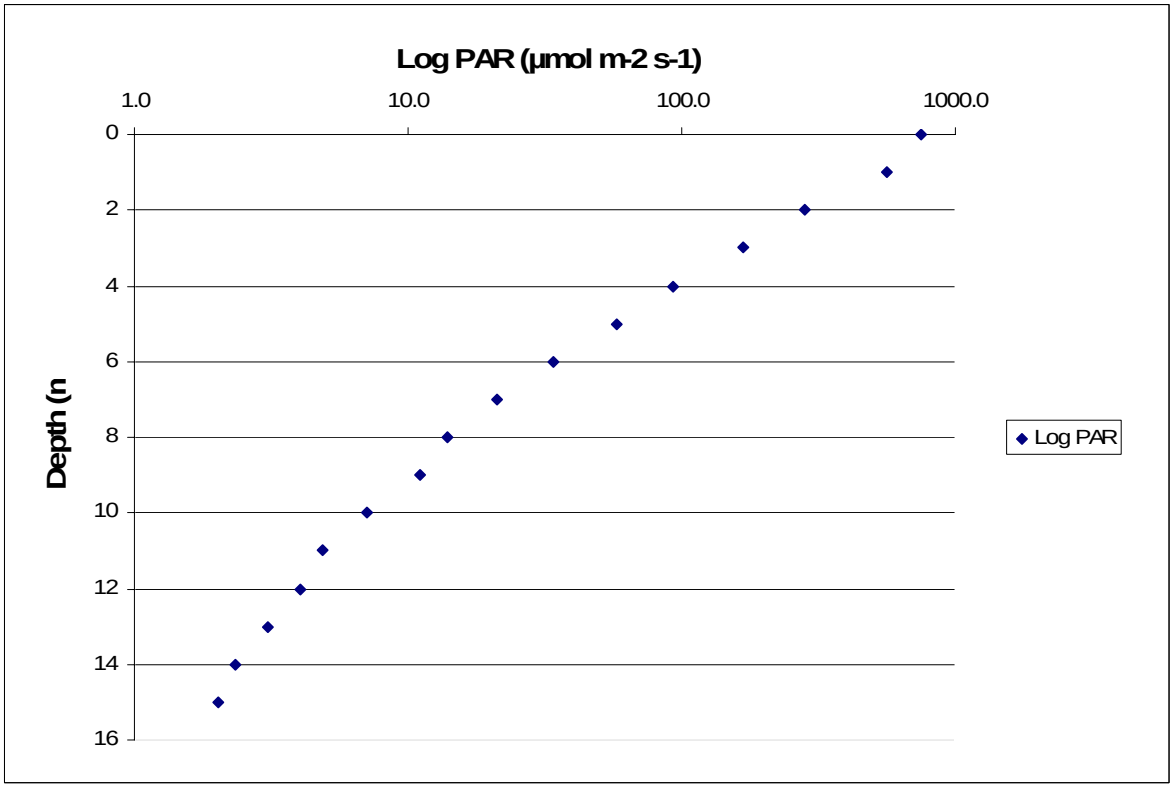


Fig 4. Photosynthetically available radiation (PAR, log transformed) by depth at station OF on 23.09.2009.

Some conclusions and comments

The water sample volume had to be reduced compared to original plans due to the high plankton biomass at the Oslofjord station, clogging filters, sieves and nets. Niskin bottles were preferred to available pumps (no strong *in situ* peristaltic pump was available) to avoid cell damage. The bottle neck was, however, not the collection of water, but the filtration for DNA/RNA. More tripod Millipore filtrations systems for 142 mm filters could solve this problem. Tubes and bottles could have been pre-labelled and a sample list available from the start to simplify the sampling. A sample list will be available during the next samplings. More time for microscopy of live material for everyone would also be valuable. In total the sampling was successful, taking in consideration of being the first BioMarKs sampling and the bad weather conditions.