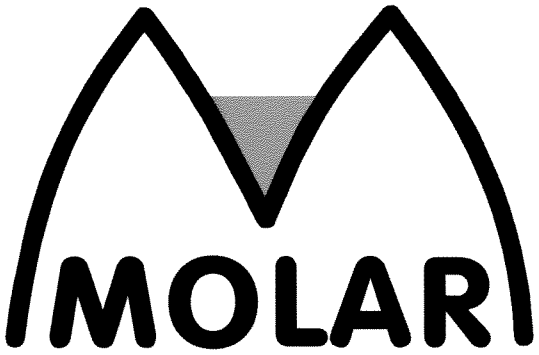

Mountain Lake Research

PROJECT MANUAL

Draft of September 1996

Measuring and modelling
the dynamic response of
remote mountain lake ecosystems
to environmental change:

A programme of **Mo**untain **La**ke **R**esearch

MOLAR

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Abstract:

The MOLAR Manual describes the practical working methods of the MOLAR project. Preparatory work and methods for sampling in the field are given in detail. Also sample handling after field work, where to send the sample material for further analysis and how to treat the results are described.

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1. Høyfjellsjøer
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1. High mountain lakes
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4. Biology

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CONTENT

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

CLIMATOLOGY AND METEOROLOGY

David Livingstone
Roy Thompson

EAWAG Zürich
University of Edinburgh

Climatology and Meteorology

1. Automatic Weather Stations

1.1 Location and Installation

Weather stations should be installed as close to the lakes as is practically possible, preferably directly at the lake shore. The mast must be well-anchored and sufficiently robust for the conditions likely to be encountered at each site (e.g. highwinds and lateral snow pressure). It may be necessary to bolt the mast to a cement foundation or to a rock base. If a large permanently-moored raft is being used for other sampling, the weather station might be able to be mounted on the raft (this may also reduce security problems). Weather stations should in general be installed on flat land well away from obstructions affecting the wind field (e.g. cliffs, trees), and the site chosen should be as representative of the lake as possible. This is particularly difficult to achieve in the case of radiation measurements made at mountain lakes subject to varying amounts of shade from the surrounding topography.

To obtain an estimate of geomorphological wind sheltering, please measure the vertical angle of the topographical horizon (with a clinometer) at the 16 points of the compass N, NNE, NE, ENE, E, ESE, SE, SSE, S, SSW, SW, WSW, W, WNW, NW and NNW. These measurements should be made twice: once at the centre of the lake surface (not necessarily identical with the deepest point of the lake) and once, for comparison purposes, at the weather station itself.Please also take one or two photographs of the weather station in situ to show the character of the surroundings.

Each site operator should take appropriate measures to ensure the security of the weather station. The measures necessary will have to be decided by the site operator basedon his/her local knowledge. At the very least, warning notices should be posted.

1.2 Meteorological Variables

The following meteorological variables should be measured at all weather stations situated at Work Package 3 sites:

Air temperature (point values, every 30 minutes)
Relative humidity (30 minute mean values)
Wind speed (30 minute mean values)
Wind direction (point values, every 30 minutes)
Short-wave (solar) radiation (30 minute mean values)
Long-wave (infra-red) radiation (30 minute mean values)
Air pressure (point values, every 30 minutes)
Precipitation (daily mean values, 07.00h - 07.00h)

The data logger should be set to measure on the hour and on the half-hour to facilitate comparison with data from other meteorological stations. However, 30 minutes should be looked upon as the coarsest sampling interval; if data can be measured at higher resolution (e.g. at intervals of 15 or 10 min), this is of coursebetter. If a thermistor chain is installed in the lake, the weather station and thermistor chain data should, if possible, be measured simultaneously. The radiation measurements are not necessary at Work Package 2 sites. Modern data loggers should allow storage of half-hourly output data from, say, 10 sensors over a period of as much as six months (86,400 data points plus date and time) without any problem. Before being sent on from each site, the raw meteorological data

must be calibrated according to the instructions of the manufacturer; uncalibrated data are not useable.

At sites where there are no plans to install a thermistor chain for the automatic monitoring of the lake temperature profile, it would be very useful if at least one temperatu re sensor measuring lake surface water temperature (at 10-30 cm below the lake surface) could be integrated into the weather station. This would allow us at least i) to relate surface water temperature to air temperature empirically, and ii) to calculate the infra-red radiation leaving the lake surface (necessary for modelling the lake heat balance).

Wind speed and direction measurements should be made 10 m above ground level (the standard meteorological height for wind measurements). This can be reduced to, say, 5 m, if it is not practicable to use a 10 m mast, since the logarithmic form of the wind speed profile means that the difference is usually relatively small between 5 and 10 m._At heights lower than 5 m, however, especially in mountainous terrain, roughness elements are likely to have a substantial effect on the wind speed. If wind measurements are being made on a raft in the lake, it would be valuable to make two simultaneous sets of wind speed measurements (say at 10 m and 3 m), which would allow the wind speed_drag coefficient over water to be determined (there is little point in doing this over land for our purposes). Make sure from the firm supplying the weather station that the anemometer will function properly in winter (problems frequently arise with snow in the cups and freezing up, for instance).

Variables other than wind speed and direction can be measured at heights much lower than 10 m; however, please make sure the sensors are mounted high enough so that they are always well above the maximum possible height of snow cover. Air temperature andrelative humidity sensors should have ventilated radiation shields. The heights of the sensors above ground level and above the surface of the lake should be noted.

If additional sensors and spare channels are available, site operators maywish to duplicate some measurements i) in case of possible malfunction of one sensor; and ii) to obtain an estimate of horizontal variability (precipitation, for instance, is very heterogeneous spatially: the possibility of using several precipitation gauges distributed over an area might therefore be worth considering, especially at Work Package 2 sites: see below).

Most practical problems with the weather station are likely to occur in winter. Anemometers ice up, snow covers the radiation gauges, the electronics become unreliable, batteries lose power, etc. etc.Measuring precipitation in winter is definitely a problem. Continuous automatic recording of precipitation using a standard unheated gauge is only feasible when the air temperature is above zero.However, heating the precipitation gauge requires a large amount of power and drains batteries rapidly, and so will not be possible at most MOLAR sites. Another possible solutionto this problem involves the use of special automatic precipitation gauges containing anti-freeze, but such sensors tend to be expensive. Thus, in general, continuous precipitation measurements will not be available in winter. Precipitation estimates at most stations during a large part of the year will therefore have to be based on totalisator-type gauges (containing a measured amount ofanti-freeze to melt snow falling into the totalisator before it is blown out again by the wind, and possibly a thin layer of oil to reduce evaporation; contact your national meteorological office for advice on this).Because precipitation tends to be spatially very heterogeneous, if possible several totalisators should be set up at different points within the lake catchment area to give an idea of the spatial variation present.This may not be feasible for reasons of cost (totalisators are not cheap), but even normal large buckets containing a measured amount of anti-freeze, fixed on a platform about 2 m above ground, and distributed around the catchment area, would be better than nothing for this purpose.

Radiation measurements in winter are also a problem, since the sensors are sometimes covered with snow, which may or may not then be blown off by the wind. One possible solution to the this problem might be to use two sensors, one receiving direct radiation, the other receiving radiation reflected upwards from the snow on the ground. However, the problem of winter measurements is less serious than it seems, since meteorological effects on a lake are much less important during periods of ice cover than otherwise.

At some sites, a permanent meteorological station belonging to some other organisation already exists within about five kilometres from the site. In this case, if the data can be made available to us, it may not beconsidered necessary to install a weather station, or it may only be necessary to install a cut-down version. Assuming all necessary meteorological variables are measured at the permanent station, such a cut-down version should at least measure wind speed and direction, precipitation and air temperature.

Calibrated data from the meteorological stations, as Excel tables or in ASCII format, should be sent in the first instance by e-mail to:

Dr. David M. Livingstone
Dept. of Environmental Physics, EAWAG
Ueberlandstrasse 133
CH-8600 Dübendorf
Switzerland
e-mail: living@eawag.ch

Variables should either be listed (i) in separate files or (ii) in table form, with one variable to a column, and with only one delimiter (tab, space or comma) between columns. Please send enough accompanying documentation to allow the data to be interpreted (e.g. starting date and time; ending date and time; measuring intervals; exact location of station; height of sensors above ground level, above lake surface level and above sea level; any information on sensor or data logger malfunctioning or otherpossible sources of bad data; the order in which the variables are tabulated and the units in which they are given).

2. Existing Meteorological Stations

A map illustrating the locations and elevations above sea level of all permanent meteorological stations within about 200 km of the MOLAR sites, with a list of the data available from these stations is requested, along withanswers to the following questions:

2.1 Data

a. Which meteorological variables have been measured (e.g. air temperature, precipitation, global radiation...)?
b. For which time period do the measurements exist (e.g. 1963-1989).
c. Are the data available free, or at reproduction cost, to researchers?
d. What is the sampling interval (10 min, hourly, daily means, monthly means...)?
e. Do you know of any really long data series (going back to, say, 1800 A.D.) from a station in your country which we may not be aware of (even if the station is situated far from the MOLAR sites)?

f. Do you know of any data series from particularly high e levations in your country (even short series)?

2.2 Researchers

Do you know the name and address of any local research workers interested in climatology that we might contact to find out more about climate data from your country?

Please send information on existing meteorological stations to:
Dr. Roy Thompson
Dept. of Geology and Geophysics, University of Edinburgh
King's Buildings, West Mains Road
Edinburgh
Scotland
e-mail: egph08@castle.edinburgh.ac.uk

3. Data on Lake Ice Cover

3.1 MOLAR sites

Any information on the ice cover of the MOLAR lakes which may be obtained during the sampling program is requested. This information includes i) date of freeze-up (defined as the first date on which the lake is completely covered with ice); ii) date of break-up (defined as the first date on which the lake is completely ice-free); iii) thickness of ice at the sampling hole.

3.2 Other Lakes

Information on the dates of freeze-up and break-up of any other lakes in your area is also requested, especially if long series of such data already exist. An effort should be made to discover if any long series of observations of freeze-up and break-up exist.

Please send information on lake ice cover to:
Dr. David M. Livingstone
Dept. of Environmental Physics, EAWAG
Ueberlandstrasse 133
CH-8600 Dübendorf
Switzerland
e-mail: living@eawag.ch

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MOLAR

ATMOSPHERIC DEPOSITION

SAMPLING AND ANALYSIS OF DIRECT DEPOSITION, SNOW PACK AND SOIL CORES

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Institute of Meteorology, Innsbruck
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ATMOSPHERIC DEPOSITION

Sampling and analysis of direct deposition, snow pack and soil cores

1. Introduction and aims of the measurements

The lakes considered in the MOLAR project are located in remote areas, far from the direct influence of human activities, so that it can be assumed that the main disturbing factors are the deposition of pollutants from the atmosphere and climatic variations. For this reason, evaluation of the input of ions from the atmosphere is of major importance.

The main aims of the measurements of atmospheric deposition are:

a. to evaluate the flux of ions and nutrients from the atmosphere to the watershed/lake surface, on a seasonal and annual basis;

b. to compare critical loads and actual loads of pollutants from the atmosphere;

c. to compare atmospheric deposition and lake water chemistry, as a starting point for studying watershed and in-lake processes which influence water chemistry.

d. to validate quantitative models for reconstruction the history of atmospheric deposition from lake sediment records.

Atmospheric deposition will be determined using several methods:

- Direct deposition
- Snow pack
- Soil cores

2. Direct deposition

Evaluation of the effects on surface waters and comparison with the critical loads require quantification of the total flux of ions from the atmosphere. This is very difficult, as there are generally marked difficulties in the evaluation of dry deposition. As an aid to clarifying the different methods of measuring atmospheric deposition, we may define the following terms:

wet-only deposition (of ions) = flux of ions from the atmosphere with rain, snow, hail. The sampling equipment used avoids collection of dust during dry periods, as the container opens automatically at the onset of precipitation.

Advantages: gives valuable information on the chemistry of atmospheric deposition.

Disadvantages: needs electric power and maintenance; does not work correctly in case of heavy snow.

bulk deposition = total flux of ions from the atmosphere during both dry and wet periodes (with rain, snow, hail). It is sampled with a continuously exposed bottle and plastic funnel.

Advantages: cheap and simple to use, does not need electric power; nor maintenance.

Disadvantages: very sensitive to dust from neighbouring areas; in calcareous soils bulk deposition gives incorrect information on pH, calcium, magnesium, potassium and alkalinity of atmospheric deposition.

dry deposition = flux of ions, gases, particles from the atmosphere during dry periods. The flux of dry deposition is due to gravity (sedimentation), impaction, interception, and is strongly influenced by:

- type of surface (broad leaves, needles, rocks, water, etc.)
- humidity of surfaces
- macro- and micro-meteorology

It is very difficult and extremely expensive to measure dry deposition; there are several techniques (see e.g. Ruijgrock *et al.* 1995), but all have limits and different degrees of difficulty. An alternative is to evaluate dry deposition through the use of large scale models, e.g. the EMEP model (Iversen 1992; Sandness & Styve 1992).

Chemical data collected on atmospheric deposition in most of the cases refer to bulk or wet-only samples. Several studies have considered a comparison between the two types of samples (Galloway & Likens 1978; Slanina *et al.* 1979; Söderlund 1982; Mosello *et al.* 1988). In general there is no relationship between the two types of samples collected at the same site. The collection efficiency of wet-only collectors is approximately 80-90% of that of bulk collectors. The reasons are a) possible delays in lid-opening as a result of difficulty in detecting the onset of precipitation; b) a lower collection efficiency for small droplets owing to the height of the dry sampler (about 2 m) above the ground or increased disturbance of the air flow.

The differences in the chemistry of bulk and wet-only samples vary in relation with the calcareous or silicic nature of the soil near the sampling site and with the relative importance of dry deposition. In calcareous areas wind-blown dust influences bulk more than wet-only samples, increasing pH and alkalinity, calcium and magnesium concentrations. The likelihood of differences is higher with low sampling frequency. In silicic areas this disturbance is much lower. Bulk samples, because of the partial collection of dry deposition, show slightly higher concentrations of sulphate, nitrate and ammonium. However, it is important to remark that the dust collected by bulk samples is not equivalent to the total dry deposition, nor in general can be assumed as a rough estimate of it.

To cater for the various needs (ions and nutrients, heavy metals, radionuclides, SCPs and organic micropollutants), it will be necessary to deploy a number of collectors. These will include:

- collectors for major ions and nutrients.
- NILU-type bulk collectors - heavy metals (except Hg), radionuclides & SCPs. These consist of a funnel (diameter 20cm) and a bottle made of polythene (PE). A PE filter is placed in the bottom of the funnel to avoid colecting local debris. For an annual rainfall of 1000 mm, the sampler will collect (on average) 0.60 L per week, or 2.6 L per month.
- IVL-type bulk collectors - Hg.
- wet and dry collectors - SCPs (Redo only).

- collectors suitable for organic micropollutants.

In all cases accurate records will be kept of the amount of precipitation that each sample represents, in order to be able to calculate fluxes.

2.1. Site selection

The site selection is the responsibility of the site operator. The selected sites should not be subject to any strong influence from local sources of pollution in the catchment that may lead to misinterpretation of the chemical data. A site should be representative of the atmospheric deposition which falls on the MOLAR lake/watershed. If the site is at some distance from the lake, the representativity of the station must be evaluated on the basis of paired samplings for short periods of time or/and using data of other stations in regional studies. Measurement of meteorological variables provides additional information of some importance; in its absence at least one double measurement of the volume of precipitation through a calibrated gauge is required. Sites with long-term series of data are preferable if the other main criteria are met.

The information required for describing each site (both lakes and atmospheric deposition sites if they are different) is:

- name and site code
- latitude, longitude, altitude
- distance from the studied lake(s)
- historical data (if available)
- presence/absence of local sources of pollution
- forest/vegetation present
- roads (paved, unpaved) and rough quantification of traffic
- geology of bedrock

2.2. Sampling for major ion and nutrient chemistry

On the basis of the considerations made in the introduction, wet-only sampling appears to be the most reliable technique for quantifying the deposition of atmospheric pollutants.

Sampling strategies will vary according to the accessibility and facility of the sampling site. In sites accessible the whole year by car and where electric power is available, the best choice is wet-only sampling on a weekly basis. In this way it is possible to evaluate seasonal variations in deposition concentrations and the time lag between precipitation and analysis is kept to a minimum.

If sites are not easily accessible, fortnightly or monthly samples are to be collected. If samples are taken less frequently than every month, they must be taken at the same times of year each year, preferably evenly spaced in time.

Difficulties in wet sampling arise with heavy snow events. In this case a bulk collector, comprising a polyethylene cylinder, 1.5 m high, raised about 0.5 m from the ground, may be used. Differences between wet-only and bulk chemistry are minimal during winter time, when the ground is covered with snow and the amount of mineral dust is negligible.

2.3. Heavy metals (except mercury) for WP2

2.3.1 Principle for heavy metal determination

The precipitation is collected with NILU-type bulk collectors which consist of a funnel and a bottle made of polyethylene. A plastic filter is placed in the bottom of the funnel to avoid collection of local debris. The collection bottles are changed weekly whereas the funnels are replaced every second month. The samples are analysed for heavy metals using inductively coupled plasma - mass spectrometry (ICP-MS). The heavy metals analysed will be Cd, Pb, Cu, Co, and Zn.

All equipment coming in contact with the samples are soaked in HNO_3 (3%) at least overnight before use. Four new sampling bottles are sent from NILU each month, whereas one new funnel is sent every second month. Four weekly samples should be returned to NILU in one batch in the beginning of a new month. The samples should be marked clearly:

MOLAR - name of site
Torunn Berg/Kjetil Tørseth
Norwegian Institute of Air Research (NILU)
P.O. Box 100 Phone: + 47 63 89 80 00
N-2007 Kjeller Telefax: + 47 63 89 80 50

2.3.2 Field operation for heavy metal determination

The sampes can easily be contaminated and it is extremely important not to touch parts of the equipment coming in contact with the sample during the field operation procedure. Plastic gloves should be worn during all handling of the bottles or other sampling equipment.

Placement of the sampler:

a. Open the plastic bag, and fold it around the funnel

b. Put on gloves, and open the plastic bag which contains the bottle

c. Unschrew the bottle cap and place it in the plastic bag

d. Schrew the funnel and the bottle together

e. Open the plastic bag which contains the plastic filter and transfer the filter into the funnel without touching it.

f. Innstall the funnel/bottle carefully in the stand.

Placement/removal of sample bottles

a. Open the first plastic bag containing the sampling bottle and fold it back

b. Put on gloves, and open the second plastic bag

c. Unschrew the bottle cap and place it in the plastic bag

d. Remove the funnel/bottle from the stand and unschrew

e. Place the old bottle in double plastic bags

f. Rinse the funnel with demineralized water

g. Schrew the funnel and new bottle together, and carefully install in the stand

h. Screw the bottle cap tightly on the bottle

i. Mark the bottle with station name and period of exposure (from - to)

j. Close the plastic bags

2.3.3 Sample pretreatment and analysis for heavy metal determination

When received at NILU, the samples in the collector bottles are acidified with concentrated HNO_3 (suprapure) to a total acid concentration of 1%. They are occacionally shaken to release any adsorbed elements on the inner walls of the bottles in a period of 2 to 3 days before transferring to storage bottles made of polyethylene. The acidified samples are subsequently stored at +5°C before analysis.

Analysis of heavy metals are carried out using ICP-MS. Calibration is accomplished by using standard solutions at 1 and 10 ng/ml. All calibration standards, blanks and samples are added 1% HNO_3 (suprapure) and 50 ng/ml Sc, In, Ho and Re (internal standards.

2.4 Mercury (Hg) determination

2.4.1 Principle for mercury (Hg) determination

The precipitation is collected with IVL-type bulk collectors on monthly basis. The rack is made of black polyethylene, whereas all parts of the collector coming in contact with the sample (funnel, filter, capillary, bottle) are made of borosilicate glass. The bulk sampler is protected from the interference of dry deposits of Hg through the capillary attached to the funnel. A filter is placed in the bottom of the funnel to avoid collection of litter and insects. The collection bottle is fitted with a wide polyethylene collar to protect it from sunlight penetration. Two collectors are placed at each station. Mercury is determined by using cold vapor atomic fluorescence spectroscopy (CV-AFS)

All the equipment which comes into contact with the sample is washed with HCl/BrCl at NILU. The sampling bottles are distributed by NILU each month to the sampling stations. For preservation, the bottles are preacidified with 2.5 ml Suprapure Hcl when sent out from NILU. The bottles are packed in double plastic bags and sent by mail to and from the sampling stations. The sample bottle should be returned to NILU together with the four weekly heavy metal bottles in the beginning of a new month. The parcel should be marked clearly:

MOLAR - name of site
Torunn Berg/Kjetil Tørseth
Norwegian Institute of Air Research (NILU)
P.O. Box 100
N-2007 Kjeller

Phone: + 47 63 89 80 00
Telefax: + 47 63 89 80 50

2.4.2 *Field operation for mercury determination*

This field operation procedure following below is the one recommended for use in the Oslo and Paris Commissions (PARCOMs) monitoring network (Munthe 1996)

Collection bottles containing the precipitation samples should be exchanged according to a fixed procedure taking great care to avoid contamination. Plastic gloves should be worn during all handling of the bottles or other sampling equipment.

a. Remove outer plastic tube

b. Prepare new collection bottle, plastic bags and container (squeeze bottle) with high purity water. All equipment needed for the bottle exchange should be placed on a plastic cover either on the ground or other available surface.

c. Open double bags of new collection bottle.

d. Carefully remove the ground glass joint connecting the bottle to the capillary. Use both hands; one for loosening the glass fittings the other for holding the funnel.

e. Remove the stopper from the new collection bottle and stopper the bottle containing the sample. Remove the bottle containing the sample from the plastic casing and put in double plastic bags.

f. Without bottle in place rinse the funnel and capillary with high purity water. If visible materials (dust, insects etc.) are present in the funnel this can be disconnected from the capillary and rinsed separately. New plastic gloves should be used if handling of the watch glass or touching the inside of the funnel is necessary. If the funnel and capillary are visibly dirty even after rinsing, they should be exchanged for newly washed pieces.

g. Remove new collection bottle from plastic bags and place in the plastic casing. Connect the ground glass fitting and check all connections. Make sure that all connections are without gaps where the silicon tubing is exposed to the precipitation sample uneccesarily.

h. Replace outer plastic tube.

i. Mark the double plastic bags containing the sample bottle with station name and period of exposure (from - to)

Field blank samples should be taken regularly (every 6 month):

- Two extra sampling bottles are brought to the site; one containing diluted Hcl (pH 3 to 4) and one empty. After removing the sample bottle (step 5) the empty bottle is installed and the diluted Hcl is poured through the funnel and capillary. The bottle is stoppered, double bagged and brought to the laboratory for analysis. The mercury content is compared to that of samples stored in a clean laboratory environment.

The sample bottles should be transported as soon as possible to NILU. If delays are unavoidable, storage outside or in wooden shed etc. are preferable to analystical laboratories with unknown history of mercury handling. The bottles containing the samples are stored in a clean room until analysis

2.4.3 Analysis of precipitation samples for total mercury

The mercury is oxidated by adding BrCl to the precipitation samples one hour before analysis. Mercury is subsequently determined by using cold vapor atomic fluorescence spectroscopy (CV-AFS) using $SnCl_2$ as reduction agent (aqueous Hg is reduced to Hg°).

2.5 Radionuclides

Precipitation will be collected using a separate NILU-type collector following the same procedures as for heavy metals, preserved in 2M high purity HNO_3, and sent (with appropriate site information, date, collection period etc,) to Peter Appleby at University of Liverpool.

2.6 SCPs

Reference is the two sections of Neil Rose's SCP protocol:

2.6.1. Bulk Deposition

SCP are to be analysed from every bulk deposition sample for WP2. i.e. weekly samples for Jorisee, Gossenkollersee, Redo, Øvre Neådalsvatn and weekly in summer and bi-weekly in winter at Lochnagar and Starolesnienske Pleso from October 1996 - March 1998. Please refer to the bulk deposition sampling protocols.

A note should be made of the total wet deposition before removing the sample and then the walls of the collector should be washed with deionised water to remove any particulate matter sticking to the walls.

If a bulk deposition sampler is used for SCP only then the whole sample should be filtered through a Whatman GF/C filter, if more analyses are to be done then a known fraction of the deposition should be filtered through a Whatman GF/C filter. As much sample as possible should be filtered for SCP. Filters should be folded once to include any particles and stored flat in an individual, labelled (site name, `Bulk deposition', dates) plastic bag. These can be sent in batches (e.g. every 2 months) to Neil Rose.

If the bulk deposition collector contains snow or ice, again, a known fraction should be sub-sampled for SCP, and melted before filtration. If no rain or snow has fallen over the sampling period then the walls of the collection vessel should be washed with deionised water and a known fraction of the resulting suspension filtered for SCP.

With each sample it is essential to know:

- The sampling interval dates
- The total amount of wet deposition that has fallen over the sampling period
- The fraction of the total that has been filtered for SCP.

2.6.2 Dry and Wet deposition

At Redo only initially for a trial period, dry and wet deposition samples will be analysed for SCP. Filters will first be analysed for organics and then passed on for SCP. If this approach fails to work, the bulk deposition sampling outlined above will be undertaken. Samples should be labelled with date, `Redo', and `dry' or `wet deposition'.

2.7 Organic Micropollutants

Reference is the procedures given by Joan Grimalt et al. in this manual. A full description of both sampling and analysis is given in the special section: "Protocols for the Analysis of Organic Micropollutants in Fish, Sediments, Wet-only depostion, Bulk deposition, Snow, Water, Air.

3. Snow pack

At Gossenöllesee, snow pack samples will analysed monthly for major ions and nutrients, heavy metals (Pb, Cd, Cu, Co, Zn), radionuclides, SCPs and organic micropollutants. For the other WP2 sites sampling will be at least once during snow season.

3.1. Sampling site

Select the sampling site according to your local knowledge of the catchment area. However, good accessibility should not be the only reason to select the site. There are several requirements a sampling site has to meet:

a. The selected site should be as representative as possible for the catchment area in respect to snow accumulation. Checking the snow depth with an avalanche sonde along various transects within the catchment may help you to get an impression on the distribution of snow.

b. The site should not be influenced by avalanches or frequent snow drift causing either additional snow accumulation or ablation.

c. The site must not be influenced by any human activity. For instance, it should be situated away from hiking or skiing routes, roads, etc.

3.2. Sampling procedure

a. Dig a snow pit at a size depending on the depth of the snow pack. For a 2 meter snow cover a pit of 1 x 2 m might be a practicable size. The wall where you will take the samples and determine snow density and stratigraphy should be

- plane: smooth it with a not rusty shovel, e.g. an avalanche shovel made of glassfiber. This will help you to recognize the vertical sequence of snow layers.
- and lie in the shadow: incident sun may rapidly alter stratigraphy or even cause local melting.

b. Mark the location of your pit. For instance, put a stick at the exact position of that pit wall where you did all your sampling („front wall"). Next time you dig another pit move ahead about 1 m from the stick. You should be sure the snow cover had not been disturbed anyhow before. Thus never step close to the front wall of the pit or throw snow there during digging the pit (only left and right of the pit).

c. Take snow samples along a vertical profile at intervals of e.g. 10 cm. Extra samples of layers with mineral (Saharan) dust or ice might be of interest and reveal additional information.

Sampling at intervals of 10 cm is especially recommended at sites with weather stations. If the height of the snow cover reaches values of 3 to 4 m sampling intervals of 20 or 30 cm may be more convenient. Bigger sampling intervals , but more than one snow pit within the catchment are recommended, if no representative site can be found.

d. Determine the density profile. It is most convenient to measure snow density at the same intervals as you take the snow samples.

e. Determine the stratigraphy of the snow cover. A detailed description of grain size, grain form, dirty layers, ice layers and lenses may help you to interpret results.

f. Optional: measurement of snow temperature at several levels. Snow temperatures in the range of -2 to 0°C indicate snow melt, that either will begin within the next time or already occurred. If snow temperature is around 0°C at all levels you should be aware that a big amount of trace substances accumulated in the snow pack will already have been released with the percolating melt water.

3.3 Precautions against sample contamination

a. All material expected to come into contact with the sampled snow has to be carefully cleaned in the lab before (with diluted HCl , soaked and rinsed with deionized water).

b. During sampling wear non fibrous cloths (inclusive a cap), mouth masks and cleaned rubber gloves. Never touch the snow to be sampled, sample bottles, tools etc. with bare hands.

c. If not stabilized keep samples frozen until analysis.

3.4 Snow density and water equivalent

In order to calculate (estimate) ionic loads, i.e. the amount of atmospheric trace substances accumulated in the snow cover, you need to know the density, respectively the water equivalent (W.E.) of the snow pack.

To measure density fill a tube of well defined volume (e.g. 500 cm^3) and weigh it with a spring balance. Density has to be determined along a vertical profile similar to sampling snow for the chemical analysis.

weight / volume	= density	[kg/m^3]
weight / area	= water equivalent	[cm]
(with area	= cross section of the tube)	

3.5 Snow samples

Estimate the amount of sample liquid you need for chemical analysis before selecting the sample bottles (fresh snow will only have a density of about 200 kg/m^3 !).

The following procedure has evolved from experiences of previous snow investigations:
The snow is cut off with a tube of stainless steel at intervals of 10 cm. The cross section of the tube fits with that of the sample bottles (about 3.5 cm for the 500 ml bottles we use). With a stopper we cram the snow into the bottle taking as many parallel samples from the respective interval as are needed to completely fill the bottle. In this way you will get enough sample liquid and - what is quite important - you will get a mean concentration of trace substances for the respective snow layer (ion concentrations may vary considerably even within a few centimeters !).

The 10 cm intervals are marked with a plate of stainless steel horizontally stuck into the snow thus guarantying that you will take the parallel samples always from the same 10 cm interval.
To put the tube and the plate you will need a hammer (glassfiber) as soon as the snow becomes harder and more dense.

3.6 Analysis

3.6.1 Major Ions and Nutrients

The analysis will follow agreed methods for water samples.

3.6.2 Heavy Metals

The analysis will follow agreed methods for precipitation and water samples.

3.6.3 Radionuclides

Snow pack samples for radionuclide analyses will be collected monthly. Sub-samples of c.250mL (sufficient to generate a total melted volume of 2L) will be taken from c.12 evenly spaced depths in a vertical profile, amalgamated and melted in storage bottles that have been cleaned and acid washed according to the same protocol as for heavy metals, preserved by acidifying to 2M strength with high purity HNO_3, and sent to Peter Appleby at Liverpool ERRC.

3.6.4 SCPs

Reference is the appropriate section of Neil Rose's SCP protocol:

Snow pack samples for SCP analysis will be collected monthly. Two litre samples (unpacked volume) will be taken from each 10cm layer of the snow pack. The snow should be melted, filtered through a Whatman GF/C filter. The filter paper should then be folded in half once (so that any filtered particles are enclosed) and stored flat in an individual plastic bag clearly labelled 'Snow' with the site name, date, sampling depth (e.g. 50-60cm) and volume (water equivalent). The bags should then be sent to Neil Rose at ECRC

3.6.5 Organic micropollutants

Reference is the procedures given by Joan Grimalt et al. in this manual. A full description of both sampling and analysis is given in the special section: "Protocols for the Analysis of Organic Micropollutants in Fish, Sediments, Wet-only depostion, Bulk deposition, Snow, Water, Air.

4. Soil cores

4.1 Introduction

At each of the WP2 sites, long-term values of the mean atmospheric flux of radionuclides will be assessed from radionuclide inventories retained in soil cores.

4.2 Sampling procedure

Soil cores will be collected using sharpened plastic tubes of 7-10 cm internal diameter. These should be driven into the soil to a depth of at least 30 cm taking care to avoid compaction, dug out and then extruded into 1 cm sections for the top 10 cm and 2 cm sections thereafter.

The cores should be from undisturbed locations selected according to the criteria:

I. there should have been no major soil disturbance for at least 30 years, and preferably longer,

II. soil types should be of a type that inhibits radionuclide migration through the soil column,

III. they should be on open level ground not subject to erosion or flooding by surface waters,

IV. the soils should be relatively compact and saturated (so as to minimise *in situ* ^{222}Rn escape),

V. the soil depth should be contain the entire fallout inventory,

VI. there should be reliable precipitation data (rain and snow).

At least three cores should be collected from each site. If there are no suitable sampling locations in the catchment itself, cores may be taken from the nearest sutable location.

4.3 Sample preparation and transport

The in situ volume of each core slice will be recorded, and the dry weight fraction determined by drying overnight following the same procedure as for the lake sediment samples. The dried samples will then be securely packed, sealed and labelled in polythene sample bags, and sent to Peter Appleby at Liverpool University ERRC.

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

SURFACE WATER

SAMPLING AND ANALYSIS PROTCOL

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Surface Water - Sampling and analysis protocol

1. Major ions and nutrients WP1, 2 and 3

The standard water samples are taken as surface grab samples from the outlet of the lake. Variability in lake properties has an annual cycle, and to study in more detail this cycle the following (minimum) sampling freqency programme will be followed:

Winter (Nov.-Dec.-Jan.-Feb.-March)	2 samples
Spring (April/May-May/June)	8 samples
Summer/Autumn (May/June-Oct.)	4 samples
Total number of samples pr. site	14 samples

Prevention of sample contamination or sample changes during storage may be critical in obtaining accurate measurements for these water samples of very low total ionic strenght. All containers used for sample collection or storage must be free of any important quantity of the determinands in relation to the lowest concentration to be measured. The containers must also be of material that will neither absorb/adsorb nor release measurable quantities of the determinand.

2. Heavy metal determination WP2

Four samples from the fish containing lakes in WP 2 (Ø. Neådalsvatn, Redo, Gossenköllesee and Jorisee), will be analysed at NILU for Hg, Cd, Pb, Cu, Co and Zn. The sample bottles will be mailed from NILU to the different participants on agreed and due time before the sampling. The porcedures for cleaning and preparation of the sample bottles will be as desceibed under 4.4 in this part of the manual. The following bottles will be sent:

- For Hg	glass bottle specially washed, preserving agent added (acidified water)
- For Cd + Pb	plastic bottle specially washed for heavy metal analysis preserving agent added (acidified water)

All water samples should be unfiltered. The samples will be taken from the lake outlet in September/October, February, May and July to cover seasonal variations.

The sample bottles should be returned to NILU and marked clearly:

MOLAR - name of lake/site
Torunn Berg/Kjetil Tørseth
Norwegian Institute for Air Research (NILU)
P.O. Box 100 Phone + 47 63 89 80 00
2007 Kjeller, NORWAY Telefax + 47 63 89 80 50

3. Heavy Metals (Speciation)

3.1 Introduction

Lake water samples are to be collected for trace metal (lead, copper) speciation and total metal (lead, copper, zinc, iron, chromium) analyses. Metal concentrations in remote lakes

are expected to be low (much lower than in rain water), so clean procedures should be followed to prevent sample contamination with metals from sampling gear or hands.

Samples should be collected at several depths from the water column of the lake: typically at 1m intervals giving up to 10 samples from shallow lakes (up to 10 m depth); greater intervals can be used for deeper lakes so that a total of 12 samples is obtained evenly spread over the water column.

Each sample consists of:

One 0.5 L sample unfiltered (bottle A)

One 0.5 L sample filtered (bottle B)

One 0.4 L sample filtered and frozen (bottle C)

A single larger sample of 2 L (unfiltered) can be collected if filtration is to be carried out later in a land-based laboratory.

3.2 Sampling gear

- Peristaltic pump, pumping rate 100-300 ml/min, with silicone (or similar) pumping tubing; the pump could be powered by a battery (or by hand which will be slow).
- Teflon tubing, 0.5 cm ID, sufficiently long to reach the bottom of the lake (~ 20 m).
- The Teflon sampling tube should be weighed down with something heavy (rock in plastic bag?) suspended from the tube or a rope (nylon) to which the tube is also attached; the tube inlet should be about 50 cm above the weight. The bottom 30 cm of the tube should be unattached from the rope to enable the inlet to move away from the weight and the rope.
- Filtrations: 47 mm filter membranes, 0.4 or 0.45 μm pore size; Oxoid or Millipore polycarbonate are okay. Polypropylene, polycarbonate, polyethylene or Teflon filter holders

3.3 Sampling procedure

Sampling is most conveniently carried out using a peristaltic pump with an extended sampling tube lowered to the desired depth. The outflow of the pump is directed into sample bottles for sampling. The filtration is then conveniently carried during sampling by attaching an in-line filtration unit to the outflow of the pump. The sampling tube is flushed prior to sample collection by pumping water from the sampling depth for at least 5 mins. The water temperature should be monitored by holding a thermometer in the water flow. First a sample is collected for pH measurement. Then bottle A is rinsed three times with pumped water (~100 ml each time is sufficient) and is filled.

Filtration

An in-line filtration unit with a pre-cleaned filter is attached to the outflow of the pump and rinsed with lake water (about 100 mL or so); then the filtrate is directed into bottles B and C which are rinsed twice and then filled; bottle B can be filled fully but bottle C not fully to allow for expansion for freezing.

Alternative filtration in laboratory: peristaltic pump driven filtration is preferable with an in-line filtration unit. Alternatively pressure filtration can be used using an all polycarbonate (or other metal-clean plastic) filtration apparatus with the outflow collected directly in a sample

bottle. It is also possible to pressurize a sample bottle with either pressurized air or nitrogen via a tube (silicone or similar) through the cap, and connect it via a second tube in the cap to an in-line filter holder. Vacuum is problematic as the filtrate should not arrive in a glass bottle.

Notes about touching sample bottles and pump tubes

Bottles are packed in plastic bags, and several bagged bottles are together in a second plastic bag. The outer bag is dirty, the inner one is clean and the bottles are very clean. The outer bag is opened and then transparent plastic gloves (clean ones) should be put on before the inner bag is opened. The bottle can be left partially in this bag whilst the cap is removed and placed inside the bag. The bottle is then rinsed and filled, then capped, the inner bag is closed and the bottle can be placed inside the partially open outer bag; the gloves are taken off and stored in a separate plastic bag for re-use, and the outer bottle bag is pulled up and closed.

The final part of the pump tube should be kept clean and should be touched only with gloves and can be rinsed with lake water. The entire pump tube should be stored in a plastic bag (large) when sampling is finished.

4. Radionuclides

Sampling will probably be carried out annually at three WP2 sites, Redo, Gossenkollesee and Neådalsvatn, by arrangement with Joan Grimalt (FBG). The object will be to determine particulate and dissolved ^{210}Pb concentrations in the water column. Using an INFILTREX II water sampler, c.500 L of lake water will be pumped through a 0.45μm filter, and the filtrate passed through an in-line Axys Environmental Systems Ltd Radionuclide Type I exchange column. On completion, the filter should be carefully folded so as to ensure no loss of the retained particulates, packed and labelled, and sent with the exchange cartridge to Peter Appleby at University of Liverpool.

5. SCPs

Reference is the appropriate section of Neil Rose's SCP protocol, as also sited below:

At twice yearly intervals (end of summer, end of winter) at least 20 L of lake water should be filtered through a Whatman GF/C filter. The filter should be folded in half once (so that any filtered particles are enclosed) and stored flat in an individual plastic bag. If necessary, more than 1 filter can be used to filter the required sample volume. Sample bags should be clearly labelled 'lake water' with the site name, date and volume filtered and sent to Neil Rose at ECRC.

If possible a sample from the outflow could also be obtained in a similar way, and labelled 'outflow water'.

Volumes required may be subject to change after the initial analyses.

[If much larger volumes are needed, it may be appropriate to use the INFILTREX II water sampler (see Organic Micropollutants), by arrangement with Joan Grimalt.]

6. Organic Micropollutants

Sampling will probably be carried out annually at three WP2 sites, Redo, Gossenköllesee and Neådalsvatn, by arrangement with Joan Grimalt (FBG). The object will be to determine particulate and dissolved concentrations of organic micropollutants in the water

column. Using an, c.500 L of lake water will be pumped through a 0.45μm filter, and the filtrate passed through an in-line Axys Environmental Systems Ltd Trace Organics exchange column.

7. Suspended Sediments

[Measurements of suspended sediment concentrations (along with speciation studies) are essential for models of the behaviour of pollutants in the water column. They would need to be done by filtering large volumes (c.500L) using the INFILTREX II water sampler, by arrangement with Joan Grimalt. Practical details need to be sorted out this summer].

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MOLAR

CHEMICAL ANALYSIS OF MAJOR IONS AND NUTRIENTS
ANALYTICAL QUALITY CONTROL

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Chemical Analysis of Major Ions and Nutrients
Analytical Quality Control

1. Chemical analyses, major ions and nutrients

1.1 Variables considered

Atmospheric deposition
The variables taken into account for each precipitation event, and their relative units, are:

a. name and site code
b. starting and ending dates of the sampling
c. volume of precipitation (mm)
d. pH
e. conductivity (μS cm^{-1} at 25°C)
f. ammonium, nitrate (μg N l^{-1})
g. sulphate (mg SO_4 l^{-1})
h. alkalinity (μeq l^{-1})
i. calcium, magnesium, sodium, potassium, chloride (mg l^{-1})

The measurement of reactive or total phosphorus, which may give indications on the contamination of the sample (e.g. by bird droppings), is optional.

Surface waters
The analytical programme diffres between WP1 and 2 with respect to heavy metals, lead, cadmium and mercury which will not be analysed under the standard procedures for WP 1. The total programme analysed in WP 1 and/or WP 2 then includes the following components with the relative units:

a. name and site code
b. sampling date and depth
c. pH
d. conductivity (μS cm^{-1} at 25°C)
e. ammonium, nitrate, total nitrogen (μg N l^{-1})
f. sulphate (mg SO_4 l^{-1})
g. alkalinity (μeq l^{-1})
h. calcium, magnesium, sodium, potassium, chloride (mg l^{-1})
i. total organic carbon (mg C l^{-1})
j. reactive aluminium, non-labile aluminium (μg l^{-1})
k. cadmium, lead, mercury (μg l^{-1})
l. total phosphorus (μg P l^{-1})

Measurement of colour is recommended where there may be influence from bogs or marsh in the watershed. Measurement of fluor is optional.

1.2 Handling of samples

The greatest care and the most accurate analytical quality control (AQC) are needed for the analyses of atmospheric deposition and water samples at/from remote sites, as in general these samples present

must be free of any important quantity of the determinands. Several checks of the cleanliness of the containers must be made, using the purest de-ionised water. Samples after collection should be stored in the dark at about 4°C and transferred to the laboratory as soon as possible.

Sample identification and documentation of the sampling must be accurately maintained for every sample. This documentation is an integral part of the sample information and must be entered into the laboratory data base. Any lack or confusion of documentation may invalidate the resulting data.

1.3 Analytical methods

The very low concentrations of these samples require the most accurate analytical techniques available. Ion chromatography (IC) is at present the most reliable technique for both anion and cation measurements; equally reliable is the use of atomic absorption spectrophotometry for the analysis of cations. In the absence of IC, wet spectrophotometric determinations may be used for anions, but they must be performed with the greatest care and applying all the internal AQC criteria (e.g. blank checking and control charts at low concentration levels).

The suggested analytical methods are summarized in table 1. See table 1 for the references on the different analytical methods. Recommendations for performing some of the measurements are given below.

pH
It must be measured in unstirred water after calibration of the electrode with two buffers covering a range of values which includes the expected value of the sample. Buffers with low ionic strength should be used. For all measurement details and for temperature correction follow the instructions accompanying the equipment.

Conductivity
The cell constant must be recalculated yearly, following the indications, e.g. APHA, AWWA, WEF (1992).

Alkalinity
The measurement of alkalinity at the very low levels normally detected in atmospheric precipitation or in high altitude lakes **requires** the use of an automatic titrator and the use of Gran's titration or the two end-point technique (Gran 1950, 1952; A.P.H.A., A.W.W.A., W.E.F. 1992). Alternatively Henriksen's technique may be used (Henriksen 1982), but the results must be corrected for the excess of acid necessary to shift the pH value from the equivalent point (5.2-5.6 in relation with inorganic carbon concentration) to the end point of 4.5. Titrations performed through the colorimetric detection of the equivalent point are in most cases affected by systematic overevaluation of values (Kramer & Tessier 1982; Kramer *et al.* 1986; Mc Quaker *et al.* 1983; Mosello *et al.* 1993).

Ion chromatographic determinations
Quantification of low concentrations of solutes requires careful AQC. Six-eight standard solutions of known concentrations are needed for the calibration every batch of analyses. As the instrument signal may not be linear, quadratic or cubic regressions must be used (Tartari *et al.* 1995). Quality controls should include:

a. test of de-ionised water
b. release of base cation from the glass of vials
c. pollution of calibration standards, easy because of the low concentration
d. cleanliness of plastic- and glass-ware

e. use and regular check of in-laboratory standards of known and stabile concentrations, in the range of those present in the samples
f. vials must be rinsed with de-ionised water and then with the sample
g. check vial glass for release of Na and K in the sample

Spectrophotometric determination
The analytical methods suggested for each variable are given in table 1.
General recommendations are, that when the calibration curve is performed on 6-8 points in standard conditions, calibration is generally stable for several months, but a check every 3-4 months is suggested.

As sample concentrations are often very low, it is important to verify the lower detection limit (LOD, defined as the smallest amount that can be detected above the noise in a procedure and within a stated confidence limit) and the lower quantification limit (LOQ) (A.P.H.A., A.W.W.A., W.E.F. 1992). The quantification of concentrations is possible only for values higher than LOQ.

Atomic absorption spectrophotometry (AAS) determination
A multi point calibration should be used for every batch of measurements. Linearity must be checked in the range of concentrations considered. It is essential to check to LOD and LOQ, and to use of internal standards of known concentrations, and control charts, to guarantee the reliability of results.

2. Analytical quality control

2.1 In-laboratory quality control

By "in-laboratory (or internal) AQC" we mean the set of rules and procedures which should be adopted to perform and check all the analytical operations, with the final aim of producing data of a pre-defined level of precision and accuracy. These rules govern many aspects of the activity of the laboratory, such as training of personnel, maintenance of laboratory equipment and facilities, choice of methods and performance of analyses, and checking and validating results (see next paragraph). A synthesis of the major points to be considered is listed in table 2. The most important part of this information (i.e. performance of the analyses, calibration, maintenance of the instruments) should be contained in a handbook which the personnel may consult easily as they work. The importance of systematic errors as part of the overall analytical error is well highlighted in inter-laboratory exercises such as intercomparisons. All the exercises performed in the framework of previous collaborations (e.g. the AL:PE project), have shown that systematic errors largely prevail over random errors (Mosello *et al.* 1995). This is usually due to faulty preparation of the calibration solutions (bad quality reagents, inaccuracy in the preparation of solutions, pollution of calibration solutions used for more than one batch of analyses).

To reduce systematic errors, and to keep them under control, is possible and is one of the aims of the program of internal AQC, which must be carefully defined by the head of each laboratory, in strict collaboration with the whole staff.

2.2 Inter-laboratory quality control

By "inter-laboratory (or external) AQC" we mean those actions aimed at achieving, maintaining and improving the laboratory AQC based on information coming from outside the laboratory, such as external standards and collaboration with other laboratories (intercomparisons).

External standards

The most common external standards are the certified reference materials (CRMs), prepared by a collective of laboratories under the control and expertise of the Community Bureau of Reference of the EU. A list of other agencies which prepare CRMs and details of the materials are given by Caroli (1993).

The concentrations of these samples are accurately determined by a group of independent laboratories, using different analytical techniques which offer a high probability of accurate results. CRMs should not be used as routine standards, but they can be used on special occasions, when high quality performance is required. They can be used for different aspects of analytical practice, for example to monitor the performance of an analytical method and demonstrate equivalence between methods, to calibrate equipment, to detect errors in the application of standardised methods.
The CRMs of interest for freshwater analyses are:

a. CRMs 398 and 399, certified concentrations Al, Ca, Cl, K, Mg, Na, P and S (Quevauviller *et al.*, 1992 a, 1992 b)
b. CRMs 408 and 409, major ions in rainwater (Quevauviller *et al.* 1993; Reijners *et al.* 1994)
c. CRMs 479 and 480 nitrate in freshwater (Quevauviller *at al.* 1996)

A catalogue of all BCR-CRMs will be sent upon request by the Community Bureau of Reference (BCR), Commission of the European Communities, Rue de la Loi 200, B-1049 Brussels.

Intercomparisons

Inter-laboratories exercises may be devoted to different aims such as the evaluation of method-performance, the certification of materials, the assessment of laboratory performance. We will consider the exercises aimed at testing the proficiency of laboratories, that is to evaluate the comparability of the results and, if possible, to point out the main causes of error. The samples used in the exercises must meet precise criteria, such as:

a. homogeneity: samples in the bottles distributed to different laboratories must have the same concentrations
b. stability in time: concentrations must not change for the duration of the exercise
c. representativeness: the considered concentrations must be in the range of interest for the studies performed by the collective of laboratories

Furthermore the organizing laboratory (laboratories) must evaluate the expected concentrations, to be compared with the results provided by the participants.

The participating laboratories must give details of the analytical methods used, to evaluate and exclude the possibility of systematic errors due to the unrealiability of a particular method. Furthermore in most cases two samples per exercise are considered, to allow an evaluation between random and systematic errors. The evaluation is made through the use of Youden's graph (Youden 1959; Youden & Steiner 1975).

In the MOLAR project two series of intercomparisons will be carried out, both dealing with major ion concentrations, pH and conductivity. The first exercise will be organized by NIVA, the second by the CNR-III, in collaboration with the Environment Institute of the Joint Research Centre of the EU, Ispra, Italy. Methodologies and frequency will be the same as those already used in exercises organized in the framework of previous collaborations (e.g. AL:PE project; Wathne *et al.* 1995).

2.3 Data checking

The following data controls should be carried out at the laboratory producing the data, and should become a routine operation for all the analyses performed. The same controls will be repeated by the MOLAR elaborating centre (surface water: NIVA Institute, atmospheric deposition: CNR-Istituto Italiano di Idrobiologia), before discussing the data.

Any data that may be in error should result in a new analysis of the sample, if possible. Comparison with previous values measured at the same site or with a range of values obtained for other sites is recommended.

Data should be sent on the diskette annexed to this document, briefly described below. If this is not possible, please use the transmission form (Tab. 3), paying attention to the recommended units.

These quality controls should be performed both on lake and atmospheric deposition data.

Ion balance

The basic assumption for this quality control is that the measurement of pH, ammonium, calcium, magnesium, sodium, potassium, bicarbonate, sulphate, nitrate and chloride accounts almost completely for the ions present in solutions. This can be incorrect in the case of lake water with pH lower than 5, where aluminium and other trace metals may be present in ionic forms. Fluoride is in most cases negligible in terms of ionic balance. On the other hand ionic balance can be influenced by the presence of high amounts of organic matter. In the case of atmospheric deposition, formic and acetic acid may have a minor role in ion balance both in urban and remote areas.

The control is based on the electro neutrality of water samples (lake or atmospheric deposition). The total number of negative and positive charges must be equal. This can be checked using milli (or micro) equivalents per litre (meq l^{-1} or μeq l^{-1}) as the concentration unit. The constants necessary to transform the units used in the MOLAR research in μeq l^{-1} are listed in table 4.

Alternatively, the ionic balance may be evaluated using an electronic sheet; in the interests of uniformity, we recommend the sheets prepared in the enclosed diskette for lake (LAKEFORM.XLS) and atmospheric deposition (RAINFORM.XLS) results.

The limit of acceptable errors varies with the total ionic concentrations and the nature of the solutions. Indicating with Σcat and Σan the concentrations (μeq l^{-1}) of cations and anions respectively, we can define percent difference as:

$$PD = 100 * (\Sigma cat - \Sigma an)/(0.5*(\Sigma cat + \Sigma an))$$

where:

$$\Sigma cat = [Ca] + [Mg] + [Na] + [K] + [H^+]$$
$$\Sigma an = Alk + [SO_4] + [NO_3] + [Cl]$$

As mentioned above, in the case of lake water with pH below 5, Al in ionic form can be important. High amounts of organic matter also need to be taken into consideration when present. To enable the ionic balance (IB) to be calculated as correctly as possible, calculations are made in two versions. In the first version (pH > 5.0), IB is calculated on the basis of all the major ions; in the second version (pH < 5.0); Al, NH_4 and TOC are also considered.

$$\Sigma cat = [Ca] + [Mg] + [Na] + [K] + [H^+] + [NH_4] + Al$$
$$\Sigma an = Alk + [SO_4] + [NO_3] + [Cl] + OA$$

OA is calculated from the TOC value taking into account weak organic acids. It may be evaluated from the following empirical equation (Oliver *et al.* 1983):

$$OA = 4.7 - 6.87 * \exp(-0.322\ TOC)$$

In normal conditions, for dilute lake water samples, a PD lower than 3% should be achieved. Higher values can indicate a lack of precision in one or more analytical techniques, or the omission of important ion/s, or both. In the case of atmospheric deposition, in the EMEP quality assurance plan (Schaug 1988) a score is given to the chemical analyses, on the basis of PD and IS (IS = Σcat + Σan; unit: μeq l^{-1}), table 5.

An example of a plot of Σcat *vs* Σan for the AL:PE lakes is shown in figure 1.

Comparison between measured and calculated conductivity

Conductivity is a measure of the ability of an aqueous solution to carry an electric current. This possibility depends on the type and concentrations of ions and on the temperature of measurement. It is defined as:

$$K = G * (L/A)$$

where G = 1/R is the conductance (unit: ohm^{-1} or siemens; ohm^{-1} is sometime written as mho), defined as the reciprocal of resistance (R; unit: ohm); A (cm^2) is the electrode surface area, L (cm) is the distance between the two electrodes. The units of K are $ohm^{-1}\ cm^{-1}$. In the International System of Units (SI) conductivity is expressed as millisiemens per meter (mS m^{-1}); this unit is also used by the IUPAC and accepted as Nordic standard. In practice the unit μS cm^{-1}, where 1 mS m^{-1} = 10 μS cm^{-1} = 10 μmho cm^{-1}, is also commonly used.

Conductivity depends on the type and concentration (activity) of ions in solution; the capacity of a single ion to transport an electric current is given in standard conditions and in ideal conditions of infinite dilution by the equivalent ionic conductance (λ_i; unit: S cm^2 $equivalent^{-1}$). Values of equivalent conductance of the main ions at 20 and 25°C are presented in table 4.

In the MOLAR research, conductivity units used are mS m^{-1} at 25°C. If other units are currently used in the different laboratories (e.g., μS cm^{-1}), the transformation is easy, while a problem does exist if a different reference temperature is used. In fact, the variation of equivalent conductance with temperature is not the same for all the ions (e.g. Pungor 1965), so that the function of conductivity with temperature will depend on the chemical composition of the solution. An example is given in figure 2, where the variation of conductivity with temperature for Lake Maggiore (buffered water), for an alpine lake (poorly buffered water) and for an an acidic sample of atmospheric deposition are compared. The ion concentrations of the three samples are presented in table 6. The different slopes of the two straight lines clearly indicate different relationships K = f (T). The values of correction of conductivity for temperature are therefore a simplification, performed assuming a "standard composition" for surface water (e.g. Rodier 1984); this can introduce a systematic error in the case of a different chemical composition, as is the case for atmospheric deposition chemistry. Of course this is also true if the correction is made automatically by the conductivity meter. For this reason the suggestion is to make the measurement as close as possible to 25°C (e.g. in the range 24-26°C), and not to try measuring at 20°C and then transforming the value to 25°C with a constant. If conductivity is also measured at 20°C, for reasons of comparison and continuity with past data or with data of other samples, please send to the elaboration centre at NIVA the values at both 20 and 25°C.

A careful, precise conductivity measurement is a further means of checking the results of chemical analyses. It is based on a comparison between measured conductivity (CM) and the conductivity

calculated (CE) from individual ion concentrations, multiplied by the respective equivalent ionic conductance (λ_i)

$$CE = \lambda_i C_i$$

The ions are those considered in the ionic balance; the values of λ_i for the different ions are given in table 4, referred to 20 and 25°C. The same values are used in the calculation performed in the electronic sheet in the diskette. The percent difference, CD, is given by the ratio:

$$CD = 100 * |(CE-CM)|/CM$$

Also in this case it is possible to score the results on the basis of CD (Schaug 1988), as shown in table 7.

At low ionic strength (below 0.1 meq l^{-1}) of high altitude lakes or atmospheric deposition samples, the discrepancy between measured and calculated conductivity should be no more than 2% (Miles & Yost 1982). Ionic strength (Ic), in meq l^{-1}, can be calculated from the individual ion concentrations as follows:

$$Ic = 0.5 \Sigma c_i z_i^2/w_i$$

where:

c_i = concentration of the i-th ion in mg l^{-1};
z_i = absolute value of the charge for the i-th ion;
w_i = gram molecular weight for the i-th ion.

For ionic strength higher than 0.1 meq l^{-1} a correction of the activity of each ion can be used, as proposed e.g. by Stumm and Morgan (1981) and A.P.H.A., A.W.W.A., W.E.F. (1992). For the routine data checking of a set of analyses, an alternative is the plot of measured *vs* calculated conductivity; the departure of some results from linearity may suggest the presence of analytical errors (Fig. 3).

Comparison between measured conductivity and ion concentrations

If we consider samples with similar ionic ratios and different ionic concentrations, a linear correlation should be expected between conductivity and the sum of cations and anions. Figures 4 and 5 show an example for the analyses performed in 1993 in the AL:PE lakes. The results which depart from the linearity must be checked with care, to see if there have been any mistakes in the analyses or in the data processing, or if the values of some ions are missing from the sum.

The linearity of the relation is lost if marked chemical differences are present between the samples; for example, a sample with pH lower than 4.5 will show, at equal total ionic concentration, higher conductivity than a buffered sample (pH above 6.0), because of the high equivalent ionic conductance of hydrogen ion compared with the other ions (Tab. 4). There will also be a lack of linearity with increasing ionic strength.

3. Mailing the results to the data centre

The diskette annexed to this document contains two electronic sheets (Microsoft Excel version 5 for Windows), respectively for the transmission of surface water (NIVA) and atmospheric deposition (CNR-III) results. The variables contained in the electronic sheet are listed in table 3, as a

transmission form of chemical data. The transmission forms, intended as units and number of decimals (if significative, due to the analytical technique), must be used for sending data if no computer facilities are available.

The electronic sheet calculates the ionic balance and the calculated conductivity. As an example the sheet for surface water contains the values measured in the AL:PE lakes in 1993. Furthermore, the relationships between (1) Σcat and Σan, (2) measured (CM) and calculated conductivity (CE), (3) CM and Σan, (4) CM and Σcat are plotted in the data sheet (Figs 1, 3, 4 and 5). Participants are invited to use chemical data already available in their laboratory, if possible of different origins (e.g. rain and lake water), to become familiar with the data check approach.

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Table 1a. Suggested analytical methods for the surface waters and deposition water. IC::ion chromatography; AAS: atomic absorption spectrophotometry.

	Suggested method	References	Alternative method	References
Ammonium	Indophenol blue spectr.	Fresenius *et al.* 1988	IC	U.S.E.P.A. 1986; Tartari *et al.* 1995
Ca, Mg, Na, K	IC	U.S.E.P.A. 1986; Tartari *et al.* 1995	AAS, ICP	A.P.H.A. 1992 (3111 B)
Sulphate	IC	A.P.H.A. 1992; Durst *et al.* 1991	Methylthymol blue spectr.	A.P.H.A. 1992 (4500-SO4-F); Coloros *et al.* 1976
Nitrate	IC	A.P.H.A. 1992; Durst *et al.* 1991	Cd reduction, Salycilate spectr.	A.P.H.A. 1992 (4500-NO3-E); Rodier 1982.
			Cd reduction, Diazoreaction	A.P.H.A. 1992 (4500- NO3-F);
Chloride	IC	A.P.H.A. 1992; Durst *et al.* 1991	Ferrycianide spectr.	A.P.H.A. 1992 (4500-Cl-E); Zall *et al.* 1956
Reactive P	Molybden blue spectr.	A.P.H.A. 1992 (4500 -P-E)		
Total P	Digestion, molybden blue spectr.	digestion: Valderrama 1981; measure: A.P.H.A. 1992 (4500 -P-E)		
Total N	Digestion, UV spectr.	digestion: Valderrama 1981; measure: A.P.H.A. 1992 (4500 -NO3-B)		
Reactive Si	Molibdosilicate, reduction to heteropoly blue	A.P.H.A. 1992 (4500-Si-E)		
Aluminium	Spectrometric method using pyrocatechol violet	ISO 10566:1994		
Aluminium fractions	Automatic Method for Fractionation and Determination of Aluminium Species in Fresh-Waters..	E.J.S. Røgeberg and A. Henriksen. Vatten 1985, **41**(1), 48 - 53		

Table 1b. International standards methods recommended in the draft ICP manual:

- EN-ISO 7887:1994 Water quality- Examination of colour.
- EN 25 813:1992 Water quality- Determination of dissolved oxygen- Iodometric method.
- EN 25 814:1992 Water quality- Determination of dissolved oxygen- Electrochemical probe method.
- EN 27 888:1993 Water quality- Determination of electrical conductivity.
- ISO 9963-2:1994 Water quality- Determination of alkalinity. Part 2. Determination of carbonate alkalinity.
- ISO 9964-1:1993 Water quality- Determination of sodium and potassium. Part 1. Determination of sodium by atomic absorption spectrometry.
- ISO 9964-2:1993 Water quality- Determination of sodium and potassium. Part 2. Determination of potassium by atomic absorption spectrometry.
- ISO 9964-3:1993 Water quality- Determination of sodium and potassium. Part 1. Determination of sodium and potassium by flame emission spectrometry.
- ISO 10523-1:1994 Water quality- Determination of pH.
- ISO 8245 Water quality- Guidelines for the determination of total organic carbon (TOC).
- ISO 10566:1994 Water quality- Determination of aluminium -Spectrometric method using pyrocatechol violet.
- For speciation of aluminium fractions, see e.g.: E.J.S. Røgeberg and A. Henriksen. An Automatic Method for Fractionation and Determination of Aluminium Species in Fresh-Waters. Vatten 1985, **41**(1), 48 - 53.
- ISO 10304-1:1992 Water quality- Determination of dissolved fluoride, chloride, nitrite, orthophosphate, nitrate and sulphate- Part 1. Method for water with low contamination.
- ISO 6878/1: 1986 Water quality- Determination of phosphorous - Part 1: Ammonium molybdate spectrometric method.
- ISO/DIS 11732: Water quality- Determination of ammonium nitrogen by flow analysis and spectrometric detection.
- ISO/DIS 13395: Water quality- Determination of nitrate and nitrite nitrogen and the sum of both by flow analysis.
- ISO/DIS 11905-1: Water quality- Determination of nitrogen - Part 1: Method using oxidative digestion with peroxodisulfate.

Information of the ISO/CEN methods listed above can be obtained from :

a. The national standardisation agencies.

b. International Organisation for Standardisation, DIN, Burggrafenstrasse 6, 10787 Berlin, Germany.

c. ISO International Organisation for Standardisation, Case Postale 56, CH-1211 Genève, Switzerland.

d. CEN European Committee for Standardisation, rue de Stassart 36, B-1050 Brussels, Belgium.

Table 2. Main in-laboratory Analytical Quality Control.

Testing facilities, organization and personnel	
Sampling and storage	
Assessment of the analytical methods used	• interlaboratory testing programs • reference materials
Statistical quality control	control chart (internal standards)
Apparatus, chemicals, reagents, and blanks	
Documentation	• methods • work-sheets • notebook
Checking of results	• ion balance • comparison between measured and calculated conductivity • relationship among ions
Reporting of results	• not computerized • computerized
Archiving of results	

Table 3. MOLAR research. Transmission form of chemical data.

	Lake	Rain	Code	Units	N. decimals	Results
Lake/site number	L	R				
Name of the lake/site	L	R				
Date starting		R	DATE			
Date ending		R	DATE			
Sampling date	L		DATE			
Number depth sampled	L					
Volume		R	Vol	mm	0	
pH	L	R	pH		2	
Conductivity 25°C	L	R	Cond	µS cm^{-1} 25°C	1	
Ammonium	L	R	NH_4-N	µg N l^{-1}	0	
Calcium	L	R	Ca	mg l^{-1}	2	
Magnesium	L	R	Mg	mg l^{-1}	2	
Sodium	L	R	Na	mg l^{-1}	2	
Potassium	L	R	K	mg l^{-1}	2	
Alkalinity	L	R	Alk	µeq l^{-1}	0	
Sulphate	L	R	SO_4	mg SO_4 l^{-1}	2	
Nitrate	L	R	NO_3-N	µg N l^{-1}	0	
Chloride	L	R	Cl	mg l^{-1}	2	
Fluoride	L		F	µg l^{-1}	0	
Total nitrogen	L		TN	µg N l^{-1}	0	
Tot. phosphorus	L		TP	µg P l^{-1}	0	
React. phosphorus		R	RP	µg P l^{-1}	0	
Tot. org. carbon	L		TOC	mg C l^{-1}	2	
Tot. Aluminium	L		*TAl	µg l^{-1}	0	
React. Al	L		*RAl	µg l^{-1}	0	
I labile Al	L		*ILAl	µg l^{-1}	0	
Labile Al	L		*LAl	µg l^{-1}	0	
Cadmium	L	R	*Cd	µg l^{-1}	2	
Lead	L	R	*Pb	µg l^{-1}	2	
Copper	L	R	*Cu	µg l^{-1}	2	
Cobalt	L	R	*Co	µg l^{-1}	2	
Mercury	L	R	*Hg	ng l^{-1}	2	

* = some of the measurements performed by the NIVA laboratory.
** = measurement performed by the NILU laboratory

Table 4. Factors to transform concentrations used in the electronic sheet in μeq l^{-1} and values of equivalent conductances.

	Code	Units	Factor to μeq l^{-1}	Equivalent conductance at 20°C kS cm^2 eq^{-1}	Equivalent conductance at 25°C kS cm^2 eq^{-1}
pH	pH		$10^6 * 10^{-pH}$	0.3151	0.3500
Ammonium	NH_4-N	μg N l^{-1}	0.07139	0.0670	0.0735
Calcium	Ca	mg l^{-1}	49.9	0.0543	0.0595
Magnesium	Mg	mg l^{-1}	82.24	0.0486	0.0531
Sodium	Na	mg l^{-1}	43.48	0.0459	0.0501
Potassium	K	mg l^{-1}	25.58	0.0670	0.0735
Alkalinity	Alk	μeq l^{-1}	1	0.0394	0.0445
Sulphate	SO_4	mg SO_4 l^{-1}	20.82	0.0712	0.0800
Nitrate	NO_3-N	μg N l^{-1}	0.07139	0.0636	0.0714
Chloride	Cl	mg l^{-1}	28.2	0.0680	0.0764
Fluoride	F	μg l^{-1}	0.05263	0.0491	0.0544

Table 5. Score of the analyses on the basis of the percent difference in ionic balance (PD) and of the total ion concentrations (IS in μeq l^{-1}; from Schaug 1988).

Sample category	**1**	**2**	**3**
$IS < 50$	$PD \leq 60$	$PD > 60$	-
$50 \leq IS < 100$	$PD \leq 30$	$30 < PD \leq 60$	$PD > 60$
$100 \leq IS < 500$	$PD \leq 15$	$15 < PD \leq 30$	$PD > 30$
$IS > 500$	$PD \leq 10$	$10 < PD \leq 20$	$PD > 20$

Table 6. Chemical composition of the sample of Lake Maggiore and of atmospheric deposition used for the evaluation of the relationship between conductivity and temperature. Temperature coefficient of the ion mobility ($°C^{-1}$, from Pungor 1965). (For units see table 4.)

Variables	Rain	L. Maggiore	Alpine lakes	% Diff. / °C
pH	4.28	8.10	6.48	
Cond	37	128	11	
H^+	52	0	0	1.54
Ca^{++}	14	958	65	2.54
Mg^{++}	8	251	11	2.54
Na^+	26	106	10	2.44
K^+	3	30	6	2.17
$N\text{-}NH_4^+$	41	0	0	2.08
Alk	0	709	17	
$SO_4^=$	79	541	44	2.30
$N\text{-}NO_3^-$	41	49	26	2.05
Cl^-	27	50	3	2.16
Σ An	147	1349	90	
Σ Cat	144	1345	92	

Table 7. Score of the analyses on the basis of the percent difference between measured and calculated conductivity (CD), in relation with measured conductivity (μS cm^{-1} at 20 °C, from Schaug 1988).

Sample category	**1**	**2**	**3**
CM ≤ 30	CD ≤ 30	CD > 30	-
CM > 30	CD ≤ 20	20 < CD ≤ 40	CD > 40

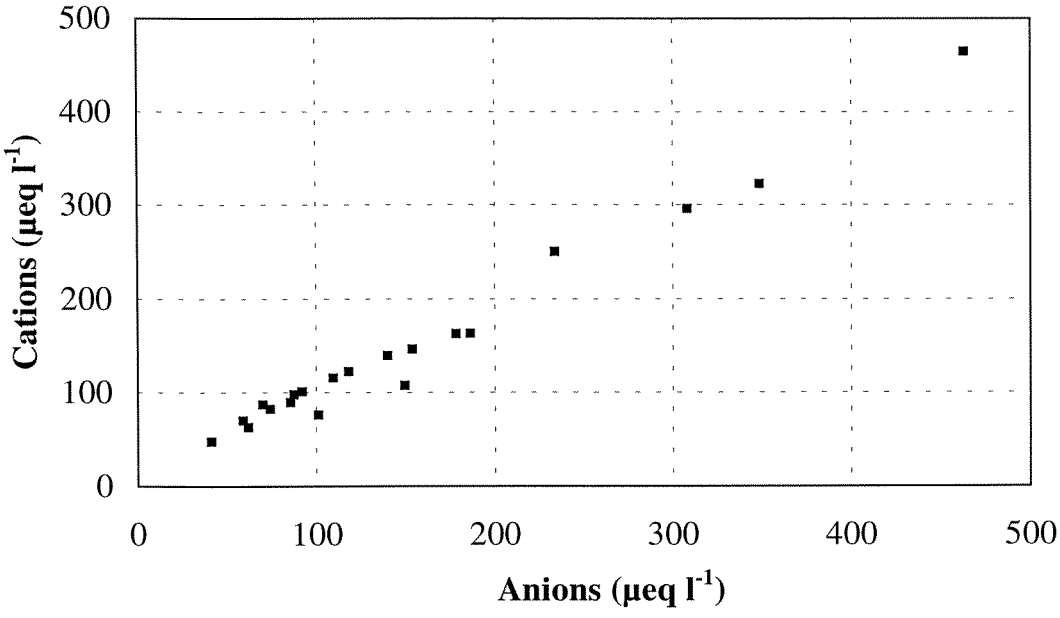

Figure 1. Plot of Σ cations *vs* Σ anions for the AL:PE lakes (data collected in 1993).

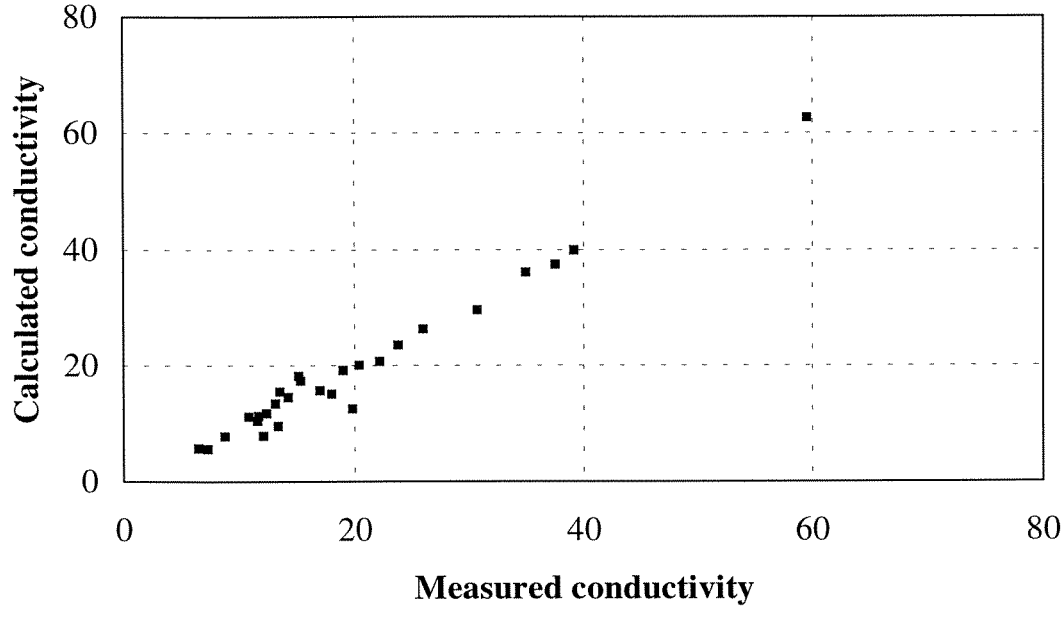

Figure 3. Plot of calculated *vs* measured conductivity (μS cm^{-1} at 25°C) for the AL:PE lakes (data collected in 1993).

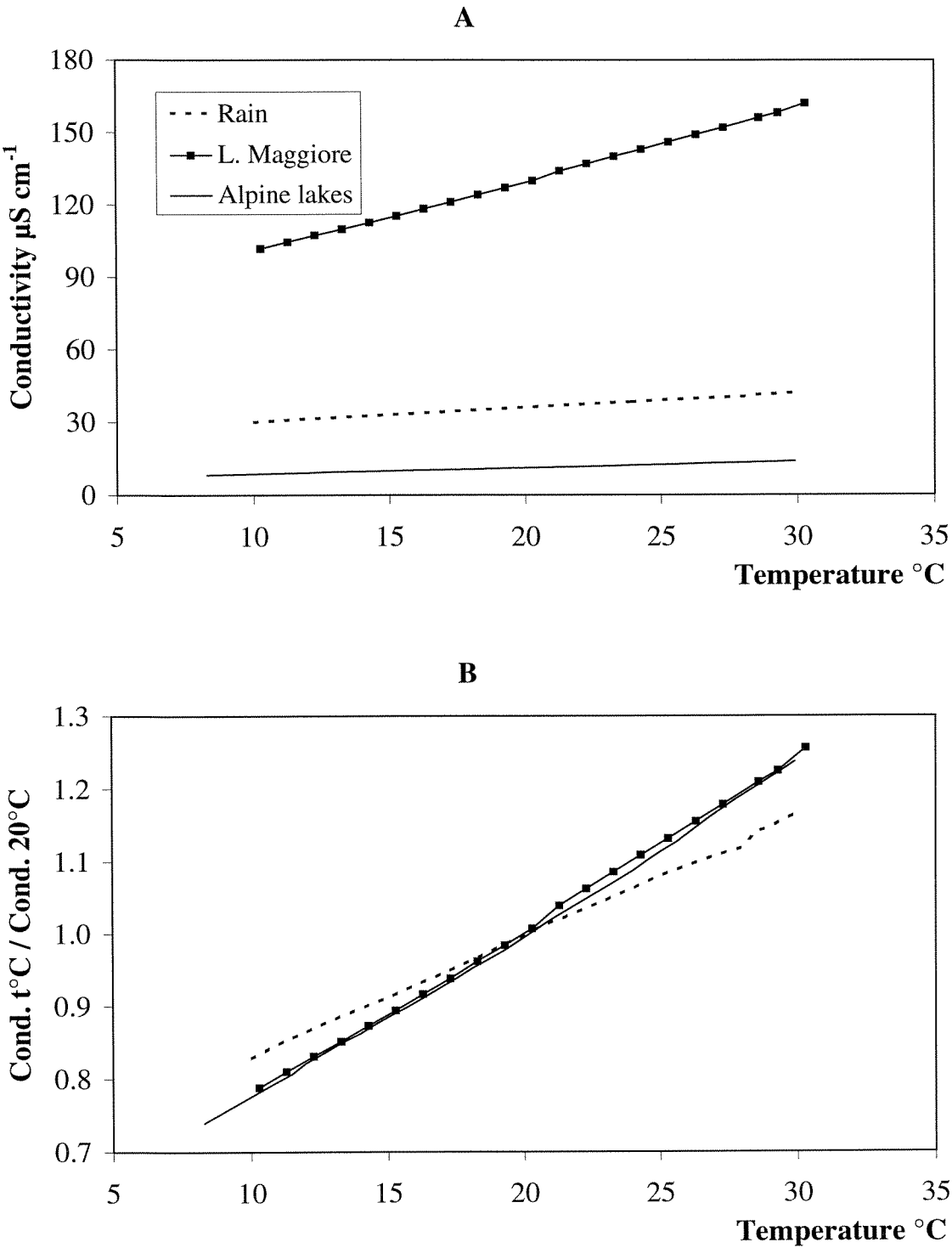

Figure 2. Relationship between conductivity and temperature of different waters. Absolute values (A) and values relative to the temperature of 20°C (B).

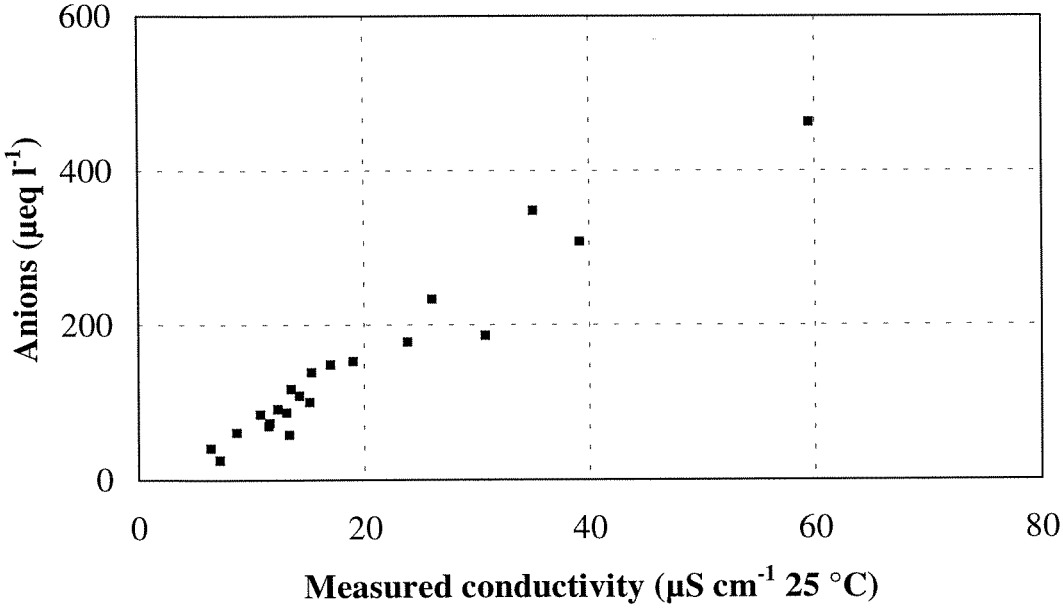

Figure 4. Plot of Σ anions *vs* measured conductivity for the AL:PE lakes (data collected in 1993).

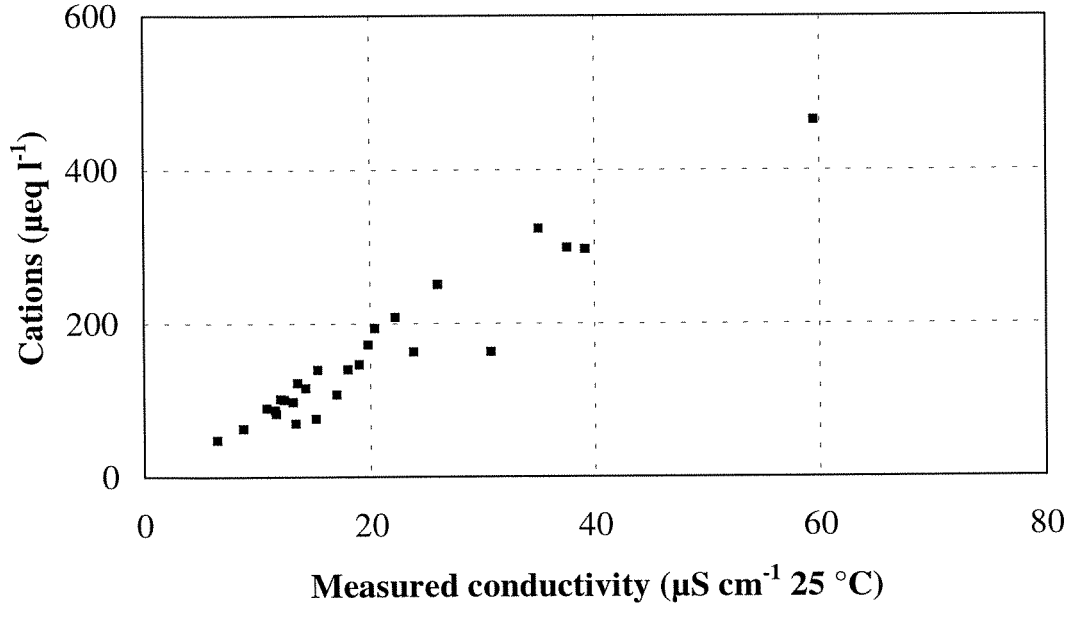

Figure 5. Plot of Σ cations *vs* measured conductivity for the AL:PE lakes (data collected in 1993).

MOLAR RAIN FORM data check and transmission

Site n°	Sampling sites	Date starting	Date ending	Volume mm	pH	Cond. µS/cm 25°C	Cond. mS/m 25°C	NH_4 µgN/l	Ca mg/l	Mg mg/l	Na mg/l	K mg/l	Alk µeq /l	SO_4 mg/l	SO_4* mg/l	NO_3 µgN/l	Cl mg/l	RP µg/l	Site n°	Sampling sites
1	Graniga	09/01/95	16/01/95	25,3	5,31	3,0	0,3	20	0,08	0,01	0,01	0,01	0	0,18	0,18	65	0,03	2	1	Graniga
1	Graniga	16/01/95	23/01/95	17,1	4,76	11,2	1,1	80	0,08	0,01	0,03	0,02	0	0,64	0,63	202	0,07	2	1	Graniga
1	Graniga	23/01/95	30/01/95	13,5	4,70	13,0	1,3	120	0,10	0,02	0,05	0,03	0	1,02	1,00	256	0,18	0	1	Graniga

Site n°	Sampling sites	Date starting	Date ending	H+ µeq/l	NH4 µeq/l	Ca µeq/l	Mg µeq/l	Na µeq/l	K µeq/l	Alk µeq/l	SO4 µeq/l	NO3 µeq/l	Cl µeq/l	S Cat. µeq/l	S An. µeq/l	% Diff.	S Ions µeq/l		Measured µS/cm 25 °C	Calculated 25°C
1	Graniga	09/01/95	16/01/95	4,9	1	4	1	0	0	0	4	5	1	12	9	12	21		3,0	2,8
1	Graniga	16/01/95	23/01/95	17,4	6	4	1	1	1	0	13	14	2	30	30	0	59		11,2	9,1
1	Graniga	23/01/95	30/01/95	20,0	9	5	2	2	1	0	21	18	5	38	45	-8	83		13,0	11,6

Figure 6. MOLAR RAINFORM.xls - EXCEL file for deposition data check and trasmisson

MOLAR LAKE FORM data check and transmission																						
Lake n°		Date	pH	Cond.	Cond.	NH_4	Ca	Mg	Na	K	Alk	SO_4	SO_4*	NO_3	Cl	F	TN	TP	TOC	TAL	RAL	ILAL	LAL
				µS/cm 25°C	mS/m 25°C	µgN/l	mg/l	mg/l	mg/l	mg/l	µeq /l	mg/l	mg/l	µgN/l	mg/l	µg/l	µgN/l	µg/l	mg C/l	µg/l	µg/l	µg/l	µg/l
1	Ø. Neådalsvatn	M 93	6,16	8,7	0,9	<5	0,38	0,09	0,76	0,12	18	0,65	0,55	13	1,04	<0.1	57	2	0,73		<10	<10	0
2	Stavsvatn	M 93	5,94	11,5	1,2	17	0,92	0,13	0,60	0,10	7	1,80	1,72	43	0,80	1	155	2	1,01		75	19	
3	L. Hovvatn	M 93	4,65	30,7	3,1		0,46	0,24	2,19	0,12		2,80	2,39	153	4,15				1,81		217	49	
9	Arresjøen	M 93	5,81	37,6	3,8	5	0,71	0,59	4,76	0,24	24	1,55	0,73	<1	8,25	<0.1	94	4	0,43		<10	<10	0
4,1	Lochnagar	M 93	5,32	23,8	2,4		0,62	0,34	2,14	0,25	11	2,79	2,47	252	3,24				0,78		53	12	41
4,2	Sandy Loch	M 93	6,09	19,0	1,9		0,83	0,23	1,89	0,15	27	2,56	2,32	98	2,35				1,08		10	9	2
4,3	L.Nan Eun	M 93	5,40	39,2	3,9		0,63	0,53	4,85	0,23	11	2,30	1,46	131	8,49				2,00		32	6	26
10	L. Maam	M 93	4,96	59,5	6,0		0,81	0,76	7,84	0,37	7	2,93	1,54	46	13,89				3,23		54	40	14
5,1	L.Paione S.	M 93	5,62	11,6	1,2	51	1,02	0,11	0,23	0,27	-1	2,21	2,20	357	0,13		408	2		61			
5,2	L.Paione Inf.	M 93	6,43	14,2	1,4	6	1,62	0,15	0,31	0,33	25	2,55	2,53	381	0,13		387	2		10			
6,1	Lago Lungo	M 93	6,43	13,5	1,3	10	1,63	0,19	0,40	0,29	30	3,37	3,35	195	0,15		244	4					
6,2	Lago di Latte	M 93	6,60	15,3	1,5	9	1,95	0,16	0,42	0,39	53	2,85	2,84	321	0,15		357	3					
7,3	Blanc	M 93	6,81	12,0	1,2		1,58	0,14	0,26			0,74	0,74	145		135				48			
7,4	Noir	M 93	7,07	19,8	2,0	0	2,60	0,35	0,32			1,14	1,14	131		44		31		8			
8	Aube	M 93	6,22	10,8	1,1	133	0,68	0,07	0,63	0,51	29	1,23	1,15	131	0,78		690	5	0,90				
11	Schwarzsee ob Sölden	M 93	5,48	13,1	1,3	21	1,17	0,22	0,31	0,12	3	3,42	3,41	150	0,10	5	172	3	0,60		23		
14	L. Escura	06/10/93	5,31	13,3	1,3	7	0,24	0,28	0,64	0,08	6	1,06	0,95	0	1,09			7	1,81				
12,1	L. Aguilo	19/10/93	5,79	6,4	0,6	7	0,44	0,16	0,21	0,08	18	0,72	0,70	42	0,18			5	1,28				
12,2	L. Redo	16/10/93	6,32	12,3	1,2	15	1,50	0,17	0,21	0,08	42	1,49	1,47	196	0,18			4	0,95				
13	La Caldera	M 93	8,05	26,0	2,6	30	3,95	0,38	0,40	0,12	195	0,89	0,84	75	0,54		244	10	3,08	32			
16	Laguna Cimera	M 93	6,06	7,2	0,7		0,62		0,70			0,75	0,71		0,36								
15,1	Starolesnianske pleso	23/09/93	4,70	15,1	1,5	17	0,73	0,10	0,19	0,08		4,04	4,02	169	0,17			9	2,75		193	17	176
15,2	Terianske pleso	23/09/93	6,80	20,4	2,0	9	3,18	0,14	0,45	0,13		3,42	3,40	510	0,18			2	0,75		9		
15,3	Dlugi Staw	23/09/93		18,0	1,8	12	2,15	0,13	0,39	0,18		3,94	3,92		0,18			2			48		
15,4	Zieloni Staw	25/10/93	6,70	22,2	2,2	13	3,08	0,24	0,63	0,25		3,75	3,73	350	0,25			5					
17	z. Chuna	25/10/93	6,57	17,0	1,7		1,21	0,25	0,53	0,14	62	2,00	1,88	162	1,21			0		5			
18	Chibini	25/10/93	7,15	35,0	3,5		0,39	0,06	6,00	1,47	251	3,18	3,09	79	0,91			0		77			

Figure 7A. MOLAR LAKEFORM.xls - EXCEL file for lake water data check and trasmisson

Lake n°		Date	Cd	Pb	Hg		H^+	NH_4	Ca	Mg	Na	K	Alk	SO_4	NO_3	Cl	F	S Cat.	S An.	% Diff.	S Ions		Cond. Measured	Cond. Calculated
			µg/l	µg/l	ng/l		µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l	µeq/l		µeq/l		µS/cm 25 °C	25°C
1	Ø. Neådalsvatn	M 93	<0.10	<0.5	<2.0		0,7		19	8	33	3	18	14	1	29		63	61	1	125		8,7	7,8
2	Stavsvatn	M 93	<0.5	<0.5	<2		1,1	1	46	11	26	2	7	37	3	23		87	70	11	157		11,5	10,5
3	L. Hovvatn	M 93					22,3		23	20	95	3		58	11	117		163	186	-7	350		30,7	29,6
9	Arresjøen	M 93	<0.1	<0.5	17,00		1,5	0	35	49	207	6	24	32		233		298			298		37,6	37,4
4,1	Lochnagar	M 93					4,8		31	28	93	6	11	58	18	91		163	178	-4	341		23,8	23,5
4,2	Sandy Loch	M 93					0,8		41	19	82	4	27	53	7	66		147	154	-2	300		19,0	19,2
4,3	L.Nan Eun	M 93					4,0		31	44	211	6	11	48	9	239		296	308	-2	604		39,2	39,9
10	L. Maam	M 93					11,0		40	63	341	9	7	61	3	392		464	463	0	927		59,5	62,7
5,1	L.Paione S.	M 93					2,4	4	51	9	10	7	-1	46	25	4		83	74	6	157		11,6	11,3
5,2	L.Paione Inf.	M 93					0,4	0	81	13	14	8	25	53	27	4		116	109	3	226		14,2	14,5
6,1	Lago Lungo	M 93					0,4	1	81	16	17	7	30	70	14	4		123	118	2	241		13,5	15,5
6,2	Lago di Latte	M 93					0,3	1	97	13	18	10	53	59	23	4		140	140	0	280		15,3	17,4
7,3	Blanc	M 93					0,2		79	12	11			15	10			102					12,0	7,9
7,4	Noir	M 93					0,1	0	130	29	14			24	9			173					19,8	12,6
8	Aube	M 93			14,00		0,6	9	34	6	27	13	29	26	9	22		90	85	3	175		10,8	11,2
11	Schwarzsee ob Sölden	M 93					3,3	2	59	18	13	3	3	71	11	3		98	87	6	186		13,1	13,4
14	L. Escura	06/10/93					4,9	0	12	23	28	2	6	22	0	31		70	59	9	129		13,3	9,6
12,1	L. Aguilo	19/10/93					1,6	0	22	13	9	2	18	15	3	5		48	41	8	89		6,4	5,8
12,2	L. Redo	16/10/93					0,5	1	75	14	9	2	42	31	14	5		101	92	5	193		12,3	11,8
13	La Caldera	M 93	0,85	1,05	0,88		0,0	2	197	31	17	3	195	19	5	15		251	234	3	484		26,0	26,3
16	Laguna Cimera	M 93					0,9		31		30			16		10			26				7,2	5,7
15,1	Starolesnianske pleso	23/09/93					20,0	1	36	8	8	2		84	12	5		76	101	-14	177		15,1	18,2
15,2	Terianske pleso	23/09/93					0,2	1	159	12	20	3		71	36	5		194					20,4	20,1
15,3	Dlugi Staw	23/09/93						1	107	11	17	5		82	0	5		140					18,0	15,2
15,4	Zieloni Staw	25/10/93					0,2	1	154	20	27	6		78	25	7		208					22,2	20,7
17	z. Chuna	25/10/93					0,3	0	60	21	23	4	62	42	12	34		108	149	-16	257		17,0	15,7
18	Chibini	25/10/93					0,1	0	19	5	261	38	251	66	6	26		323	349	-4	671		35,0	36,1

Figure 7B. MOLAR LAKEFORM.xls - EXCEL file for lake water data check and trasmisson

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

WATER COLUMN PROFILING

Jordi Catalan

University of Barcelona

Water Column Profiling (Work Package 3)

1. Sampling objective

The main objective of water column sampling in work package 3 is to provide data for: (a) modelling the coupling between weather conditions, lake physics and lake biogeochemistry; and (b) studying the seasonal variability in mountain lakes with enough resolution to improve our understanding of organisms as environmental indicators.

2. Sampling design

Two sets of variables have to be considered: those fundamental for resolving the seasonal change of the lake, and for validating the models, which will be recorded at regular intervals; and those variables that can be measured occasionally in order to increase the potentiality of the models, and to investigate the causes of some of the responses observed in the master variables. Therefore, we will consider a regular sampling, which is mandatory for all sites in work package 3, and an occasional sampling with lower sampling frequency and with some optional variables.

3. Sampling point

Profiles should be carried out in the deepest part of the lake.

4. Regular sampling

The measurements during regular sampling are temperature, oxygen, pH, conductivity, chlorophyll, and ice-cover description during winter. Most of the quantitative modelling will be based on those measurements, hence they require a minimum sampling frequency, sampling depths and common methods which are stated below.

4.1. Frequency

The recommended minimum sampling is monthly during the ice-free season and at least three times during the ice-cover period. This winter minimum sampling is required for studying the oxygen consumption in the lake, as a proxy for total metabolism. Therefore, in case of carrying out only these three winter surveys, please, avoid initial conditions with clear ice, where primary production in the water column is still possible; and also the melting period, when a lot of water is entering the lake disturbing the oxygen profile. Because these two transition periods are quite interesting from the point of view of the partitioning of the seasonal variability, we strongly encourage to carry out extra surveys during these periods, if possible.

In case of very severe sampling restrictions, the very minimum required sampling to have some idea of the seasonal dynamics is three times during ice-free season and twice during ice-cover period. Nevertheless, please consider that with these data any model will be hardly applied.

4.2. Sampling depths

If submersible probes are available for in situ measurements of some variables, then a minimum of every meter recording should be carried out. For measurements requiring analytical work, the number of sampling depths depends on the maximum depth of the lake. For lakes shallower than 15 m a minimum of 5 depths regularly spaced are required; for deeper lakes a minimum of 10 depths. Regular distribution of the samples greatly facilitates the numerical analysis of the variance in the

lake and interlake comparison. If you think "but I know my lake and ....", then add to the regular mesh those sampling depth you already know are "key points" for your system. In lakes with a fluctuating level, use as reference points distances to the bottom.

Water from the selected depths can be sampled either with limnological bottles or with a pump. Nevertheless, take into account that pumping can enhance gas exchange if bubbling occurs during the process, therefore, it is not recommended when oxygen is going to be determined using BOD bottles.

4.3 Temperature

This measurement requires a higher resolution than chemical measurements, we suggest a 0.5 m resolution for shallow lakes and 1 m for deeper lakes. Measurements should be reliable to 0.1 ^{0}C, which is provided by most thermistors. Periodical checking of the thermistor readings throughout the sampling period with a precision thermometer is necessary, although most commercial devices are usually quite stable now. A thermistor chain recording at the same rate than weather station is necessary for developing the physical models, otherwise those lakes without sufficient temporal temperature record will be used only for validating some of the models.

4.4 Light

The minimum requirement is the depth of Secchi disk disappearance, any other measurement added will be welcome, but keep measuring Secchi disk.

4.5 Oxygen

If an oxymetre with a long cable is available, vertical profiles should follow the same sampling depths as temperature. If not, measurements from the chemistry sampling depths have to be made. In this latter case, BOD special bottles have to be used, and measurements can be made either with an oxymetre directly in the bottle, or by Winkler titration (iodometric method). Special care is needed when subsampling, it has to be carried out before any other subsampling to avoid bubbling inside the sampler, the outlet tube of the sampler has to be placed just above of the bottom of the BOD bottle; and before the sample is collected the contents of the sample bottle should be displaced at least three times by the flowing water.

WARNING: We are looking for very comparable measurements to follow the seasonal dynamics, in particular, we want to estimate the oxygen consumption during winter, which in some lakes it can represent a drop in concentration of only 0.1 mg l^{-1} $month^{-1}$. Therefore, it is quite important to check the calibration of the oxymetre or the accuracy of the Winkler titration. With the iodometric method a higher precision can be obtained, but accuracy depends a lot on the experience of the analyst, the error titrating can be very large. Therefore, we recommend electrode readings, if titration is not performed by experienced analyst. Cross checking of some samples with both methods is also helpful.

Troubles with oxymetres can mainly come from mechanical problems (electrode membrane integrity, stirring, electronic failure at low temperatures) and calibration. Each device has its own system of calibration, some of them are quite automatic, other are more manual. In any case, it is important to check that the calibration has been done properly, in especial, for those working at high altitude, correction by pressure changes has to be considered. Checking the calibration in the field is important; and it is mandatory to use the same stirring method for both calibration and profiling. A solution without oxygen (zero point) can be easily obtained by adding to a glass of water an small amount (half tea spoon) of sodium sulphite powder. A saturated solution can be obtained bubbling air

(you can use an aquarium bubbler with batteries) during half an hour through a water sample from the lake surface. Remember to keep the sample close to the original temperature in the lake, in order to avoid significant saturation changes caused by cooling or heating.

4.6 Conductivity and pH

For these measurements follow the water chemistry protocol from work package 1. pH readings from multi-parameter profilers will be not accurate enough unless you stop for quite a while at each depth. pH readings should be comparable with pH measurements for other purposes in the project.

4.7 Chlorophyll

Filter one to several litres of water (depending on final absorbance readings) through glass fibre filters (Whatman GF/F). If not processed immediately, the filters have to be stored frozen, individually packed with aluminium foil and folded in such a way that direct contact between the face of the filter containing the algae and the foil is avoided. Extraction must be carried out just before measurements, avoid storing the pigment solution because alteration of a significant amount of pigments occurs in a day. Using acetone, extraction must include either strong sonication (ultrasound bath used in microbiology are not enough) or mechanical grinding using a glass or Teflon homogeneizer. The extract must be clarified by filtration or centrifugation, usually the former performs better. Then, measure the absorbance in a 1 cm length cell at the following wave-lengths: 750, 663, 647, 630, 480, 430, 410 nm. The 750 wavelength is for checking the turbidity of the extract, hence, it must be as low as possible, and never a significant proportion respect to other wavelength readings, if that happens clarify the solution again by filtration or centrifugationn. For calculations, we propose to use the equations of Jeffrey and Humphrey (1975) because they provide the most accurate specific coefficients for Chl-c

Calculations :

Chl-a (µg/l) = (11.85 (A663-A750) - 1.54 (A647-A750) - 0.08 (A630-A750)) * VOLacet(ml) / VOLfil (l)
Chl-b (µg/l) = (-5.34 (A663-A750)+ 21.03 (A647-A750) - 2.66 (A630-A750)) * VOLacet(ml) / VOLfil (l)
Chl-c (µg/l) = (-1.67 (A663-A750) - 7.60 (A647-A750) + 24.52 (A630-A750)) * VOLacet(ml) / VOLfil (l)

Carotenoid index: (A480-A750) / (A663-A750)
Phaeopigment index: (A430-A750) / (A410-A750)

Reference: Jeffrey and Humphrey (1975), Moss (1967) and Strickland and Parsons (1968).

Please, be careful in the evaluation of the volume of solvent where pigments are dissolved, errors at this point are critical for adequate quantification, most common problems can be evaporation or volume retention during different steps of the extraction. If evaporation is kept null, then the best procedure is to use the initial volume added.

In case of very low chlorophyll levels, fluorimetric methods can be used, but please calibrate them with the above stated method. If you prefer to use other alternative methods, because of the routines in your lab or other personal requirements, please check your method against the one above stated a few times along the surveys, and send the results with your readings.

4.8 Ice-cover description

During winter sampling, a description of the physical structure of the snow and ice cover is required in order to apply dynamic models later on. Minimum measurements must be total thickness and water level in the hole drilled for sampling, these allow for an estimation of the mean density of the cover. An stratigraphical description can be made following very simple conventions such as:

hardness degree:

very soft:	you can intrude into the snow layer your hand closed
soft:	you can intrude into the snow layer four extended fingers wearing gloves
medium:	you can intrude into the snow layer only one finger
hard:	you can intrude into the snow layer a pencil
very hard:	you can intrude into the snow layer a knife

free water:

dry:	you are unable of making a snow ball
moist:	you are able of making a snow ball
wet:	handing some snow you can see the water
very wet:	handing some snow you can appreciate the water moving
slush:	handing a piece of snow water runs out from your hand

grain type:

new snow
partially melted snow
granular rounded
granular rounded with melting
granular with facets

An example of their application to mountain lakes can be found in Catalan (1989).

5. Occasional sampling

These includes data necessary for understanding and modelling the seasonal variability but at a lower temporal resolution than the variables in the regular sampling. The set of mandatory variables for the occasional sampling are: major ions, nitrate, ammonium, reactive soluble phosphorus (RSP), total phosphorus (TP), phytoplankton (diatoms and chrysophytes) and zooplankton (cladocera). Furthermore, some optional data can increase significantly the capabilities of some models, although they are not strictly necessary, these optional variables include: nitrite, total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), particulate organic nitrogen (PON), dissolved and particulate organic carbon (DOC, POC), silicate, iron and manganese. On the other hand, recording of lake level fluctuations is desirable, and outflow measurements are valuable data for some models.

5.1 Frequency

Each site have to make his own decision about the frequency of the occasional sampling and which optional measurements will be added. At least one measurement of the mandatory variables in summer and one in winter are desirable.

5.2 Sampling depths

Number of samples is also a site by site decision; priority is for the depth 1 m above sediment.

5.3 Phytoplankton (diatom and chrysophytes) and zooplankton

Please follow the protocols in work package 1.

5.4 Major ions and nutrients

Please, follow the water chemistry protocol for work package 1. For the optional measurements, methods are free in order to encourage the measurements in the different labs involved.

References:

Catalan, J. 1989. The winter cover of a high-mountain Mediterranean lake (Estany Redó, Pyrenees). Wat. Res. Research 25: 519-527.

Jeffrey, S.W. and Humphrey, G.F. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae, and natural phytoplankton. Biochem. Physiol. Pflanzen., 167: 191-194.

Moss, B. 1967. A spectrophotometric method for estimation of percentage degradation of chlorophyll to phaeopigments in extracts of algae. Limnol. Oceanogr. 12: 335-340.

Strickland, J. D. H. and T. R. Parsons. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Ca. 167.

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MOLAR

PROTOCOL FOR INVERTEBRATE SAMPLING: RECENT FAUNA PALAEOLIMNOLOGICAL DATA

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Invertebrate Sampling:
Recent Fauna and Palaeolimnological data

1. Invertebrate sampling - Field and laboratory methods

Based on experience from the last two decades, including the ALPE programmes, benthic invertebrates have proved to be very useful as key organisms in mapping the degree of damage due to atmospheric pollutants as well as a tool in the monitoring of lake and stream ecosystems (Raddum and Fjellheim 1984, Fjellheim & Raddum 1990, Raddum et al. 1994). In the MOLAR programme the Institute of Zoology, University of Bergen, will co-ordinate sampling, data processing and evaluation of the benthic invertebrate data. Linked to the Norwegian Institute of Water Research we will also form a part of the programme center of the project.

2. Sampling protocol

The sampling frequency varies between the different sites. Please contact the site operator for further details.

3. Contemporary fauna - field methods

Samples must be taken from the profundal zone, the littoral zone, and outlet stream, and, if possible, from the inlet stream. In the littoral zone quantitative samples should be taken from 5 meters depth and qualitative samples using a kick net should be taken from the upper littoral zone. The mesh size used for washing all the samples should be 250 µm. All samples should be conserved in ethanol; alcohol concentration of the sample being approximately 70%.

3.1 Quantitative samples

Six parallel quantitative samples should be collected from two different depths: the deepest part and 5 m, preferably using a Kajak corer (Kajak 1971) or modified versions of this sampler. If diatom coring is done in the same locality, a set of core samples should be taken near this site.

3.2 Qualitative lake samples

The lake littoral must be sampled qualitatively using the "kick method" (Frost et al. 1971). If the littoral zone is heterogeneous, for example with stones and macrophytes, samples should be taken from the different habitat types.

3.3 Qualitative stream samples

Qualitative "kick samples" should be taken from two sites: one at the outlet of the lake and one 200 - 300 m downstreams of the lake. If the lake has a defined inlet, an additional sample should be taken from this locality.
Drift net samples

Sampling chironomid pupal exuviae using drift nets is optional, but highly recommended due to the great help the exuviae provide in species identification. Preferably two drift nets should be used; one placed at the outlet of the lake sampling the lake fauna, and one placed 200 meters downstream of the

outlet sampling the river fauna in addition to the lake fauna. The nets should be left operating for as long as possible.

3.4 Lake transect sampling

In some cases transect sampling for algae and invertebrates will be performed by divers.
The transect will cover depths from the littoral to the profundal. As this sampling is optional, no standardized methods are given. Sampling may be quantitative, using a core sampler or qualitative, using a sweep net.

4. Comtemporary fauna - laboratory methods

The samples taken with the Kajak corer from the littoral and profundal zones are quantitative and all animals must be sorted out from the sediments under a binocular. The kick samples are qualitative and should be sorted under a binocular for 1 hour (if necessary). In cases with high densities of chironomids, additional time should be used to get a representative material of this group. The chironomid larvae should be mounted in Hoyer's solution on microscopic slides and identified as far as possible.

5. Historical data - field methods

The core sampling should be carried out with the same equipment as the diatom palaeolimnological samples. The core should be sectioned into slices of 5 mm or less.

6. Historical data - laboratory methods

The sediment samples should be stored fresh in plastic bags at 4o C. Subsamples will be taken at 2 or 5 mm intervals. Each subsamples should be weighted before sorting of head capsules and, after at least 100 head capsules are sorted out, it should be reweighted in order to find the concentration of head capsules. The sediments should be deflocculated in 10 % KOH heated to 60^{o} C for 15 minutes and sieved through a sieve with mesh size between 90 and 105 µm. Head capsules will be picked out from a Bogorov sorting tray and slide-mounted in a permanent medium.

7. Data transmission

Taxonomic lists and site data, preferably in ASCII or EXCEL format should be sent to:

Gunnar G. Raddum
University of Bergen,
Institute of Zoology
Allégt. 41 N-5007 Bergen
Norway

8. References

Fjellheim, A. & Raddum, G. G. 1990. Acid precipitation: Biological monitoring of streams and lakes. - The Science of the Total Environment 96: 57-66.

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MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

MICROBIAL (PELAGIC) FOOD WEBS - 1st LEVEL

SAMPLING PROTOCOL FOR SITE OPERATORS
Work Package 1.

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Microbial (Pelagic) Food Webs - 1st Level

Sampling protocol for site operators Work Package 1.

1. Objective

Sampling for a quantitative assessment of the biomass of main components of pelagic assemblage, including: bacteria (BAC), heterotrophic nanoflagellates (HNF), ciliates (CIL), picocyanobacteria (PICY), phytoplankton (PHY), small (ZOOS) and large (ZOOL) zooplankton.

2. List of materials and equipment necessary (at the site, sampling from three depths):

- boat (inflatable), anchor, line
- volume sampler (bottle type) with a rubber tube discharge, plankton net # 40 μm, quantitative plankton net # 200μm
- calibrated vessel 250 - 500 ml
- 3 bottles with filtered formaldehyde for BAC + HNF
- 3 bottles with formaldehyde-cacodylate for PICY
- 3 bottles for CIL with Lugol-reagent
- 3 bottles for PHY
- Lugol solution
- formaldehyde
- 4 bottles for zooplankton (ZOOS and ZOOL)
- polystyrene box for preserved samples

3. Handling of samples in the lab

- CIL samples should be elaborated within two weeks. If this is not done in your lab and you must send them to another partner, another fixative must be added within 5 days after sampling: add 50 ml of 40% formaldehyde to 500 ml sample, mix and clear with 1 ml of 45% (w/v) sodium thiosulphate. Then they can be elaborated within 2 months.

- BAC + HNF and PICY samples should be elaborated within less than 1 month. If this is not done in your lab, send them to another partner immediately after each sampling

4. Sampling strategy

Samples will be taken four times during each of the two ice-free periods - in 1996 (July to September) and 1997 (July to September). The sampling should be synchronised with sampling for chemical analyses (organic carbon, phosphorus, nitrogen, alkalinity, conductivity, pH), and with temperature profiling. The sampling will be performed from the boat (may be an inflatable one) using a large volume (3 litres or more) sampler of van Dorn type. The sampler will be used for sampling all components but the large zooplankton. A quantitative net (200 # μm mesh size) of AL:PE type will be used for sampling large zooplankton. Samples will be preserved, transported to the lab and elaborated later or sent to another partner for elaboration (will be specified for particular determinations and particular sites).

5. Basic sampling technique

The sampler should be well washed (at other place than you will sample!), free of dust, precipitate etc (this is more important than sterility!) and located in the boat in a plastic bag or a clean box. The boat must be fixed at the sampling point either by an anchor or, at small lakes, using a line stretched from one shore to the opposite one. Always start with taking samples of BAC, HNF, CIL, PICY and PHY (from one sampler) before the other measurements are done. Try to avoid contamination from boat sides, from the anchor and lines, by plankton nets, sediment, littoral plants etc. Take one sample with the upper end of the sampler 1 m below the surface, mix the contents and fill (through a well washed rubber tube at the lower end of the sampler) the sampling bottles for BAC + HNF, PICY, CIL and PHY (as described further), the rest of the sampler contents may be used for chemical analyses. Then take another sample of BAC + HNF, PICY, CIL and PHY from a layer near bottom, with the lower end of sampler 1 m above the bottom. In the lakes deeper than 6 m, a third layer to be sampled for BAC, HNF, PICY, CIL and PHY is recommended: take the third sample from a middle layer between surface and bottom (or from the thermocline, if any). Then sample ZOOS with the same sampler and ZOOL with nets.

6. Sampling bottles

Preferentially, plastic screw-cap bottles should be used. The polypropylene (PET) flasks, well washed, are excellent for work in hard field conditions. They are light, transparent, unbreakable, they tight well and their wall is not permeable for iodine.

DO NOT USE POLYETHYLENE BOTTLES for PHY and CIL samples, iodine would penetrate through the walls!

Add the measured amounts of respective fixatives into bottles before each sampling (do not prepare in advance for the whole season). Using bottles with the fixative inside is a compromise for harsh sampling conditions. Microbes might be damaged during a short exposure to concentrated fixative. Pour samples quickly into bottles and mix gently while pouring.

The volume ratio of fixative and sample is obligatory - you may use slightly different sample volumes than prescribed, but then adjust the volumes of fixatives respectively.

6.1 **BAC + HNF** (in one sampling bottle)

Use 105 ml bottles with 5 ml 37-40% formaldehyde in each. Pour 100 ml of sample into a calibrated well washed vessel and transfer into a bottle with formaldehyde, mix and close. Label: lake, depth, date, BAC. During transport protect against light and sudden changes of temperature in a polystyrene box, then store in the darkness at 5°C. Samples *must be elaborated within one month* if autotrophic and heterotrophic part of nanoplankton should be distinguished (because of fading autofluorescence of chlorophyll). Later, only bacteria could be assessed.

- Reagents needed at sampling: 37-40% formaldehyde *prefiltered* through membrane or nuclepore filter with 0.1 - 0.3 μm pores. Attention: it is recommended to use special glassware and filtration apparatus etc. for handling formaldehyde and not to use it for "live" samples!

- Elaboration (principle): (a) numbers of bacteria and heterotrophic nanoflagellates - concentration on Poretics or Nuclepore filters (with different porosity for different sizes of organisms), stained by DAPI and counted in epifluorescent microscope, (b) sizing

(measurement of cell sizes) in similar preparations like (a), bacteria by the semiautomatic image analysis system, HNF with an ocular micrometer.

6.2 PICY

Use 100 bottles with 5 ml of 20% formaldehyde-cacodylate in each. Measure 95 ml of sample with calibrated vessel, pour into bottle and mix immeediately. Label: lake, depth, date, PICY. Transport and storage the same as for BAC+HNF. Samples *must be elaborated within one month* if autotrophic part of picoplankton should be distinguished (because of fading autofluorescence of chlorophyll).

- Reagents needed at sampling: 20% formaldehyde-cacodylate:

  (i) sodium cacodylate buffer 0.1M pH 7.2: dissolve 2.14 g of sodium cacodylate in deionized and 0.2 µm filtered water to make 50 ml,
  add 2.5 ml of diluted HCl (1ml of 36-38% HCl in 60.3 ml of deionized and 0.2 µm filtered water)
  and make up to 100 ml with deionized and filtered water

  (ii) mix 100 ml of sodium cacodylate buffer with 100 ml of 40% formaldehyde stabilized with methanol

- Elaboration (principle): concentration on filters, staining with DAPI, counting in epifluorescent microscope and sizing by image analysis or ocular micrometer.

6.3 CIL

Use 580 ml bottles with 5 ml of Lugol reagent. Measure 500 ml of sample with calibrated vessel, pour into bottle, and mix immediately. Label: lake, depth, date, CIL. Transport and storage same as for BAC + HNF + PICY. Elaboration necessary within 2 weeks, the fixative does not preserve ciliates sufficiently to be quantitatively elaborated later. For a later elaboration or sending to other partners, add 50 ml of 40 % formaldehyde and 1 ml of 45 % (w/v) sodium thiosulphate to the Lugol fixed sample (this should be done within 5 days after sampling).

- Reagents needed at sampling: Lugol solution:
  solution A - dissolve 10 g KI in 20 ml dist. water, <u>then</u> add 5 g of cryst. iodine
  solution B - 10 % acetic acid
  Mix solution A and B in the ratio 2:5 and store at least one day before use. The reagent can be stored for long periods.
  Sodium thiosulphate: dissolve 45 g $Na_2S_2O_3$ in approx. 90 ml of deionized water and add up to 100 ml.
  40 % formaldehyde

- Elaboration (principle): (a) numbers and taxonomic groups in sedimentation chambers in an inverted microscope, (b) cell volumes measured in the same preparations with an ocular micrometer

- For a detailed taxonomic study the same samples could be used, postfixed with Bouin's fixative (to final conc. 5%) and stained with protargol.

6.4 PHY

Use plastic screw-cap bottles (PET, not polyethylene!) of more than 500 ml capacity. Pour at least 500 ml of sample (need not be measured exactly) into the bottle, add approx. 0.5 ml of Lugol reagent (the resultant colour should be that of "tea"). Store at a dark place. Label: lake, depth, date, PHY. Check the colour of the sample each two weeks and add additional reagent if the colour faints. Elaboration recommended within 2 months.

- Reagents needed at sampling: Lugol solution, the same as for CIL.

- Elaboration (principle): counting, taxonomic determination and sizing will be performed in one sample in sedimentation chambers using an inverted microscope with phase contrast. For taxonomic determination of some phytoplankton groups, the samples preserved by formaldehyde (BAC + HNF + PICY) additionally might be used.

6.5 ZOOS

Take another sample from each sampling depth, filter the contents of the whole sampler using a 40 µm plankton net, repeat until at least 10 litres are filtered. Rinse the net as described below for ZOOL. Store the sample in a plastic bottle of 50-100 ml capacity, preserve with formaldehyde to the final concentration 4% v/v. The bottle must be filled up at least to two thirds! Label: ZOOS, lake, depth, volume filtered.

- Elaboration (principle): taxonomic determination, counting and sizing.

6.6 ZOOL

will be sampled by quantitative vertical hauls, using a quantitative net, mesh # 200 µm. Tow the net from 1 - 2 m above the bottom to the surface, the towing speed being about 0.3 m per second. After each haul the inner surface of the net must be rinsed carefully by lowering the net (bucket closed) into the water. Then the contents of the bucket is emptied into a sampling bottle. Repeat until all animals are washed into the bottle. Write down the towing length and the number of hauls per sample.

Sample 1: For enumeration and sizing. The sampling bottle is of 100 - 250 ml capacity. Preserve with formaldehyde as described above for ZOOS. Label: ZOOL, Lake, Date, Length (m) and number of the tows, net opening diameter (cm).

- Elaboration (principle): counting, taxonomic determination and sizing

Sample 2: For determination of the total biomass. Optional. The technique and handling will be specified individually with each site operator.

Sample 3: For determinations and taxonomy (both ZOOS and ZOOL). Qualitative sample using the # 40 µm net: take vertical and long oblique hauls in order to obtain a rich sample. Preserve with formaldehyde. Label: Lake, date, qualitative sample 40#.

List of lakes sampled and labs for elaboration

lake	BAC	PICY	HNF	CIL	PHY	ZOOS	ZOOL
ONeadals.	HBI	HBI	HBI	HBI	FSCV	FSCV	FSCV
Stavsvatn	HBI	HBI	HBI	HBI	FSCV	FSCV	FSCV
Lochnag.	HBI	HBI	HBI	HBI	FSCV	FSCV	FSCV
Pai.Super.	HBI + CNR	CNR	HBI + CNR	CNR	CNR	CNR	CNR
Goessenk.	UIBK	UIBK	UIBK	UIBK	FSCV	FSCV	FSCV
La Cald.	UGR.ES	UGR.ES	UGR.ES	HBI	UGR.ES	UGR.ES	UGR.ES
Redo	FBG	FBG	FBG	FBG	FBG	FBG	FBG
Starolesn.	HBI	HBI	HBI	HBI	FSCV	FSCV	FSCV
Dlugi St.	HBI + FWB	HBI	HBI	FWB	FSCV	FWB	FWB
Chuna	HBI	HBI	HBI	HBI			
Jorisee	UZUR	UZUR	UZUR	UZUR + HBI	UZUR	FSCV	FSCV

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MICROBIAL (PELAGIC) FOOD WEBS - 1st LEVEL

LABORATORY PROTOCOL
Work Package 1.

Vera Straškrabová,
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Miroslav Macek

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Microbial (Pelagic) Food Webs

Laboratory protocol - microbiology 1st level - Work Package 1

1. Objective

Quantitative assessment of biomass of the main components of pelagic assemblage - elaboration of preserved samples: BAC + HNF, PICY, CIL.

2. Bacterial abundance and biomass (BAC)

Formaldehyde preserved samples (final concentration 2% vol/vol, i. e. 5 ml of 40% formaldehyde in 100 ml sample) are used. 2 - 10 ml subsamples (depending on the abundance) are filtered through 0.2 µm pore-size black polycarbonate filters (Poretics or Nuclepore) and stained with the fluorochrome 4',6-diamino-2-phenylindole (DAPI, final concentration 0.2% wt/vol) according to Porter & Feig (1980). Solution of DAPI 0.001 g/10 ml is used, 50µl of solution per 2 ml of subsample. Samples are inspected in an epifluorescent microscope equipped with the filter set for DAPI (specification of filter depends ona type of the microscope). At least 400 bacteria are counted on at least 10 fields of the filter. Bacterial numbers are calculated as follows:

$$N = \frac{A \times n}{a \times 0.95\ v}$$

where:

N ..... number of bacteria per ml of sample
A ..... filtration area (of the filter)
n ..... number of bacteria counted on 10 fields
a ..... area of 10 fields
v ..... volume of sample filtered (0.95 is a correction factor for the fixative added)

Between 400 and 600 cells are sized by semiautomatic image analysis system and volumes calculated, as described by Psenner (1993). Pay specific attention to the appropriate evaluation of biomass of filamentous bacteria, which are frequently found in alpine lakes.

Bacterial biomass will be calculated according to the allometric relationship between cell volume (V) and carbon content (C) reported by Norland (1993):

$C = 120 \times V^{0.72}$ (in fg per cell)

Data requested for the final evaluation from different sites are:

- lake, sampling date, depth
- bacterial numbers in millions per ml
- cell volume (mean ± SD), cell length (mean ± SD), cell width (mean ± SD)

Save also the files with raw data (!) from sizing by image analysis (the best would be in Excell). They can be used e.g. for evaluation of the contribution of different size classes to total bacterial biomass.

3. Autotrophic picoplankton (PICY)

Usually dominated by unicellular cyanobacteria, it might be a very important contributor to the carbon metabolism of planktonic communities of oligotrophic freshwater ecosystems (Stockner 1988). Picocyanobacteria can be easily quantified due to the presence of specific pigments via epifluorescence microscopy (for details about appropriate filter sets see MacIsaac & Stockner, 1993). Elaboration necessary within 2 weeks after sampling! Depending on the picocyanobacteria concentration, 2 - 10 ml of formaldehyde-cacodylate preserved sample are concentrated onto black 0.2 µm pore-size filters (Poretics, Nuclepore or Anopore). Try to have a homogeneous distribution of cells on the filter. PICY are enumerated using their specific autofluorescence of phycoerythrin (green excitation, filter set for Zeiss Axioplan - 510-560/ FT580/LP590). For calculation of numbers, use a factor of 0.95 as a correction for the volume of added fixative. In case of high abundance of picocyanobacteria, their volumes will be sized by the image analysis (see bacteria) based on their cell autofluorescence. Cell volumes of PICY will be transformed to carbon using a conversion factor of 200 fg C μm^{-3} (Weisse 1993).

Data requested for final elaboration:

- lake, sampling date, depth
- number of picocyanobacteria per ml
- if measured: cell volume (mean ± SD), cell length (mean ± SD), cell width (mean ± SD)

4. Heterotrophic flagellate abundance and biomass (HNF)

Elaboration necessary within 2 weeks after sampling! 10 - 50 ml of formaldehyde preserved sample (the same as for bacteria) are concentrated onto 1 µm pore-size black polycarbonate filters (Poretics or Nuclepore), stained with DAPI and counted in an epifluorescent microscope (see bacteria). Each individual has to be checked for presence of autofluorescing plastids (switching from filter set for DAPI to filter set for chlorophyll autofluorescence and back) to differentiate heterotrophic (aplastidic) from autotrophic (plastidic) forms. The same filter is used for sizing: at least 50 individuals per sample are measured with a calibrated ocular micrometer (cell lengths and widths). Mean cell volumes are calculated using geometrical approximations of prolate spheroids. For biomass estimations based on preserved samples, we assume a conversion factor of 220 fg C μm^{-3} (Borsheim and Bratbak 1987).

Data requested for final elaboration:

- lake, sampling date, depth
- number of HNF per litre
- cell volume (mean ± SD), cell length (mean ± SD), cell width (mean ± SD)

5. Ciliate abundance and biomass, taxonomic determination (CIL)

Lugol fixed samples should be elaborated within two weeks or postfixed by formaldehyde + thiosulphate (see sampling protocole, Sherr & Sherr, 1993). Ciliate numbers and taxonomic groups will be assessed in sedimentation chambers with the aid of inverted (Uterm\hl) microscope. Lengths and widths of individuals will be measured with a calibrated ocular micrometer and volumes calculated by approximation to prolate spheroids. If there are no ciliates found per 50 ml, no further search is done.

For calculating numbers, correction factor for the volume of fixative added will be 0.99 for Lugol-fixed samples and 0.89 for the additionally postfixed samples.

Live cell volumes may be calculated by mutiplying the volumes of preserved animals by a factor of 1.4 suggested for Lugol preserved ciliate cells (M]ller & Geller, 1993). Formaldehyde does not produce additional shrinkage (Wiackowski et al. 1994).

Ciliate cell organic carbon may be estimated using a conversion factor of 140 fg μm^{-3} (Putt & Stoecker, 1989).

For a detailed taxonomic study, samples can be further treated as follows: sample is let to sediment and a concentrated sediment is fixed with Bouin's fixative (Montagnes & Lynn, 1993, Protocols in Protozoology, 1992). Concentrated samples are filtered through 1.2 or 3 μm pore-size nitrocellulose filters (Millipore) and protargol staining is performed directly on filters (Montagne & Lynn, 1987, Skibbe, 1994).

Data requested for final elaboration

- lake, sampling date, depth
- number of ciliates per litre
- taxonomic structure (number of specimens in various taxonomic groups or not classified)
- cell volume (mean ± SD), cell length (mean ± SD), cell width (mean ± SD)

At high abundances, give the cell size data separately for taxonomic groups.

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List of persons responsible for microbiological analyses 1st level

Partners	bacteria	picocyanobact.	heterotr. nanofl.	ciliates
HBI	Karel Simek	Karel Simek	Petr Hartman	Mirek Macek
FBG	Marisol Felip	Marisol Felip	Marisol Felip	Marisol Felip
UGR-ES	Manuel Villar + Isabel Reche	P.Sánch.Castillo + P.Carrillo	P. Carrillo	Isabel Reche
UIBK	Birgit Sattler + Anton Wille	Birgit Sattler + Anthon Wille	Birgit Sattler + Anthon Wille	Johannes Fried
CNR	Crist. Callieri	Crist. Callieri		Crist. Callieri
UZUR	Brigitte Hinder	Isabel Baur	Isabel Baur	
FWG				K.Wiackowski

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MOLAR

MICROBIAL (PELAGIC) FOOD WEBS - 2nd LEVEL

FIELD AND LABORATORY PROTOCOL FOR EXPERIENCED LABS

Work Package 1.

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Microbial (Pelagic) Food Webs - 2nd Level

Field and laboratory protocol for experienced labs, WP1.

1. Objective

Assessment of fluxes among the pelagic components. The protocole is not strict and will be adapted according to specific conditions of particular lakes. Alternative methods are recommended. The measurement of fluxes will be performed only in several lakes of WP 1: Ovre Neadalsvatn, Lochnagar, Redo, La Caldera, Gossenkoellesee, Jorisee, Dlugi staw, Starolesnianske.

For constructing a model (carbon flow chart) of pelagic food webs, which should characterize "an average situation in the lake during the second half of ice-free period", the data of biomasses (1st level) and fluxes must be generated *from particular depths and days simultaneously.* The measurements should be carried on during several short periods of intense investigations - e.g. three days in one week, and during 2 - 3 weeks per two ice-free seasons, if possible.. It is not planned to construct a model of seasonal dynamics. This could not be achieved in the whole set of remote lakes included in WP1. Just two lakes of the set have a well equiped laboratory (with electricity) located close to the lake, where such measurements could be carried on regularly during the whole season.

2. Phytoplankton primary production

2.1 Principle

^{14}C-method is used. Incorporation of ^{14}C by phytoplankton cells as well as bacterial incorporation of photosynthetically produced extracellular organic C, plus concentration of photosynthetically produced and released organic C not utilized by bacteria, is measured. Only the photosynthetically produced carbon, which was released by respiration during the incubation could not be assessed. If possible, the fractions of primary production are separated by differential filtration:

a. Incorporation by phytoplankton on 0.8 - 2 μm filter (polycarbonate filters: Nuclepore, Poretics, are necessary, which retain phytoplankton but not bacteria)

b. incorporation by bacteria (from the filtrate of A) on 0.2 μm filter

c. labelled organic carbon in the filtrate of B.

Depending on the structure of phytoplankton at particular lake and date, the appropriate porosity of algal filter (A) should be chosen. In some cases, separation of phytoplankton and bacteria may be impossible due to overlap in the sizes of phytoplankton and bacteria or due to very low primary production. Then the sum of "particulate" production should be measured (A+B) on 0.2 μm filters of any material (polycarbonate, glass-fiber, cellulose-acetate etc.).

In field conditions when differential filtration would be difficult, total labelled organic carbon might be measured (A+B+C) in a subsample before filtration, instead of using filtrate (C).

2.2 Sampling and incubation strategy

Thermal stratification of the lake and the depth of euphotic layer (2 x Secchi depth) should be determined. The layers sampled should include 0.5 m plus four other layers within the euphotic layer.

In the lakes with not pronounced stratification or in very shallow lakes, the number of layers sampled may be reduced.

Note that primary production, concentration of chlorophyll, bacterial production and elimination are to be measured at identical depths.

All samples should be exposed in the same layers, from which they were sampled, for 2 - 4 h (preferentially 10 a.m. to 2 p.m.). Actual values of primary production, valid for particular light and temperature conditions in each depth during measurement, are thus obtained (similarly as the values of bacterial production and elimination). These data cannot be used for the estimation of primary production in the whole lake and whole season. If an estimate of total production in the lake is desirable, primary production from each sampled depth should be measured at different light intensities. The procedure is described in a separate Protocol for measuring photosynthetic rates by using the ^{14}C method and estimation of primary production (L. Camarero). Using this procedure, it is necessary to have data on primary production extrapolated to the actual light intensity and temperature in the layers where bacterial production and elimination is determined.

2.3 Incubation

- Collect a sample from each depth

- For each depth, fill two dark and two light quartz-glass or PET bottles (100-250 ml) plus one bottle for C_{inorg} determination, plus another bottle for Chl.*a* determination (for the determination of C_{inorg} and Chl.*a* see the last two sections

- Keep the bottles in dark (e.g. in insulated plastic container) until start of incubation Dispense 0.5 Mbq of ^{14}C-bicarbonate in small volume (50-200 ml) per bottle. Amount of radioactivity can be adjusted (depending on expected rate of photosynthesis and C_{inorg} concentration) to get appropriate values of d.p.m./filter

- Expose bottles in the depths of sampling, after exposition stop incubation by addition of formalin (1 % final concn). Formaldehyde can be avoided if the time period between incubation and filtration is very short

- Store bottles in dark and cold until filtration

2.4 Filtration and C_{org} separation

- From each bottle remove a 1-ml subsample for determination of total activity and transfer it into scintillation vial containing 1 ml of β-phenetylamine (or Carbosorbe) - (T)

- Filter a defined volume (50-100 ml) of incubated subsamples through algal filter, collect the filtrate. The porosity of filter must be chosen according the locality sampled, the filter should retain phytoplankton but not bacteria - see above:Principle - (A).

- Filter the collected filtrate through 0.2-mm (bacterial - B) filter, collect the filtrate. (alternatively: only one filter A+B from a non-filtered sample will be filtered - see above: Principle)

- Vacuum must not exceed 20 kPa (0.2 atm) during the filtrations

- To the 10 - 20 ml aliquote of the 0.2-mm filtrate add in a small volume (10-100 ml) HCl to the final concentration of 0.01 N (C)
(alternatively: use an aliquote from non-filtered sample - see above: Principle - A+B+C)

- Bubble the acidified filtrate with air for 60 min to remove inorganic ^{14}C. Alternatively, let aliquotes stand in open vials overnight.

- Neutralize the aliquote with NaOH added in a small volume

- Transfer 5-10 ml of the neutralized filtrate into scintillation vial, add appropriate volume of the liquide scintillator capable of mixing with water

- Determine ^{14}C radioactivity (d.p.m.) of all filters, and of neutralized aliquotes.

2.5 Calculations

- Depending on the volumes incubated, volumes filtered, and volumes used for aliquotes calculate for each bottle:

  A = radioactivity retained on the algal filter per liter of original sample
  B = radioactivity retained on the bacterial filter per liter of original sample
  C = radioactivity of acidified filtrate (dissolved C_{org}) per liter of original sample
  T = total added radioactivity per liter of original sample

  (alternatively, you may have either:
  A, B, (A+B+C), T .....then C can be calculated
  or: (A+B), C, T ..... then B cannot be calculated)

- Calculate relative values of primary production and exudation as $A_{rel} = A/T$ etc.

- Correct values in light bottles for (blank) values in dark bottles $A_{rel} = A_{rel}(light) - A_{rel}(dark)$

- Using values of C_{inorg} calculate absolute values of primary production and of exudation:
  $A_{abs}[\mu gC \cdot l^{-1} \cdot h^{-1}] = (C_{inorg} + {}^{14}C_{inorg}) \cdot 1.06 \cdot A_{rel}$
  $B_{abs}[\mu gC \cdot l^{-1} \cdot h^{-1}] = (C_{inorg} + {}^{14}C_{inorg}) \cdot 1.06 \cdot B_{rel}$
  etc.

where C_{inorg} is concentration of dissolved inorganic carbon and $^{14}C_{inorg}$ is the final concentration of ^{14}C bicarbonate added (it can be neglected if $C_{inorg} >> {}^{14}C_{inorg}$)

A+B+C	=	**gros primary production**
A	=	**net primary production**
B+C	=	**extracellular production (exudation)**
B	=	**part of extracellular production used by bacteria**

2.6 Inorganic carbon determination

Inorganic carbon (DIC) can be determined directly by using an inorganic carbon analyzer or estimated from pH and alkalinity (using Gran titration). It is important to prevent samples from any contact with air during storage before DIC analysis or pH determination (to avoid any CO_2 exchange).

See Protocol for measuring photosynthetic rates by using the ^{14}C method and estimation of primary production (L. Camarero) for detailed description of the procedure for DIC calculation based on pH and akalinity.

2.7 Chlorophyll determination

Spectrophotometric method is used.
Lakewater is filtered through GF/C filter (1 - 3 l according to Chl concn) and analyzed immediately or freezed.

Extraction of pigments is performed with 4 - 5 ml acetone 90% by grinding the filter or using a sonicator. Extraction vial should be cooled and extraction time minimized to avoid evaporation of acetone. After grinding, the remains of the filter are removed by centrifugation or filtration through a fiberglass filter. The supernatant (or the first filtrate) is then filtered through 0.1 μm Anopore filter for a complete clarification. Turbidity is checked by measuring the absorbance at 750 nm (it should not exceed 0.002).

Absorbance is then read at 663, 647 and 630 nm.
Chlorophyll.*a* concentration is computed with the equation (Jeffrey & Humphrey 1975, *Biochem. Physiol. Pflanzen* **167**: 191-194):

$$\text{Chl.}a\ [\mu g \cdot l^{-1}] = [11.85\ (A663 - A750) - 1.54\ (A647 - A750) - 0.08\ (A630 - A750)] \times \frac{\text{VOLacet[ml]}}{\text{VOLfilt [l]}} \times lc$$

where:
VOLacet = volume of the acetone extract
VOLfilt = volume of lakewater filtered
lc = length of the spectrophotometric cuvette in cm

3. Bacterial production

3.1 Principle

Incorporation of (i)^{3}H-thymidine or (ii)^{3}H-leucine by bacteria is used for estimating production: (i) external thymidine is supposed to be taken up at a rate proportional to the rate of nucleotides synthesis (de novo synthesis of thymidine by the cells is neglected), (ii) leucine is supposed to be taken up at a rate proportional to the rate of protein synthesis.

3.2 Sampling

- Determine the thermal stratification of the lake under study and the depth of euphotic layer(2 × Secchi depth).

- Make a decision about the depths to be sampled (0.5 m + four further depths within the euphotic layer). Note that bacterial production and primary production are to be measured at identical depths. In the lakes with not pronounced stratification of bacterial parameters, the number of depths sampled may be reduced.

- Collect water samples, avoid change of temperature of samples between sampling and the beginning of incubation (if necessary, keep water samples in thermally insulated boxes, bottles, etc.). Begin the incubation within 60 min after sampling.

3.3 Incubation (the same for ^{3}H-thymidine and ^{3}H-leucine)

- For each sample, prepare 6 incubation vials (e.g. washed plastic scintillation vials), three of them will be blanks (in the case of uniformly low blank values the number of blanks can be reduced).

- To the blank vials, dispense appropriate volume of 40-% formaldehyde (50 ml per 1 ml of the incubated subsample; final concentration 2 %)

- To all vials, add a small volume (10-100 ml) of working solution of ^{3}H-thymidine (^{3}H-TdR) or ^{3}H-leucine (^{3}H-Leu). The same volume add also directly into scintillation vial (in duplicate) for determination of the total activity (and concentration) of ^{3}H-TdR or ^{3}H-Leu present during the incubation. The added volume depends on the concentration of the working solution of ^{3}H-TdR or ^{3}H-Leu, on the incubation volume and on the required final concentration (see the last section).

- **The final concentration** of both ^{3}H-thymidine and ^{3}H-leucine is **25 nmol·l^{-1}.** A lower concentration can be used only if it has been demonstrated (in preliminary saturation experiments) that incorporation is already saturated at the lower concentration (to check saturation, use e.g. concentrations of 2-5-10-15-20-25-50 nmol·l^{-1} of ^{3}H-TdR or ^{3}H-Leu).

- Start the incubation by the addition of a 10-ml subsample of water sample. The volume can be increased to get higher filter-counts.

- Incubate samples **in the dark at *in-situ* temperature**. The samples where temperature differs less than by 2 °C, may be incubated at the same temperature. For each incubation temperature use a separate incubation bath with temperature adjusted to the temperature of the sample (e.g. polystyrene box filled with water taken from the sampling depth can be used). Alternatively, subsamples can be incubated directly *in-situ*, in the sampling depth .

- Incubate for **30-60 minutes.** The incubation period can be extended (up to several hours) only if it has been demonstrated (in preliminary experiments) that the time course of incorporation is linear over the whole period of incubation.

- Stop the incubation by the addition of 40-% neutral formaldehyde (the same volume as it has been used in blanks).

3.4 Extraction

- Between the end of incubation and extraction, keep the subsamples in cold (~ 4 °C). Extraction should be completed as quickly as possible, at latest during the next day.

- There are two extraction procedures:

  a. *Cold TCA extraction* (specific for macromolecules = DNA + RNA + proteins): This procedure can be used for both ^{3}H-TdR and ^{3}H-Leu incorporation, because the incorporation of the radioactivity from ^{3}H-TdR into proteins and from ^{3}H-Leu into nucleotides is low during the short incubation times used (£60 min).

b. *Hot TCA extraction* (specific for proteins) may be alternatively used for ^{3}H-Leu incorporation .

3.4.1 Cold TCA extraction

- Filter subsamples through 0.2-mm polycarbonate filter (Nuclepore, Poretics etc. - using the cellulose-nitrate or cellulose-acetate filters may result in a very high blank values!).
- Rinse filters 10 □ with 1 ml of ice-cold 5-% trichloroacetic acid (TCA).
- Vacuum must not exceed 20 kPa (0.2 atm) during the filtration and the TCA extraction.
- Place filters in scintillation vials, add scintillation cocktail suitable for tritium counting.
- Count for ^{3}H.

3.4.2 Hot TCA extraction

- Use 5-% TCA instead of formaline for blank vials and for incubated subsamples.
- Heat the 5-% TCA killed subsamples to 80 °C for 15 min.
- Filter subsamples through 0.2-mm polycarbonate filters
- Vacuum must not exceed 20 kPa (0.2 atm) during the filtration and the TCA extraction.
- Rinse filters 2 □ with 3 ml of ice-cold 5-% TCA and then 2 □ with 2 ml of ice-cold 80-% ethanol.
- Place filters in scintillation vials, add scintillation cocktail suitable for tritium counting.
- Count for ^{3}H.

3.5 Calculation of the incorporation rate of ^{3}H-TdR or ^{3}H-Leu

- Correct d.p.m. values (d.p.m. per filter) for blanks
- Convert d.p.m. values to pmol·l^{-1}·h^{-1} of ^{3}H-TdR or ^{3}H-Leu

$$Incorporation\ rate\left[pmol\ /\ l.h\right]=\frac{d.p.m./\ filter\ \left(corrected\ for\ blank\ values\right)}{60\times Spec.Act.\ \left[TBq\ /\ mmol\right]\times Incub.volume\left[ml\right]\times Time\left[h\right]}$$

Example:

d.p.m./filter:	5 000
Specific activity:	1.81 MBq·mmol^{-1}
Incubation volume:	10 ml
Time:	60 min

Incorporation rate = 5 000 / (60 □ 1.81 □ 10 □ 1) = 4.6 pmol·l^{-1}·h^{-1}

- Bacterial production in terms of cells produced per unit of volume and time can be calculated using appropriate conversion factor. The conversion factor should be determined experimentally. Alternatively, for ^{3}H-TdR theoretical conversion factor of 2□10^{18} cells □ (mol of incorporated ^{3}H-thymidine)$^{-1}$ can be used.

3.6 Preparation of work solutions of 3H-thymidine and 3H-leucine

- In sterile vessel, dilute original ^{3}H-TdR or ^{3}H-Leu preparation with bidistilled water to achieve appropriate concentration (calculation of the dilution factor see below).

- By means of a syringe assembled with a sterile disposable filter holder with 0.2-mm filter, dispense appropriate portions of the diluted ^{3}H-thymidine into sterile plastic tubes (adjust volume of ^{3}H-thymidine in vials to that which will be used in one experiment).

- Store at -20 °C.

Calculation of the dilution factor:
Dilution factor depends on radioactive concentration and specific activity of the original ^{3}H-TdR or ^{3}H-Leu preparation, on the volume in which you want to add work solution into incubation vials, on incubation volume and on the desired final concentration of ^{3}H-thymidine during incubation:

$$Dilution\ factor = \frac{Rad.concn.\,[MBq\,/\,ml] \times Added\ volume\,[\mu\ell]}{Spec.act.[TBq\,/\,mmol] \times Incubation\ volume\,[ml] \times Final\ concn.[nmol\,/\,l]}$$

Example (^{3}H-TdR):

Added volume:	50 ml
Specific activity:	1.81 MBq·mmol^{-1}
Radioactive concentration of original preparation:	37 MBq·ml^{-1}
Incubation volume:	10 ml
Final concentration:	25 nmol·l^{-1}

Dilution factor = 37·50/(1.81·10·25) @ 4.1

Preparation of the work solution:
1 : 4.1 ; e.g. 1 ml of original preparation of ^{3}H-TdR + 3.1 ml of bidistilled H_2O

4. Estimation of bacterivory

Fluorescently labelled bacteria (FLB) stained by DTAF will be used (Sherr & Sherr 1993). Protozoa, especially HNF and ciliates, are recognized as major consumers of bacteria in most of freshwater pelagic ecosystems. Since both these protozoan groups are strongly size-selective, size class distribution of FLB offered should mimic very tightly the size class distribution of bacteria in a studied lake ecosystem. There are basically two approaches available:

4.1 Time-integrated grazing impact of all (nano- and micro-) bacterivores

This approach is based on disappearance rate of FLB (constituting cca 1 - 5 % of natural bacterial concentration) with time. This method will be more convenient for those who do not have enough experience with measurements of direct uptake of FLB by protozoans (2nd approach). Water samples (at least 250 ml) with an addition of FLB will be exposed in duplicates *in situ* or at least at *in situ* temperature for 1-2 days. Subsamples (20-30 ml) will be taken at the start and end of the exposure and preserved by formaldehyde (2% final concn). In 5-10 ml subsamples filtered through 0.2 ∝m pore-size black filters, FLB will be enumerated using the same filter set as for acridine orange. Along with the FLB counting, natural bacterial concentration in the same subsamples has to be quantified (DAPI staining, see procedure for bacterial counting). To calculate the rate of cell disappearance, a linear model which takes into account also changes in natural bacterial abundance will be used.

$$G = (F_0 - F_T) \square \text{ where } N = \frac{}{2} \text{ and } F = \frac{}{2}$$

G	bacteria grazed during a period T
N_0 and N_T	natural bacterial abundance at time 0 and time T, respectively
F_0 and F_T	FLB abundance at time 0 and time T, respectively.

For details see Salat and Marasse (1994).

Elimination rate (e) can be estimated as G/N_0 per time.
An estimate of bacterial production rate (p) could be calculated as follows:

$$p_{ap} = \frac{\ln N_T - \ln N_0}{T} \qquad p = p_{ap} + e$$

where:

p_{ap} = apparent production rate ("net" rate, if elimination is not considered)

4.2 Direct uptake of FLB

Experiments are conducted in at least 250 ml samples using 30 min. incubation. Tracer amount of FLB added should account for cca 10-20% of natural bacterial abundances. Subsamples (30-50 ml) from time zero and 30 min. after tracer addition are fixed by adding 0.5 % of alcaline Lugol solution immediately followed by 2% formaldehyde (final concn) and several drops of 3% sodium thiosulphate to clear the color of Lugol (Sherr & Sherr 1993). To determine flagellate and ciliate grazing rates, at least 50 HNF and 30 ciliates should be inspected per sample fixed after 30 min (the same procedure as the determination of HNF numbers, using DAPI stain and switching between filter sets for DAPI and for acridine orange-DTAF). Samples from zero time are also inspected to avoid a potential bias due to attachment of non-ingested tracers on protozoan surfaces. To estimate total protozoan grazing rate, average uptake rates of HNF and ciliates are multiplied by their *in situ* abundances.

* Alkaline Lugol: dissolve 10 g of KI in 20 ml dist. water, then add 5 g of cryst. iodine (sol. A), dissolve 5 g of sodium acetate in 50 ml dist. water (sol. B), then mix A + B

These two methods do not provide same results. During frequent measurements throughout the season, at average, the estimates of bacterial elimination by grazing based on both methods are comparable, though they differed (to both sides) in particular samples. The same is valid for a comparison of bacterial production based on thymidine uptake and with the estimate based on changing bacterial numbers (see approach 1).

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MEASURING AND MODELLING THE DYNAMIC RESPONSE
OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO
ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

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PROTOCOL FOR MEASURING PHOTOSYNTHETIC RATES BY USING THE ^{14}C METHOD AND ESTIMATION OF PRIMARY PRODUCTION

MICROBIAL FOOD WEBS 2nd LEVEL
Work Package 1

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Protocol for Measuring Photosynthetic Rates by Using the ^{14}C Method and Estimation of primary Production

Pelagic Food Webs. Microbiology 2nd level. Work Package 1.

1. Measuring photosynthetic parametres: photosynthesis vs. irradiance (P-I) curves

In trophic dynamic theory (Lindeman 1942 *Ecology* **23**:399-418), primary production is the chemical energy in an ecosystem that is the direct product of photosynthesis. Some few in situ measurements of C uptake by phytoplankton are insufficient to extrapolate the instantaneous photosynthesis rates thus obtained to estimate the primary production of a lake. In particular, changes in the irradiance along the day or during the whole season are ignored. A more adequate approach is to measure the photosynthetic parametres of the phytoplankton communities to model photosynthesis as a function of the incident radiation. The actual irradiance values (either measured or estimated from astronomic-meteorological-geographic models) corrected by the light absorbance coefficient of the lakewater may thus be used to obtain better estimates of the primary production during a given period (see the *Computing primary production* section).

The photosynthetic parametres are estimated from the light saturation curves or photosynthesis vs. irradiance (P-I) curves: several water samples are incubated under a light gradient to measure the photosynthetic C fixation at different light levels. The curve so obtained has two parts: a light dependent initial slope, and a light saturated asynthotic part. For very high radiation levels a third segment with negative slope caused by photoinhibition may occur. Several equations have been proposed to model P-I curves. The following two equations (Platt *et al.* 1980 *J. Mar. Res.* **38**:687-701; Gallegos & Platt 1981. In *Physiological bases of phytoplankton ecology,* Platt, T. (ed.). *Can. Bull. Fish. Aquat. Sci.* **210**) include parametres with a real physiological meaning:

model without photoinhibition

$$P^B = P^B_{max} \times tanh\left(\frac{\alpha^B I}{P^B_{max}}\right)$$

model with photoinhibition

$$P^B = P^B_{max} \times \left(1 - exp\left(\frac{-\alpha^B I}{P^B_{max}}\right)\right) \times exp\left(\frac{-\beta^B I}{P^B_{max}}\right)$$

where:

P^B = photosynthetic rate (mg C $\times$ mg^{-1} Chl*a* $\times$ h^{-1})
P^B_{max} = maximum potential photosynthetic rate (mg C $\times$ mg^{-1} Chl*a* $\times$ h^{-1})
α^B = initial slope (µE $\times$ mg Chl*a* $\times$ mg^{-1} C $\times$ h^{-1})
β^B = photoinhibition slope (µE $\times$ mg Chl*a* $\times$ mg^{-1} C $\times$ h^{-1})
I = irradiance (µE $\times$ m^{-2} $\times$ s^{-1})

The superscript *B* indicates that the values are normalized by biomass, using chlorophyll *a* concentrations.

For realistic estimations of primary production, P-I curves should be inferred from incubations under full sun light, that is, under a natural wavelenght light spectrum including PAR, UVA and UVB.

2. An *in situ* incubator for determination of P-I curves

Figure 1 shows a simple design of an in situ incubator. An appropriate number of cylindric baskets made of 2 cm white plastic grid, and of a size adequate to contain the incubation bottles are mounted on a grey PVC plate, in which holes have been drilled to allow the access to the inner part of the baskets (Fig. 1a). A lower PVC cover holds the bottles during incubation. A light gradient is obtained by shading the baskets with a different number of layers of grey plastic mosquito-net of 1 mm 8meshsize: a roll of mosquito-net (of a length suficient to produce the number of layers desired) and several disks ot the top are placed inside each basket (Fig. 1b). Each single layer filters 40-50% of the incident light. Percentage of light attenuation in each basket must be calibrated by using an spherical light sensor. It is recommended that the light gradient includes at least 5 points at irradiances below 100-200 $\mu E\ m^{-2}\ s^{-1}$ (for an accurate calculation of the initial slope α), and at least 3 more points above (to compute P_{max}). During incubations, irradiance must be measured continuously to compute the average incident light. Incubations must be conducted around noon in a clear sky day. The incubator is placed horizontally in the lake water near the surface, either in a shallow point or hanging on a pole from a nylon thread to avoid shading (Fig 1c).

3. Field protocol for incubations

- Water samples must be collected by using opaque samplers and stored in dark bottles until further processing, which should start as soon as possible (ideally, just after collection). The collection depths, refered to the Secchi depth (SD), are: surface, 0.5×SD, 1×SD, 1.5×SD and 2×SD. In deep lakes, the deep chlorophyll maximum should be also sampled.

The next steps must be performed under dim light:

- Stir well the sample bottle and dispense a subsample in a glass bottle of a volume large enough to allow for all the necessary incubation replicates. Take also subsamples for Chl*a* and DIC determinations (see *Chlorophyll measurement* and *DIC determination* sections) .

- Add the ^{14}C solution (see the *Preparation of the ^{14}C solution* section) in an approximate amount of 10-15 µCi per 100 ml of sample and stir well. (REMARK: this is an orientative amount. The activity to be added may vary from site to site. In any case, the activity added must be enough to obtain a number of counts in the samples incubated at optimum irradiance significantly higher than that in the dark (blank) samples).

- Take two 100 µl aliquotes of the inoculated sample and dispense them into two radioassay vials, in which 500 µl of ß-phenyl-ethyl-amine (ß-PEA) have been previously dispensed. Mix well. This is to determine the exact amount of activity initially added to the samples (DPMini).

- Distribute the sample into 50-150 ml quartz-glass or polyethylene (PET) incubation bottles (REMARK: toxicity of PET bottles used in photosynthesis measurements has been reported by Holm-Hansen & Helbling, 1993, *Science* **259**:534). For P-I curves, a minimum number of 8 bottles is needed. Make sure that no air bubbles are left in the bottle.

- Attach the bottles to the incubation system in a way in which shading (as caused by stoppers, other bottles, etc.) is avoided, and place the ensemble into a dark box.

As soon as possible, take the samples to the incubation site. The recommended incubation lenght is no longer than 2-3 hours. After incubation, recover the samples and take them inmediately to the dim light working place into the dark box.

4. Recovering of the ^{14}C fixed by photosynthesis

The recovering of the labelled C fixed by photosynthesis can be done in two ways: either filtration or acid bubbling:

4.1 Filtration method

- Stir well the sample bottles and filter the sample through a 25 mm diameter fibre-glass filter Wathman GF/F (or equivalent). The vacuum pressure during filtration must be low enough to ensure that no cell breakage occurs. The exact volume of each bottle must be known.

- Rinse the filter first with 20 ml of HCl 1% and then with 20 ml of distilled water.

- Place the filter on the bottom of a radio-assay vial.

4.2 Acid bubbling method

- Stir well the sample bottles and dispense 5 ml of sample into a glass radioassay-vial

- In a fume hood or a well ventilated place, add 100 µl of HCl 0.5 N.

- Bubble with air in a vacuum device (Fig. 2) for at least 30 min. A CO_2 trap must be placed at the vacuum pump outlet to retain $^{14}CO_2$ released during acidification. As an alternative, the acidified samples may be left open in the fume hood for at least 24 h to remove non-fixed ^{14C}, but in this case $^{14}CO_2$ is released to the atmosphere. Furthermore, it must be checked that the amount of fixed ^{14}C (which is expected to be low) is well above that of inorganic ^{14}C which could be left without bubbling.

The acid bubbling method is also used to determine the extracellular organic carbon (EOC) produced during photosynthesis. In this case, the samples must be previously filtered trough a GF/F filter (or equivalent). Make sure that no cell breakage occurs during filtration by using low vacuum pressure.

REMARKS: DO NOT use plastic scintillation vials for acid bubbling. Plastic adsorbs significant amounts of labelled inorganic C which can not be eliminated by acidification. In ultra-oligotrophic mountain lakes, this method could not be sensitive enough to determine photosynthetic EOC release. A test should be run in all lakes before it is adopted as a routine method.

5. Scintillation counting

- For counting filters and initial samples, add 5 ml of scintillation cocktail for aqueous samples, and allow for a complete absorbtion of the cocktail by the filter for at least two hours before counting.

- For counting acid bubbled water samples, add 15 ml of scintillatin cocktail for aqueous samples. Mix energetically and allow for stabilisation for at least two hours before counting.

Samples should be counted to 10,000 scintillations or 10 min, whichever comes first. Counts per minut (CPM) are converted into disintegrations per minut (DPM) by the two channels method and counting efficiency correction, according to the especifications of each counter.

6. Calculation of photosynthetic rates

Calculations to convert DPM to photosynthetic rates (P = mg C / mg Chl*a* / h) are as follows:

$$P = \frac{1.06 \times DPMsample \times \left(\left(DPMini / \left(2.217 \times 10^6 \times As \times Vini \right) \right) \times DI^{14}C + DIC \right)}{\left(Vsample / Vini \right) \times DPMini \times t \times Chla}$$

where:

1.06	= ^{14}C assimilation factor
As	= specific activity of the ^{14}C labelled solution, in μCi ml^{-1}
Chla	= chloropyll *a* concentration, in mg/l
DPMsample	= DPM of incubated samples
DPMini	= DPM of initial samples
DIC	= dissolved inorganic carbon in the water sample, in mg/l
$DI^{14}C$	= dissolved inorganic carbon in the ^{14}C labelled solution, in mg/l
t	= incubation time, in hours
Vsample	= volume of sample filtered or bubbled, in ml
Vini	= volume of initial sample, in ml

7. Preparation of the ^{14}C solution

The radiotracer is generally supplied as a $H^{14}CO_3$ solution with high activity (of the order of 1 mCi ml^{-1}). It can be directly added to the samples by using a micropipette. The inconveniences are the difficulty of handling small volumes, and the conservation of the solution: once the sterile vial is open the solution must be used quickly or kept deep frozen to avoid any loss of activity. An alternative is to prepare a diluted solution using an adequate buffer, distribute it in srew-cap vials of a ready-to-use volume and autocleave them. It is also possible to obtain low activity ^{14}C solution in sterile vials of small volume from the International Agency for ^{14}C Determination (The Water Quality Institute, 13 Agem Allè, DK-2970 Hoersholm, Denmark. Fax +45 42 867273), but it is much more expensive. The main inconvenience of using a dilute solution is that the buffer contains a significant amount of unlabelled dissolved inorganic C which is added to the generally DIC-poor samples from mountain lakes.

To prepare the dilute working solution from the commercial high activity solution:

- Buffer solution: In a 500 ml volumetric flask, dissolve 0.6 g of HCO_3Na in 400 ml of distilled water. Add 1.7 ml of NaOH 1 N and fill up to 500 ml. Check the pH. It must be between 9.5 and 9.7 If necessary, adjust pH by adding some drops of NaOH 1 N.

- Working ^{14}C solution: Open the sterile vial or ampoule with high activity solution and dispense it by syringe into a volumetric flask of an adequate volume. With the same syringe, rinse the sterile vial (and the cap or tip) several times with buffer and dispense into the flask. Then, bring it to the final volume with the buffer solution. A final specific activity As = 50 μCi ml^{-1} is recommended: 500 ml of sample should then be inoculated with 1 ml of working ^{14}C solution to obtain the required incubation sample activity. $DI^{14}C$ concentration of this working solution is 172 mg l^{-1}.

- Distribute the working solution in ready-to-use volumes into autocleavable, screw-cap glass vials, and autocleave at 130 °C during 30 min. DO NOT OPEN the vials until they are to be used.

8. Chlorophyll measurement

The simplest way to measure Chl*a* is the spectrophotometric method:

- Filter at least 2-3 l of lakewater trough a GF/F filter. Analize or freeze the filter immediately after filtration.

- Extract the pigments in a suitable vial with 4 - 5 ml of acetone 90% by grinding the filter or using a sonicator. During extraction, cool the extraction vial with ice and keep the extraction time to the minimum to avoid evaporation of acetone.

- After grinding, sediment the remains of the filter by centrifugation or filter trough a fiber-glass filter. Filter the supernatant (or the first filtrate) trough a 0.1 μm Anopore filter for a complete clarification. Check turbidity by measuring the absorbance of the extract at 750 nm. Absorbance must be 0.000 ± 0.002

- Read the absorbance of the extract at 664 and 630 nm

Chl*a* concentration (in μg l^{-1}) is computed with the equation (Jeffrey & Humphrey 1975 *Biochem. Physiol. Pflanzen.* **167**:191-194):

$$Chla = \left(11.47 \times Abs_{664} - 0.40 \times Abs_{630}\right) \times \frac{Vext}{Vf \times lc}$$

where:

Vext = volume of the extract, in ml
Vf = volume of lakewater filtered, in l
lc = length of the spectrophotometer cuvette, in cm

9. DIC determination

DIC can be determined directly by using an inorganic carbon analyzer or estimated from pH and alkalinity, after correction by temperature and ionic strength. For measuring pH and alkalinity in very soft waters, see recommendations in the chemical analysis protocols. In particular, Gran titration is strongly recommended for alkalinity determination. Both positive and negative alkalinity values may be calculated with this method, and DIC may therefore be estimated even in acidic waters by using the formula shown below. It is important to prevent samples from any contact with air during storage,

and pH determination or DIC analysis, in order to avoid any CO_2 exchange. This caution is unnecessary during alkalinity analysis. DIC (in mg l^{-1}) is computed as:

$$DIC = \frac{\left([Alk] - 10^{(-pKw'+pH)} + 10^{(-pH)}\right) \times 10^3 \times 12}{\alpha_1 + 2\alpha_2}$$

where:

$[Alk]$ = alkalinity, in eq l^{-1}
pH = pH of the sample corrected for the incubation temperature:

$$pH = pH_{lab} + 0.0114\left(t_{lab} - t_{is}\right)$$

where:

pH_{lab} = pH measured in the lab
t_{lab} = temperature of the sample in the lab, in °C
t_{is} = in situ temperature, in °C

α_1 and α_2 are the ionization fractions, and are calculated as:

$$\alpha_1 = 1 \Big/ 10^{(pK_1'-pH)} + 10^{(pH-pK_2')} + 1$$

$$\alpha_2 = 1 \Big/ 10^{(pK_1'+pK_2'-2pH)} + 10^{(pK_2'-pH)} + 1$$

and pK_w', pK_1', and pK_2' are the logarithm transformations of the dissociation constants of water, carbonic acid and bicarbonate respectively, corrected for temperature and ionic strength. They are computed as:

$$pK_w' = \frac{4470.99}{T} + 0.01706\,T - 6.09 - \frac{0.5\sqrt{\mu}}{1+\sqrt{\mu}}$$

$$pK_1' = \frac{3404.71}{T} + 0.03279\,T - 14.84 - \frac{0.5\sqrt{\mu}}{1+\sqrt{\mu}}$$

$$pK_2' = \frac{2902.39}{T} + 0.02379\,T - 6.50 - \frac{2\sqrt{\mu}}{1+\sqrt{\mu}}$$

where:

T = incubation temperature, in °K
μ = ionic strength

The ionic strength may be estimated from the conductivity of the water sample, by interpolating the values given in the following table:

$Cond_{25}$ (µS cm^{-1})	70	400	1000	5000
μ	0.001	0.005	0.010	0.050

For conductivities lower than 70 µS cm^{-1}, the effect of ionic strength may be neglected.

10. Computing primary production

Computer programs (Fee 1990 *Can. Tech. Rep. Fish. Aquat. Sci.* **1740**: v + 27 pp.) are available for calculating the fraction of primary production attributable to phytoplankton in the lake. Using α^B, P^B_{max}, Chl*a*, the light absortion coeficient of lakewater, surface irradiance, and hypsographic data as input parametres, the programs calculate:

- Daily and annual photosynthesis rates
- Separate estimates of photosynthesis in the mixed layer and below the thermocline
- The total mass of carbon fixed in the waterbody

Irradiance input data may be measured values or may be computed by the program from the latitude of the lake, day of the year, and empirical constants describing the light absorbance properties of the atmosphere and average cloudiness.

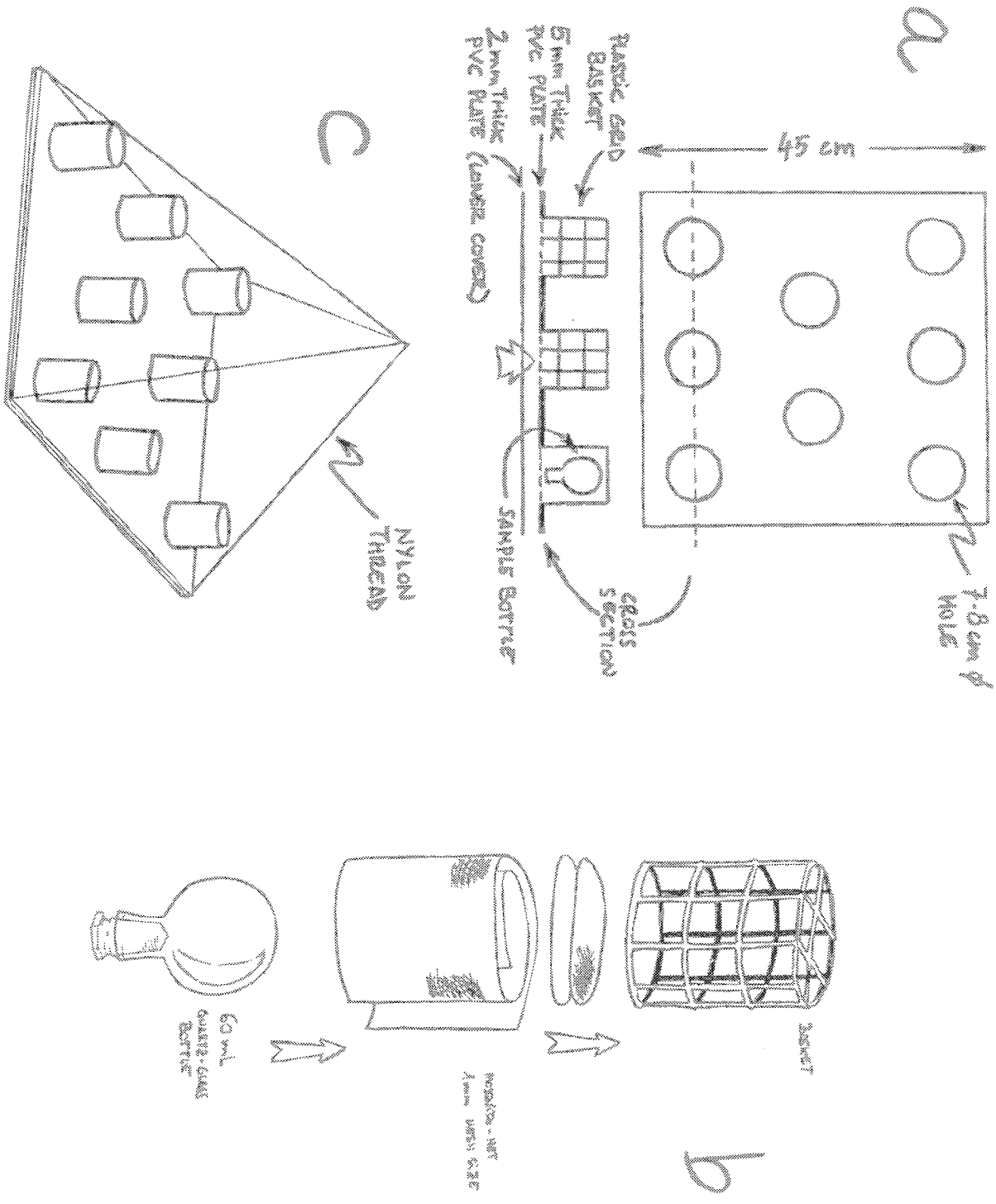
a
45 cm
Plastic grid basket
5 mm Thick PVC plate
2 mm Thick PVC plate (lower cover)
Sample bottle
Cross section
1.6 cm ϕ hole
c
Nylon thread
b
60 mL Sample glass bottle
Basket

Figure 1

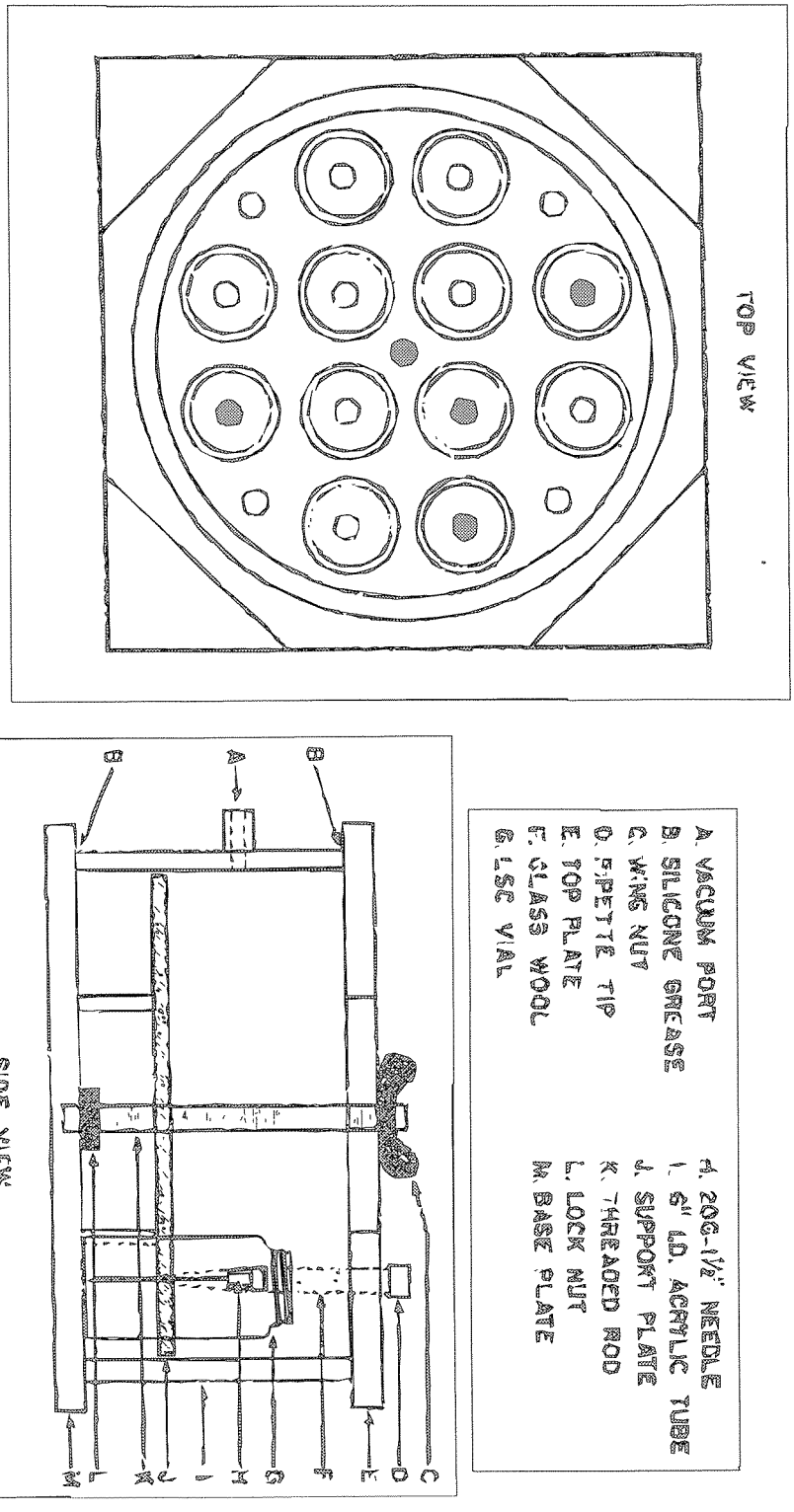

Figure 2 (after Shearer *et al.* 1985 *Can. Tech. Rep. Fish. Aquat. Sci.* **1341**: iv + 58 pp)

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

ZOOPLANKTON

SAMPLING AND LABORATORY PROTOCOL FOR SITE OPERATORS
Work Package 1.

Jan Fott

Charles University, Prague

Zooplankton - Protocol for WP1 - Sampling large zooplankton (ZOOL) with nets

(This is a more detailed description of the sampling methods mentioned briefly in the paragraph **ZOOL** of the protocol **"MICROBIAL (PELAGIC) FOOD WEBS - 1st LEVEL"** supplied earlier (May 19, 1996), which includes also the protocol for sampling small zooplankton (**ZOOS**).

1. Sites, site operators and analysts to whom preserved samples will be sent

Chuna (T. Moiseenko > Fott) - Øvre Neådalsvatn, overlap with WP3 (Lien > Fott) - Stavsvatn (Lein > Fott) - Lochnagar (Rose > Fott) - La Caldera (Cruz- Pizarro) - Lago Redo, overlap with WP3 (Catalan > Cruz Pizarro) - Lago Paione Superiore (Mosello > Manca) - Gossenköllesee, overlap with WP3 (Psenner > Fott) - Dlugi Staw (Galas > Pianowska) - Starolesnianske Pleso (Stuchlik > Fott) - Jorisee (Hanselmann - Fott).

(Note that all **dry samples** for the determination of biomass (org. C) will be sent to Prague).

2. Objective

Sampling pelagic zooplankton for quantitative assessment of the biomass of its components.
The biomass (as organic carbon) will be determined by combination on sizing (estimates based on measurements) and direct determination of organic carbon in the large zooplankton fraction.
The samples will be used also for description of seasonality in pelagic zooplankton communities.

3. Equipment and materials

- boat (inflatable or other), anchor, line
- plankton nets:
  a. AL:PE net (1): quantitative pelagic net, Apstein type, #200 μm
  b. AL:PE net (2): qualitative pelagic, #40 μm
- formaldehyde, plastic bottles 100 - 250 ml for preserved samples
- plastic bottle 1 - 2 litres for live zooplankton, cooling box - for the direct determination of biomass

4. Sampling procedure

4.1. Large pelagic zooplankton, quantitative sample for counting and sizing

Quantitative sample from the open water, using the net (1). The boat will be anchored close to the maximum depth. Tow the net from 1 - 2 m above the bottom to the surface, the towing speed being about 0.3 m per second (time in seconds = length in m x 3). After each haul the inner surface of the net must be rinsed carefully by lowering the net (bucket closed) into the water. Then the contents of the bucket is emptied into a sampling bottle. Repeat until all animals are emptied into the bottle. Take several hauls in order to get rich material. Write down the towing length and the number of hauls per sample. Preserve with formaldehyde to the final concentration of 4%. The sampling bottle must be almost full. Label: ZOOL, lake , date, length (m), number of tows, net opening diameter (cm) - or: AL:PE net (1), if you use a net supplied from Prague.

Note: This sample is taken also for WP3 (take only once at Ovre Neadalsvatn, Redo and Gossenkollesee).

4.2 Large pelagic zooplankton, quantitative sample for determination of biomass (org. C).

Take a quantitative sample using the net (1) as before. Put the live sample into a transparent plastic bottle ~ 1000 ml capacity, filled with lake water.

Check if the sample contains mud or debris, in this case discharge and repeat sampling.
Note the length and number of hauls.

Transport cool to the laboratory and go on with the treatment as soon as possible. Zooplankton should stay alive.

4.3 Pelagic zooplankton, all sizes, qualitative sample for taxonomy

Qualitative sample using the net (2): take vertical and long oblique hauls in order to obtain a rich sample. Preserve with formaldehyde. Label: Lake, date, qualitative, #40.

Note: This sample is taken also for WP3 (take only once at Ovre Neadalsvatn, Redo and Gossenkollesee).

Note to the preservation of all plankton samples: Preserve with formaldehyde to the final concentration of 4%. Do not leave too much air in the bottles, after preservation the bottles should be almost full. Be sure that all sampling bottles are tight and put the preserved samples into a plastic bag anyway. Good field bottles for 40% formaldehyde are plastic "shampoo" bottles - they use to be tight.

Overlap with WP3: For Ovre Neadalsvatn, Gossenkollesee and Redo, see also "Sampling zooplankton, WP3".

5. Laboratory treatment (by site operators) of the live zooplankton samples (for determination of biomass as org. C)

5.1 After return to the laboratory

Pour the sample on a white dish, remove macroscopic objects like leaves, insects etc. if present. Write your observations on the state of the sample. Discard the sample if the animals are in an advanced stay of decay (It can be tolerated if some animals do not move). **NOTE A.**

Pour the sample into a filter unit equipped with a pre-weighed circle of nylon netting, apply gentle vacuum, wash with distilled water, suck through with air. Remove the circle from the unit, fold with the zooplankton layer inside, put into a clean labelled vial, dry at 60° C in a dust-free oven (usually overnight). **NOTE B, C.**

Close the vial when the sample is dry. The dried samples, well closed, can be kept in room temperature. **NOTE D.**

Each sample (vial) must be labelled properly and the accompanying protocol must contain this information: Site, date, diameter of the net mouth, length of the tows (m), number of tows, notes on the state of the sample, weight of the circle (#.### g), other comments if appropriate.

5.2 Mailing

Mail to Prague (Dept. of Hydrobiology, Charles University, Vinicna 7, CZ-128 44 Prague 2). Please declare a value of the package of several ECU - if you write zero value, custom officers in Prague are confused and make troubles.

5.3 Analysis

In Prague the samples will be checked for purity, weighed, and either the whole samples or subsamples will be analysed for organic carbon.

6. Notes

a. The success in getting the animals in a good state to the laboratory depends on such factors as: density of the animals in the transport bottle, temperature during the transport, duration of the transport. Things are easy when your laboratory is close to the site.

b. The recommended procedure as they use it at Pallanza: Circles 4.7 cm diameter with a number written on an "ear" on its edge. The filtering unit Sartorius or Millipore, the glass sinter disc removed. The nylon circle buckles to form a dish where zooplankton accumulates. The folded circle with retained zooplankton is clamped by a non-corrosive clip and put into a vial. The vials are clean glass vials with plastic caps, like scintillation vials or similar ones.

c. At the very best zooplankton remains wrapped in the nylon filter but if some animals fall into the vial the sample is not lost for analysis.

d. Before you leave for the MOLAR site please check the procedure with zooplankton from your home lake or the pond in the garden of your Institute.

e. FSCU (Dept. of Hydrobiology) offers sending pre-weighed circles (4.7 cm or other diameter) in vials, ready for use, upon request.

Please contact Evzen Stuchlik by e-mail (stu@beba.cesnet.cz) or fax (+42 2 299713).

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

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MOLAR

SAMPLING RECENT ZOOPLANKTON

PROTOCOL FOR SITE OPERATORS
Work Package 3.

Jan Fott

Charles University, Prague

Protocol WP3 - Sampling recent zooplankton

1. Sites, site operators and analysts to whom the samples will be sent

Øvre Neådalsvatn, overlap with WP1 (Lien > Fott) - Lago Redo, overlap with WP1 (Catalan > pelagic samples to Cruz Pizarro, littoral samples to Brancelj) - Gossenköllesee, overlap with WP1 (Psenner > Fott) - Terianske Pleso (Sporka > Fott) - Jezero Ledvicah (Brancelj) - Saanajarvi (Korhola), Hagelsee (Lotter > Hofmann)

2. Objective

Sampling pelagic and littoral zooplankton with the emphasis to:

a. description of the communities and their seasonality
b. species leaving subfossil remains in the sediment

3. Equipment and materials

- boat (inflatable or other), anchor, line

- plankton nets:
  a. AL:PE net (1): quantitative pelagic net, Apstein type, #200 µm
  b. AL:PE net (2): qualitative pelagic, #40 µm
  c. AL:PE net (3): qualitative littoral, #100 µm

- formaldehyde, plastic bottles 100 - 250 ml

4. Sampling procedure

4.1 Large pelagic zooplankton, quantitatively (ZOOL)

Quantitative sample from the open water, using the net (1). The boat will be anchored close to the maximum depth. Tow the net from 1 - 2 m above the bottom to the surface, the towing speed being about 0.3 m per second (time in seconds = length in m x 3). After each haul the inner surface of the net must be rinsed carefully by lowering the net (bucket closed) into the water. Then the contents of the bucket is emptied into a sampling bottle. Repeat until all animals are emptied into the bottle. Take several hauls in order to get rich material. Write down the towing length and the number of hauls per sample. Preserve with formaldehyde to the final concentration of 4%. The sampling bottle must be almost full. Label: ZOOL, lake , date, Length (m), number of tows, net opening diameter (cm) - or: AL:PE (1) net, if you use a net supplied from Prague.

Note: This sample is taken also for WP1 (take only once at Øvre Neådalsvatn, Redo and Gossenköllesee).

4.2 Pelagic zooplankton, all sizes, qualitatively

Qualitative sample using the net (2): take vertical and long oblique hauls in order to obtain a rich sample. Preserve with formaldehyde. Label: Lake, date, qualitative sample #40.

Note: This sample is taken also for WP1 (take only once at Ovre Neadalsvatn, Redo and Gossenkollesee).

4.3 Littoral zooplankton, qualitatively

Qualitative sample using the net (3): throw the net repeatedly from the rocky shore and tow it along solid surfaces (rocks), partially touching them (but avoid loosing the net by getting it caught on a rock). The sample will contain a certain amount of debris. Preserve like the previous sample and label: Lake, date, littoral.

Note: This is a sample from a shallow water < 2 m.

Note to the preservation of all plankton samples: Preserve with formaldehyde to the final concentration of 4%. Do not leave too much air in the bottles, after preservation the bottles should be almost full. Be sure that all sampling bottles are tight and put the preserved samples into a plastic bag anyway. Good field bottles for 40% formaldehyde are plastic shampoo bottles - they use to be tight enough.

Note to the sampling from ice: Sampling zooplankton from thick ice may be limited by the possibilities of making holes of sufficient diameter.

Overlap with WP1: For Øvre Neådalsvatn, Gossenköllesee and Redo, see also WP1 protocol "Sampling large zooplankton with nets".

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

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MOLAR

PROTOCOL FOR SEDIMENT CORING & SUB-SAMPLING

Nigel Cameron

University College London

MOLAR Protocol for Sediment Coring & Sub-Sampling

Cores are to be taken for both WP2 and WP3 and Gossenköllesee, Øvre Neådalsvatn and Redo are to be cored for both. At these sites 5 cores will be needed as only one back-up will be needed to cover both WP's. One core from each site only will be dated and not all sites will be analysed for SCP. Please consult carefully with BOTH sections below and with the Site Operators sheets to determine exactly which cores and analyses are required.

1. Work Package 2

Laboratories responsible for coring
For MOLAR WP2, only the following sites will be cored:

Site	**Laboratory**
Jorisee	UZURICH
Gossenköllesee	ILIMNOL
Redo	FBG
Øvre Neådalsvatn	ECRC

Gossenköllesee, Øvre Neådalsvatn and Redo are also to be cored for WP3.

Techniques
Cores are needed to cover the last 150 years. In AL:PE, cores 30-40 cm in length were found to be sufficient for this purpose. Gravity corers (e.g. Glew) was found to be satisfactory. However, any corer that takes undisturbed cores of sufficient length (and gives enough sediment mass per 2 mm slice - see below) is suitable. It is important to stress the need for a good undisturbed sediment / water interface so that the surface layer(s) are known to be intact. Laboratories responsible for coring should check with Joan Grimalt about the suitability of their coring apparatus for taking cores for organics.

Two cores should be taken.

Extrusion
The cores should be extruded vertically, if possible in the field. Extrusion should be:

2 - 5 mm slices for 0 - 5cm
5 mm slices for 5cm - base of the core.

Core 1. The 'Master' core. This should be the less disturbed and longer core and will be used for all analyses if possible. ^{210}Pb and organics analysts will amalgamate received samples if necessary.

The core should be sub-divided for (i) organics, and (ii) the other analyses. Consequently, sectioning should be with stainless steel or teflon utensils previously rinsed with Milli-Q water and acetone. This rinsing should also be done between samples. Samples are best obtained from the centre of the tube to avoid `smearing' and this is best achieved by using two sampling rings of differing sizes.

The samples for organics analysis should be double wrapped in aluminium foil. The foil should also be rinsed in Milli-Q water and acetone. A paper label should be placed between the two foil sheets and the samples frozen as soon as possible. Please refer to the 'Organics Protocol'.

The second half of the sediment should be stored in sealed, labelled plastic bags and kept cool until further analysis can be undertaken. Dry weight (DW) and loss-on-ignition (LOI) analyses should be done by the laboratory responsible for the coring. The samples can then be dried before sub-sampling

for SCP (0.1 - 0.2g dry mass). The rest of the sample can then be sent for ^{210}Pb dating after which they will be passed on for metals analysis.

Core 2. This is a back-up core. It should also be extruded in 2mm intervals and stored cool, in sealed plastic bags. DW and LOI analyses should be undertaken on this core.

All samples should be clearly labelled with site name/code, core code and sediment level.

Sediment storage and transport
Sediment samples for organics analysis should be stored and transported frozen. This includes transport of the samples to the analytical laboratory (Joan Grimalt, CSIC, Barcelona).

Dry-weight and loss-on-ignition analyses on both sediment cores should be undertaken by the coring laboratories. Once these have been done satisfactorily, the remaining sediment can be dried (in a clean environment) and stored until needed.

The dried sediment should be sub-sampled and the required sediment weights (see above) sent for SCP analysis (Neil Rose, ECRC, London) ^{210}Pb dating and metals (Peter Appleby, ULIV, Liverpool).

2. Work Package 3

Laboratories responsible for coring

Site	Laboratory
Gossenköllesee[1, 2]	ILIMNOL
Jezero Ledvici[2]	NIB / ECRC
Terianske Pleso	ECRC
Saanajärvi[2]	UHEL
Hagelsee[2]	EWAG
Øvre Neådalsvatn[1, 2]	ECRC
Redo[1, 2]	FBG

[1] - Also WP2.
[2] - For SCP

Techniques
As for WP2 gravity corers will be used as only records covering the last 200 years are needed.
4 cores need to be taken at every site so that there is sufficient material for all analyses. Cores will be cross-correlated using dry weight and loss-on-ignition profiles.

Extrusion
Cores should be extruded in 2 mm intervals, this can be done by transporting to a laboratory if necessary.

Core 1
DW/LOI analysis should be done. The rest of the wet sediment should be weighed into labelled plastic bags (also label the bag with the sediment weight), sealed and sent for chironomid analysis.

Core 2
Wet sediment sufficient for DW and LOI analysis (0.5g) and grain-size analysis (0.5g) should be sub-sampled. The rest of the sample should be weighed into a labelled plastic bag (also label the bag with

the sediment weight), sealed and frozen as soon as possible and sent for pigment analysis after which it will be sent on for cladocera analysis.

Core 3

0.5g of wet sediment should be sub-sampled for diatom and chrysophyte analysis (check to see whether this is by 2 separate laboratories!). DW and LOI should then be undertaken on the sediment after which the sediment can be dried in a clean environment. 0.1 - 0.2g of the dried sediment should then be sent for SCP analysis (check list to see which sites) and the remainder can be sent for ^{210}Pb dating. After dating the sediment will be passed on for magnetics analysis.

Core 4

This core should be extruded in 2mm intervals and stored wet in a cool place as a back-up.

Sediment storage and transport

Sediment samples for pigments analysis should be stored and transported frozen. This includes transport of the samples to the analytical laboratory (Andrea Lami, CNR, Pallanza).

Dry-weight and loss-on-ignition analyses on all sediment cores should be undertaken by the coring laboratories (ILIMNOL, ECRC, UHEL, EWAG, FBG). Sub-samples of the appropriate amount (see above and confirm with analytical protocol sheets) of wet or dry sediment should be sent to the responsible laboratories for the analyses (see site sheets for details):

Analysis	Site							
	Gossenkollersee	Jezero Ledvici	Terianske Pleso	Saanajavi	Hagelsee	Øvre Neådalsvatn	Redo	Cimera
Dry Weight	ILIMNOL	ECRC / NIB	ECRC	UHEL	EWAG	ECRC	FBG	MADRID
Loss-on-ignition	ILIMNOL	ECRC / NIB	ECRC	UHEL	EWAG	ECRC	FBG	MADRID
Grain Size	ILIMNOL	ECRC	FSCU	UHEL	EWAG	ECRC	FBG	/
Magnetics	ULIV	ULIV	ULIV	ULIV	ULIV	ULIV	ULIV	ULIV
Diatoms	ILIMNOL	NIB	IZSAS	UHEL	EWAG	ECRC	FBG	MADRID
Chironomids	PLÖN	ECRC (NHM)	IZSAS	UHEL	ECRC	UIBZI	FBG	MADRID
Chrysophytes	ILIMNOL	ILIMNOL	ILIMNOL	ECRC	ILIMNOL	ECRC	FBG	MADRID
Cladocera	FSCU	NIB	FSCU	UHEL	FSCU	FSCU	NIB	NIB
Dating	ULIV	ULIV	ULIV	ULIV	ULIV	ULIV	ULIV	ULIV
SCP	ECRC	ECRC	/	ECRC	ECRC	ECRC	ECRC	/

Contact:

ILIMNOL	Roland Schmidt
UHEL	Atte Korhola
EWAG	Andy Lotter
ECRC - (SCP)	Neil Rose
- (The rest!)	Nigel Cameron
FBG	Jordi Catalan
ULIV	Peter Appleby
NIB	Anton Brancelj
IZSAS	Ferdinand Sporka
UIBZI	Gunnar Raddam
FSCU	Jan Fott
PLÖN	Wolfgang Hoffmann

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

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PROTOCOL FOR GRAIN SIZE MEASUREMENTS OF SEDIMENT CORE SAMPLES

Michael Sturm

EAWAG/ETH, Dübendorf

Protocol for Grain Size Measurements of Sediment Core Samples

1. Introductionary note

Grain size measurements of sediments are important to understand the sedimentological history of a lake. They provide information on the formation of sediments and on the stability or irregularities of a given sedimentary record. However, techniques of grain size measurements are numerous and overspan a whole range of techniques from very simple, low cost methods to highly sophisticated, high precision and expensive techniques.

As some of the MOLAR laboratories may not be suffiently equipped with appropriate instruments, two methods for grain size determinations are recommended within the following GrainSizeManual.

2. General

Grain sizes of unconsolidated sediments can very basically be divided into three different fractions (all grain size values in this manual are given in [µm], which describes the largest diameter of a given particle):

a. Very fine particles, showing particle diameters of
<2 µm = clay fraction

b. medium particles, showing particle diameters of
2 to 63 µm = silt fraction

The silt fraction may be further divided into three sub-fractions:

fine silt	= 2 - 10 µm
medium silt	= 10 - 20 µm
coarse silt	= 20 - 63 µm

c. Coarse particles, showing particle diameters of
>63 µm = sand fraction

3. Sample preparation

For any technique of grain size determination sediment samples have to be either wet or freeze dried. Using freeze dried samples makes it a bit easier at the end, to calculate percentages of different size fractions.

There is one paramount rule in the grain size business: **NEVER USE OVEN-DRIED OR AIR-DRIED SAMPLES FOR GRAIN SIZE ANALYSES.** Grain size results will then always be arbitrary and wrong.

For most lake sediments it is appropriate to use bulk sediment samples for grain size determination, e.g. without eliminating the organic material from the samples prior to analysis.

Commonly sample preparation is simple for most of the different methods: An aliquot (1 to 5 g) of the wet or freeze dried sample has to be dispersed into a Calgon® solution. This is to avoid

koagulation and the formation of larger flocs of smaller particles. To enhance homogeneous dispersion of particles, the suspension should subsequently be placed into an ultrasonic bath or be homogenized with an ultrasonic stirrer.

3.1 **Sieving technique** (particle fractions of <10 µm to >63 µm)

This basic, low cost grain size determination technique requires three to four measuring sieves, a centrifuge and an ultrasonic bath or an ultrasonic stirrer. If samples are very coarse, one may use additional sieves for sand fractions (e.g. mesh sizes of 125 µm, 250 µm etc.). This 'quick and dirty' technique, does not allow to determine the amount of clay fraction (<2 µm) in a sample; additionally, it is rather inaccurate determining particle fractions between 10 to 30 µm (more accurate determination of small size fractions without automated instrumentation could be performed by time- and labor-intensive methods such as pipette- or Atterberg-cylinders; quicker analyses with an automated sizing method see below).

- Take 5 g of freeze dried sample (or an equivalent amount of wet sample)and disperse sample into 250 ml of a Calgon® solution of 0.01%
- Homogenize the suspension with an ultrasonic stirrer for 3 minutes (US-bath 10 minutes)
- Place three measuring sieves of mesh sizes of 10 µm, 20 µm and 63 µm over a beaker with a volume of approx. 2 l
  (additional sieves with mesh sizes of 30 µm, 40 µm and 50 µm are optional, but would require larger initial sample sizes)
- Rinse sample suspension carefully through the sieves with as little as possible additional water until size fractions are clearly separated on top of the individual sieves
- Keep the effluent water (e.g. with the particles <10 µm) in the beaker
- Carefully transfer particles from sieves into preweighted beakers, ovendry samples and determine weights of individual size fractions of 10-20 µm, 20-63 µm and >63 µm
- Transfer effluent with particles <10 µm into a centrifuge for 10 minutes with 5000 rpm
- Determine weight of residual matter and calculate the amount of particles <10 µm

3.2 **Automated particle sizing technique (particle fractions of <0.5 µm to >100 µm)**

This sizing technique, based on the principles of Stokes's equation on vertical particle velocities in aqueous media, uses a SEDIGRAPH®, which is an automated, microprocessor controlled particle size analysator, to determine continuous particle distributions between 0.5 µm and 100 µm. Required time for an analysis of size fractions between 1 µm and 63 µm is about 20 minutes. Results can be directly compared to pipette- and Atterberg-techniques. To compare results with Laser® particle sizer, values have to be transformed from mass % (SEDIGRAPH®) to volume % (Laser® instruments).

3.2.1	i	If sample is free of sand (>63 µm) take 1-2 g of freeze dried sample (or an equivalent amount of wet sample) and dispers into 250 ml of a Calgon® solution of 0.01%
	ii	Homogenize the suspension with an ultrasonic stirrer for 3 minutes and transfer dispersed suspension into SEDIGRAPH® for further analyses
	iii	Use continuous digital or analog data output of SEDIGRAPH® for calculation of particle sizes
3.2.2.	i	In case that samples contain considerable amounts of coarse particles (>63 µm), take 2-5 g of freeze dried sample (or an equivalent amount of wet sample) and dispers into 250 ml of a Calgon® solution of 0.01%
	ii	Homogenize the suspension with an ultrasonic stirrer for 3 minutes
	iii	Place one measuring sieve of mesh size of 63 µm over a beaker of approx. 1-2 l
	iv	Rinse sample suspension carefully through the sieve with as little as possible additional water until size fraction >63 µm is clearly separated on top of sieve
	v	Carefully transfer particles from sieve into preweighted beaker, oven-/air-dry sample and determine weight of fractions >63 µm
	vi	Keep the effluent water (e.g. with all particles <63 µm) in the beaker
	vii	Transfer effluent with particles <63 µm into a centrifuge for 10 minutes with 5000 rpm
	viii	Transfer residual matter into 250 ml of a Calgon® solution of 0.01% and perform like above (2A.)

4. Data preparation

Results from SEDIGRAPH® (or similar automated techniques) should be graphically presented both as histogram and as sum curve of the grain size distribution.

Moreover, the results from SEDIGRAPH® (or similar automated techniques) should be used to calculate the following size fractions and some statistical values of the grain sizes of an individual sample:

size fractions:	<1 µm, 1-2 µm, 2-4 µm, 4-8 µm, 8-16 µm, 16-32 µm, 32-63 µm, >63 µm (or additional sand fractions if available)
statistical values:	median and mean diameter, sorting, skweness.

5. Final note

It is essential that the samples taken for grain size analyses are kept wet or freeze-dried until grain size analyzes is performed. Again (see above): air-dried or oven-dried material cannot be used for grain size analyses. Just to add: grinded samples can also not be used.

In order to obtain homogenized and comparable data of grain size analyses from the different MOLAR laboratories I recommend that a list of grain size measuring techniques is compiled, where each Institute states, which technique is available and/or can be used for the project. Keep

in mind that grain sizes should be measured for all the samples, which are taken of cores from the different MOLAR-sites.

Table 1 The most common size fractions

Sizes of sediment particle fractions (diameter values)

[mm]	[mm]	[µm]	phi-units [-log2 mm]	WENTWORTH-units
1/1024	0.00098	1	10.0	clay
1/512	0.00195	2	9.0	fine silt
1/256	0.00391	4	8.0	fine silt
1/128	0.00781	8	7.0	fine silt
1/064	0.01563	16	6.0	medium silt
1/032	0.03125	31	5.0	coarse silt
1/016	0.06250	63	4.0	coarse silt
1/008	0.12500	125	3.0	fine sand
1/004	0.25000	250	2.0	medium sand
1/002	0.50000	500	1.0	coarse sand
1	1.00000	1000	0.0	coarse sand
2	2.00000	2000	-1.0	fine gravel
4	4.00000	4000	-2.0	fine gravel
8	8.00000	8000	-3.0	medium gravel
16	16.00000	16000	-4.0	medium gravel
32	32.00000	32000	-5.0	coarse gravel

For further information contact:
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Equipment for Grain Size Analysis atEAWAG.
sieves [20 µm to 500 µm)
pipette-technique
ATTERBERG-technique
SEDIGRAPH®
LASER ®PARTICLEANALYZER
COULTER COUNTER®
SEM

MEASURING AND MODELLING THE DYNAMIC RESPONSE
OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO
ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

SEDIMENT CLADOCERA

PROTOCOL FOR ISOLATION, PREPARATION OF SLIDES AND COUNTING
Work Package 3.

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Sediment Cladocera: isolation, preparation of slides, counting

1. Equipment

Precision balance
Stirrer hot-plate
Compound microscope and/or good quality stereomicroscope
Counter

2. Isolation of cladoceran remains from the sediment

1. Weigh about 3 g wet sediment precisely, transfer into an Erlenmeyer flask (100 - 250 ml), add 50 ml of 10% KOH
2. Heat 10 - 15 minutes on a stirrer hot-plate (avoid boiling up!)
3. Fill up with tap water (cooling) and screen on a 40 μm sieve, washing well with use of a polyethylene wash bottle
   OPTIONAL (if carbonates are present)
   Put the residue from the sieve into a 100 ml beaker, add 10% HCl and repeat (3)
4. Transfer the residue into a labelled vial

3. Preparation of slides and counting

1. Put the residue into a calibrated 10 ml centrifuge tube
2. Let it settle (or centrifuge)
3. Reduce the volume to 2 - 10 ml by removing the supernate water
4. Mix well and take a subsample of 0.5 ml; distribute the subsample on 4 slides (the subsample should contain at least about 200 chydorids and 200 planktonic Cladocera)
5. Cover with 24x32 slips and count at about 100x magnification
6. After counting return all material back to the labelled vial
   OPTIONAL
   a. Fractions >100 μm and 100>(fraction)>40 μm are separated by screening and steps 1 - 6 are carried out separately. This improves the counting of large but scarce remains (e.g. ephippia of Daphnia).
   b. Staining or use of phase contrast is left to the discretion of the analyst
   c. Glycerol may be added in order to prebent drying up.

4. Expression of the results

Calculation of numbers per gram wet weight of the sediment is carried out according to Frey (1986) tab. 1, "total exuviae". Data on "lithography" (wet density, dry weight, loss on ignition) may be used for recalculation per unit volume, dry weight or organic matter).

5. Permanent slides

Permanent slides will be produced for intercalibrations of taxonomy and circulated among the members of the group. Two methods of making permanent slides are suggested:

a. The fast method: (1) let the subsample on the slide dry, (2) add a drop of 96% alcohol, (3) mount in EUPARAL

b. The method using Chlorazol Black for staining chitin and mounting in PVA: The technique as recommended by Mirka Prazakova was circulated among all members of the group.

6. References

Frey D., 1986: Cladocera Analysis.- p. 227 - 257 in: Berglund B.E. (ed.), Palaeohydrological changes in the temperate zone in the last 15 000 years. Subproject B. Lake and mire environments. Volume II. Specific methods. International Geological Correlation Programme, Project 158.

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MOLAR

MOLAR DIATOMS SAMPLING PROTOCOLS: LIVING COMMUNITIES, TRAPS

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Diatom Sampling Protocols: Living Communities, Traps

Sampling of living diatom communities (epilithon and, where appropriate, the plankton) and sediment diatom assemblages will be carried out at sites in work packages 1 and 3. Within these work packages the aims of this research have been set out.

Work Package 1

- To assess the seasonal variability in diatom communities in relation to seasonal changes in the physical and chemical environment

Work Package 3

- To assess seasonal variability in diatom communities by intensive sampling at small number of key sites (cf. above)

- To establish the relationship with other groups of 'fossilisable' organisms (chrysophytes, cladocerans, chironomids) and measured environmental variables

- To assess underlying trends and natural variability in climate from the fine detail analysis of the uppermost sediment at key sites

As set out in the MOLAR proposal and discussed at the Prague meeting the sites involved in these work packages and diatomists responsible for the analyses are tabulated below. Local site operators are responsible for carrying out the more frequent sampling.

1. Methods

1.1 Diatom communities

Sampling living diatom communities to assess their seasonal variability

i) In WP 1 this will involve sampling of each lake 2-3 times during the ice free period. Methods for epilithon sampling will follow those used in the ALPE programme (see below).

ii) In WP 3 sampling of diatom communities will be carried out at monthly intervals with one detailed transect to assess within lake variability and all benthic habitat types (epilithon, epiphyton, epipelon, epipsammon).

iii) In addition to sampling the epilithon, diatom plankton, when present, will also be sampled.

The chrysophyte sampling protocol is outlined elsewhere by Roland Schmidt.

1.2 Diatom community sampling: field methods for epilithon & plankton

Diatom epilithon is removed from stones, visibly uncontaminated by sediment, taken from c. 40 - 50 cm water depth along a c. 10 - 20 m stretch of shoreline. Sampling sites close to possible point sources of water quality variation, eg. inflow streams, should be avoided. Stones in shallower water (< 30 cm) should also be avoided since these are more likely to dry out as a result of fluctuations in lake level.

Algal growth on the stone is detached from the whole of the upper surface using a toothbrush and by repeated washings with distilled water from a wash bottle. The sample is collected in a 1 litre capacity polythene water sample bottle or similar via a polythene funnel. Three stones should washed into the bottle in this way to give a single mixed sample. At least three such composite samples should be taken on each sampling visit so that between sample variability can be inspected. Samples should be preserved by addition of a few drops of Lugols Iodine immediately after collection.

Living diatom material, both periphyton and plankton should be preserved with Lugol's Iodine. However, where SEM is planned the group from ILIMNOL argued that formaldehyde is a preferable preservative as they believe that Lugol's Iodine can damage silica surfaces.

Sampling of diatom plankton will follow standard techniques (Lund *et.al.* 1958). Initially it was suggested that where a planktonic diatom community is present, 250 ml or more of lake water would be collected from the surface water above the deepest point of the lake. Given that phytoplankton develop at different levels in the water column it was suggested that an amalgamation of water from the whole water column would be better approach. This could either be collected using a tube sampler, a tube with a pump or Rutner bottle. Where possible samples can be taken along with the depth profiles for water chemistry (see also Jan Fott's suggestions for efficient phytoplankton sampling and analysis).

Samples are to be preserved using Lugol's Iodene. Diatom cells are concentrated by settling, as detailed in the reference. A qualatative assessment (count of c. 100 valves) for diatom frustules with and without chloroplasts should be made (using either a wet mount on a microscope slide, or using an inverted microscope) before cleaning the material for detailed taxonomic and quantitative work.

1.3 Variation in diatom communities with depth: transect work

In the highly transparent waters of mountain lakes the photic zone extends to greater depths than in many other lake types and in some MOLAR sites may extend to the deepest point of the lake. Therefore at the WP 3 sites a single transect, during the summer period of 1996, will be made to sample all diatom communities and their variability in relation to depth.

The protocol is outlined jointly from the previous experience of the Barcelona & UCL laboratories.

The transect will be carried out preferably on the side of the lake with the lightest aspect. However, in addition to the need to sample on a side of the lake which is comparable with other lakes (lightest aspect), it may also be desirable to include the greatest diversity of benthic diatom habitats (substrates), for example epilithon, epiphyton, epipsammon, epipelon. Where possible divers will be employed to carry out the sampling. Alternatively a rope transect can be laid out with a shore station, anchor and buoys (Raven 1988). Sampling would then be made from a boat using an Ekman grab. The sampling interval will depend on the gradient of the lake bed, so rather than sampling at prescribed horizontal intervals, (eg. horizontal intervals of c. 2-5 m), it may be more appropriate to sample at approximately even vertical intervals. The spacing of the transect samples will vary with the depth of the photic zone and maximum depth of the study lake. However, a *minimum* of 5 sampling stations with samples from all the benthic habitats is required. As indicated above, the means of sampling will vary between groups and may include: a boat and transect line using an Ekman grab; divers picking samples and using some type of closed-chamber epilithon sampler (see protocol for reference); or at Lake Redo the use of a robot sampler. Replicate samples of each substrate should be taken at each sampling station, in order that within station variability can compared against between station variation. Where divers take the samples it will be necessary to have some form of closed chamber sampler to remove epilithon from bedrock (brush & syringe Barcelona design, cf. Flower 1985). Epipsammon should be sampled according to Round (1965).

2. Diatom preparation, identification and counting: living communities, trap & fossil assemblages

All samples will be prepared by the laboratory who will carry out the diatom analysis. Preparation will follow the methods used in the ALPE project (Battarbee 1986, Wathne *et.al.* 1995)

Preparation and counting of diatoms from sediment cores will follow standard procedures (Battarbee 1986). Cleaned diatoms are identified and counted under oil immersion at a magnification of c. x 1000 or x 1200 usually under phase contrast, bright field or DIC illumination In cores diatom cell concentrations are determined using the microsphere method of Battarbee & Kneen (1982), for the core samples a minimum of 100 valves will be counted in contiguous samples (Renberg 1990).

Sediment coring protocols are discussed elsewhere, diatom and chrysophte sub-samples will be taken from the dated mastercore, which will be sliced at 2 mm intervals throughout the core.

3. Taxonomic harmonisation

Taxonomic harmonisation will be achieved through workshops (the first meeting took place in London 13-14 June 1996, jointly with cladoceran and chironomid analysts) and the exchange of diatom material in the form of published references, descriptions, microscope slides, photographs and material for SEM examination.

It would be desirable and useful to document the expertise we have built up in ALPE and MOLAR with the production of a diatom iconograph which could be circulated, at least initially, amongst the MOLAR diatom group.

An analytical quality control exercise (Kreiser & Battarbee 1987, Munro *et.al.* 1990) will be carried out. To some extent the lakes fall into 2 types, those with planktonic diatom floras and those with mainly benthic diatom floras. The idea was put forward that a group of 3 analysts (Sanna, Andre, Karin) dealing with the former would exchange and count slides and a group of 3 analysts dealing with the latter (Sergi, Elena, Nigel) would exchange and count slides. A comparison would then be made between the 3 counts for each of the 6 sites. In this way differences in taxonomic concepts can be identified quickly.

4. Sediment Trapping

4.1 Rationale for sediment trapping & diatom/chrysophyte work

Sediment traps provide a useful means of investigating the relationship between living diatom and chrysophyte communities and the records of these communities in recent sediment (eg. Cameron 1995). The rationale for their use is set out below.

i) Traps provide a link in space between diatom communities and lake sediment diatom assemblages.

ii) Traps enable short term events to be resolved. Where these events give a weak signal being either of too short a duration or of too low an intensity, for example where too few valves accumulate to register in the stratigraphic record, traps may provide the only clear record.

iii) Traps provide a continuous relative measure of the composition of the diatom mixture arriving at the sediment during any exposure period. They are a simple means of estimating relative diatom productivity as opposed to the measures of standing crop given by sampling living communities.

iv) Resuspension of sediment can be monitored by sediment traps. Comparison in time & space (not concerned so much with this in these well mixed lakes) between traps of species composition, live to dead cell ratios, and dry weight of sedimenting material collected may allow estimation of the intensity of resuspension.

4.2 Sediment trap design

Sampling will be at the intervals indicated in the site protocols for WP3, ie. monthly in the ice-free period and once at the end of the ice-covered period.

i) There are various pre-existing designs for sediment trap arrays, and to some degree for the traps themselves, amongst the groups. However, it is not felt that this limited diversity of design is critical and therefore can be retained.

ii) The traps themselves must be simple, cylindrical traps (having no modifications that would influence sediment collection, such as funnel shaped mouths etc.). The exception to this will be the marine sediment traps used in Lake Redo, but here Sergi Pla will employ a comparable, cylindrical trap array in addition to the marine traps. The design of the traps will follow the recommendations of a review article on sediment trap technique (Bloesch & Burns 1980, also Blomqvist & Hakanson 1981). Andy Lotter will circulate a copy of the former article to those of the diatom/chrysophyte group who do not have a copy. A key element of the recommendations for cylindrical traps is that the ratio height:width of the trap mouth (aspect ratio) must be greater than 5.

iii) A single array of traps will be employed in each lake. This will be suspended c. 1.5 m to 3 m above the lake bed and close to the coring point or deepest point of the lake. The trap array could have replicate cylinders (3 or 4) so that between trap variability can be examined. However, it may be preferable to amalgamate SM, given that the monthly amounts of sedimenting material are likely to be low and that in many published studies between trap variability has been shown to be small. Our primary aim is to have enough SM for analyses. An alternative would be to use larger traps, so long as the aspect ratio is the same (see ii above). It should be noted that at three lakes: Ovre Neadalsvatn, Lake Redo and Gossenkollersee; sediment trapping at bi-annual intervals is also required by WP2. Site operators should be aware of this when sampling and distributing sedimenting material collected at the intervals required by WP2.

iv) It will be useful to continue trapping during the period of ice cover. Consideration should be given to avoiding dragging of the traps by ice. Traps may be placed underneath the ice in the winter once ice has formed or the marker buoy may be a subsurface float located by a pole.

v) It should be possible to make comparisons of sediment flux between and within lakes at different times. Hence the use of similar trap designs.

vi) Wet sediment will be examined for live/dead/broken cells prior to cleaning and counting. Wet mounts can be made as for plankton counting using either a microscope slide & coverslip or inverted microscope and counting chamber. This will provide a measure of sediment resupension prior to taxonomic work on cleaned slides. The purpose of this is to gain an approximate idea of sources of sedimenting material eg. from resuspension vs. recently living cells. A percentage count of the classes live/dead/broken cells or valves will be adequate.

vii) Relatively large cylindrical traps are preferable as sampling intervals are quite short and productivity may be low. In addition to diatoms and chrysophytes, sediment dry weight, loss-on-ignition and in Gossenkollersee, Redo, and Ovre Neadalsvatn ^{210}Pb analysis will be made for WP2

viii) For diatom counting of sedimenting material aswell as core assemblages we should routinely count chrysophyte cyst numbers so that both cyst and diatom concentrations can be counted and if required cyst to valve ratios can be calculated. The microsphere technique (Battarbee & Kneen 1982) was reiterated as the method for calculating fossil concentrations. Copies of this publication and the ECRC protocol for preparing diatom samples and slides have been distributed to those who required this information.

^{210}Pb analysis will be made for WP2.

MOLAR sites & diatomists involved in WP1 & WP3

Site	Diatomist	MOLAR WP 1	MOLAR WP 3
Chuna	Nadia Solovieva	X	
Øvre Neådalsvatn	Nigel Cameron	X	X
Stavsvatn	Nigel Cameron	X	
Lochnagar	Nigel Cameron	X	
La Caldera	P.Sanchez-Castillo	X	
Redo	Sergi Pla	X	X
Paione Superiore	?Aldo Marchetto	X	
Dlugi Staw	Barbara Kaweka	X	
Starolesnianske Pleso	Elena Stefkova	X	
Terianske Pleso	Elena Stefkova		X
Gossenköllesee	Karin Koinig	X	X
Jezero Ledvicah	Milijan Sisko		X
Saanajärvi	Sanna Sorvari		X
Hagelsee	Andy Lotter		X
Laguna Cimera (secondary site)	Manolo Toro	X	x

5. References

Battarbee, R.W. (1986) Diatom analysis. In: *Handbook of Holocene palaeoecology & palaeohydrology*. Ed. Berglund, B.E., John Wiley, Chichester.

Battarbee, R.W. & Kneen, M.J. (1982) The use of electronically counted microspheres in absolute diatom analysis. *Limnol. & Oceanogr.*, 27, 184-188

Cameron, N.G. (1995). The representation of diatom communities by fossil assemblages in a small acid lake. *Journal of Palaeolimnology* 14: 185-233.

Bloesh, J. & Burns, N.M. (1980) A critical review of sedimentation trap technique *Schweiz Z. Hydrol.*, 42, 15-54

Blomquvist, S. & Hakansson, L. (1981) A review of sediment traps in aquatic environments. *Arch. Hydrobiol.*, 91, 101-132

Flower, R.J. (1985) An improved epilithon sampler and its evaluation in two acid lakes. *Br.phycol.J* 20: 109-115

Lund, J.W.G., Kipling, C. & Le Cren, E. D. (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiol.* 11, 143-170

Munro, M.A.R., Kreiser, A.M., Battarbee, R.W., Juggins, S., Stevenson, A.C., Anderson, D.S., Anderson, N.J., Berge, F., Birks, H.J.B., Davis, R.B., Flower, R.J., Fritz, S.C., Haworth, E.Y., Jones, V.J., Kingston, J.C. & Renberg, I. 1990. Diatom quality control and data handling. *Philosophical Transactions of the Royal Society, London* B 327, 257-261.

Raven, P.J. 1988. Occurrence of *Sphagnum* moss in the sublittoral of several small oligotrophic lakes in Galloway, southwest Scotland. *Aquatic Botany* 30 233-230

Renberg, I. (1990) A 12600 year perspective of the acidification of Lilla Öresjön, southwest Sweden. *Phil. Trans.R. Soc. Lond. B* 327, 357-361

Round, F.E. (1965) The epipsammon: a relatively unknown algal association. *Br. Phycol. Bull.* 2, 456-462.

Wathne, B.M., Patrick, S.T., Monteith, D.M. & Barth H.(Eds.) (1995) *AL:PE 1 Report.* EC Ecosystems Research Report 9.

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SEDIMENT TRAPS FOR MEASURING DEPOSITION OF ^{210}Pb AND SCP

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Sediment traps for measuring annual deposition of ^{210}Pb and SCPs

1. Sampling methods

The rationale is to collect both SCPs and ^{210}Pb which settle to the the lake bottom The amount of material in the traps will be compared with data resulting from soil inventories and total material in the sediment record. We expect to measure a gross flux composed of atmospheric and catchment input and resuspended material from the lake bottom. To distinguish to a certain degree between these two components we will use a trap 2 m beneath lake surface and one 2 m above the bottom of the lake. Two time periods will be analyzed: the winter season (ice-covered period), and the summer season (open water situation).

All possible disturbancies which could interact with the sedimentation of particles must be avoided, especially coring must be done very carefully.

In addition to these simple traps which have a small effective catch area, a large marine trap will be exposed in Estany Redò, with higher sampling frequency during the summer months.

2. Trap design

The design of the simple traps is the same as used for the chrysophyte sampling: it consists of an array of 4 tubes (plexiglass or PVC, best made of opaque material), similar to those used for sediment coring. The lenght to diameter ratio should be around 10, the diameter approximately 60 mm. The tubes are closed at the lower end with a tight cap and 4 of them are fixed on a small platform with a central stick which gives the array the necessary rigidity. Details of the design are available at Mondsee (Roland Schmidt; he will also be able to prepare those traps at a modest price).

The mooring system (see the coresponding section for traps in WP3, by Nigel Cameron) must allow a save retrieval of the traps, and should not disturb the sediment surface. Also fishing and other sampling activities etc. must be considered before fixing the mooring system.

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CHRYSOPHYCEAN ANALYSIS

SILICEOUS CYSTS, SCALES, PLANKTON SAMPLES

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Chrysophycean analysis
Siliceous cysts, scales, plankton samples

1. Sediment Cores

1.1. Coring and subsampling

Procedures followed are according to diatom analysis.

1.2. Preparation

1.2.1 Chemical treatment

Procedures followed are according to diatom preparation (Battarbee, 1986).

1.2.2 LM

Procedures followed are according to diatom preparation (Battarbee, 1986), spiking with microspheres (Battarbee & Kneen, 1982).

1.2.3 SEM

Chemically cleaned suspensions as used for diatoms (without microspheres!) are diluted to an appropriate density, evaporated on cover slips, mounted on SEM stubs and sputtered.

Battarbee, R. W. & M. J. Kneen 1982. The use of electronically counted microspheres in absolute diatom analysis. Limnol. Oceanogr. 27, 184-188.

Battarbee, R. W. 1986. Diatom analysis. In: Berglund, B. E. (ed.), Handbook of Holocene Paleoecology and Paleohydrology. John Wiley & Sons Ltd., Chichester: 527-570.

1.3 Identification

1.3.1 Cysts

Facher, E. & R. Schmidt 1996. A siliceous chrysophycean cyst-based pH transfer function for Central European lakes. Journal of Paleolimnology (in press).

Duff, K. E.; Zeeb B. A. & J. P. Smol 1995. Atlas of Chrysophycean cysts. Developments in Hydrobiology 99. Kluwer Academic Publishers, Dordrecht, 189 pp.

Unknown cyst types are described according to:

Cronberg, G & C. D. Sandgren 1986. A proposal for the development of standardised nomenclature and terminology for chrysophycean statospores. In: Kristiansen, J. & R. A. Andersen (ed.), Chrysophytes: aspects and problems. Cambridge University Press, Cambridge: 317-328.

1.3.2 Scales and bristles

Asmund, B. & J. Kristiansen 1986. The Genus *Mallomonas*. Opera Botanica 85: 1-128.

Takahashi, E. 1978. Electron Microscopical Studies of the Synuraceae (Chrysophyceae) in Japan, Taxonomy and Ecology. Tokia, University Press, Tokyo: 1-143.

1.4 Counting and quantification

a) Microsphere/diatom/cyst relations and absolute numbers are evaluated in LM.
b) Since cyst types cannot be identified in LM, identification and counting has to be done in SEM.
c) Recommended number of cysts to be counted in SEM and LM: 200.
Magnification: LM: 1000x -1250x (oil immersion, high resolution phase contrast objectives)
SEM: 4800x

The absolute number of each cyst type is calculated by the following equations:

a) $$total\ number\ of\ cysts\,(LM) = \frac{microspheres\ introduced * cysts\ counted\ (LM)}{microspheres\ counted\ (LM)}$$

b) $$number\ of\ cyst\ type = \frac{\%\ of\ cyst\ type\,(SEM) * total\ number\ of\ cysts\,(LM)}{100}$$

1.5 pH reconstruction

As for diatoms, the pH is calculated by using WA regression and calibration (WACALIB). Cyst optima and tolerances are listed in Facher & Schmidt (1996).

2 Sediment trap samples

2.1 Trap type

A simple Bloesch-type (plastic tubes of 60 cm in length and 6 cm in diameter; 6 tubes per trap) is used.

Bloesch, J. & N. M. Burns 1980. A critical review of sedimentation trap technique. Schweiz. Z. Hydrol. 42: 15-55.

2.2 Sampling

Half of the water can be removed carefully. The rest is filled into plastic bottles and fixed with formol.

2.2.1 Sampling intervals

A pilot study on lake Gossenkölle indicated, that because of low accumulation rates in high alpine lakes, sampling intervals during summer less than monthly were insufficient. Since cyst production under ice is extremely low, traps were left during winter in the water and sampled after ice break.

2.3 Preparation

Sediment trap samples are centrifuged and washed carefully (Formol fixation). For further preparation see 1.2.

2.4 Identification, Counting and Quantification

See 1.3 and 1.4.

Application: Facher E. & R. Schmidt 1996. Application of chrysophycean sediment trap data and a cyst-based pH transfer function of annually laminated sediments (Lake Plesné, Czech Republic). Beih. Nova Hedwegia (in press).

3. Plankton samples

For cyst/taxa relation plankton samples are required.

3.1 Sampling

By the use of a Schindler sampler and/or nets (mesh size 10 µm and 50 µm). Living cells should be investigated. Samples fixed with Formol.

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PIGMENT AND CNS IN SEDIMENTS

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Pigments and CNS in sediments

Work Package 3

1. Field and shipping instructions

During the field work, the sediment should be protected against direct sun light and excessive warming. After slicing the core, the sediment samples should be deep frozen (-20°C) as soon as possible and shipped frozen to Pallanza.

As regards pigment stability, the frozen samples are stable for ca. 6 months. Sediment samples could be sent in an "ice-cream" like box with dry ice. Warning: Be sure that the ink or label on the bags are resistant to very low temperature such as with dry ice.

We need some basic data from site operator on each sample: Water content and Loss On Ignition at 550C.

The amount of sediment needed to perform pigment and CNS determination is ca. 3 g of fresh sediment.

2. Laboratory Methods

Algal and bacterial pigments will be extracted (overnight, at 10°C, in the dark and under nitrogen) from ca. 1 - 2 g wet sediments using 90% acetone and then centrifuged at 3000 rpm for 10 minutes in 15 ml glass centrifuge tubes. Duplicate samples will be extracted with HPLC-grade Acetone 90%.

Chlorophyll derivatives (CD) and total carotenoids (TC) will be extracted with 90% acetone and expressed as in Guilizzoni et al. (1983) and Züllig (1982), respectively.

Specific pigments will be determined by ion-pairing, reverse-phase HPLC (modified from Mauntoura & Llewellyn, 1983, and Hurley, 1988) and expressed as nanomoles per gram of organic matter. The ion pairing (tetrabutyl ammonium phosphate 10^{-3} M) allows for greater resolution of the dephytolated acidic chloropigments (Chl c, chlorophyllide a, and pheophorbide a). The equipment employed consisted of a gradient pumping system and dual channel variable wavelength UV-VIS detector (set at 460 nm and 656 nm for carotenoids and chloropigments, respectively) controlled by a computer (Beckman System Gold). An auto-sampler for sample injection was connected through a precolumn to a reverse-phase C18 ODS column (5 µm particle size; 250 mm a 4.6 mm i.d.). After sample injection (330 µl of acetone extract not dried in a rotary evaporator), a gradient program that ramped from 85% mobile-phase A (80:20, by vol. methanol:aqueous solution of 0.001 M ion-pairing and 0.001 M propionic acid) to 100% mobile-phase B (60:40, acetone:methanol) in 30 minutes with a hold for 20 minutes provided sufficient resolution of all pigments of interest. Flow rate from 1 ml min-1 to 2 ml min-1. The column was re-equilibrated between samples by linear ramping to 85% mobile-phase A for 5 minutes and maintenance for 10 minutes before sample injection. With this procedure, we are able to separate zeaxanthin from lutein and ϑ-carotene from phaeophytin *a*. Analysis of replicates sediment samples yielded at C.V. of 4.5% - 11.5%, depending on pigments.

Identification of all pigments was confirmed by comparing the absorption spectral characteristics and chromatographic mobility of pigments isolated from sediments with those obtained from TLC analysis (Züllig, 1982; Guilizzoni et al., 1986), commercial standards (Sigma Chemical Co.), standards kindly donated by Hoffmann-La Roche of Basle, water samples of known phytoplankton

composition, and published values (Foppen, 1971; Davies, 1976; Züllig, 1982; Mantoura & Llewellyn, 1983). Standard of okenone was obtained from culture of Chromatium okenii kindly provided by Dr. H. Züllig. Spectra will be obtained with a Perkin-Elmer Lambda 6 spectrophotometer.

Concentrations of pigments will be determined, following Mantoura & Llewellyn (1983), on the basis of molar extinction coefficients at the detection wavelengths. The molar extinction coefficient El%460 and E1%656 is derived from the E1% max reported in Davis (1976) and Mantoura & Llewellyn (1983).

Total carbon and nitrogen will be determined on dry sediment using a CNS analyzer (Carlo Erba). The inorganic C will be measured with the CNS analyser on a sub-sub.sample previously ignited at 550°C. Total nitrogen is essentially organic N, since inorganic N is usually negligible (<2% dry weight).

3. References

Davies, B.H. 1976. Carotenoids. In: T.W. Goodwin (Ed). Chemistry and Biochemistry of Plant Pigments, Academic Press London, New York, San Francisco, Vol. 2: 38-165.

Guilizzoni, P., G. Bonomi, G. Galanti & D. Ruggiu. 1983. Relationship between sedimentary pigments and primary production: evidence from core analyses of twelve Italian lakes. Hydrobiologia 103: 103-106.

Guilllizzoni, P. A. Lami, D. Ruggiu & G. Bonomi. 1986. Stratigraphy of specific algal and bacterial carotenoids in the sediments of Lake Varese (N. Italy). Hydrobiologia 143: 321-325.

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MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

PROTOCOL FOR SAMPLING FOR SPHEROIDAL CARBONACEOUS PARTICLE ANALYSIS

Neil Rose

University College London

Protocol for Sampling for Spheroidal Carbonaceous Particle Analysis

SCP analysis will be undertaken on sediment core, sediment trap, snow pack, lake water and bulk deposition samples in WP2, and on sediment cores for new sites in WP3.

All SCP samples should be sent to:

Neil Rose,
Environmental Change Research Centre,
University College London,
26 Bedford Way, London WC1H 0AP, U.K.

e-mail: nrose@geog.ucl.ac.uk
Tel: +44 0171 387 7050 (ext. 5543)
Fax: +44 0171 380 7565

1. Sediment

0.1 - 0.2 g of dried sediment should be sent from every level of the extruded sediment core. Samples should be sent in individual plastic bags, clearly labelled with site name and sediment depth.

Cores will be analysed from:
WP2- Jorisee, Gossenkollersee, Redo & Øvre Neådalsvatn
WP3- Jezero Ledvicah, Saanjarvi & Hagelsee

It is important that there is a good sediment water interface and that the cores are extruded following the coring protocol.

2. Sediment traps

SCP will be analysed from the monthly sediment trap samples from Redo, and from any other sediment traps deployed in WP2 sites (e.g. bi-annually in Lochnagar, Gossenkollersee). Please refer to the sediment trapping protocol.

Again, 0.1 - 0.2 g of dried sediment is required (if possible) and all samples should be clearly labelled with site and most importantly the period of deployment (e.g. 3rd Sept 1996 - 4th October 1996). It is also important to know the total dry weight of sediment collected in the sediment traps over the sampling interval.

3. Snow

SCP are to be analysed from snow pack samples taken monthly (if possible) from WP2 sites. The sampling should follow the snow pack protocol. 2 litres (unpacked volume) of snow should be sampled from every 10 cm layer of the snow-pack. The snow should be melted and then filtered through a Whatman GF/C filter. Fold the filter in half once, so that any filtered particles are enclosed and store each filter paper flat in an individual plastic bag. Clearly label each plastic bag with site name, date, `Snow' and snow depth (e.g. 50-60 cm). The bags should then be sent to Neil Rose at ECRC.

It is also important to include the following information with the samples. Date and location of sampling and the volume of snow melted and filtered for SCP for each layer.

NB. Snow analyses for SCP are new and it maybe that the volume required will change after initial analyses have been undertaken.

4. Bulk Deposition

SCP are to be analysed from every bulk deposition sample for WP2. i.e. weekly samples for Jorisee, Gossenkollersee, Redo, Øvre Neådalsvatn and weekly in summer and bi-weekly in winter at Lochnagar and Starolesnienske Pleso from October 1996 - March 1998. Please refer to the bulk deposition sampling protocols.

A note should be made of the total wet deposition before removing the sample and then the walls of the collector should be washed with deionised water to remove any particulate matter sticking to the walls.

If a bulk deposition sampler is used for SCP only then the whole sample should be filtered through a Whatman GF/C filter, if more analyses are to be done then a known fraction of the deposition should be filtered through a Whatman GF/C filter. As much sample as possible should be filtered for SCP. Filters should be folded once to include any particles and stored flat in an individual, labelled (site name, `Bulk deposition', dates) plastic bag. These can be sent in batches (e.g. every 2 months) to Neil Rose.

If the bulk deposition collector contains snow or ice, again, a known fraction should be sub-sampled for SCP, and melted before filtration. If no rain or snow has fallen over the sampling period then the walls of the collection vessel should be washed with deionised water and a known fraction of the resulting suspension filtered for SCP.

With each sample it is essential to know:

- The sampling interval dates
- The total amount of wet deposition that has fallen over the sampling period
- The fraction of the total that has been filtered for SCP.

5. Dry and Wet deposition

At Redo only initially for a trial period, dry and wet deposition samples will be analysed for SCP. Filters will first be analysed for organics and then passed on for SCP. If this approach fails to work, the bulk deposition sampling outlined above will be undertaken. Samples should be labelled with date, `Redo', and `dry' or `wet deposition'.

6. Lake water

At twice yearly intervals (end of summer, end of winter) (see lake water sampling protocol) at least 20 litres of lake water form the outlet of the lake should be filtered through a Whatman GF/C filter. The filter should be treated as above. If necessary, more than 1 filter can be used to filter the required sample volume. Sample bag should be labelled with site name, `lake water', and the volume filtered.

Volumes required may be subject to change after initial analysis.

7. Analytical Methods

For sediment material the technique described in Rose (1994) will be used for SCP analysis. This involves the sequential removal of unwanted sediment fractions by chemical attack. HF, HNO_3 and HCl are used to remove silicates (biogenic and mineral), organic material and carbonates respectively. A known fraction of the resulting suspension is evaporated onto a cover-slip and counted under a microscope at x400 magnification.

All other samples (snow, bulk deposition, lake water) should be sent as GF/C filters. These readily dissolve in HF although a white BF_3 precipitate can sometimes form. This is dissolved using HCl leaving a suspension of particulates. Because of this precipitate formation, small GF/C filters should be used wherever possible and especially for the smaller sample volumes (e.g. bulk deposition).

8. References

Rose, N.L. (1994) A note on further refinements to a procedure for the extraction of carbonaceous fly-ash particles from sediments. J. Paleolim. 11: 201-204.

PLEASE REMEMBER THE FOLLOWING.....

- CLEARLY LABEL THE SAMPLES
- SUPPLY ALL THE REQUESTED INFORMATION FOR EVERY SAMPLE (e.g. volumes, dates etc) WHEN IT IS SENT
- INFORM Neil Rose IF ANY SAMPLES ARE MISSING

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

PROTOCOLS FOR THE ANALYSIS OF ORGANIC MICROPOLLUTANTS IN

SEDIMENTS
WET-ONLY DEPOSITION
DRY DEPOSITION
BULK DEPOSITION
SNOW
WATER
AIR

Joan O. Grimalt
Pilar Fernandez
Rosa Vilanova
Lourdes Berdie

Department of Environmental Chemistry (CID-CSIC). Barcelona. Catalonia.

1. SEDIMENT CORES

1.1 Compounds to be determined.

* Hexachlorobenzene
* DDTs (namely, pp'-DDE)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH: Parent compounds: fluorene, phenanthrene, anthracene, fluoranthene, acephenanthrylene, pyrene, benzo[*a*]fluorene, benzo[*ghi*]fluoranthene, cyclopenta[*cd*]pyrene, benz[*a*]anthracene, chrysene+triphenyene, benzo[*b*+*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[7,1,2,3-*cdef*]chrysene, indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene, dibenz[*aj*]anthracene, dibenz[*ah*]anthracene, dibenz[*ac*]anthracene, benzo[*b*]chrysene, coronene.

  Methyl derivatives: 3-methylphenanthrene, 2-methylphenanthrene, 4H-cyclopenta-[*def*]phenanthrene, 4-methylphenanthrene, 1-methylphenanthrene, dimehtyl-phenanthrenes (10 individual isomers), retene, methylfluoranthenes/pyrenes (7 individual isomers).

  Sulphur derivatives: naphto[1,2-*b*]thiophene, dibenzothiophene, naphto[2,1-*b*]-thiophene, 4-methyldibenzothiophene, 3+2-methyldibenzothiophene, 1-methyldibenzothiophene, benzo(*b*)naphto[1,2-*d*]thiophene, benzo(*b*)naphto-[2,1-*d*]thiophene, benzo(*b*)naphto[2,3-*d*]thiophene.

1.2 Other measurements.

* Total organic carbon

1.3 Materials and reagents.

-Cutting tools
-Tweezers
-Aluminum foil
-Sulphuric acid for analysis grade
-Distilled water
-Milli-Q water
-Potassium hydroxide (for analysis)
-*n*-Hexane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Dichloromethane (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-*Iso*-octane (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-alumina (70-230 mesh, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

1.4 Cleaning.

1. The material to be used for core sampling and sub-sectioning must be:
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40°C for drying.

2. Prior to sampling they have to be rinsed with Milli Q water and acetone.

1.5 Sampling and sub-sectioning.

1. After sampling the core must be kept upright to avoid any disturbance of the sediment profile.

2. The sediment sections (0.5-1 cm) for trace organic analyses should be obtained from the central core area to avoid contamination from the walls and cross-contamination from other core sections.

3. The collected sediment in each section should be wrapped in aluminum foil. Two aluminum sheets must be used: one in direct contact with the sediment (previously rinsed), another to wrap the first. This last one should be labelled. A paper label than a water-resistant mark is preferred. Sediment storage in glass jars is not recommended because of breaking during frozening and de-frozening and transport to the laboratory.

1.6 Storage.

Sample sediments should be stored at -20°C after sub-sectioning. These frozening conditions should also be kept during transport.

1.7 Sample amount.

0.1-1 g. of sediment are needed.

1.8 Transport.

Samples should be sent within boxes containing dry ice to the address indicated above. The contact persons are also the same as indicated above.

1.9 Analyses (extraction and fractionation).

About 0.1-1 g of wet sediment are extracted by sonication with methanol (1 x 20 ml; 20 min) to separate the interstitial water. Subsequent extractions are performed with (2:1, v/v) dichloromethane-methanol (3 x 20 ml; 20 min). The extracts are combined and spiked with d_{10}-pyrene, d_{12}-benzo[*ghi*]perylene and PCB 30). The combined extracts are vacuum evaporated to 10 ml and hydrolyzed overnight with 20 ml of (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the

solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigourous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

1.10 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

1.11 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 80 to 150°C at 10°C/min and to 300°C at 4°C/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

1.12 Quantitation.

Authentic standards of hexachlorobenzene pp'DDE and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

1.13 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

2. WET-ONLY DEPOSITION

2.1 Compounds to be determined.

* Hexachlorobenzene
* Hexachlorocyclohexanes (namely a and g isomers)
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

2.2 Other measurements.

* Weight of total particles
* Total organic particulate carbon (when sufficient material will be collected)

2.3 Materials and reagents.

-Dry and wet+dry deposition sampler.
-Millipore 47-mm filtration apparatus
-0.45 mm PTFE fiber glass filters
-Octadecylsilane membrane extraction disks (47 mm diameter, 0.5 mm thickness). Each disk contains about 500 mg C_{18} bonded silica.
-Glass bottles
-Test tubes
-Pasteur pipettes
-Tweezers
-Aluminum foil
-Sodium sulphate (analysis grade)
-Milli-Q water
-Ethyl acetate (for trace organic analysis, Merck)
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

2.4 Cleaning.

Plastic should be avoided in any instance.

1. Tweezers and the dry and wet+dry reservoirs should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40°C for drying.

2. Glass bottles and the Millipore 47-mm filtration apparatus should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-emptied, cleaned with tap water, Milli-Q water and acetone.
-left to dry and the open parts covered with aluminum foil.

3. The glass fiber filters will be pre-cleaned by the Department of Environmental Chemistry.

4. The membrane extraction disks should be washed with (1:1) dichloromethane:ethyl acetate (2 x 10 ml) and then with methanol (2 x 10 mL). BEFORE ALLOWING THE DISK BECOME DRY, the water sample should be poured into the filtration apparatus.

5. The Pasteur pipettes were kiln-fired at 400°C.

2.5 Filtration.

-Record the water volume collected.
-Record the filter label (filters are pre-weighted in the Department of Environmental Chemistry).
-Take the filter with the tweezers and place it in the filtration apparatus.
-Pour the water in the upper part and connect the vacuum.
-Add more water until total volume. Stir adequately to insure that all the suspended particles in the reservoir are transferred to the filtration apparatus. If needed Milli-Q water can be added to the dry+wet reservoir to complete particle transfer.
-Store the filter as indicated below.

2.6 Adsorption on membrane extraction disks.

-Transfer the filtered water to a glass bottle.
-Rinse the filtration apparatus with Milli-Q water.
-Take the disk with the tweezers and place it in the filtration apparatus.
-Check whether the upper and lower parts of the apparatus are well assembled.
-Connect the apparatus to vacuum.
-Wash the membrane extraction disk with (1:1) dichloromethane:ethyl acetate (2 x 10 ml)
-Wash the membrane extraction disk with methanol (2 x 10 mL).
-BEFORE ALLOWING THE DISK BECOME DRY, pour the water into the filtration apparatus.
-Water extraction should be slow. Adjust vacuum for a flow of 2 liters/hour.
-Never allow the membrane extraction disk go to dryness during water adsorption. If by accident the membrane goes to dryness the rest of the water should be adsorbed with a new one (which should be cleaned as previously described).
-Store the membrane wrapped in aluminum foil. DO NOT BEND.

2.7 Storage.

-BEND the FILTER with the tweezers. The two parts containing the particulate material should face each other.
-Double wrap it in aluminum foil.
-DO NOT BEND the MEMBRANE EXTRACTION DISK, just wrap it in aluminum foil.

-Record the filter label and give a sample identification. This information together with period of collection, date, site, temperature range during collection and precipitation should be indicated in a sample protocol form.
-Store the wrapped and labelled filters and membrane extraction disks at -20°C.

2.8 Blank.

1. Prepare a blank by filtration and membrane disk extraction of 2 liters of Milli-Q water in the same place where the water samples are analyzed.

2. Use one of the filters and membrane extraction disks sent by the Department of Environmental Chemistry for this purpose.

3. Record the filter and membrane extraction disk labels.

2.9 Transport.

Samples should be sent within boxes containing dry ice to the address indicated above. The contact persons are also the same as indicated above.

2.10 Analyses (extraction)

The filters are freeze-dried, weighed and placed in test tubes. A mixture (*ca.* 10 ml) of (2:1) dichloromethane:methanol is added and the test tube is sonicated for 20 min. The solvent is decanted by centrifugation. The procedure is repeated two more times. The combined extracts are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

The membrane disks are introduced in the filtration apparatus. 5 Ml of methanol are introduced. The suspension is left to stand 3 min and then drawn under vacuum. 5 Ml of ethyl acetate are added to the disk, let to stand for 3 min and drawn through under vacuum. 5 Ml of dichloromethane are added, they are also let to stand for 3 min and drawn through under vacuum. The eluates are combined and dried over anhydrous sodium sulfate to remove the excess of water. Then, they are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

2.11 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

2.12 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column

(5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 80 to 150°C at 10°C/min and to 300°C at 4°C/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

2.13 Quantitation.

Authentic standards of hexachlorobenzene, a-, g- and d-hexachlorocyclohexane, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

2.14 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

Sample protocol form

Sample identification	Site	Date	Period of collection	Temperature range	Filter label

3. DRY DEPOSITION

3.1 Compounds to be determined.

* Hexachlorobenzene
* Hexachlorocyclohexanes (namely a and g isomers)
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

3.2 Other measurements.

* Weight of total particles
* Total organic particulate carbon (when sufficient material will be collected)

3.3 Materials and reagents.

-Dry and wet+dry deposition sampler.
-Millipore 47-mm filtration apparatus
-0.45 mm PTFE fiber glass filters
-Octadecylsilane membrane extraction disks (47 mm diameter, 0.5 mm thickness). Each disk contains about 500 mg C_{18} bonded silica.
-Glass bottles
-Tweezers
-Test tubes
-Pasteur pipettes
-Aluminum foil
-Sodium sulphate (analysis grade)
-Milli-Q water
-Ethyl acetate (for trace organic analysis, Merck)
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

3.4 Cleaning.

Plastic should be avoided in any instance.

1. Tweezers and the dry and wet+dry reservoirs should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40°C for drying.

2. Glass bottles and the Millipore 47-mm filtration apparatus should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-emptied, cleaned with tap water, Milli-Q water and acetone.
-left to dry and the open parts covered with aluminum foil.

3. The glass fiber filters will be pre-cleaned by the Department of Environmental Chemistry.

4. The membrane extraction disks should be washed with (1:1) dichloromethane:ethyl acetate (2 x 10 ml) and then with methanol (2 x 10 mL). BEFORE ALLOWING THE DISK BECOME DRY, the water sample should be poured into the filtration apparatus.

5. The Pasteur pipettes were kiln-fired at 400°C.

3.5 Filtration.

-Add a known water volume to the dry reservoir.
-Record the filter label (filters are pre-weighted in the Department of Environmental Chemistry).
-Take the filter with the tweezers and place it in the filtration apparatus.
-Stir the dry reservoir to resuspend all the collected particles.
-Add this water to the filtration apparatus.
-Check whether the upper and lower parts of the apparatus are well assembled.
-Connect the vacuum.
-Keep stirring the dry reservoir and pouring more water into the filtration apparatus until total volume.
-Examine carefully the dry reservoir to detect non transferred particles.
-If needed, add more Milli-Q water to the dry reservoir and repeat the filtration procedure.
-Store the filter as indicated below.

3.6 Adsorption on membrane extraction disks.

-Transfer the filtered water to a glass bottle.
-Rinse the filtration apparatus with Milli-Q water.
-Take the disk with the tweezers and place it in the filtration apparatus.
-Connect the apparatus to vacuum.
-Wash the membrane extraction disk with (1:1) dichloromethane:ethyl acetate (2 x 10 ml)
-Wash the membrane extraction disk with methanol (2 x 10 mL).
-BEFORE ALLOWING THE DISK BECOME DRY, pour the water into the filtration apparatus.
-Water extraction should be slow. Adjust vacuum for a flow of 2 liters/hour.
-Never allow the membrane extraction disk go to dryness during water adsorption. If by accident the membrane goes to dryness the rest of the water should be adsorbed with a new one (which should be cleaned as previously described).
-Store the membrane wrapped in aluminum foil. DO NOT BEND.

3.7 Storage.

-BEND the FILTER with the tweezers. The two parts containing the particulate material should face each other.
-Double wrap it in aluminum foil.

-DO NOT BEND the MEMBRANE EXTRACTION DISK, just wrap it in aluminum foil.
-Record the filter label and give a sample identification. This information together with period of collection, date, site, temperature range during collection and precipitation should be indicated in a sample protocol form.
-Store the wrapped and labelled filters and membrane extraction disks at -20°C.

3.8 Blank.

1. Prepare a blank by filtration and membrane disk extraction of 2 liters of Milli-Q water in the same place where the water samples are analyzed.

2. Use one of the filters and membrane extraction disks sent by the Department of Environmental Chemistry for this purpose.

3. Record the filter and membrane extraction disk labels.

3.9 Transport.

Samples should be sent within boxes containing dry ice to the address indicated above. The contact persons are also the same as indicated above.

3.10 Analyses (extraction)

The filters are placed in test tubes. A mixture (*ca.* 10 ml) of (2:1) dichloromethane:methanol is added and the test tube is sonicated for 20 min. The solvent is decanted by centrifugation. The procedure is repeated two more times. The combined extracts are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

The membrane disks are introduced in the filtration apparatus. 5 Ml of methanol are introduced. The suspension is left to stand 3 min and then drawn under vacuum. 5 Ml of ethyl acetate are added to the disk, let to stand for 3 min and drawn through under vacuum. 5 Ml of dichloromethane are added, they are also let to stand for 3 min and drawn through under vacuum. The eluates are combined and dried over anhydrous sodium sulfate to remove the excess of water. Then, they are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

3.11 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

3.12 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column

(5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 80 to 150°C at 10°C/min and to 300°C at 4°C/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

3.13 Quantitation.

Authentic standards of hexachlorobenzene, a-, g- and d-hexachlorocyclohexane, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

3.14 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

Sample protocol form

Sample identification	Site	Date	Period of collection	Temperature range	Filter label

4. BULK DEPOSITION

4.1 Compounds to be determined.

* Hexachlorobenzene
* Hexachlorocyclohexanes (namely a and g isomers)
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

4.2 Other measurements.

* Weight of total particles
* Total organic particulate carbon (when sufficient material will be collected)

4.3 Materials and reagents.

-Bulk deposition sampler.
-Millipore 47-mm filtration apparatus
-0.45 mm PTFE fiber glass filters
-Octadecylsilane membrane extraction disks (47 mm diameter, 0.5 mm thickness). Each disk contains about 500 mg C_{18} bonded silica.
-Glass bottles
-Tweezers
-Test tubes
-Pasteur pipettes
-Aluminum foil
-Sodium sulphate (analysis grade)
-Milli-Q water
-Ethyl acetate (for trace organic analysis, Merck)
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

4.4 Cleaning.

Plastic should be avoided in any instance.

1. Tweezers and the dry and wet+dry reservoirs should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40°C for drying.

2. Glass bottles and the Millipore 47-mm filtration apparatus should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-emptied, cleaned with tap water, Milli-Q water and acetone.
-left to dry and the open parts covered with aluminum foil.

3. The glass fiber filters will be pre-cleaned by the Department of Environmental Chemistry.

4. The membrane extraction disks should be washed with (1:1) dichloromethane:ethyl acetate (2 x 10 ml) and then with methanol (2 x 10 mL). BEFORE ALLOWING THE DISK BECOME DRY, the water sample should be poured into the filtration apparatus.

5. The Pasteur pipettes were kiln-fired at 400°C.

4.5 Filtration.

-Record the filter label (filters are pre-weighted in the Department of Environmental Chemistry).
-Take the filter with the tweezers and place it in the filtration apparatus.
-Stir the reservoir to resuspend all the collected particles.
-Pour this water into the filtration apparatus.
-Check whether the upper and lower parts of the apparatus are well assembled.
-Connect the vacuum.
-Keep stirring the reservoir and pouring more water into the filtration apparatus until total volume.
-Examine carefully the reservoir to detect non transferred particles.
-If needed, add more Milli-Q water to the reservoir and repeat the filtration procedure.
-Store the filter as indicated below.

4.6 Adsorption on membrane extraction disks.

-Transfer the filtered water to a glass bottle.
-Rinse the filtration apparatus with Milli-Q water.
-Take the disk with the tweezers and place it in the filtration apparatus.
-Connect the apparatus to vacuum.
-Wash the membrane extraction disk with (1:1) dichloromethane:ethyl acetate (2 x 10 ml)
-Wash the membrane extraction disk with methanol (2 x 10 mL).
-BEFORE ALLOWING THE DISK BECOME DRY, pour the water into the filtration apparatus.
-Water extraction should be slow. Adjust vacuum for a flow of 2 liters/hour.
-Never allow the membrane extraction disk go to dryness during water adsorption. If by accident the membrane goes to dryness the rest of the water should be adsorbed with a new one (which should be cleaned as previously described).
-Store the membrane wrapped in aluminum foil. DO NOT BEND.

4.7 Storage.

-BEND the FILTER with the tweezers. The two parts containing the particulate material should face each other.
-Double wrap it in aluminum foil.
-DO NOT BEND the MEMBRANE EXTRACTION DISK, just wrap it in aluminum foil.

-Record the filter label and give a sample identification. This information together with period of collection, date, site, temperature range during collection and precipitation should be indicated in a sample protocol form.
-Store the wrapped and labelled filters and membrane extraction disks at -20°C.

4.8 Blank.

1. Prepare a blank by filtration and membrane disk extraction of 2 liters of Milli-Q water in the same place where the water samples are analyzed.

2. Use one of the filters and membrane extraction disks sent by the Department of Environmental Chemistry for this purpose.

3. Record the filter and membrane extraction disk labels.

4.9 Transport.

Samples should be sent within boxes containing dry ice to the address indicated above. The contact persons are also the same as indicated above.

4.10 Analyses (extraction)

The filters are placed in test tubes. A mixture (*ca.* 10 ml) of (2:1) dichloromethane:methanol is added and the test tube is sonicated for 20 min. The solvent is decanted by centrifugation. The procedure is repeated two more times. The combined extracts are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

The membrane disks are introduced in the filtration apparatus. 5 Ml of methanol are introduced. The suspension is left to stand 3 min and then drawn under vacuum. 5 Ml of ethyl acetate are added to the disk, let to stand for 3 min and drawn through under vacuum. 5 Ml of dichloromethane are added, they are also let to stand for 3 min and drawn through under vacuum. The eluates are combined and dried over anhydrous sodium sulfate to remove the excess of water. Then, they are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

4.11 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

4.12 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack,

Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 80 to 150°C at 10°C/min and to 300°C at 4°C/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

4.13 Quantitation.

Authentic standards of hexachlorobenzene, a-, g- and d-hexachlorocyclohexane, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

4.14 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

Sample protocol form

Sample identification	Site	Date	Period of collection	Temperature range	Filter label

5. SNOW

5.1 Compounds to be determined.

* Hexachlorobenzene
* Hexachlorocyclohexanes (namely a and g isomers)
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

5.2 Other measurements.

* Weight of total particles
* Weight of dissolved+colloid organic matter
* Total organic particulate carbon (when sufficient material will be collected)

5.3 Materials and reagents.

-Metal bucket
-Metal shovels
-Metal corers
-Tweezers
-Glass bottles
-Test tubes
-Pasteur pipettes
-Aluminum foil
-Millipore 47-mm filtration apparatus
-0.45 mm PTFE fiber glass filters
-Octadecylsilane membrane extraction disks (47 mm diameter, 0.5 mm thickness). Each disk contains about 500 mg C_{18} bonded silica.
-Sodium sulphate (analysis grade)
-Milli-Q water
-Ethyl acetate (for trace organic analysis, Merck)
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

5.4 Cleaning.

Plastic should be avoided in any instance.

1. Tweezers, shovels, corers, buckets and other metal pieces should be:
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)

-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40°C for drying.

2. Glass bottles and the Millipore 47-mm filtration apparatus should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-emptied, cleaned with tap water, Milli-Q water and acetone.
-left to dry and the open parts covered with aluminum foil.

3. The glass fiber filters will be pre-cleaned by the Department of Environmental Chemistry.

4. The membrane extraction disks should be washed with (1:1) dichloromethane:ethyl acetate (2 x 10 ml) and then with methanol (2 x 10 mL). BEFORE ALLOWING THE DISK BECOME DRY, the water sample should be poured into the filtration apparatus.

5. The Pasteur pipettes were kiln-fired at 400°C.

5.5 Filtration.

-Let the collected snow melt in the metal bucket.
-Record the water volume collected.
-Record the filter label (filters are pre-weighted in the Department of Environmental Chemistry).
-Take the filter with the tweezers and place it in the filtration apparatus.
-Pour the water in the upper part and connect the vacuum.
-Add more water until total volume. Stir adequately to insure that all the suspended particles in the reservoir are transferred to the filtration apparatus. If needed Milli-Q water can be added to the bucket to complete particle transfer.
-Store the filter as indicated below.

5.6 Adsorption on membrane extraction disks.

-Transfer the filtered water to a glass bottle.
-Rinse the filtration apparatus with Milli-Q water.
-Take the disk with the tweezers and place it in the filtration apparatus.
-Check whether the upper and lower parts of the apparatus are well assembled.
-Connect the apparatus to vacuum.
-Wash the membrane extraction disk with (1:1) dichloromethane:ethyl acetate (2 x 10 ml)
-Wash the membrane extraction disk with methanol (2 x 10 mL).
-BEFORE ALLOWING THE DISK BECOME DRY, pour the water into the filtration apparatus.
-Water extraction should be slow. Adjust vacuum for a flow of 2 liters/hour.
-Never allow the membrane extraction disk go to dryness during water adsorption. If by accident the membrane goes to dryness the rest of the water should be adsorbed with a new one (which should be cleaned as previously described).
-Store the membrane wrapped in aluminum foil. DO NOT BEND.

5.7 Storage.

-BEND the FILTER with the tweezers. The two parts containing the particulate material should face each other.
-Double wrap it in aluminum foil.
-DO NOT BEND the MEMBRANE EXTRACTION DISK, just wrap it in aluminum foil.
-Record the filter label and give a sample identification. This information together with period of collection, date, site, temperature range during collection and precipitation should be indicated in a sample protocol form.
-Store the wrapped and labelled filters and membrane extraction disks at -20°C.

5.8 Blank.

1. Prepare a blank by filtration and membrane disk extraction of 2 liters of Milli-Q water in the same place where the water samples are analyzed.

2. Use one of the filters and membrane extraction disks sent by the Department of Environmental Chemistry for this purpose.

3. Record the filter and membrane extraction disk labels.

5.9 Transport.

Samples should be sent within boxes containing dry ice to the address indicated above. The contact persons are also the same as indicated above.

5.10 Analyses (extraction)

The filters are placed in test tubes. A mixture (*ca.* 10 ml) of (2:1) dichloromethane:methanol is added and the test tube is sonicated for 20 min. The solvent is decanted by centrifugation. The procedure is repeated two more times. The combined extracts are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

The membrane disks are introduced in the filtration apparatus. 5 Ml of methanol are introduced. The suspension is left to stand 3 min and then drawn under vacuum. 5 Ml of ethyl acetate are added to the disk, let to stand for 3 min and drawn through under vacuum. 5 Ml of dichloromethane are added, they are also let to stand for 3 min and drawn through under vacuum. The eluates are combined and dried over anhydrous sodium sulfate to remove the excess of water. Then, they are vacuum evaporated to nearly dryness and redissolved to a small volume (*e.g.* 50 ml) of *iso*-octane for instrumental analysis.

5.11 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The

injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

5.12 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 80 to 150°C at 10°C/min and to 300°C at 4°C/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

5.13 Quantitation.

Authentic standards of hexachlorobenzene, a-, g- and d-hexachlorocyclohexane, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

5.14 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

Sample protocol form

Sample identification	Site	Date	Period of collection	Temperature range	Filter label

6. WATER (DISSOLVED+COLLOID AND PARTICULATE MATTER)

6.1 Compounds to be determined.

* Hexachlorobenzene
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

6.2 Other measurements.

* Weight of total particles

6.3 Materials and reagents.

-Tweezers
-Glass bottles
-Test tubes
-Pasteur pipettes
-Aluminum foil
-Infiltrex 2 Pump
-1 mm GF/B glass fiber filters (14.2 cm diameter)
-XAD-2 resin columns
-Sodium sulphate (analysis grade)
-Milli-Q water
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

6.4 Cleaning.

Plastic should be avoided in any instance.

1. Tweezers and the dry and wet+dry reservoirs should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40°C for drying.

2. Glass bottles should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-emptied, cleaned with tap water, Milli-Q water and acetone.

-left to dry and the open parts covered with aluminum foil.

3. The glass fiber filters and the Pasteur pipettes are kiln-fired at 400°C.
4. Prior to sampling the XAD-2 columns are cleaned with 200 ml of methanol and 200 ml of dichloromethane. Then, further 200 ml of dichloromethane are collected to determine blank levels. If blanks are acceptable the columns are left moist with methanol not being allowed to dry out to prevent cracking of the extraction material. Columns should be stored in the refrigerator. Cleaning must be continued until low blank levels are achieved.

6.5 Sampling

The pump is introduced in the water column. The water is impelled through the filter and XAD2 column. Flow rate should be between 300-400 ml/min and the volume sampled about 100 liters.

6.6 Storage

The filters should be wrapped in aluminum foil and frozen (-20°C) until analysis. The XAD-2 columns should be stored in the refrigerator but never freezed. These columns should be extracted within one week after sampling.

6.7 Analysis of particulate organic matter

The filter is freeze dried in an oil-free freeze-drier and weighed. Then, it is cut in small pieces and spiked with PCB 30, octachloronaphtalene, d_{10}-pyrene and d_{12}-benzo[*ghi*]perylene. The mixture is extracted by sonication with (2:1) dichloromethane:methanol (3 x 20 ml, 20 ml). The extract is vacuum evaporated to 2 ml and hydrolyzed overnight with 20 ml of (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigourous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

6.8 Analysis of dissolved+colloidal organic matter

The columns are eluted in the inverted direction to sampling with 200 ml of methanol and 200 ml of dichloromethane. The methanol fraction is extracted with 3 x 30 ml of *n*-hexane. This *n*-hexane extract is combined with the dichloromethane eluate and spiked with PCB 30, octachloronaphthalene, d_{10}-pyrene and d_{12}-benzo[*ghi*]perylene. The combined extracts are hydrolyzed with 20 ml (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigourous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

6.9 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

6.10 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 80 to 150°C at 10°C/min and to 300°C at 4°C/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

6.11 Quantitation.

Authentic standards of hexachlorobenzene, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

6.12 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

7. AIR

7.1 Compounds to be determined.

* Hexachlorobenzene
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

7.2 Other measurements.

* Weight of total particles

7.3 Materials and reagents.

-Glass microfiber (GF/A) 1.6 mm (20.3 x 25.4 cm)
-Polyurethane foam density 0.022 g/cm^3
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-*n*-Hexane (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Hydrochloric acid (25% w/w; for analysis; Merck)
-neutral silica gel (Kieselgel 40, 70-230 mesh, Merck)
-alumina (aluminum oxide 90 active, 70-230 mesh, Merck)

7.4 Cleaning.

1. The filters are kiln-fired at 400°C for 12 h and then wrapped into solvent rinsed aluminum foil until use.

2. The individual polyurethane plugs (height 7.5 cm, diameter 5 cm) are compressed/decompressed in water and acetone (10 times). Then, they are Soxhlet extracted with acetone (24 h), dried in a vacuum dessicator containing phosphorous pentoxide. Each plug is wrapped with aluminum foil and stored in a clean Teflon sealed glass jar.

3. Silica gel and alumina are extracted with (2:1, v/v) dichloromethane:methanol in a Soxhlet apparatus for 24 h. After Soxhlet evaporation they are respectively heated at 120°C and 350°C for 12 h. A total of 3% (w/w) of Milli-Q grade water is then added to the chromatographic absorbents for deactivation.

7.5 Sampling

The sampling system is composed by a high-vol MCV pump Mod. CAV-P, a filtering system equipped with Wathman GF/A filters and a Teflon column filled with 2 foam plugs. Air volumes of 40 m^3 are collected at a flow rate of 20 m^3/h. After air sampling the polyurethane plugs are removed from the Teflon columns, wrapped in aluminum foil, and stored in Teflon sealed glass jars at -20°C.

7.6 Analyses (extraction and fractionation)

An internal standard containing perdeuterated naphthalene and anthracene are added to the polyurethane plugs before Soxhlet extraction with 700 ml of *n*-hexane for 24 h. The extract is vacuum evaporated to 0.3 ml and then fractionated by column chromatography. A column containing 2 g of alumina is used. The aliphatic and aromatic hydrocarbons are respectively eluted with 3 ml of *n*-hexane and 6 ml of (4:1, v/v) *n*-hexane:dichloromethane. These fractions are concentrated under a current of nitrogen to a small volume (0.1 ml) and reconstituted to 2 ml with *n*-hexane.

Filters are stored at -20°C until analysis. Before extraction the above described internal standard mixture is added and then the filters are cut into *ca.* 1 cm^2 pieces. These pieces are Soxhlet extracted with 375 ml of (2:1, v/v) dichloromethane:methanol for 24 h. The extract is vacuum evaporated to 0.3 ml and fractionated by column chromatography following the same procedure described above.

7.7 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

7.8 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 80 to 150°C at 10°C/min and to 300°C at 4°C/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

7.9 Quantitation.

Authentic standards of hexachlorobenzene, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

7.10 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

PROTOCOL FOR DATA FLOW WITHIN THE MOLAR PROJECT

Einar Heegaard
H. John B. Birks

Botanical Institute, University of Bergen

1. Data-flow within the MOLAR project

The Molar project will result in an enormous amount of data from very different scientific fields. These data will eventually be stored and systematized in a data-base. The aim of this data-base is to make all the data available in combinations suitable for the scientists' purposes.

1.1 The path for the data from sampling to the data-base

- The field operators will send the collected material either to laboratories or to the scientists responsible according to the MOLAR protocol.

- The laboratories will send their results to the scientists responsible for the particular scientific field.

- The scientists responsible will check these data and store them until they are entered into the MOLAR data-base.

- The data-base managers will contact each of the scientists responsible in turn to agree on the specific format of the data-files and to collect the data for the data-base.

NB! All data that will be stored in the data-base must be thoroughly checked by the scientist responsible for this particular field.

1.2 How should these data be sent?

- The data files (matrices) are recommended to be sent by e-mail.
- XL-format is preferred for the data files.

1.3 What should the files (matrices) look like?

- Rows: The first row includes the titles of the columns, the parameters to be recorded. The second row includes the units of these parameters. The following rows contain the successive records.

- What parameters are to be recorded, the resolution, the units, the nomenclature, and abbreviations of these parameters must be decided by the responsible scientists. If abbreviations are used then a key for these abbreviations must be obtainable and they must be used by all those working within the same scientific field.

- Columns: The first column shall contain unique codes for that particular recording. The other columns shall contain the parameters measured.

- The codes for each sample (recording, row in column) shall be written in the first column and must include the lake code (MOLAR protocol), date of sampling (M.D.Y., 07.11.96 = 11th July 1996), the name of the recorder, analysts or laboratories (Surname only). The sampling techniques require a code. In addition the depth of the sample points should be coded if the samples are stratified.

- The purpose of these codes is to create unique name for each record and to describe the sample procedure. Thus more codes might be necessary. To find out which codes should be used in addition

to these mentioned here contact the responsible scientists, who can contact the data-base group if problems arise.

1.4 Comments following the files

- A note should follow each file to describe the sampling and any deviations from the sampling procedure described in the MOLAR protocol.

The data-base group:
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Einar.Heegaard@bot.uib.no

MEASURING AND MODELLING THE DYNAMIC RESPONSE OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

PROTOCOL FOR FISH SAMPLING:

TEST FISHING
FISH PHYSIOLOGY
FISH HISTOLOGY
HEAVY METALS
ORGANIC MICROPOLLUTANTS

Bjørn Olav Rosseland
Sigurd Rognerud
Jean-Charles Massabuau
Rudolf Hofer
Joan Grimalt

Norwegian Institute for Water Research, Oslo
Université de Bordeaux I et CNRS, Arcachon
University of Innsbruck

Protocol for fish population sampling

Bjørn Olav Rosseland, NIVA, Norway

1. Test fishing

1.1 SNSF gillnet series

In AL:PE 1 and 2, the gillnet series that was design for the Norwegian monitoring programme (Rosseland *et al.* 1979) has been used (Wathne *et al.* 1995). Eight individual bottom gillnets of different mesh sizes (Table 1) or a set of three gillnets, each as a combination of these 8 mesh sizes (SFT 1983), has been used either alone or in combination.

The new series consist of three gillnets, 32 X 1.5 m, each net containing the 8 mesh sizes mixed and randomised in units of 4m. The same type of nets as in the single net SNSF series (thread size, colour etc.) are used, as well as the producer of the nets. Because of the different catch efficiency of the series, the old (8 single nets) was given a Catchability of 1, compared to 0.46 of the multimesh size series (3 nets).

Table 1. The SNSF gillnet series, containing 8 gillnets of given mesh size and threadthickness. (After: Rosseland *et al.* 1979). In the series of 8 single nets, each individual gillnet is 26 X 1.5 m, and have a dark red colour. In the SNSF multimesh-size series, 3 nets are a unit, 32 X 1.5 m each net containing a combination with 4 m panels of each mesh size. Each of the 3 nets in the unit have the individual mesh sizes in different order. The catchability of the 8 single nets are 1.0, compared to 0.46 of the 3 net series. The gillnets are produced by Lundgrens Fiskredskapsfabrik AB, Stockholm, Sweden.

Mesh size mm.	10	12.5	16.5	22	25	30	38	46
Thread thickness mm.	.15	.15	.15	.15	.15	.15	.15	.17

1.2 The "Nordic" multimesh-size series

Multimesh-size gillnet series has also been used in other Nordic countries. In Sweden, a monitoring program was initiated in 1983 to study the long-term effects of liming on fish populations (Degerman *et al.* 1988). In this program, a multimesh-size gillnet series containing 14 randomly distributed panels of 4 x 1.5 m (total gillnet length 42 m) of mesh sizes from 6.25 to 75 mm was used (Hammar and Filipsson 1985).

In 1990, a co-operation between Norway, Sweden and Finland was established to develop sampling protocols for monitoring of fish populations in acidified lakes. As a part of this, a new "Nordic multimesh-size gillnet series" was developed, which since then has been tested against the "national standard" for each country. In Finland, the comparative studies started in 1993 (Kurkilathi and Rask, 1995), and in Norway in 1992 (Jensen and Hesthagen 1996, SFT 1993). The Nordic series contains

12 different mesh sizes, between 5 and 55 mm,, in panels of 2.5 m, height 1.5 m and total length of 30 m per net, Table 2.

Table 2 The Nordic multimesh-size gillnet series, contains 12 mesh size, each panel being 2.5 m long and 1.5 m in height, total gillnet length of 30 m. The mesh sizes and thread thickness are given. The gillnets are produced by Lundgrens Fiskredskapsfabrik AB, Stocholm, Sweden.

Mesh size mm.	5	6.25	8	10	12.5	16	19.5	24	29	35	43	55
Thread thickness mm.	0.1	0.1	0.1	0.12	0.12	0.15	0.15	0.15	0.15	0.18	0.20	0.25

In 1992 (SFT 1993, Figure 2), a comparative study on 8 brown trout and 5 Arctic charr (*Salvelinus alpinus* L.) populations in Norway, found the comparative CPUE values between the old SNSF series and the Nordic series to be:

Brown trout: $Nordic_{CPUE} = 0.79 * SNSF_{CPUE} + 3.18$ ($P<0.005$)
Arctic charr: $Nordic_{CPUE} = 1.62 * SNSF_{CPUE} + 5.05$ ($P<0.01$)

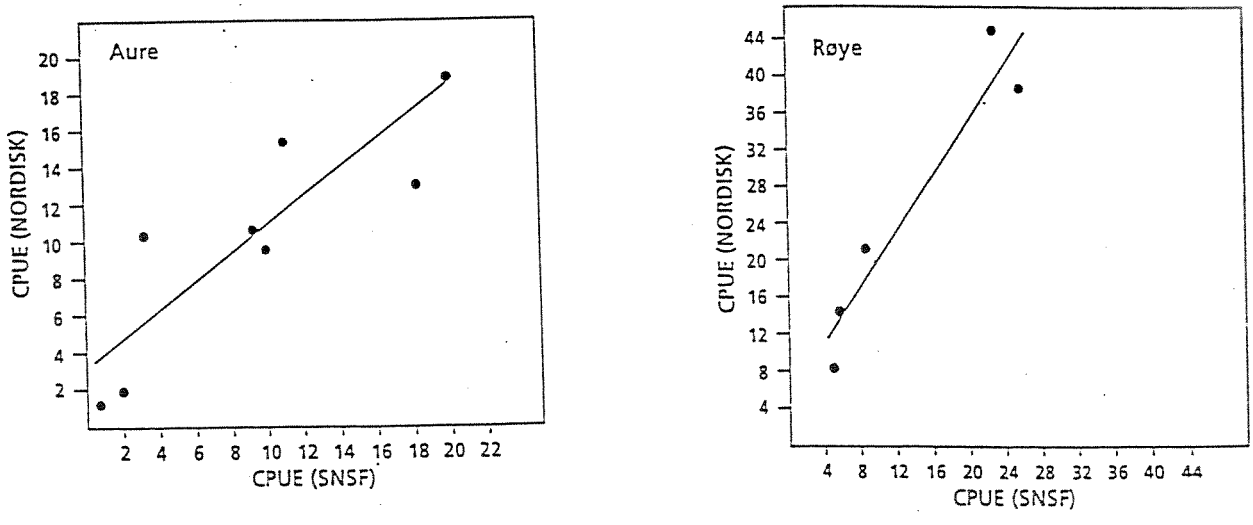

Figure 2 A comparison between the Catch per Unit Effort (CPUE) of the SNSF series and the Nordic series in lakes with populations of brown trout (Aure) and Arctic charr (Røye). From SFT (1993).

In another comparative study using the SNSF and the Nordic series, the conclusion was stated:, "Over a limited range of mesh sizes and fish sizes (5-20 cm fish), the selectivity of the two types of net do not differ much" (Jensen and Hesthagen 1996).

Based on these results, there seems to be no problem by either the SNSF series or the Nordic series as standard gillnets in the MOLAR.

1.3 Gillnet-setting

The gillnets are set perpendicular to the shore, avoiding steep-slope shore to bottom areas. Gillnets in the "old" series are set at random as for mesh size, whereas the "new" series always will have all eight mesh sizes represented at a certain point.

Both in the AL:PE 1 and 2 projects, the period between August 15 to October 15 have been selected for testfishing, for reasons given in Wathne *et al.* (1995). This is the main (and only) period for providing data to describe the population structure of salmonids. However, in the MOLAR project, physiological and histological data from other periods of the ice free season are needed. Sub-sampling of fish will therefore take place in some localities from early summer to late autumn.

1.4 Analytical program

Each fish must be given a specific number which follow all subsamples to be analyzed. Data from individual fish must be sent to NIVA. Due to differences in age determination of fish from some lakes in the AL:PE 2 project, age determination of all fish used in the MOLAR project should be undertaken by one laboratory.

For each individual fish, the following parametres must be noted:

- lake
- date
- species
- length, in mm, measured from snout to lower part of tail.
- weight, in gram.
- sex and gonadal maturation, from stage I - VII
  - I - II juveniles
  - III - V recruit spawners
  - VI spawning
  - VII/.. postspawners
- flesh colour; white, pink or red.
- stomach fullness, classified from 0 - 4.
- stomach content (if possible), conserved in 70% alcohol and classified in main invertebrate groups (not fully analysed in AL:PE 2).
- scale samples **from brown trout only** for age determination, taken from the area between the sideline organ and dorsal and pectorial fin.
- otolith samples for age determination **(all species)**, using the "burning technique" described by Christensen (1964). If age differ when determined by otolith and scales, otolith age is considered as the true age.
- growth, determined by:
  - length at catch as a function of true age.
  - back calculation of growth (brown trout only), using the methods of Dahl (1910) and Lee (1920).

Otholiths from single fish should be sent to NIVA for age determination. The results are processed in data bases at NIVA

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2. References

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Protocol for fish physiology

Jean-Charles Massabuau, CNRS, Arcachon, France

1. Blood ionic composition:

To avoid intercalibrations, all blood ion analyses will be performed at CNRS, Arcachon, France. CNRS will send a complete package to all MOLAR Fish Groups containing sampling and storing equipment.

1.1 Sampling equipment

In one package for one lake, you have:

- 10 syringes, 2 ml
- 10 yellow needles
- 10 pink needles
- 10 plastic pipettes
- 6 ml of heparin
- 20 tubes for centrifugation (1.5-2.0 ml)
- 10 tubes for blood transfer (2.2 ml). Each tube is numbered.
- No hematocrit tubes. The idea is that if you have a centrifuge for doing hematocrit, you have the corresponding tubes.
- plastic gloves
- 16 microcollection kits for blood and plasma hemoglobin contents.

Each kit contains either 1.25 or 2.5 ml of Drabkin solution. It is toxic as it contains cyanide. So take care although the risk is really minimum with these kits. You can use the provided gloves. See procedure on the video and on the back side of the general cooking list.

1.2 Blood Analysis Protocol (6 very fresh fish)

-Take a very freshly catched fish, check and note its number
- Sample its blood gently!
- Separe some blood for an hematocrit (if you can do it)
- Separe 10 µL of blood for the hemoglobin measurement by using the provided kit. Do not freeze. The kit is already numbered. Note it
- Centrifuge about 1.5-2 ml of blood
- Collect the plasma with a plastic pipette
- Separe 10 µL of plasma for the hemoglobin measurement by using the provided kit. Do not freeze. The kit is already numbered. Note it.
- Put the remaining plasma in the numbered 2.2 ml tubes and note the corresponding numbers
- Keep the plasma tubes in the cold. Freeze as soon as possible.

Contact us before sending

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1.3 MOLAR Scanning and Electronic Microscopy of fish gills (cooking list for 5 fish)

In one package for one lake, you must have:

- **tweezers** and **scissors** *You must have your own material*
- razor blades
- 3 x 10 ml syringes
- 2 empty bottles numbered 2 and 3
- 1 field dissection table
- 6 small transfer tubes (numbered)
- a kit to prepare extratemporaneously 100 ml of cacodylate buffer
- 1 ampula (10 ml) of glutaraldehyde
- 1 ampula (2 ml) of osmium tetroxide (**Take care !!!!!, toxic, to use with latex gloves**)
- latex gloves
- plastic pipettes
- 1 syringe with 6 ml of distilled water
- 50 ml of ethanol 70 %

On the field, prepare solutions 1 and 2 below, **USE LATEX GLOVES**:

Cacodylate buffer: *Solution 1 in bottle 1,*
- *Reciepe.* To prepare a few hours before the test fishing.
You have a plastic bottle with 97.5 ml of distilled water in it. There are 1.07g of sodium cacodylate in the small plastic can that is scotched on it. Empty it in the distilled water and put also the can (and its plug) in the water. Sheake it to dissolve the powder. Add the 2.5 ml of HCl 0.2 N provided in the 2 ml syringe. Sheake it. The volume of acid has been calculated to adjust the pH of the solution at 7.4.
Solution 2 in bottle 2: break one 10 ml ampula of glutaraldehyde and mix with 10 ml of cacodylate buffer by using 10 ml syringes,
Solution 3 in bottle 3: break one 2 ml ampula of osmium tetroxide and mix with 6 ml of distilled water by using 10 ml syringes.

1.4. Sampling protocol; (5 very fresh fish)

-Take a very freshly catched fish, check and note its number
-Dissect the first gill arch, put it on the small "field dissection table" provided.
-Section it in portions that contain from 2 to 4 pairs of filaments, the arch tissue must be cut with the provided razor blades leaving the pairs of filaments attached by the septum.
-Take at least 6-8 samples per animal.
-Put them in 1 tube, add 2 ml of solution 2 and leave 2 hours.
-**Two hours later**, empty the tube with a plastic pipette, filled with solution 1 once, empty the tube, filled with solution 1 again.
Leave it like that for 12-24 hrs at 10 °C in theory (in fact in a fresh place and at least in the shadow). No more than 24 hrs!!!!!
-12-24 hrs empty the tubes with the plastic pipettes and add 1.5 ml of solution 3.
Leave it like that for 1 hr

-1 hr later, empty the tube, rinsed 10 times in ethanol 70 % (empty the tube, fill the tube gently, shake the tube gently, empty....)
-Finally, store the samples in ethanol 70 %. Seal the tubes and send them. Do not freeze. Contact us before sending

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Protocol for fish histology

Rudolf Hofer, Institute of Zoology, UIBK, Austria

1. Sampling the fish

- Fish are caught by gill nets. If possible fish should not be exposed for more than 3-4 hours in gill nets and **only live fish** can be used for histological samples.

- Select **20 males**, preferably in the age of 5-8 years, and keep them until dissection under aerated conditions and at temperatures similar to those of the lake. Females should be only used if not enough males are available (during autumn the liver of females is more affected by vitellogenesis than by environmental parameters). Minimum number of fish: 12 males.

2. Dissection of the fish

Dissect the fish immediately at the lake site.

You will get a complete set containing:

a. Tubes with formalin for tissues (all tissue samples of one fish are collected in one tube)
b. Empty tubes for otoliths
c. Figures for fish dissection
d. Protocol form

* Kill the fish by a blow on the head (not too hard) and record its weight and total length. If no balance is available in the field and a transport of live fish to the laboratory under optimum conditions is impossible, take only the length of the fish.
  The dissection should be performed within 10 min after you have killed the fish! In consequence, kill the first fish and dissect it immediately, then kill the second fish and collect the tissues, and so on.

* Cut the second gill arch of one side by scissors and transfer it to formalin. Don't squeeze the gill filaments!

* Open the body of the fish, dissect a portion of the liver and transfer it to formalin (about 0.5 cm^3, not more than 1 cm^3). Don't squeeze the tissue!

* Cut carefully the caudal portion of the kidney by a scalpel (see Figure 1) without squeezing the tissue.

* After you have collected the tissue of all fish, dissect the otolith bones of the fish and store them in a tube. If you are not familiar with otolith dissection cut the head of the fish, sign it with its number, freeze it and send it to Innsbruck.

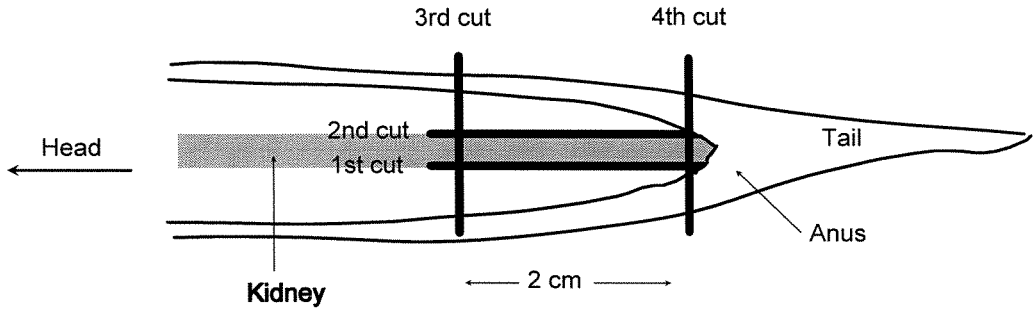

Fig. 1 Preparation of the fish kidney

3. Storage and transport of samples

All samples should be stored at **room temperature** and can be sent to Innsbruck by normal post.

IMPORTANT

Use only live fish
Tissues sampling within 10 min after you have killed the fish
Don't squeeze the tissues
Do not freeze the tissue samples !!!

4. Protocol form

Lake:
Date of sampling:
Maximum time at which fish were exposed in gill nets:
Maximum time between capture and dissection:
Conditions during storage of live fish:
Problems:
Responsible person:

No. of fish	Sex	Total body length (mm)	Total body weight (g)	Remarks
1	Male	231	66.3	Skin lesions
2	Male	192	48.0	Parasites .....

The samples should be returned to: Rudolf Hofer, Innsbruck

Rudolf Hofer
University og Innsbruck
Institute of Zoology
Technikerstrasse 25, A-6020 Innsbruck, Austria

Phone: 43 512 2186183
Fax: 43 512 2182930
Rudolf.Hofer@uibk.ac.at

Protocol for heavy metals in fish

Sigurd Rognerud, NIVA, Norway

1. Sampling

To get an estimate of adjusted means with an acceptable statistical significance it is necessary to collect 25 fish of different length in the intreval 15-30 cm from each lake. Record total length, weight and sex. Collect scales or otoliths for age estimates. Remove approximately 40g of bone and skinnless dorsal axial muscle, liver and kidneys from each fish, put it separatly in polyethylene bags and store it in the freezer.

2. Shipment

All samples must be carefully packed and shipped to NIVA in a frozen condition. Inform the reciever so the package could be picked up before it thaws.

3. Metal analyses

Approximately 1g muscle is digested in a 4: 1 nitric-perchloric acid mixture under pressure. After dissolution, samples are analyzed for mercury by a atomic absorption spectrophotometer equipped with a hydrid generator (detailed description given in Fjeld & Rognerud 1993). Lead and cadmium are analysed by atomic absorption spectrometry using a graphite furnace.

The frozen samples should be returned to NIVA after direct contact and agreement by either phone call, Fax or E-mail:

Sigurd Rognerud[1)] or Leif Lien[2)]
NIVA
P.O. Box 173 Kjelsås
N-0411 Oslo, Norway
Phone: [1)] 47-62576400 [2)] 47-22185100
Fax: [1)] 47-62576653 [2)] 47-22185200
sigurd.rognerud@niva.no
leif.lien@niva.no

4. Reference

Fjeld, E. and Rognerud, S. (1993). Use of path analyses to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. *Can. J. Fish. Aquat. Sci.* **50**: 1158 - 1167.

Protocol for organic micropollutants in fish

Joan Grimalt, FBG, Barcelona, Spain

1. ORGANOCHLORINATED COMPOUNDS IN FISH TISSUES (MUSCLE AND LIVER)

1.1 Compounds to be determined.

* Hexachlorobenzene
* Hexachlorocyclohexanes (namely a and g isomers)
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* Polycyclic aromatic hydrocarbons (PAH; 63 individual compounds)

1.2 Other measurements.

* Water and lipid content

1.3 Materials and reagents.

-Self adhesive labels
-Pen
-Big box to keep the samples when dissected
-Field fridge
-Glass bottles
-Recipient for cleaning and rinsing tissues
-Latex gloves (only for cleaning the material in the soap, for safety reasons). No to dissect the fishes.
-Plastic tray.
-Scalpel
-Tweezers
-Scissors
-Aluminum foil
-Sulphuric acid for analysis grade (Merck)
-Potassium chloride (for analysis, Merck)
-Distilled water
-Lake water
-*Iso*-octane (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran MA01, ref. 7555.1000, alkaline, Merck)

1.4 Cleaning.

Correct cleaning of the material is a very important step in the analysis of organic compounds. Plasticizers are present in most recipients and material of common use (*e.g.*, gloves, bottles -distilled water is usually kept in plastic bottles-, ...). The hands and hair can also be a source of organic compounds in the sample. For this reason the cleaning procedure needs to be followed carefully during sampling and analysis.

1. Find a flat horizontal surface to work comfortably.
2. Put the gloves on.
3. Fill the plastic tray with EXTRAN solution (20 g per litre of distilled water).
4. Clean the glass bottle with this solution.
5. Rinse the glass bottle with lake water (3 times)
6. Fill the glass bottle with lake water.
7. Spread an aluminium sheet over the grass.
8. Put in the tray the material to be used and clean it.
9. Once cleaned, rinse it with the lake water stored in (6).
   IMPORTANT: Do not throw the first rinsing water nor the cleaning mixture in the catchment. Store it in a recipient and throw it when being back in the lab. EXTRAN products can be discarded in the inorganic salt recipient for residues of the lab.
10. Put the material on the aluminium sheet spread in (7)
11. Cover the clean material with aluminium if not to be used immediately.

1.5 Dissection protocol (see also "The working procedure for sampling of fish blood and tissue")

1. Prepare small sheets (*ca.* 10 x 10 cm) of aluminium foil to be used as boxes.
2. Hold the fish taking the fins with the tweezers.
3. Open the fish longitudinally from the anus to the head base with the scissors.
4. Make two transversal cuts on the head base.
5. Take the liver carefully with the tweezers.
6. Put the liver in an aluminium box.
7. With a scalpel, cut along the fish length just above the lateral line. Cut verticaly in the anterior and posterior region. Then, starting from the anterior, peel off the skin with a tweezer. Cut in the middle. Separe the flesh from the body by cutting from the back. Put the muscle in an aluminium foil and add a sticker.
8. Put the muscle in an aluminiun box.
9. Write the fish code down on two self adhesive labels.
10. Stick the labels on the boxes.
11. Wrap together with an aluminium sheet the boxes containing the liver and muscle of the same sample and label this second sheet with permanent marker pen.
12. Put the boxes in a basket.
13. Store them in the field fridge.

1.6 Sample amount.

5 g. of muscle. 0.5 g of liver.

1.7 Transport.

The fish tissues, protocol form and the list of analyses performed to each fish should be placed in a box containing dry ice and sent to Barcelona. Before mailing contact directly with Joan Grimalt, Pilar Fernandez or Rosa Vilanova:

Department of Environmental Chemistry (CID-CSIC)
Jordi Girona, 18
08034-Barcelona
Catalonia. Spain
Phone: 34 3 400 61 22 or 34 3 400 61 00
Fax 34 3 204 59 04
e-mail: jgoqam@cid.csic.es

1.8 Analyses (extraction).

The tissues are freeze-dried in an oil-free freeze-drier and Soxhlet extracted with (4:1) *n*-hexane-dichloromethane for 18 hours. An aliquot (10%) of this extract is used to measure total extractable lipid weight after evaporation to dryness. Water content is determined by weight difference prior and after freeze-drying.

1.9 Analyses (organochlorinated compounds in muscle and liver).

The extract is reduced to 2 ml of *n*-hexane and cleaned up with agitation with sulphuric acid. After vigourous stirring in a Vortex (2 min) the two layers are decanted by centrifugation for removal of the sulphuric acid. The *n*-hexane extract is neutralized by washing (three times) with Milli-Q water for pH neutralization. The *n*-hexane is then concentrated under vacuum to a small volume, *e.g.* 50 ml, for instrumental analysis.

1.10 Analyses (polycyclic aromatic compounds).

After lyophilisation, the sample is saponified with 6 N NaOH and then extracted with (4:1) *n*-hexane-dichloromethane to obtain the total organic extract. The extract is vacuum evaporated to 0.5 ml and fractionated by column chromatography using columns filled with 5% water deactivated silica (top) and alumina (bottom). The first fraction (*n*-hexane) is discarded. The second fraction (*n*-hexane:dichloromethane) is collected and evaporated to dryness (vacuum rotary evaporation and nitrogen stream) to dryness for PAH analysis.

1.11 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a DB-5 column (5% phenyl-95% methylpolysiloxane, 25 m length, 0.25 mm i.d., 0.25 mm film thickness; J&W Scientific, Folsom, CA, USA). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310°C, respectively. Oven temperature is programmed from 60 to 300°C at 6°C/min with a final holding time of 10 min. The make up gas is nitrogen (60 cm/s).

1.12 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310°C at 4°C/min, held for 10 min. Injection and transfer line temperatures are 280 and 300°C, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

1.13 Quantitation.

Authentic standards of hexachlorobenzene, a-, g- and d-hexachlorocyclohexane, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The polycyclic aromatic hydrocarbons are analyzed by reference to standards of fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*k*]fluoranthene, pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene and dibenzo[*ah*]anthracene. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

1.14 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used. Transfer line and ion source temperatures are 300 and 120°C, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.

Protocol for sampling procedure of fish blood and tissue

The following working protocol is based on the agreed sampling procedure made by the MOLAR Fish Group. A videotape showing all fish sampling procedure for blood and tissue was made by CNRS during the Fish Group Workshop in Arcachon, France, June 1996.

Two peoples should attend. Before starting, prepare aluminium foil, paper and dissection tools, and the content of the packages sent by the different laboratories.

1- ***Blood sampling*** **(6 fish).** Heparanized a 2 or 5 ml syringe. Take the blood sample from the ventral aspect of the tail. You should get 1.5-2 ml of blood.
- Put off the needle and empty an hematocrit tube, seal it with the hematocrit paste, centrifuge.
- Collect 10 µL of blood for the hemoglobin measurement by using the provided kit. Do not forget to wipe the excess blood from outside of capillary pipette. Note the number written on the kit. Wrap it in an aluminium foil
- Place the remainder of the blood in a 1.5-2 ml centrifuge tube and separe the plasma from the pellet (red blood cells and leucocytes; 9-10 min and 10 000 rpm).
- Collect 10 µL of plasma for the hemoglobin measurement by using the provided kit. Note the number written on the kit. Wrap it in an aluminium foil
- Collect plasma in the plasma transfer tubes **FROZEN**

2- ***Weigth*** the fish and ***measure*** its length from the noose to the end of the tail

3- ***Excise the 1st gill arch*** for the scanning and electron microscopy **(5 fish)**. Cut 6 to 8 segments with the razor blade by using the small dissection table. Cut the bones. Place the samples in the corresponding tubes, notice at what time you did it and the corresponding number. Already note the time at which you will have to change the bath. Follow procedure described by CNRS!

4- ***Excise the 2nd gill arch*** for histology. Put it in formalin. **(20 fish)**.

5- If the ***gall bladder*** is fairly full of bile, empty it with a 1ml syringe. Fill a centrifuge tube, tag it **(20 fish) FROZEN**

6- ***Cut the liver:*** One little piece in formalin **(20 fish)**. Cut the remaining in two pieces. One must be used for the heavy metals **(25 fish)** and placed in a plastic bag **FROZEN**. The other one is for the organics **(20 fish)** and it must be kept in aluminium. Tag the aluminium staff with a sticker. **FROZEN**

7- ***Kidney***: In the posterior part of the open abdomen, cut 1 cm of kidney and put it in formalin with the liver **(20 fish)**. In the anterior part, cut 5 cm of kidney for the heavy metal **(25 fish)** and put it in a plastic bag **FROZEN**

8- ***Muscles***. With a scalpel, cut along the fish length just above the lateral line. Cut verticaly in the anterior and posterior region. Then, starting from the anterior, peel off the skin with a tweezer. Cut in the middle. Separe the flesh from the body by cutting from the back. Put one piece in the plastic bag for the heavy metals **(25 fish) FROZEN**. Put the other one in an aluminium foil for the organics **(20 fish)**, add a sticker **FROZEN**

9- ***Scales.*** Turn the animal onto the other side. Eliminate the mucus and then collect some scales with a scalpel or your knife. Put the scales in the special tiny envelope. Don't forget to fill the corresponding questionnary.

10- ***Otoliths***. Take your best knife and open the roof or the head in order to see the brain. Sample the otoliths. You need two big ones. Put them in a plastic bags that you place into the envelope. If you can not find them, keep the head and send it like that to NIVA.

11- Congratulation, you have finish this fish. There is no more noise from the centrifuge but did you collect the plasma? Measure the hematocrit? Check the bath for the scanning and electron microscopy? You noted the fish number. Nothing to do ??? Start with a new fish....

- fish histology, Austria: gill, liver, kidney (x 20) in vials with formalin
- heavy metals, Norway: muscle, kidney, liver (x 25) in plastic bags, frozen
- organics, Spain: muscle, liver, bile (x 20) in aluminium, frozen
- blood analysis, France: (x 6) plasma frozen, hemoglobin kits and hematocrit
- scanning and electronic microscopy, France: gill pieces (x 5) ethanol

Samplig protocol forms for lakes Gossenkollesse, Redo and Øvre Neadalsvatn.

Fish Code	Fish weight	Fish length	Sex	Age	Remarks
(1)					
(2)					
(3)					
(4)					
(5)					
(6)					
(7)					
(7)					
(8)					
(9)					
(10)					
(11)					
(12)					
(13)					
(14)					
(15)					
(16)					
(17)					
(18)					

(19)					
(20)					

Samplig protocol forms for the lakes where only 5 fish specimens will be taken.

Fish Code	Fish weight	Fish length	Sex	Age	Remarks
(1)					
(2)					
(3)					
(4)					
(5)					

List of analyses available for each fish

Fish Code	Fish Population	Blood ionic composition	Scanning electron microscopy of gill filaments	Electronic microscopy of gill filaments	Fish histology	Heavy metals	Organics	Remarks
(1)								
(2)								
(3)								
(4)								
(5)								
(6)								
(7)								
(8)								
(9)								
(10)								
(11)								
(12)								
(13)								
(14)								

List of analyses available for each fish

Fish Code	Fish Population	Blood ionic composition	Scanning electron microscopy of gill filaments	Electronic microscopy of gill filaments	Fish histology	Heavy metals	Organics	Remarks
(15)								
(16)								
(17)								
(18)								
(19)								
(20)								
(21)								
(22)								
(23)								
(24)								
(25)								

ANNEX A

MOLAR Sites, status, operators and steering group representative

MOLAR sites
status, operators and steering group representative.

Site nr.	Site name	Status	Site operator	Steering Group Repr.
1	Ø. Neådalsvatn	WP 1, 2, 3	L.Lien	B.O. Rosseland
2	Stavsvatn	WP 1	L.Lien	B. Wathne
4.1	Lochnagar	WP 1, 2	N. Rose	S. Patrick
5.1	Lago Paione Superiore	WP 1	R. Mosello	R. Mosello
11.2	Gossenköllesee	WP 1, 2, 3	R. Psenner	R. Psenner
12.2	Lago Redo	WP 1, 2, 3	J. Catalan	J. Catalan
13	La Caldera	WP 1	L. Cruz-Pizzarro	J. Catalan
15.1	Starolesnianske Pleso	WP 1, 2	E. Stuchlik	J. Fott
15.2	Terianske Pleso	WP 3	F. Sporka	J. Fott
15.3	Dlugi Staw	WP 1	J. Galas	J. Fott
17	Chuna	WP 1	T. Moisenko	B. Wathne
19.2	Jezero Ledvicah	WP 3	A. Brancelj	S. Patrick
21	Saanajävi	WP 3	A. Korhola	S. Patrick
22	Hagelsee	WP 3	A. Lotter	R. Psenner
23	Jorisee	WP 1, 2	K. Hanselmann	R. Psenner
24	Laghetto Inferiore	WP 1	O. Gianora	R. Mosello
Site nr.	**Site name**	**Status**	**Site operator**	**Steering Group Repr**
6.2	Lago di Latte	Secondary	D. Tait	R. Mosello
8	Etang d'Aube	Secondary	J.-C. Massabuau	J. Catalan
9	Arresjøen	Secondary	S. Rognerud	B. Wathne
11.1	Schwarzsee ob Sólden	Secondary	R. Schmidt	R. Psenner
16	Laguna Cimera	Secondary	M. Toro	J. Catalan
20	Limgambergtjern	Secondary	S. Rogenrud	B. Wathne

AL:PE sites where data is kept in the database

Site nr.	Site name	Status/Data	Site operator	Steering Group Repr.
3	Lille Hovvatn	AL:PE 1, 2	L.Lien	B. Wathne
4.2	Sandy Loch	AL:PE 1, 2	N. Rose	S. Patrick
4.3	Loch nan Eun	AL:PE 1, 2	N. Rose	S. Patrick
5.2	Lago Paione Inferiore.	AL:PE 1, 2	R. Mosello	R. Mosello
6.1	Lago Lungo	AL:PE 1, 2	D. Tait	R. Mosello
7.1	Lac Rond	AL:PE 1	G. Blake	R. Mosello
7.2	Lac Combeynod	AL:PE 1	G. Blake	R. Mosello
7.3	Lac Blanc	AL:PE 1, 2	G. Blake	R. Mosello
7.4	Lac Noir	AL:PE 1, 2	G. Blake	R. Mosello
10	Lough Maam	AL:PE 2	N. Rose	S. Patrick
14	Lago Escura	AL:PE 2	J. Catalan	H.J.B. Birks
12.1	Lago Aguilo	AL:PE 2	J. Catalan	J. Catalan
15.4	Zieloni Staw	AL:PE 2	J. Galas	J. Fott
18	Chibini	AL:PE 2	T. Moisenko	B. Wathne
19.1	Zgornje krisko jezero	AL:PE 2	A. Brancelj	S. Patrick

ANNEX B.

MOLAR SAMPLES AND RESULTS. Handling addresses for site operators and analysts

MOLAR SAMPLES AND RESULTS

HANDLING ADDRESSES FOR SITE OPERATORS AND ANALYSTS

Method	Type of sample/result	Format of data	To be sent to
Climatology and metorology	• Calibrated data from met. station • Lake ice cover	• EXCEL or Ascii	• David M. Livingstone Dept. of Environmental Physics, EAWAG Ueberlandstrasse 133 CH-8600 Dübendorf, Switzerland Phone: 41 1 8235540 Fax: 41 1 823 5210 e-mail: living@eawag.ch
	• Information on existing met. stations		• Roy Thompson Dept. of Geology and Geophysics University of Edinburgh King's Buildings, West Mains Road Edinburgh, Scotland Phone: 131-650-4907 (direct line) Fax: 131-668-3184 e-mail: egph08@castle.edinburgh.ac.uk
Atmospheric deposition	• Major ion and nutrients results • Site information	• MOLAR EXCEL form	• Rosario Mosello Istituto di Idrobiologia Largo Tonolli 50, I-28048 Italy Phone: 39 323 556571 Fax: 39 323 556513 e-mail: mosello@iii.to.cnr.it
	• Heavy metal samples		• Torunn Berg or Kjetil Tørseth Norwegian Institute for Air Research Box 100, 2007 Kjeller, Norway Phone : 47 63 898249 Fax: 47 63 898050 e-mail: torunn.berg@nilu.no
	• Organic micropollutants		• Joan O. Grimalt Department of Environmental Chemistry Centro de investigacion Desarrollo Jordi Girona 18-26, Barcelona, Spain Phone: 34 3 2040600 / 34 3 2050063 Fax: 34 3 2045904 e-mail: jgoqam@CID.CSIC.es
Snow pack	• Major ion and nutrients results	• MOLAR EXCEL form	• Uli Nickus Inst. of Meteorology, Innrain 52 A-6020 Innsbruck, Austria Phone: 43 512 507 5456 Fax: 43 512 507 2924 e-mail: ulrike.nickus@uibk.ac.at
	• Organic micropollutants		• Joan O. Grimalt Department of Environmental Chemistry Centro de investigacion Desarrollo Jordi Girona 18-26, Barcelona, Spain Phone: 34 3 2040600 / 34 3 2050063 Fax: 34 3 2045904 e-mail: jgoqam@CID.CSIC.es

Method	Type of sample/result	Format of data	To be sent to
Snow pack	• Spheriodal carbonaceous particles		• Neil Rose Environmental Change Research Centre, University College London, 26 Bedford Way, London WC1H 0AP, U.K. Phone: 44 171 387 7050 (ext. 5543) Fax: 44 171 380 7565 e-mail: nrose@geog.ucl.ac.uk
	• Heavy metals		• Stan Van den Berg Oceanography Laboratories, Univ. of Liverpool Liverpool L69 3BX, UK Phone: 44 151 7944096 Fax: 44 151 7944099 e-mail: sn35@liv.ac.uk
Surface water	• Major ion and nutrients results	• MOLAR EXCEL form	• Bente M. Wathne NIVA, Brekkevein 19 Box 173 Kjelsås, 0411, Oslo, Norway Phone: 47 22 185211 Fax: 47 22 185200 e-mail: bente.wathne@niva.no
	• Heavy metal samples		• Torunn Berg or Kjetil Tørseth Norwegian Institute for Air Research Box 100, 2007 Kjeller, Norway Phone : 47 63 898249 Fax: 47 63 898050 e-mail: torunn.berg@nilu.no • Stan Van den Berg Oceanography Laboratories, Univ. of Liverpool Liverpool L69 3BX, UK Phone: 44 151 7944096 Fax: 44 151 7944099 e-mail: sn35@liv.ac.uk
Water column profiling	• Physical, chemical and biological results	• ASCII or EXCEL	• Jordi Catalan University of Barcelona. Dep. of Ecology Diagonal 645, 08028 Barcelona, Spain Phone: 34 3 402 1512 Fax: 34 3 411 1438 e-mail: catalan@portohos.bio.ub.es
	• Heavy metals	• Samples	• Stan Van den Berg Oceanography Laboratories, Univ. of Liverpool Liverpool L69 3BX, UK Phone: 44 151 7944096 Fax: 44 151 7944099 e-mail: sn35@liv.ac.uk
Invertebrates	• Taxonomic list and site data	• ASCII or EXCEL	• Gunnar G. Raddum University of Bergen, Institute of Zoology Allégt. 41 N-5007 Bergen, Norway Phone: 47 55 212236 Fax: 47 55 329362

Method	Type of sample/result	Format of data	To be sent to
Microbial food webs	• 1st and 2nd level results		• Vera Straškrabová Hydrobiological Institute ACCR, Na sádkách 7 CZ-370 05 Ceske Budejovice, Czech Rep. Phone: 42 38 45484 Fax: 42 38 45718 e-mail: verastr@dale.entu.cas.cz
Photosynthetic rates primary production	• Results		• Vera Straškrabová (see above)
Sediment coring	• Results (for analysis see list in maual)		• Neil Rose Environmental Change Research Centre, University College London, 26 Bedford Way, London WC1H 0AP, U.K. Phone: +44 171 387 7050 (ext. 5543) Fax: +44 171 380 7565 e-mail: nrose@geog.ucl.ac.uk
	• Organic micropollutants		• Joan O. Grimalt Department of Environmental Chemistry Centro de investigacion Desarrollo Jordi Girona 18-26, Barcelona, Spain Phone: 34 3 2040600 / 34 3 2050063 Fax: 34 3 2045904 e-mail: jgoqam@CID.CSIC.es
Diatoms, livivng communities and traps	• Results (for analysis see list in maual)		• Nigel Cameron Environmental Change Research Centre, University College London, 26 Bedford Way, London WC1H 0AP, U.K. Phone: 44 171 387 7050 (ext. 5543) Fax: 44 171 380 7565 e-mail: ncameron@geog.ucl.ac.uk
Chrysophycean analysis	• Results		• Nigel Cameron (see above)
Pigments and CNS in sediments	• Sediment slice (The same sample will be sent toJan Fott for analysis of zoplankton remains)		• Andrea Lami Istituto di Idrobiologia Largo Tonolli 50, I-28048 Italy Phone: 39 323 556571 Fax: 39 323 556513 e-mail: lami@iii.to.cnr.it
Spheriodal carbonaceous particles	• Dried sediment • Sediment traps • Snow • Bulk deposition • Dry + wet deposition • Lake water		• Neil Rose Environmental Change Research Centre, University College London, 26 Bedford Way, London WC1H 0AP, U.K. Phone: 44 171 387 7050 (ext. 5543) Fax: 44 171 380 7565 e-mail: nrose@geog.ucl.ac.uk
Chironomids	•		• Steve Brooks, Dept. of Entomology, The Natural History Museum, Cromwell Rd. London SW7 5BD, UK Phone: 44 171 938 8905 Fax: 44 171 938 8937 e-mail: sjb@nhm.ac.uk

Method	Type of sample/result	Format of data	To be sent to
Zooplankton	• Samples/results		• Jan Fott Dep. of Hydrobiology,Charels Univ. Vinicna 7, 128 44 Prague 2, Czech Republic Phone: 42 2 24915520 Fax: 42 2 299713 e-mail: fott@beba.cesnet.cz
Soil coring	• Samples		• Peter G. Appleby Dep. of Mathematical Sciences, University of Liverpool, L69 3BX, UK Phone: 44 151 794 4020 Fax: 44 151 794 4061 e-mail: appleby@liverpool.ac.uk
Fish	• Age determination • Test fishing results	• Otholiths	• Bjørn Olav Rosseland NIVA, Brekkevein 19, Box 173 Kjelsås, 0411 Oslo, Norway Phone: 47 22 185110 Fax: 47 22 185200 e-mail: bjoern.rosseland@niva.no
	• Physiology	• Blood samples	• Jean - Charles Massabuau / Jean Forgue Lab. de Neurobiologie et Physiologie Comparées Université de Bordeaux I et CNRS Place du Dr Peyneau, 33120 Arcachon France Phone: (33) 56 22 39 25; Fax: (33) 56 83 03 50; e-mail: massabuau@lnpc.u-bordeaux.fr
	• Electron microscopy	• Gill filaments	• Suzanne Dunel-Erb Centre d'Ecologie et Physiologie Energetiques 23 rue Becquerel, 67087 Strasbourg, France Phone: 33 88 10 69 00 Fax: 33 88 10 69 06
	• Histology	• Fish tissue	• Rudolf Hofer University of Innsbruck, Inst. of Zoology Technikerstrasse 25 A-6020 Innsbruck, Austria Phone: 43 512 507 6183 Fax: 43 512 507 5358 e-mail: Rudolf.Hofer@uibk.ac.at
	• Heavy metals	• Muscle, liver, kidney	• Sigurd Rognerud or Leif Lien NIVA, Brekkevein 19 Box 173 kjelsås,Oslo, Norway Phone: 47 62 576400/+47 22 185153 Fax: 47 62 576653/+47 22 185200 e-mail: sigurd.rogenrud@niva.no e-mail: leif.lien@niva.no
	• Organic micropollutants	• Muscle and liver	• Joan O. Grimalt Dep.Env.Chem. Centro de investigacion Desarrollo Jordi Girona 18-26, Barcelona, Spain Phone: 34 3 2040600 / 34 3 2050063 Fax: 34 3 2045904 e-mail: jgoqam@CID.CSIC.es

ANNEX C.
MOLAR Methodological responsibilities

Molar: methodological responsibilities

Method	Steering Group Repr.	Associated responsible scientists
Meteorology	R. Psenner	D. Livingstone
Deposition	R. Mosello	
Major water/snow chemistry	B. Wathne/R. Mosello	
Chemical AQC	R. Mosello	
Water column profiling	J. Catalan	
Organic micropollutants	J. Grimalt	
Metals	B. Wathne	S. Rognerud
1st level microbiology	V. Straskabova	
2nd level microbiology	V. Straskabova	
Invertebrates	G. Raddum	
Zooplankton	J. Fott	
Diatoms	R. Battarbee	N. Cameron
Chironomids	G. Raddum	S. Brooks
Chrysophytes	R. Battarbee	R. Schmidt/N. Cameron
Cladocera	J. Fott	A. Brancelj
Biological transects	R. Battarbee/J. Catalan	
Sediment coring	R. Battarbee	N. Rose/N. Cameron
Sediment core dating	R. Battarbee	P. Appleby
Sediment core analyses	R. Battarbee	N. Rose/N. Cameron/P. Appleby/R. Schmidt/ G. Raddum/A. Brancelj/A. Lami
Sediment trap sampling	R. Psenner/J. Catalan	N. Rose
Sediment trap analyses	R. Psenner/J. Catalan	N. Rose/N. Cameron
Soil coring	R. Psenner	P. Appleby
Soil core analyses	R. Psenner	P. Appleby
Fishing	B-O Rosseland	
Fish analyses	B-O Rosseland	R. Hofer/J-C Massabuau
Climate data collation	R. Battarbee	R. Thompson
Modelling	J. Catalan	R. Wright/L. Hakanson/V. Straskabova/ H.J.B. Birks/R. Battarbee/P. Appleby
Database	H.J.B. Birks	
Statistics	H.J.B. Birks	
Reports & Publications	B. Wathne/S. Patrick	
Administration	S. Patrick	

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