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.

Vera Straškrabová,
Miroslav Macek,
Karel Šimek,
Jan Fott

Hydrobiological Institute, Ceske Budejovice
Charles University, Prague

Microbial (Pelagic) Food Webs - 1st Level

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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
Staroleśn.	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