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

LABORATORY PROTOCOL
Work Package 1.

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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), carbon content per cell (mean ± SD)

Note: cell volumes and carbon contents per cell must be calculated from each pair of individual length and width measurements.

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 and ANF)

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) *of HNF*
- number of ANF per litre
- cell volume (mean ± SD), cell length (mean ± SD), cell width (mean ± SD) of ANF

Note: In principal, nanoflagellates include cells 2 - 20 μm, but with a clear dominance of cells < 5 μm. However, all nanoflagellates within this

size range (2 -20 µm) should be counted and measured.

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

Lugol fixed samples should be elaborated within two weeks or postfixed by formaldehyde + thiosulphate (see sampling protocol, Sherr & Sherr, 1993). In case of scarce ciliate presence a concentration of the sample is needed. A minimum of 500 ml water sample (plus fixatives) should be left undisturbed for one week in a 600 ml cylinder, stoppered with parafilm. By gentle aspiration remove the upper water volume with a 50 ml pipette concentrating to 100 ml.

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 *25* ml of concentrated sample, 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. Depending on the sedimentation chamber used a correction for the concentration must be calculated.

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 (Möller & 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.

6. References

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