European Mountain lake Ecosystems: Regionalisation, diaGnostics & socio-economic Evaluation

EMERGE

08

MICROBIAL (PELAGIC) FOOD WEBS

Laboratory protocol - microbiology

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1. Objective

Quantitative assessment of biomass of the main components of pelagic assemblage - elaboration of preserved samples (Mo – MOLAR data, Em – EMERGE data): BAC (Mo, Em), FIL (Em), HNF (Mo), CIL (Mo).

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). 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)
- bacterial carbon biomass in sample in μg C per litre

Note 1: 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.

Note 2: if the sample contains too many heterotrophic filaments, the above described procedure (viz. microscopic counting, sizing and calculation of C content per cell) is performed only for bacterial cells **shorter than 5 μm.** The biomass of filaments is assessed separately using a different methodical approach– see the following section 3. Filaments (FIL).

3. Biomass of heterotrophic filaments (FIL)

The same samples (preserved by formaldehyde) like for BAC (see section 2), filtered and stained by DAPI were used. In some cases, the samples might contain high number of heterotrophic filaments (check for chlorophyll autofluorescence like for HNF – see section 4), which could not be assessed by the procedure described previously (section 2). These filaments can be bacteria, actinomycetes, fine fungal filaments etc. Filaments are defined as elongated microorganisms longer than 5 μm.
The same filter like for BAC can be used. In case of very dense filaments and detritus, a filtration through filter of higher porosity (0.4 to 1 μm) could be used, but it must be controlled for possible biomass losses.
Diameter of filaments is measured microscopically and, accordingly, filaments are grouped into several size classes (e.g. 2 classes with diameters 0.3 and 0.7 μm). The cumulative length of filaments of each class was assessed by the line-intercept method (Newman 1966). The procedure is modified for a digital image analysis system (Nedoma et al. in press, manuscript available upon request by <nedoma@hbu.cas.cz>).
The cumulative length of filaments of a particular class (randomly arranged) was calculated from the number of intercepts between the filaments and test bars of known length according to the equation:

$$CLF_i = \pi/2 \times N/T \times A_F \times 10^{-6}/V_F$$

where
CLF_i [m ml^{-1}] …… the cumulative length of filaments of a particular class
N/T [μm^{-1}] ……...the ratio of the number of intercepts and the cumulative length of test bars
A_F [μm^2] …….the effective filtration area of the filter examined
V_F [ml] ……...the volume of the water sample filtered

Conversion of cumulative length of filaments to carbon is calculated as follows:

- calculate carbon content per cell of 2 µm cell length and cell width corresponding to the diameter of respective class (i) of filaments using allometric relationship according to Norland (see section 2. BAC) …….. cC_i [fg per 2 µm cumulative length]
- calculate carbon content in the biomass of respective class (i) of filaments in the sample as follows:

$$C_i\ [\mu g\ l^{-1}] = 0.5\ CLF_i \times cC_i$$

- calculate carbon content of all classes of filaments in the sample as follows

$$C_{FIL}\ [\mu g\ l^{-1}] = \sum C_i$$

Data requested for the final evaluation from different sites are:
- lake, sampling date, depth
- diameters of classes and respective length of filaments in m per ml of sample for each class
- total carbon biomass of heterotrophic filaments in sample in µg C per litre

4. Heterotrophic and autotrophic 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
- total carbon biomass of HNF in µg C per litre

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 protocole, 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 cilinder, 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 (Utermöhl) 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 (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)
- total carbon biomass of HNF in μg C per litre

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

6. References

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