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

EMERGE

15

SEDIMENT CLADOCERA

PROTOCOL FOR ISOLATION, PREPARATION OF SLIDES AND COUNTING

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Sediment Cladocera: isolation, preparation of slides, counting

The procedure is a modified version of the former MOLAR protocol by J. Fott and W. Hofmann. The modifications are based on the experience with material from high mountain lakes of the project MOLAR.

Equipment

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

1. Full cores from new areas (Romania, Bulgaria) and Ladove (Tatras).

Isolation of cladoceran remains from the sediment

1. Samples used for pigment extraction will be analysed subsequently for cladoceran remains. The samples will be mailed to respective laboratories as dry sediment in small tubes, together with a file containing data on their original wet weight. The amount of material will correspond to about 3 g wet sediment.
   Alternatively (when no pigment analyses are carried out), weigh precisely 2-3 g wet sediment and take into analysis.
2. Transfer the sample into an Erlenmeyer flask (100 - 250 ml) with a glass cover, add 50 ml of 10% KOH.
3. Heat 10 - 15 minutes on a stirrer hot-plate (avoid boiling up!)
4. 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 (4)

4. Transfer the residue into a labelled vial. If there is a delay before further analysis, add some drops of formaline to discourage growth of bacteria and fungi.

Preparation of slides and counting

Subsampling, when necessary, can be done at this stage. Subsampling is appropriate for those remains, numbers of which exceed 400 in the sample; the number counted should not be less than 200. The less abundant remains (e.g. ephippia of *Daphnia*) must be counted in the whole sample. Sediments of high mountain lakes are often so poor in cladoceran remains that whole samples must be counted for all remains.
Subsampling: Fill up the suspension to 50 ml in a spherical, wide-neck flask. Agitate well, altering the direction of agitation several times. Take subsamples with a piston macro-pipette of 1– 5ml volume (cut off the narrow opening of the pipette's tip).

1. Put the sample (or subsample) into a centrifuge tube and let it settle or centrifuge.
2. Reduce the volume to about 0.5 - 1 ml by removing the supernate water
3. Distribute the suspension on slides, cover with 24x32 slips and count at about 100x magnification.

OPTIONAL
a. After the step (2) stain the suspension in glycerol – alcohol solution of Chlorazol-Black and, after evaporation of water and alcohol, count the stained remains in glycerol (see the procedure below) or,
b. add glycerol just in order to prevent drying up.

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).

Staining, permanent slides

Staining is recommended for better identification of remains. 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 (according to W. Hofmann):
(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 method used at the Department of Hydrobiology, Faculty of Science, Charles University, Prague (adopted and tested by Miroslava Prazakova)

Additional equipment and chemicals

1. Multiwell tissue-culture plate, "wells" of about 2 ml volume
2. Chlorazol Black E (stains chitin)
3. Glycerol : 70% Alcohol (1:1) with Chlorazol Black (saturated solution)
4. Polyvinyl alcohol (PVA) solution, with Chlorazol Black

PVA powder	8 g
Aceton 70%	28 ml
Glycerine	20 ml
Lactic acid	20 ml
Distilled water	40 ml

The 70% aceton is added slowly and with stirring to the PVA powder. Mix 20 ml water with 20 ml glycerine and 20 ml lactic acid. Stir this into the PVA-acetone paste. Add the remaining 20 ml of water drop by drop with constant stirring. Heat the cloudy mixture on a water bath about 10 minutes until it clears. Sometimes filtration through a glass wool or glass fibre filter is necessary. Dissolve some Chlorazol-Black powder in 70% acetone and stir a small amount into the clear PVA mixture until a dark violet colour is reached. The optimum concentration may vary according to the material to be stained.

Procedure

Transfer the particles (after the KOH treatment and washing) into a depression of a tissue-culture plate, let settle, partially remove the supernate and add about 8 drops of (3). Let stand uncovered about 3 days. Water and alcohol evaporates, the stained remains are in glycerol. At this stage the remains may be counted.

Making permanent slides: Put a drop of glycerol with the stained remains on a slide, add a drop of (4), and put on a cover slip. Maintain the slides in horizontal position until the mounts harden; this can take several weeks.

2. Cores (top & bottom) from survey lakes

Samples from the top 0.5 cm and from a prae-industrial layer (≈ 15 cm) will be analysed.

For details of coring, amalgamating, subsampling and mailing se the EMERGE coring protocol. The results will be expressed per unit wet weight, dry weight or as relative abundance. The amount of material taken into analysis will be decided by the analyst, the number of remains should be between 200 – 400, or as much as possible (the entire sample counted). In other respects follow the guidelines stated above.

Reference

Frey D. G, 1986: Cladocera Analysis. - p. 667-692 in: Berglund B.E. (ed.): Handbook of Holocene Palaeoecology and Palaeohydrology. John Wiley & Sons Ltd.