

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  $\mu\text{m}$  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  $>100 \mu\text{m}$  and  $100 > (\text{fraction}) > 40 \mu\text{m}$  are separated by screening and steps 1 - 6 are carried out separately. This improves the counting of large but scarce remains (e.g. ehippia 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 prevent 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.