New technique of sample preparation of Quaternary lake sediments for palynological and chironomid (Insecta: Diptera) analyses

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Abstract. Almost six-years’ experience of my work on isolation and study of macrofossils of chironomid larvae showed that the conventional technique of lake sediment sampling has significant drawbacks. This paper describes the author’s method of sample preparation of lake sediments to isolate and determine macrofossils of Chironomidae larvae for paleoclimatic reconstruction. The advantage of the proposed technique is better washing of sediment samples, which facilitates a more complete sampling of the macrofossils, as well as its applicability for palynological analysis.

1. Introduction
In limnology, chironomid larvae have long been used as indicators of various habitat types [1-4] and paleoclimatic changes [5-9]. The standard technique of lake sediment samples treatment [9] for studying macrofossils of buried chironomid larvae (whole head capsules and mentums) has several drawbacks.

During five years of studying bottom sediments of lakes in Eastern Siberia we have developed and tested a new method of sample preparation for chironomid analysis. Using the “alkaline” method, Brooks et al. [9] noted, that from 2-10 cm³ of wet sediment 50 to 100 head capsules could be recovered (depending on the type of lake and the sedimentation characteristics).

In our studies, the standard sample volume for oligotrophic alpine lakes was 0.5-1 cm³ of wet sediment. Low content of oxygen in water is a limiting factor for chironomid larvae – content of their head capsules in sediments of eutrophic and mesotrophic lakes is usually much lower than in oligotrophic ones.

Therefore, in our studies of sediment cores from eutrophic and mesotrophic lowland lakes, the volume of each wet sediment sample was twice as large and amounted to 1-2 cm³. The content of head capsules of chironomid larvae in the samples taken by us for analysis significantly exceeded the statistical average content due to better washing of head capsules from the sediment after treatment with hydrofluoric acid.
2. Results and Discussion

In the first step of our proposed method the collected samples of the wet sediment are placed in 15 ml plastic tubes. Then concentrated hydrofluoric acid (HF) is poured into the tubes up to half of their volume. The acid is poured in small portions; at the same time, with each new portion of acid, the sediment is stirred thoroughly with a plastic stick (Figure 1, a).

![Figure 1](image)

**Figure 1.** Inventory used to isolate macrofossils of chironomid larvae from lake sediment samples.

The contents of the tubes are stirred vigorously with a plastic stick to reduce foaming of the mixture. To prevent ebullition, the tube is half immersed in a container with ice or cold water. On the next day, the tubes are centrifuged for 3 minutes at 2500 g. The hydrofluoric acid is decanted and the test tubes with the precipitate are filled with distilled water. The precipitate is thoroughly mixed, then the tubes are centrifuged again. The procedure is repeated 3-4 times until the universal indicator shows a neutral medium in the supernatant. If fresh sediment shows traces of ostracods or shellfish, the sample is pretreated with a 10% solution of hydrochloric acid (HCl) in the manner described above to remove carbonates. Calcium compounds react violently with concentrated hydrofluoric acid to form a white, water-insoluble salt, calcium fluoride (CaF₂). Large amounts of this salt crystals can make it difficult to view washed samples and distort the data when estimating the amounts of coarse and fine fractions of the organic residue.
Acid treatment of samples is carried out to remove the mineral components, which makes the sludge more friable and less sticky. This ensures better washing of samples on 90–100 µm sieves made of two-layer silk bolting cloth (Figure 1, h). The fine fraction washed out of the sieve can be successfully used for spore-pollen analysis. For this purpose, each sample is washed over a container (Petri dish) from which the washed fraction is poured into its corresponding tube, and then centrifuged.

The resulting volume of each sample is recorded for subsequent assessment of the decomposition degree of the sample’s organic component. The degree of decomposition (DD) can be a very important characteristic. By DD we mean the percentage ratio of the volume of non-structured matter (particle size <<100 µm) in the sediment to the volume of larger (>>100 µm) non-humified plant and animal debris.

The detritus remaining on the sieve is washed off with a stream of water from a plastic wash bottle (500 ml) into a “common” Petri dish (D≤8 cm), on the bottom of which a 0.5 cm² cage sheet is glued on the back side (Figure 1, f-g). The organic sediment can be taken from such dishes, in rows of squares of 0.5 cm² (see Figure 1, f). This will help to find out the volume of detritus examined when it is obvious that the number of macrofossils of chironomid larvae is very large and it makes no sense to view the entire volume of organic matter from the sediment sample. For viewing detritus, we use Petri dishes (6 and 8 cm diameter) with a cage sheet glued to the bottom on the back side. A small amount of detritus from the “common” dish is transferred using a Pasteur pipette to a lined dish with a larger diameter; the detritus is evenly distributed over the cup and the sample is viewed under a binocular microscope at ×28 magnification.

As a rule, the detritus content in the sediments of high-altitude oligotrophic lakes is insignificant. Much larger amounts are observed in the sediments of meso- and eutrophic lakes of plains and lowlands – such samples should be viewed in several steps, taking 0.5-2 ml from the main sediment volume (Figure 1, f) with a Pasteur pipette (Figure 1, e).

Often the isolation of macrofossils of chironomid larvae is complicated by the high content of plant detritus in the samples. Some head capsules and their remains may simply not be seen due to their small size or severe deformity; they may also be enclosed in clumps of detritus or out of focus, floating on the water surface. When examining cores from meso- and eutrophic lakes, where the content of chironomid head capsules is usually low; washed sediment samples (detritus) were examined by us 2-3 times in Petri dishes of different diameters for a more complete isolation of macrofossils. As a rule, all identifiable chironomid larval macrofossils, contained in each core horizon, should be isolated and counted, that guarantees the most accurate interpretation of analysis data.

Head capsules are selected using a dissecting needle (Figure 1, c), an Eppendorf Research pipette (Figure 1, b) (0.5 ml) and fine entomological needles - minutiae (Figure 1, d) into a drop of glycerol on a well-filled slide (Figure 1, i). The examined detritus of each sample was concentrated in a crucible (Fig. 1, l) and using a plastic Pasteur pipette was transferred to appropriate tubes – this material can be used to study plant macroinvertebrates, zooplankton (Cladocera, Cyclopidae, etc.), macrozoobenthos and other invertebrates (Oribatida, Coleoptera, etc.) of the studied water bodies. After centrifugation all detritus (without macrofossils of chironomid larvae) was taken for subsequent calculation of DD.

When calculating and determining the selected macrofossils, ten head capsules/mentums are placed on a glass slide with a flat surface in a drop of glycerol (Figure 1, j) with thin needles and viewed under a microscope at a magnification of ×200 and ×400.

The halves of the head capsules and mentum of each species are counted separately from the whole ones. To estimate chironomid larval abundance in the sediment horizons, the total number of split mentum and head capsule larvae of each individual species is divided by two and added to the total number of whole macrofossils of the species. The resulting number of whole macrofossils of each larval form is converted to the volume unit largest among all the horizons examined. The participation of species in the assemblage of the chironomid fauna of each horizon is expressed as a percentage.
3. Conclusion
The proposed method was tested in the study of bottom sediments of some lowland and high-mountain lakes in the south of Eastern Siberia. Its principal difference is the use of hydrofluoric acid (HF) instead of KOH when processing samples of lake sediments. Dissolving the mineral component of sediments, we obtain a more friable, well-sorted, pure organic material, including macrofossils of invertebrates and plants, as well as pollen and spores.

The main advantage of the proposed technique is better washing of samples, which facilitates more complete sampling of macrofossils of chironomid larvae and other invertebrates (Cladocera, Cyclopidae, Oribatida, Coleoptera, etc.) from sediment samples. Moreover, the finer fraction washed out of the sieve can be successfully used for spore-pollen analysis. The proposed technique is suitable for both hydrobiologists and palynologists studying biotic and climatic successions of the Pleistocene-Holocene age.

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References
[1] Balushkina E V 1976 Chironomids as indicators of water pollution Methods of biological analysis of fresh water (Leningrad: Nauka Press) 106–8
[2] Ferrington L 2007 Global diversity of non-biting midges (Chironomidae; Insecta – Diptera) in freshwater Hydrobiol. 595 447–55
[3] Saether O A 1975 Nearctic chironomids as indicators of lake typology Ver. Internat. Ver. Theor. Ang. Limnol. 19 3127–33
[4] Saether O A 1979 Chironomid communities as water quality indicators Hol. Ecol. 2 65–74
[5] Enushchenko I V 2014 Non-biting midges (Diptera, Chironomidae) as indicator of climate changes on the East Siberia Vladimir Ya. Levanidov’s Biennial Memorial Meetings (Vladivostok: Dalnauka Press) 6 pp 206–10
[6] Nazarova L B and Brooks S J 2004 Chironomids (Diptera: Chironomidae) in paleoclimatic changes Euras Ent J 3(4) 300–6
[7] Brodin Y W 1986 The postglacial history of Lake Flarken, southern Sweden, interpreted from subfossil insect remains Int Rev Ges Hydrobiol 71 371–432
[8] Walker I R, Smol J P, Engstrom D R, Birks H J B 1991 An assessment of Chironomidae as quantitative indicators of past climatic change Can. J. Fish. Aquat. Sci. 48 975–87
[9] Brooks S J 1997 The response of Chironomidae (Insecta: Diptera) assemblages to Late-glacial climatic change in Kråkenes Lake, Western Norway Quarter Proc 5 49–58