3. METHODS

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Chemical analyses of the waters: Water samples were taken from surface layer (to ca. 0.5 m of depth) and from over the bottom layer (at ca. 5 m), four times in 1990 (April, June, August, October), three times in 1991 (April, August, October). In 1992 and 1993, samples were collected once a month from February to October. In 1990 and 1991, the samples were analysed for concentrations of calcium and total dissolved phosphate phosphorus and organic Kjeldahl nitrogen (total and dissolved). In 1992 and 1993 analyses were made also of pH and concentrations of nitrate nitrogen, ammonium nitrogen and iron. After filtration through Whatman filter of GF/C type, analyses were made of phosphate (SRP) by using molibdate-blue method (Standard Methods 1960), ammonia (NH_4) – indophenol blue method and nitrate ($N\text{-}NO_3$) – with addition of phenolodisulphonic acid. Dissolved phosphorus (DP) was assessed after filtration of water samples and mineralised with perchloric acid (1 ml/50 ml of sample), whereas dissolved nitrogen (DKN) – by using Kjeldahl method after mineralisation of water samples. Calcium content was determined by using complexometric method with EDTA addition. Total phosphorus was assessed in the non-filtered water samples after their mineralisation with perchloric acid, and total nitrogen – by Kjeldahl method (TKN). The sum of $N\text{-}NO_3$ and TKN was assumed to be total nitrogen. Iron in filtered and non-filtered water samples was determined by using phenantroline method (Golterman 1969).

Primary productivity, chlorophyll: Measurements of gross primary produc-

tion and decomposition (daily uptake of oxygen *in situ*) of plankton were made by using oxygen method of transparent and dark bottles after 24-hour-exposition at three depths (1, 2 and 4 m). It was assumed that the measurements represent gross production and decomposition in 0–4 m water layer of the central part of the lake. Chlorophyll *a* and phaeophityn contents were also analysed from the surface down to the bottom at every 1 m. Water samples were filtered through GF/C Whatman's filters, and then concentrations of chlorophyll *a* and phaeophityn were assessed according to Goltерman (1969). Simultaneously, measurements were made of dissolved oxygen by using oxygen electrode YSI, water temperature and Secchi's disc transparency.

Phytoplankton: Sampling and analysing procedures used in 60-ties, 70-ties as well as in the present studies were similar: water samples were taken with a 5-litre sampler at each meter of depth from the surface to the bottom, at the deepest part of the lake. Subsamples of about 100 ml of the water were taken up, stirred and preserved with Lugol's solution and formalin. In 1970–1974, samples were collected at monthly intervals from March or April to October or November, and in 1990–1991 – 3–5 times during that period. The samples were elaborated using sedimentation method under the microscope. Additionally, in 1992 and 1993, detailed analysis of picoplankton (the finest size fraction of algae 0.2–0.3 μm) was performed by using the method given by Jasser (1993).

Macrophytes: Vegetation of Lake Flosek was examined in 1991–1994. In July 1991, assessment of species composition and identification of communities was made by using Braun-Blanquet's method (Scamoni 1967). This comprised the plants growing around the lake and in littoral. Similar observations were made in two consecutive years. From November 1991 to May 1994, taxonomic composition and biomass of periphyton and floating algae (metaphyton) were assessed. Except fallen tree branches, no other available substrate for periphyton algae exist in Lake Flosek. Samples of periphyton algae were taken from 10 cm^2 of branch surface, and those of floating algae – from a water column of 1 l volume. Every month, 20 samples of each type from different parts of littoral were collected. In the laboratory, taxonomic composition of the algae was assessed under the microscope, and then, in order to determine dry weight of the algae, the samples were dried at 105°C over 12 hours and weighed. Dry weight of periphyton algae was recalculated per 1 m^2 of substrate, while that of floating algae – per 1 m^2 of littoral. Moreover, water pH and conductivity were measured inside the peatmoss mat and among algae of the littoral zone.

Zooplankton (crustaceans and *rotifers)*: Sampling procedure used in each of the study periods, i.e. in 70-ties and 1990–1993, was the same. Water samples were taken from the deepest part of Lake Flosek with use of 5-litre sampler, at every 1 m from the surface to the depth of 5 m. The samples were then concentrated with a net of about 30 μm pore size and merged into one sample representative for average conditions in the water column. In the 70-ties (see: Hillbricht-Ilkowska *et al.* 1977) as well as in 1992 and 1993, samples were taken once a month from March to October, while in 1991–1992 – less frequently, i.e. 3–5 times a year. Samples of zooplankton were preserved with Lugol's solution and formalin, and then examined by routine methods. The body weights of rotifers were determined on the basis of the rela-

tionship between body (lorica) length and fresh weight according to Ruttner-Kolisko (1977), and those of crustaceans – using length/weight regressions according to Bottrell *et al.* (1976).

Zoobenthos: Investigations of bottom fauna of Lake Flosek were conducted in 1963 and 1964, then continued with various frequency in the years 1971–1974. Twenty years after liming (1990–1994), the samples were taken 3 times a year: in spring (April), in the period of summer stagnation (July or August), and in autumn (October). In all the study periods, samples were taken with use of a pneumatic bottom sampler of Kajak's type. As liming affected mainly the bottom fauna occurring beyond the littoral zone, samples were not taken from the shallow waters (1.5 m). Thereby, sampling was confined to the central part of the lake (ca. 5 m) and sites of about 3 m deep. The samples collected were rinsed on a net of 0.4 × 0.4 cm pore size and preserved with formalin solution. The material was sorted in the laboratory, and size and weight of invertebrates were determined. Biomass (fresh weight) was assessed through weighing individuals on a torsion balance or, as in the case of *Chironomidae*, *Chaoborus flavicans* Meig., from the relationship between body length and fresh weight of individuals (Konstantinov 1969).

Bottom sediments: In 60-ties and 70-ties (pre-liming, liming and 1–4 years after treatment) the water contents, organic matter and calcium concentration in mid-lake sediments were analysed by Rybak (1969) (see also: Hillbricht-Ilkowska *et al.* 1977). After 20 years studies were carried out in spring 1993 and summer 1992 and 1993. Sediments were taken from three different sites located along the longitudinal axis of the lake. I and III site comprised ~3 m depth and weakly decomposed sediments of littoral character, and II site – semi-liquid sediments from the central part of the lake (depth of ~5 m). Phosphorus fractions were analysed in two layers: 0–5 cm and 20–25 cm using the method given by Psenner *et al.* (1993), and described in detail by Rzepecki (1997). The method enables to distinguish the following phosphorus fractions: P-labile, P bound to Fe and Mn, P bound to Al, P bound to Ca, and P-residual – bound to organic compounds and not exchanged under natural physico-chemical conditions. Analyses of element contents in the sediment layers 0–5 and 20–25 cm were made by using flame atomic absorption technique after soft digestion. Water content of the sediments was assessed from the weight difference between fresh and dried (at 105°C over 24 hours) sediments. Phosphate content of water samples and extracts obtained in the course of fractionating the sediments was determined using molibdate-blue method. Furthermore, studies on phosphate exchange between the sediments and water were performed through experimentation with cores of the bottom sediments (of a thickness 15–20 cm) taken from the above mentioned three sites of Lake Flosek. Methods employed have been described by Rzepecki (1997).