POLSKA AKADEMIA NAUK
INSTYTUT BIOLOGII DOŚWIADCZALNEJ IM. M. NENCKIEGO

POLISH ACADEMY OF SCIENCES
THE NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

POLSKIE ARCHIWUM HYDROBIOLOGII

POLISH ARCHIVES OF HYDROBIOLOGY

VOL. XXI
No. 3-4

Founded by
A. LITYŃSKI, M. BOGUCKI

Editor
R. Z. KLEKOWSKI

PWN • WARSZAWA 1974

POLSKIE ARCHIWUM HYDROBIOLOGII
jest kontynuacją ARCHIWUM HYDROBIOLOGII I RYBACTWA

POLISH ARCHIVES OF HYDROBIOLOGY
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Nakł.: 680 (566+114) ark. wydawn. 22,25, ark. druk. 13+0,625 kredy + 0,5 wkł. Oddano do składania 29.VIII 1974 r. Podpisano do druku w kwietniu 1975 r. Druk ukończono w kwietniu 1975 r. Papier druk. sat. III kl. 80 g. Warszawska Drukarnia Naukowa. Zam. 604. B-56

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	315–333	1974

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DISTRIBUTION OF MICROORGANISMS IN THE WATERS OF THE KORTOWSKIE LAKE

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ABSTRACT

The paper presents the results from microbiological studies conducted between 1966–1968 in the Kortowskie Lake, at the southern Station No. 1, where in summer deoxidized waters of hypolimnion were drained off from the depth of 13 m deep with a bream and sucking pipe and at the northern Station No. 2, which was beyond the sucking pipe operating range. In the time of investigations the total number of the bacterioplankton in 1 ml of water at the two experimental stations was in the range from 120,000 to 9000,000 cells/ml. The number of heterotrophic bacteria ranged from 50 to 4000 cells/ml and yeasts in general did nod exceed the number of 30 cells/ml of water. There was no regularity in the seasonal occurrence of these microorganisms in the water. In the time of summer stagnation bacteria were on the whole more abundant in thermocline and at the bottom of the lake while in winter they appeared in greater numbers in the surface waters. Yeasts were scarse in all the water layers. The operating bream and sucking pipe at the Station No. 1 did not have any essential effect upon either the content of the microorganisms in water or the oxygenation of water in that part of the lake.

1. INTRODUCTION

The quantity of microorganisms in the water bodies is subject to considerable fluctuations depending on various seasons of the year (Kuznetsov 1934, 1970, Henrici 1938, Salmanov 1959, Stepanek, Pokorny 1960, Kozhova, Kazantseva 1961, Genovese et al. 1963, Gerletti, Santolini 1968, Olah 1969 a, b, Jones 1971, Pokorný 1971, Niewolak 1973), on particular days (Ocevski 1958, Olah 1970) and even during the period of 24 hours (Paluch et al. 1963). These fluctuations in the number of microorganisms were attributed to: bactericidal effect of ultraviolet rays (Minder 1918, Zih 1932, Paluch et al. 1963), seasonal temperature changes (Allen 1969), oxygen content in water (Goldman et al. 1968), phytoplankton development and utilization by microorganisms the substances secreted by live algae (Fogg et al. 1965, Schegg, Ruschke 1970), or the bactericidal effect of those substances (Butterfield, Purdy 1931, Harder, Opperman 1953, Collins 1957, Sieburth 1959, Daubner 1966).

The role played by zooplankton in the limitation of bacterial population in the water bodies was also taken under consideration (Salmanov 1959, Goldman et al. 1968). The thermocline maximum of bacterial population in summer (Bere 1933, Ocevski 1960, 1966, Collins 1970, Kuznetsov 1970) is correlated with the presence of greater numbers of the decayed phytoplankton remnants in the thermocline. The more numerous appearance of microorganisms at the bottom layers of water is ascribed to the convection streams, the troubling of the bottom sediments and gathering of detritus at the bottom of the water bodies (Kuznetsov 1970, Cappenberg 1972). The excessive development of microorganisms in the bottom water layers gives in result a reduced oxygen content in the stagnation periods (Kuznetsov 1970), which decreases the economic value of the lakes. Some attempts are made to counteract those negative effects by oxygenation of the lakes (Mercier 1957) or by removal of deoxidized hypolimnion (Hooper et al. 1952, Olszewski 1959).

In the climatic conditions of the Mazurian Lake District studies on the removal of deoxidized hypolimnion have been carried on since 1956 in the Kortowskie Lake near Olsztyn. The postulate of the experiment was to improve the oxygen conditions by reconstruction of the thermic system.

It had been expected (Olszewski 1959) that the homothermy produced in this lake, in result of the draining of the cold waters of hypolimnion off, will bring about constant circulation and consequently a stronger oxygenation of the deeper layers of the water. Between 1966–1968 quantitative changes in the bacterial microflora and yeasts have been investigated against the background of general environmental conditions in the Kortowskie Lake.

2. MATERIALS AND METHODS

Lake. The Kortowskie Lake (Fig. 1 A) lies west of the outskirts of the town of Olsztyn (53° 46′ N, 21° 27′ E). The surface area of the lake is 89.7 ha, average depth 5.9 m. In the configuration of the lake basin two parts are singled out: the southern part (maximal depth 17.2 m) and the northern part (maximal depth 15.7 m). The two parts are separated by a shallow, 6–7 m deep, extending over approximately one third of the total surface area of the bottom of the lake. On its

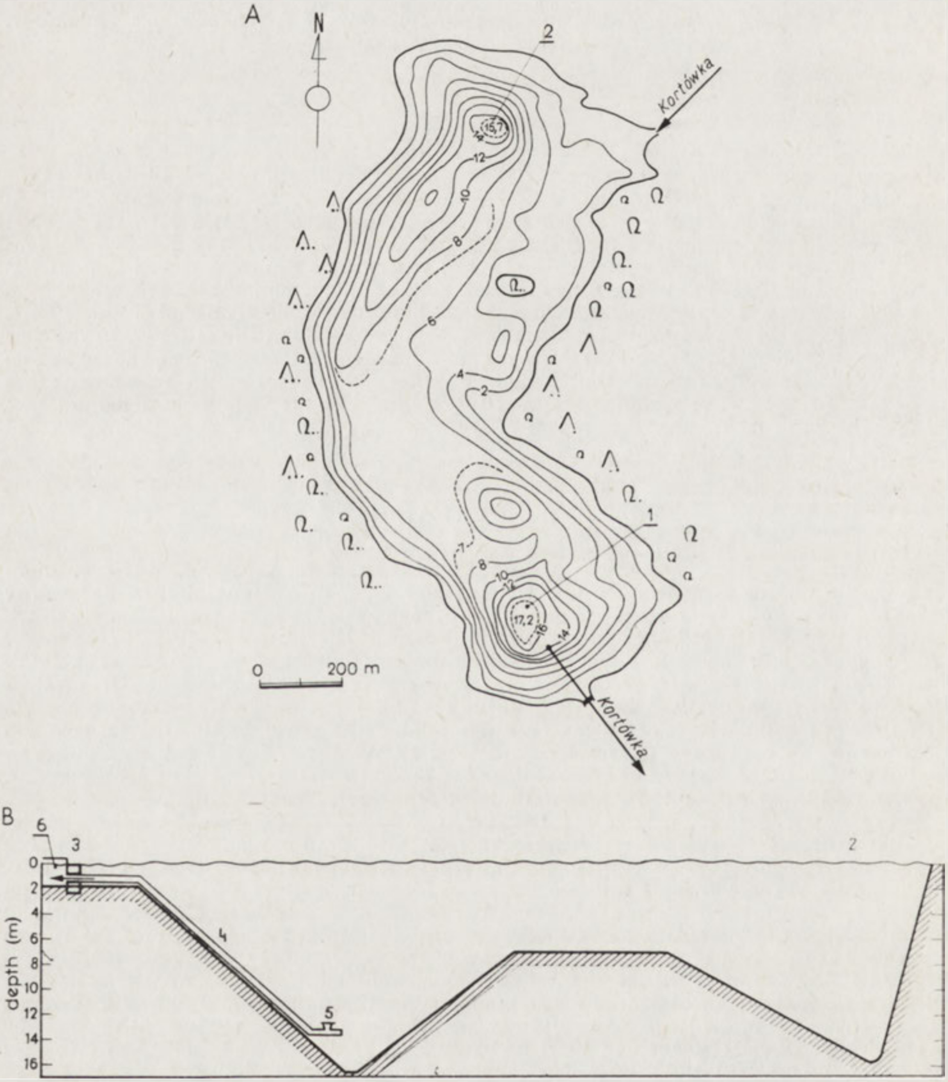


Fig. 1. Location sketch of the Kortowskie Lake (A), schematic longitudinal section of the lake (B). 1, 2 — sampling stations, 3 — weir, 4 — bream and sucking pipe, 5 — inlet, 6 — outlet

north side the Kortowskie Lake is connected by a canal with a larger body of water, the Ukiel Lake. Nearby, there is also the outlet of the main drain conveying a part of the sewage from the town of Olsztyn. On the south side of the Kortowskie Lake there is the inlet of a stream draining off the excess of its waters into the Łyna River.

Description of the experiment. In 1956 a wooden bream and sucking pipe (Fig. 1 B), 135 m long, 0.4 × 0.4 m transverse section, was introduced at a depth of 13 m into the southern part of the Kortowskie Lake (Olszewski 1959). By holding back by means of a weir the outflow of warm, well-oxygenated surface waters, in the period of summer stagnation, and due to 0.5 m difference between the water levels of the lake and the flowing out stream, a spontaneous outflow of the cold, deoxygenated deep-waters occurs by itself. The northern part of the lake is beyond the range of the experiment and to certain degree is used for comparison. During the experimental period the bream and sucking pipe was in work from June 1 to July 29, 1966 and from June 23 to September 20, 1967.

Physico-chemical determinations. In the period between April 27, 1966 and April 17, 1968, the temperature of water was measured once or twice a month at the southern Station No. 1 and the northern Station No. 2 from the surface to the bottom of the water by means of a mercury-in-glass thermometer in the Ruttner bathometer. Clarity of water was measured with a Secchi disc and the pH with a Lovibond comparator. The oxygen content in water was determined, using the method of Winkler, in the Institute of Hydrobiology and Water Conservation at the Academy of Agriculture and Technology in Olsztyn.

Microbiological studies. Simultaneously with physico-chemical measurements samples of water were collected for microbiological determinations at the Station No. 1 from the depths of: 0.01, 0.3, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, and 16.0 m deep, at the Station No. 2 from the depths of: 0.01, 0.3, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 m deep. The surface samples from the depth of 1 cm deep were collected into sterile tubes, aseptically, the samples from deeper water layers and the bottom, at the depth of 20 cm above the bottom, were sampled by means of the Isachenko apparatus using exchangeable 100–150 ml glass flasks (Rodina 1965). The period of time from the moment of sampling till the analysis did not exceed 4 hours. During the period of investigations meteorological observations were carried on.

The collected water samples were used for determination of: 1° the total number of bacterioplankton using the method of direct counting on the MF type of membrane filters with pores of 0.275 μm in diameter (Membranfiltergesellschaft GmbH, Göttingen), sterilized prior to the test by boiling in fresh distilled water. For filtering 1 ml of the tested water was used diluted in proportion 1:10 with NaCl physiological solution. Bacteria on the filters were stained with 5% solution of erytrosine in 5% phenol solution, on filter paper on the Petri plates, for three hours, and then they were counted directly under the microscope on the filters in a drop of the immersing oil; 2° the number of heterotrophic bacteria in broth-agar after 7-day incubation at 20°C; 3° the number of yeasts in malt-agar after 5-day incubation at 25°C. All the determinations were performed parallely in three inoculations and the obtained results were converted to 1 ml of water.

3. RESULTS

METEOROLOGICAL OBSERVATIONS

The results from meteorological observations carried out by the Meteorological Department at the Academy of Agriculture and Technology in Olsztyn at the time of the water samples collection from the Kortowskie Lake are presented in Fig. 2 A and B. In the days immediately preceding the collection of samples the speed of the wind rarely exceeded 5m/sec velocity and this did not give in effect any significant troubling of the waters. Heavy rainfalls in the preceding day before, the sample collection occurred only three times — in April and August 1966 and in December 1967 and the measurements did not exceed 10 mm. On the other hand, during the week preceding the sample collection from the lake, such amounts of the rainfall and even greater occurred quite often in the spring and summer and exceptionally in the autumn.

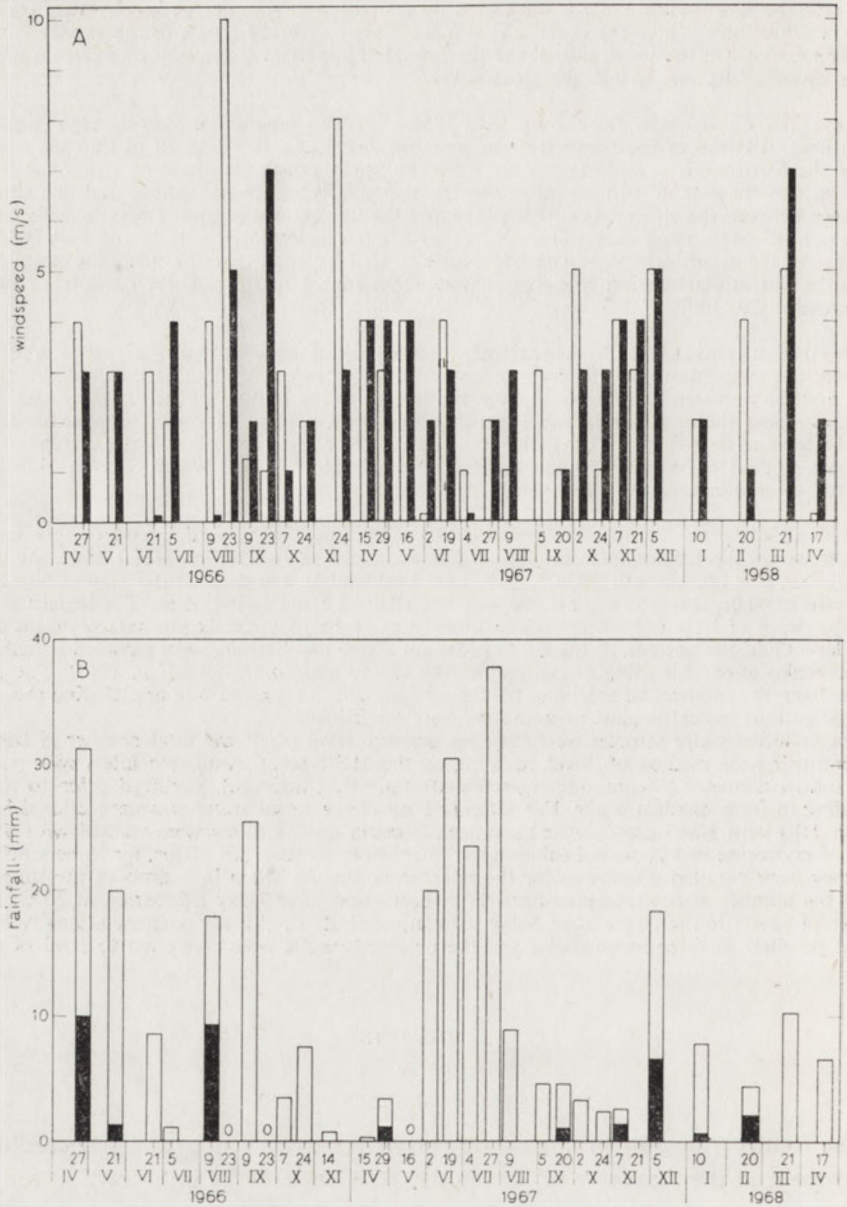


Fig. 2. Windspeed (m/sec) (A), recorded during 1 and 2 days before sampling; rainfall (mm) (B), recorded during 1 and 7 days before sampling. Black bars — during 1 day, plain bars — during 2 or 7 days

PHYSICO-CHEMICAL DETERMINATIONS

The temperature of water (Fig. 3 A–D). In 1966 distinct thermic stratification characteristic for the beginning of summer stagnation were stated at both stations in the Kortowskie Lake on May 21; in 1967 this occurred already on April 29.

The greatest differences in the water temperature of the surface and bottom layers were found during the summer stagnation on June 21, 1966. They were 13.2°C at the Station No. 1 and 13.6°C at the Station No. 2. In 1967, the maximal differences in the temperatures of the surface and bottom water layers, 13.1°C at the Station No. 1 and 14.0°C at the Station No. 2, were recorded on August 9. In the autumns 1966 and 1967 the homothermy began in October and lasted till December, i.e. until the freezing of the lake. The lake was covered with ice from December till the first days of April. In January 1968 a typical winter stratification was observed in the Kortowskie Lake with the lowest temperatures beneath the ice cover and higher at the bottom of the lake.

Oxygen content (Fig. 4 A–D). A very characteristic feature observed at both stations in the Kortowskie Lake was a complete lack of dissolved oxygen in the hypolimnion during the summer stagnation and a marked deficit of it in the autumn. The homooxygeny became stabilized but in October and lasted till December. In the time of winter stagnation in 1968 a striking deoxygenation was observed in the lower water layers where the value of oxygen content has decreased to 0.3 O_2 mg/l. The opening of the bream and sucking pipe at the Station No. 1 on June 1, 1966 and on June 23, 1967 did not improve considerably the oxygen conditions in the water at the bottom of this part of the lake.

The pH of water (Fig. 5 A and B). The pH of the lake water was in the range of 7.5–8.4 in the surface water layer and 7.2–8.0 in the bottom layers. During the vegetative period the pH values were higher.

Clarity of water (Fig. 6 A and B). The clarity of water measurements were based on the visibility of the Secchi disc in the water of the Kortowskie Lake and showed wide fluctuations during the period of investigations. The visibility was lowest in the spring–summer period ranging from 1.5 to 2.6 m and its maximum in winter was 3.5 m.

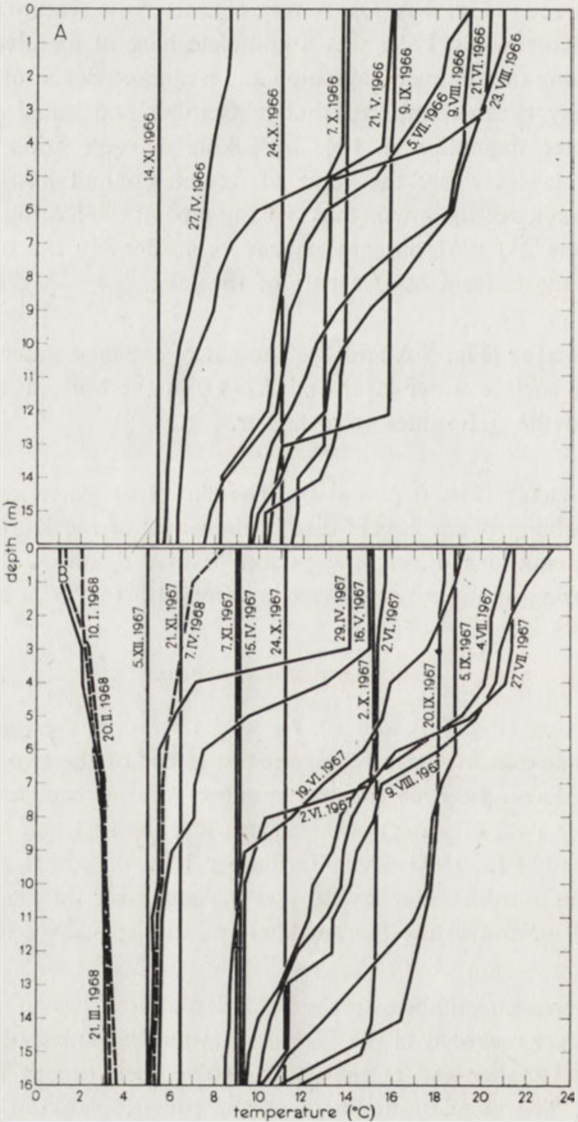
MICROBIOLOGICAL STUDIES

Bacterioplankton (Fig. 7 A and B). No cyclic seasonal changes in the numbers of the bacterioplankton in water were noted at either of the two sampling sites in the Kortowskie Lake. In 1966 greatest numbers of the bacterioplankton (up to 9000,000 cells per 1 ml of water) occurred in May, the first half of August, and in October, the smallest (250,000–400,000 cells per 1 ml of water) in the second half of August (in the bottom water layers) and in September (in the surface water, at the depth of 0.3 m, and in the thermocline) and at the Station No. 1 also in November.

In 1967 the greatest numbers of the bacterioplankton (up to 9000,000 cells per 1 ml of water) were recorded in the period from the beginning of May till the end of June, also in October and at the end of November (up to 7000,000 cells per 1 ml of water). The smallest numbers of the bacterioplankton (100,000–300,000 cells per 1 ml of water) occurred in September and in the first half of November. Similar quantities of the bacterioplankton were noted also beneath the ice in March 1968.

After the opening of the bream and sucking pipe (at the Station No. 1) a considerable decrease and after its closing a similarly strong increase in numbers of the bacterioplankton was recorded at both sampling sites.

At the Station No. 1 during the summer stagnation, in the period from May 21 to September 23, in 1966 and at more or less the same period in 1967 a characteristic vertical stratification of the bacterioplankton was observed showing its occurrence in greatest numbers (up to 9000,000 cells per 1 ml of water) in the thermo-



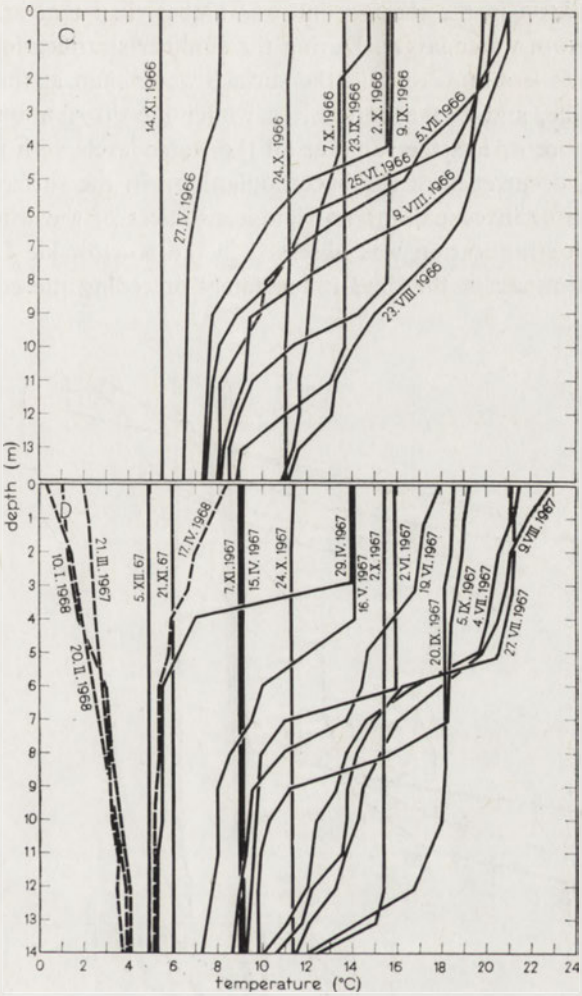


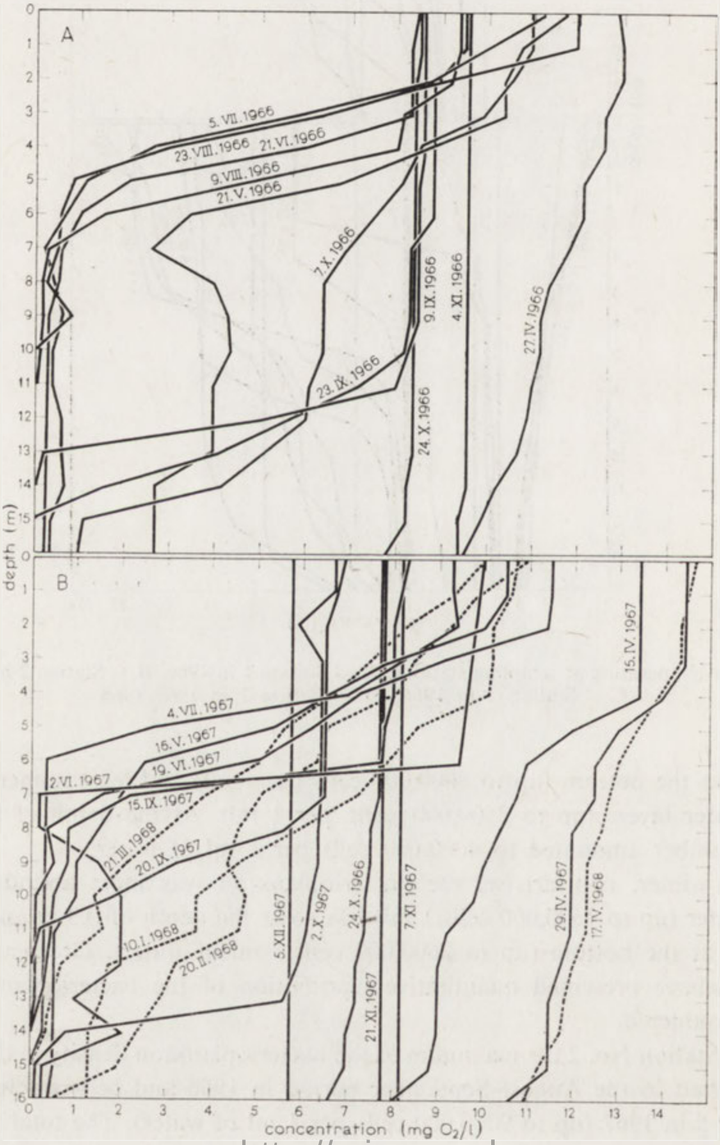
Fig. 3. Water temperature at sampling stations. A — Station 1 in 1966, B — Station 2 in 1967–1968, C — Station 2 in 1966, D — Station 2 in 1967–1968

cline and at the bottom (up to 8000,000 cells per 1 ml) and least numerous in the surface water layers (up to 2500,000 cells per 1 ml). At the depth of 0.3 m the average number amounted to 4000,000 cells per 1 ml of water.

In the winter, contrarivise, the bacterioplankton was most abundant in the surface water (up to 4500,000 cells/1 ml) less so at the depth of 0.3 m and the least numerous at the bottom (up to 2600,000 cells/1 ml of water). Greater deviations from the above presented quantitative distribution of the bacterioplankton were rarely encountered.

At the Station No. 2 the maximum of the bacterioplankton density in the thermocline occurred in the August–September period in 1966 and between July 25 and September 5 in 1967 (up to 9000,000 cells per 1 ml of water). The total number of the bacterioplankton in the thermocline was higher than that at the depth of 0.3 m or at the bottom water layers. During the summer stratification period the bacterioplankton was less numerous in the surface water than at the depth of 0.3 m, in the thermocline, and at the bottom. In winter the distribution of the bacterioplankton was more or less the same at all the water levels with perhaps a slightly more numerous occurrence of the bacterioplankton in the surface water.

In the period of investigations no significant effect of the windspeed upon the bacterioplankton stratification was observed in the Kortowskie Lake. Even at the windspeed of 10 m/sec in the days immediately preceding the collection of water



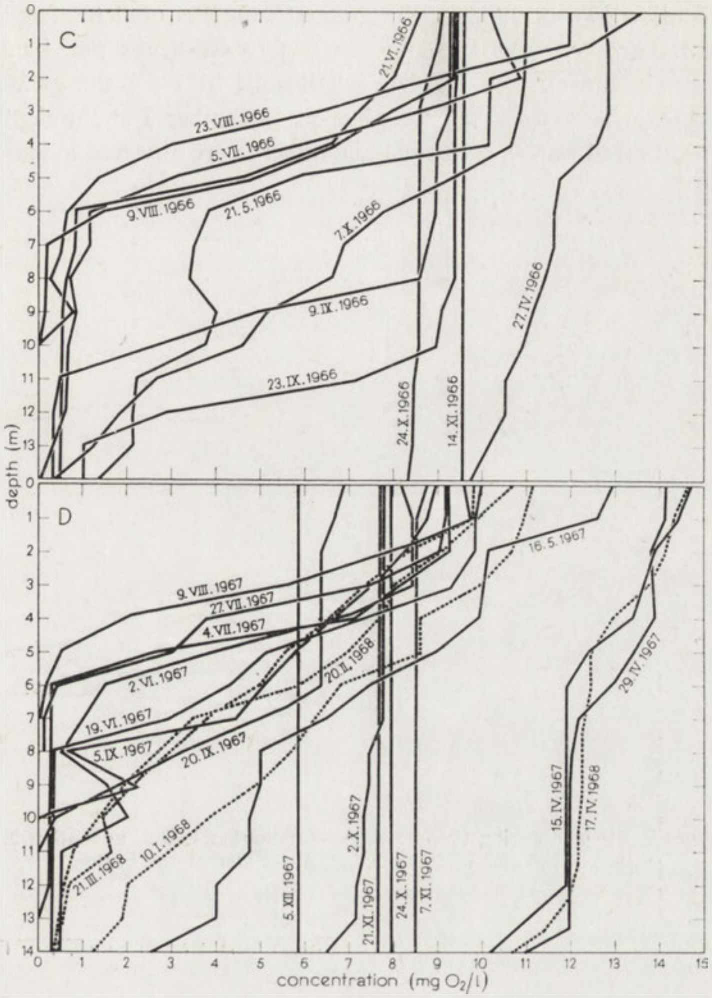


Fig. 4. Dissolved oxygen concentration at sampling stations. Denotations as in Fig. 3

samples on August 23, 1966, the differences between the bacterioplankton contents in the waters 1 cm beneath the water surface and at the depth of 0.3 m were still quite significant.

Heterotrophic bacteria (Fig. 8 A and B). At the two sampling sites in the Kortowskie Lake no cyclic seasonal changes in the numbers of the heterotrophic bacteria were noted. At the Station No. 1 in 1966 heterotrophic bacteria were most abundant in June (up to 3600 cells per 1 ml of water) and least numerous in the autumn (90–200 cells/1 ml). In 1967 heterotrophic bacteria occurred in small numbers in all the water layers; higher density was recorded in August (up to 1500 cells/1 ml) lower — in the early spring, at the beginning of June, in September and October (50–350 cells per 1 ml of water).

At the Station No. 2, in 1966, the greatest numbers of heterotrophic bacteria occurred in the first ten days of September (up to 4000 cells per 1 ml of water) the smallest — in the autumn (75–350 cells/1 ml). In 1967, the greatest number of the heterotrophic bacteria was recorded on November 7 at the depth of 0.3 m (1800 cells per 1 ml of water), such smaller numbers were observed in various periods

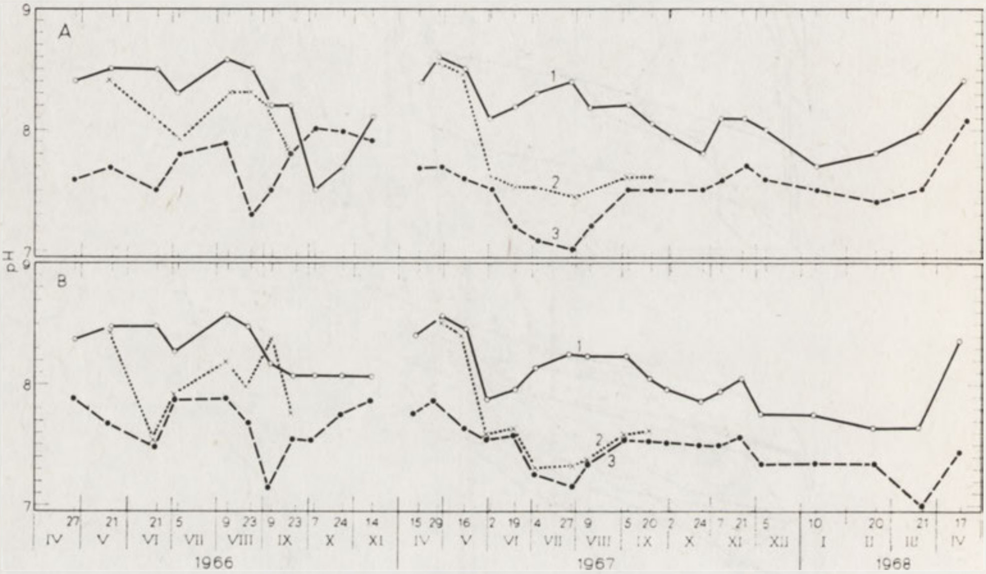


Fig. 5. pH values in the lake during 1966–1968. A — at Station 1, B — at Station 2. 1 — surface water, 2 — water in thermocline, 3 — water at the bottom

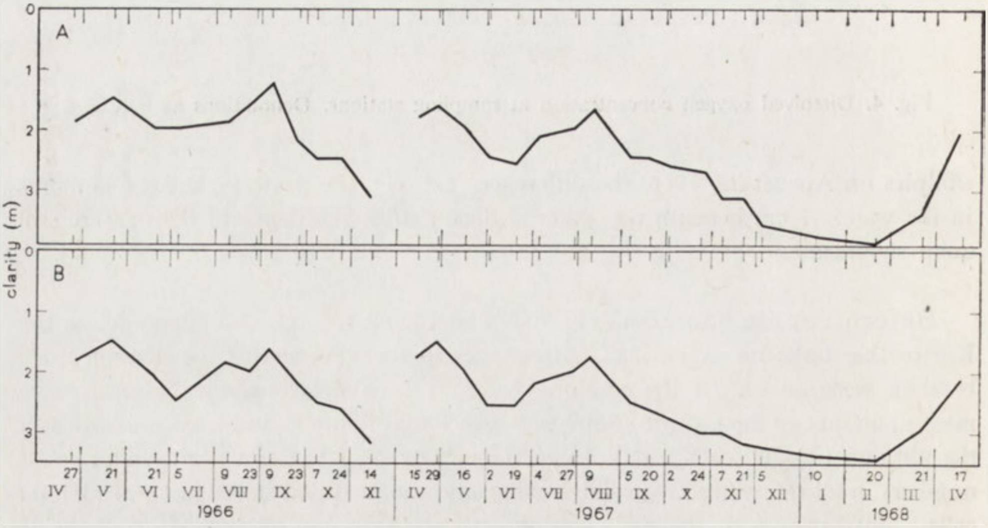


Fig. 6. Clarity of water. A — at Station 1, B — at Station 2

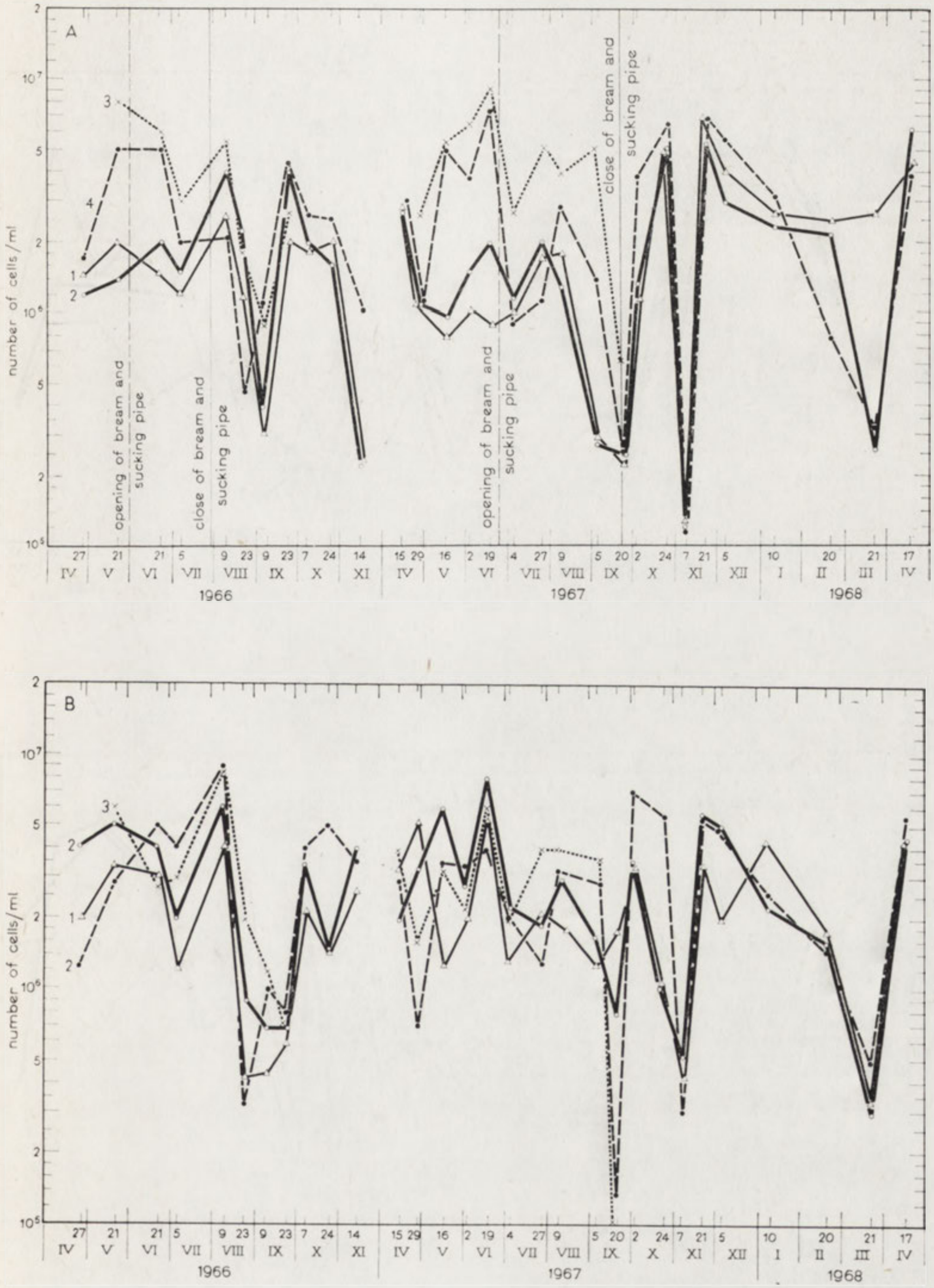


Fig. 7. Number of bacterioplankton in the water of the Kortowskie Lake. Each point is an average of 3 parallel repetitions (semi-log scale). A — sampling Station 1, B — sampling Station 2. 1 — surface water, 2 — at the depth of 0.3 m, 3 — in thermocline, 4 — water at the bottom

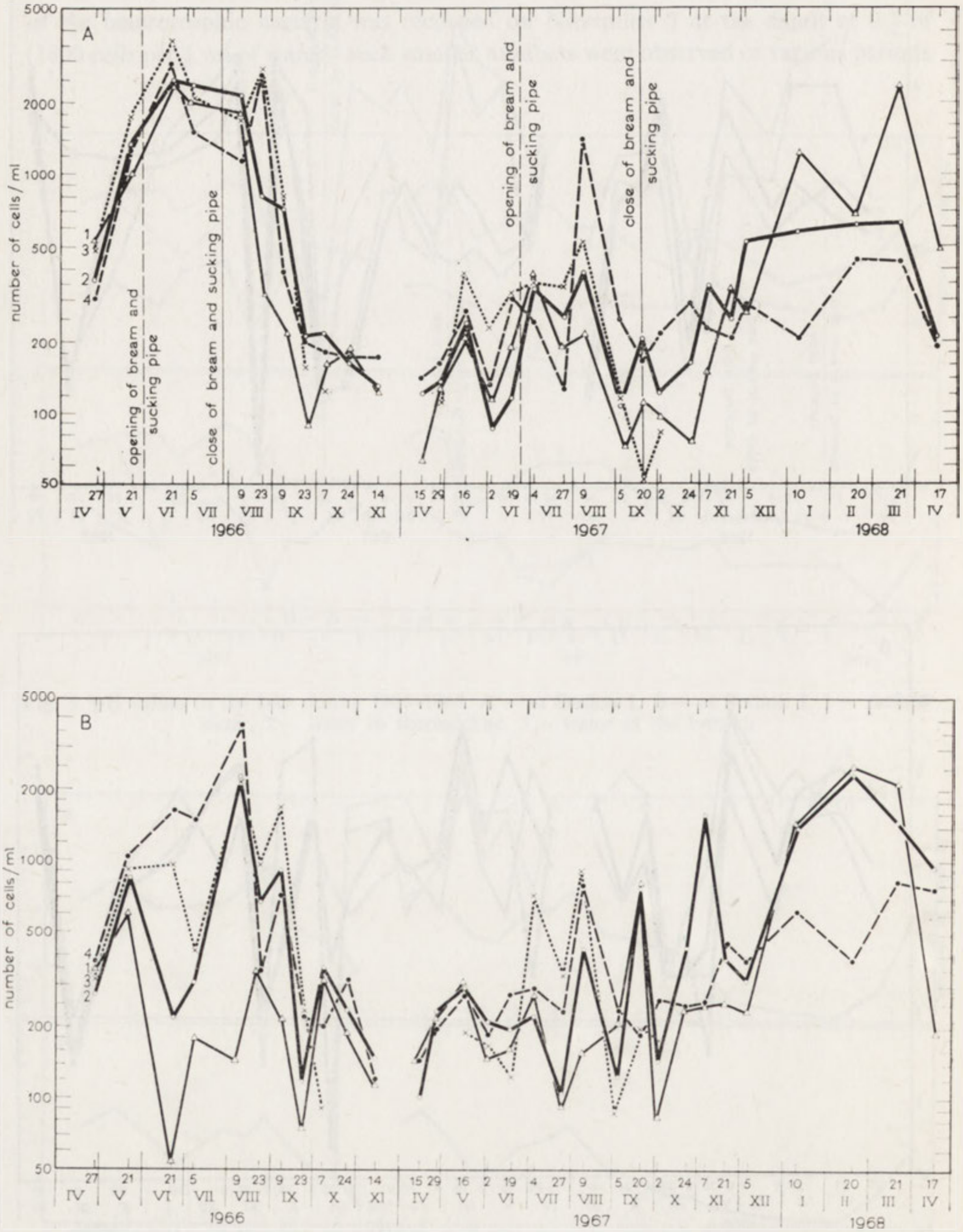


Fig. 8. Number of heterotrophic bacteria in the Kortowskie Lake. Each point is an average of 3 parallel repetitions (semi-log scale). Denotations as in Fig. 7

during the summer (85–100 cells per 1 ml of water). In the early spring they were very scarce.

After the opening of the bream and sucking pipe at the Station No. 1 in some of the various water layers an increase was observed and after the closing of the pipe a decrease in the numbers of the heterotrophic bacteria. At the same time at the Station No. 2 the situation was reverse after the opening of the bream and sucking pipe there was a decrease and after its closing — an increase in the numbers of the heterotrophic bacteria.

At both sampling sites in the Kortowskie Lake during the summer stratification the heterotrophic bacteria were more abundant in the thermocline and at the bottom and less numerous in the surface water and at the depth of 0.3 m. Greater deviations from this type of distribution occurred rarely. For instance, at the Station No. 1 in July 1967, the number of heterotrophic bacteria was greater in the surface water (400 cells/1 ml) and smaller in the water at the bottom (250 cells/1 ml). A similar stratification of the heterotrophic bacteria occurred also at the Station No. 2 in September 1967. In winter 1968 at both sampling sites heterotrophic bacteria were more numerous in the surface water (2500 cells/1 ml) than in other water layers.

The statistical evaluation of the results from investigations concerning the number of heterotrophic bacteria in broth-agar and some physico-chemical factors showed in some cases a highly significant correlation between them (Table I). A highly significant positive correlation, at the $\alpha_1 = 0.01$ level ($n = 30$, Zieliński 1972), has been stated between the number of heterotrophic bacteria and oxygen content in the bottom water layers in the Station No. 2 ($r_{xy} = +0.79$) and between the number of heterotrophic bacteria and the pH of various water layers in the Station No. 1 (r_{xy} ranges from $+0.55$ to $+0.60$).

Table I. Correlation coefficients of some physico-chemical factors and heterotrophic bacteria density of the Kortowskie Lake

Factors	Sampling station					
	1			2		
	water from					
	0.3 m depth	thermocline	near bottom	0.3 m depth	thermocline	near bottom
Temperature	+0.29	−0.31	+0.12	+0.24	−0.30	+0.01
Dissolved oxygen	−0.38	+0.11	−0.16	+0.15	−0.74*	+0.79*
pH	+0.60*	+0.55*	+0.60*	+0.21	+0.08	+0.01
24 hrs	+0.05			+0.05		
Windspeed						
48 hrs	+0.03			+0.05		
24 hrs	+0.01			+0.01		
Rainfall						
7 days	+0.05			+0.05		

* Correlation significant at 0.01 level.

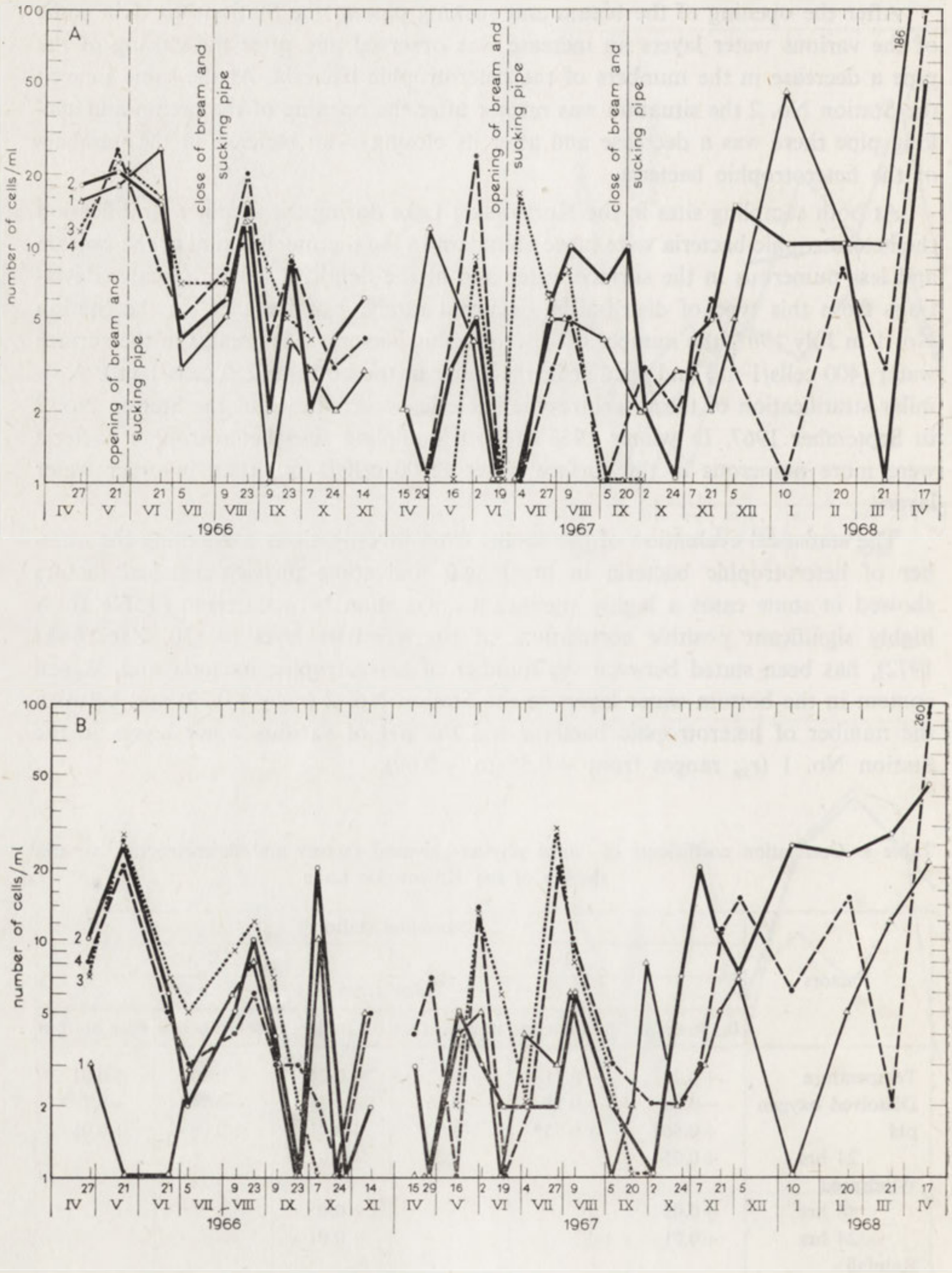


Fig. 9. Number of yeasts in the water of the Kortowskie Lake. Each point is an average of 3 parallel repetitions (semi-log scale). Denotations as in Fig. 7

The significant negative correlation was found between the number of heterotrophic bacteria and oxygen content in the thermocline at the Station No. 2 ($r_{xy} = -0.73$). The correlation in other cases (i.e. between the number of heterotrophic bacteria and temperature, clarity of water, windspeed and rainfall at both sampling sites and the pH at the Station No. 2) is not significant.

Yeasts (Fig. 9 A and B). Yeasts in the water of the Kortowskie Lake were scarse. Most frequently they were found in numbers not exceeding a few or several cells per 1 ml of water. At both sampling sites in 1966 slightly greater numbers of yeasts were observed in the spring (May), in 1967 in summer (in June and July). The maximal number of yeasts in the water of the Kortowskie Lake was recorded in the spring 1968 (265 cells/1 ml at the Station No. 1 and 185 cells/1 ml of water at the Station No. 2). The differences between the content of yeasts in the surface water, at the depth of 0.3 m, in the thermocline and in the bottom water layers were insignificant.

At both sampling sites in 1966 after the opening of the bream and sucking pipe a decrease and after its closing, an increase in the numbers of yeasts was observed in nearly all the water layers. In 1967, contrarivise, after the opening of the bream and sucking pipe the content of the yeasts in water has increased slightly and after the closing of the pipe, on the whole, a decrease in the content of yeasts was observed.

4. DISCUSSION

The results from microbiological studies in the Kortowskie Lake indicate an insignificant water pollution in the lake. The total number of the bacterioplankton (120,000–9000,000 cells per 1 ml of water) was approximately 5 times higher than in the eutrophic Jeziorak Lake in the Iława Lake District (Niewolak 1973, 1974) and 10 times higher than in the Leginskie Lakes, belonging to the eastern group of lakes in the Mazurian Lakeland (Czeczuga, Czerpak 1967). In general, such great amounts of the bacterioplankton occur more frequently in the dam reservoirs (Paluch 1963, Kuznetsov 1970).

The quantities of the heterotrophic bacteria noted in the Kortowskie Lake (50–4000 cells per 1 ml of water) are typical for the waters slightly polluted (Cabejszek et al. 1960). The quantities of yeasts (1–30, exceptionally 260, cells per 1 ml of water) likewise corresponded to the values given for waters with a low degree of pullution (Van Uden, Ahearn 1963, Ahearn et al. 1968, Meyers et al. 1970).

In the seasonal occurrence of the microorganisms in the Kortowskie Lake the presence of the accessive sources of carbon and energy supply plays an important role. The spring increase in the microorganisms population is connected with the inflow of the allochtonic organic substances during the early spring thaw and rains. This phenomenon was observed likewise in other water bodies (Straškrabova-Prokešova 1966). Later on, in summer, planktonic organisms are of greater importance.

Fluctuations in the number of the microorganisms population in summer may be caused by a stronger proliferation of zooplankton feeding on bacteria (Widuto 1972) and also by the periods of development and decay of algae secreting toxic metabolic substances. These kinds of phenomena are often cited in the literature. Paluch, Dobrzański (1957) have observed in an artificial body of water at Kozłowa Góra the disappearance of bacteria simultaneously with the growth of algae and an increase in the number of bacteria together with decay of phytoplankton after the bloom.

The increase in the number of the investigated microorganisms in the water of the Kortowskie Lake in August 1966 and July and August 1967 could have been caused by the enrichment of the lake by allochtonic organic substances during the rain in the time preceding the collection of samples. On the ot her hand the opening or closing of the bream and sucking pipe in 1966 and 1967 was not likely to have any greater effect on their occurrence in the waters of the lake. Anyhow, in the experimental period similar changes in the numbers of the bacterioplankton and yeasts were observed at both sampling sites. The number of the heterotrophic bacteria has increased after the opening of the bream and sucking pipe at the Station No. 1 which was a quite unexpected phenomenon. On the contrary, due to the removal of the biogenous salts by the sucking pipe (Olszewski 1959, Widuto 1972) a decrease in the number of all the groups of microorganisms could have been rather expected at this sampling site.

The thermocline maximum of the density of the bacterioplankton and heterotrophic bacteria population in the period of the summer stagnation is a characteristic feature of the Kortowskie Lake. A similar stratification of those microorganisms has been observed by, among other authors, Bere (1933) in the Crystal Lake (Wisconsin), Ocevski (1960, 1966) in the Dojran and Ostrovo Lakes (Macedonia), Collins (1970) in the lakes in Great Britain, Kuznetsov (1970) in the Glubokoye Lake (USSR). This phenomenon is attributed to the differences in the specific gravity of the water masses due to a sudden drop of water temperature and accumulation of greater quantities of the organic remnants of the decayed algae.

Great quantities of the bacterioplankton and heterotrophic bacteria occur in summer also in the waters at the bottom of the Kortowskie Lake. In literature similar distribution of those microorganisms is ascribed (Kuznetsov 1970, Cappenberg 1972) to the troubling of the bottom sediments and accumulation of detritus in the lower layers of the hypolimnion. No greater effect of the wind upon the stratification of the microorganisms in the Kortowskie Lake was observed at that time. In winter, the occurrence of greater numbers of the bacterioplankton and heterotrophic bacteria and sometimes also yeasts in the surface waters of the Kortowskie Lake and less numerous at the bottom can be explained by the photosynthetic processes beneath the ice on the lake (Czeczuga 1960, Pokorný 1971, Niewolak 1974).

In results of the abundant development of the bacterioplankton in summer there is a continuous oxygen deficit in the waters at the bottom of the Kortowskie Lake at both sampling sites. The quantity of oxygen consumed in respiration processes by 8000,000 cells of the bacterioplankton in June 1967 at the Station No. 1 and 9000,000 cells in August 1966 at the Station No. 2 may be in the range of 2.31 and 2.60 O_2 mg/l per 24 hrs or 69.3 and 78.0 per month, respectively. This calculation is based on the assumption that one bacterial cell uses $12 \cdot 10^{-12}$ O_2 mg/l per 1 hour at the water temperature of 15°C. These values are given by Deufel (1967) for the bacterioplankton in the Lake of Constance.

The opening of the bream and sucking pipe in 1966 and 1967 did not affect significantly the oxygenation of the water layers at the bottom of the lake in the Station No. 1 for that reason probably that the pipe comprises within its range only a small area of the hypolimnion. As reported by Widuto (1972) a part of the bottom area (28.2% at the Station No. 1 and 41.0% at the Station No. 2) was then still inaccessible to fish.

5. SUMMARY

Between 1966–1968 microbiological studies of the water in the Kortowskie Lake were carried on at the Station No. 1 (southern part of the lake), where a bream and sucking pipe was laid on in order to remove deoxygenized waters from the hypolimnion, and at the Station No. 2 (northern part of the lake), which was beyond the range of the experimental area.

Meteorological observations and measurements of the temperature of water, oxygen content, reaction and clarity of water, etc. were performed simultaneously. In the microbiological studies the number of the bacterioplankton, heterotrophic bacteria and yeasts have been taken under investigation.

The total number of the bacterioplankton in the waters of the Kortowskie Lake was in the range of 120,000 to 9000,000 cells/ml, the number of the heterotrophic bacteria ranged from 50 to 4000 cells/ml, yeasts rarely exceeded the number of 30 cells/ml. An intensified increase in the numbers of these microorganisms begins usually in spring (May) afterwards no cyclic seasonal changes were observed in their occurrence in the water.

In the period of the summer stagnation, in general, the maximal quantities of bacteria occur in the thermocline and the minimal — in the surface water. Yeasts are scarse in all the water layers. In autumn the quantitative distribution of the investigated microorganisms is more or less uniform at all the water levels. In winter they are, generally, more numerous in the surface water.

In result of an abundant development of microorganisms in summer in the water at the bottom of the Kortowskie Lake there is a continuous oxygen deficit. The opening of the bream and sucking pipe at the Station No. 1 in 1966 and 1967 did not have a significant effect on the content of microorganisms and oxygenation of water in that part of the lake.

6. STRESZCZENIE

W latach 1966–1968 przeprowadzono badania mikrobiologiczne wody jeziora Kortowskiego, na stanowisku 1 (południowym), gdzie zaistalowany jest rurociąg, którego zadaniem jest usuwanie odtlenionych wód hypolimnionu, oraz na stanowisku 2 (północnym), które znajduje się poza zasięgiem eksperymentu. Jednocześnie prowadzone były obserwacje meteorologiczne, mierzona była temperatura wody, zawartość tlenu, odczyn wody i przezroczystość. W badaniach mikrobiologicznych uwzględniano ogólną liczbę bakterioplanktonu, bakterie heterotroficzne i drożdżaki.

Ogólna liczba bakterioplanktonu w wodzie jeziora Kortowskiego wahała się od 120 tys. do 9 mln komórek/ml, bakterie heterotroficzne występowały w ilościach rzędu 50–4000 komórek/ml, drożdżaki rzadko przekraczały 30 komórek/ml. Silniejszy wzrost liczebności tych drobnoustrojów rozpoczyna się zwykle wiosną (maj), później brak jest cyklicznych zmian sezonowych ich występowania w wodzie. W okresie stagnacji letniej maksymalne ilości bakterii występują na ogół w metalimnionie, minimalne w wodzie powierzchniowej. Drożdżaki nielicznie w różnych warstwach wody. Jesienią rozmieszczenie badanych drobnoustrojów jest mniej więcej równomierne w całej toni wodnej. Zimą występują one na ogół liczniej w wodzie powierzchniowej.

Następstwem licznego rozwoju drobnoustrojów w wodzie przydennej jeziora Kortowskiego latem, jest ciągły deficyt tlenu. Uruchomienie rurociągu na stanowisku 1 w 1966 i 1967 r. nie wpływało w sposób istotny na zawartość drobnoustrojów i natlenienie wody w tej części jeziora.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	335–342	1974

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PHOSPHATASE ACTIVITY OF AQUATIC BACTERIA *AZOTOBACTER AGILIS*. PART I. INFLUENCE OF THE AGE OF CULTURE, PH AND SUBSTRATE CONCENTRATION

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ABSTRACT

Azotobacter agilis, a strain of bacteria capable of hydrolysis of phosphoric acid esters, was isolated from water of Oława River. The influence of the age of culture, pH and substrate concentration upon the intensity of acidic and alcalic phosphatase was studied in laboratory conditions.

1. INTRODUCTION

Phosphorus is indispensable for all living organisms and its presence in surface waters is considered to be of paramount ecological significance. It is a component of nucleic acids and of highly energetic compounds, including adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP). This element is instrumental in all the basic metabolic processes, in energy storage and transportation, in processes of synthesis of enzymes, etc.

The importance of phosphorus for algae is quite exceptional, as it stimulates and even determines the limits of their development. Therefore the presence in water of compounds making phosphorus readily available is considered to be the main cause of mass development of algae and of increasing eutrophization.

Phosphorus can be found in surface waters in many different organic and mineral forms. According to Hutchinson (1957), organic phosphorus appears in those waters in higher concentrations than mineral phosphorus. The same is true for sea waters (Armstrong 1965). Availability of phosphorus for algae differs for its various compounds. In principle, it is absorbed only in form of orto-phosphates. Other compounds can be used only if they are appropriately transformed. This can occur in purely chemical, as well as in biochemical ways. The mechanism of those reactions is not fully understood yet, though we know that they play a significant role in the increase of the degree of eutrophication. The study of biochemical processes is made difficult mainly by the great variety of the ways in which different sources of phosphorus are utilized and metabolized by microorganisms.

One of the many interesting ways of mineralization of phosphorus compounds is the hydrolysis of phosphoric acid esters, with simultaneous release of phosphate ions. The reaction occurs under the impact of phosphatases produced by many species of microorganisms.

According to many reports, among them there are also some species of algae which possess phosphatases and thus are able to decompose organic phosphorus compounds and then to utilize the products of such hydrolysis.

The first report of the presence of acidic phosphatase in such algae as *Anabaena cylindrica*, *Anacystis nidulans* (Cyanophyceae), *Chlorella vulgaris* (Chlorophyceae) and *Monodus subterraneus* (Xanthophyceae) was by Talpasayi (1962). Bone (1971) experimented with fresh-water Cyanophyceae *Anabaena flos-aquae* and he proved the presence of alcalic phosphatase in them. The fact of the presence of phosphatases in many species of algae was also confirmed by Kuenzler, Perras (1965), Fitzgerald, Nelson (1966), Reichardt (1971) and Overbeck (1962). It was proved by those experiments that algae can utilize such phosphorus compounds as glicerophosphate, lecitine, nucleic acids, glucose-6-phosphate and phospholipids.

In opposition to the generally recognized capacity of bacteria to biodegradation of organic compounds, informations about their participation in mineralization of organic phosphorus compounds are scant. The aim of the present work was to prove the presence of phosphatases in surface water bacteria and to study the changes in the activity of those enzymes under the influence of certain environmental factors.

A proof of their ability to release the phosphate ion from organic phosphorus compounds would confirm the presence of an additional link in the fertilization of surface water with mineral phosphorus, enhancing the mass development of algae.

2. MATERIAL AND METHODS

ISOLATION OF EXPERIMENTAL MATERIAL

Water from Oława River was sown on agar substrate with an addition of 1% solution of disodium fenolftalein diphosphoric acid salt. After 72 hours incubation at 22°C the colonies grown on the surface of agar were exposed to ammonium vapors. Bacterial colonies which did not produce phosphatases remained colourless in such conditions, while those with phosphatases released free fenolftalein during incubation which in alcalic environment made the colonies pink-hued; the latter were taken up for further investigations.

IDENTIFICATION OF THE EXPERIMENTAL STRAIN D_2

Pure bacterial cultures were derived by normal methods from the phosphatase colonies. For investigations of phosphatase activity strain D_2 was used; it was subject to an identifying procedure to establish its systematic position.

SUBSTRATE AND METHOD OF CULTIVATION OF STRAIN D_2

The agar substrate used for propagation and obtaining of materials for enzymatic studies was composed of the following ingredients: KNO_3 — 1.0 g, $MgSO_4 \cdot 7H_2O$ — 0.2 g, NaCl — 0.5 g, $FeSO_4 \cdot 7H_2O$ — 0.01 g, pepton — 5 g, glucose — 5.0 g, beta natrium glicerophosphate — 0.5 g, aqua dest. — 1000 ml, substrate pH was 7.5. The substrate was sterilized three times.

Phosphatase bacteria were sown on agar thus prepared and the culture was kept at 22°C during 48 hours.

THE MAKING OF BACTERIAL SUSPENSION

The bacterial culture was submarsed in physiological salt solution and homogenized in Poter-Silber electrical homogenizer for 10 minutes. Such suspension was reduced to a constant density, yielding an extinction of 0.5 after dissolution. The measurement was taken by means of a photocolorimeter produced by Specol at 450 nm.

IDENTIFICATION OF THE ACTIVITY OF ALCALIC PHOSPHATASE (AP)

The activity of alcalic phosphatase (AP) was identified by the method described in Bodansky (1932, 1933). As substrate was used 0.016 M of beta natrium glicerophosphate in 0.048 M of natrium veronal, at pH 8.6. The volume of the sample was 5 cm³, incubation time was 60 minutes at 37°C. The incubat contained 40 μ of substrate moles and 0.5 cm³ of bacterial suspension of constant density. For each identification a control sample was prepared, in which the bacterial suspension was immediately deproteinized, and a blank sample with 0.5 cm³ of water instead of the bacterial suspension. After incubation the amount of released inorganic phosphorus in clear filtered substance was identified by the method of Fiske, Subbarow (1925). As the unit of activity was adopted the amount of enzyme releasing 1 mg of phosphorus after 1 hour of incubation at 37°C in the conditions defined by the employed method.

IDENTIFICATION OF ACTIVITY OF ACIDIC PHOSPHATASE (AcP)

Activity of acidic phosphatase was identified by the method of Bodansky (1932, 1933). As substrate was used 50.3 mM of natrium beta glicerophosphate at pH 5.0. The other details of the procedure were the same as in AP activity identification.

PROTEIN IDENTIFICATION

Homegenized bacterial suspension was centrifuged at 10,000 rpm and protein was identified in clear supernatans by spectrophotometric method as described in Warburg, Christian (1941). Absorption was measured at 280 and 260 nm.

CHECKING OF EFFICIENCY OF BACTERIAL CULTURES AFTER VARIOUS INCUBATION INTERVALS

Phosphatase bacteria were sown on substrates in the above described manner. AP and AcP activity was identified after 1, 2, 3, 4 and 5 days.

DEFINING OF pH INFLUENCE

Investigating the influence of pH on phosphotase activity of bacteria, on one hand the role of agar pH was observed, and on the other hand the influence of short-term impact of various pH degrees on phosphotase activity of bacteria cultivated on neutral substrate was determined.

Bacterial substrate with pH 7.0 was modified by means of 1 n HCl and 1 n NaOH to pH ranging from 5.0 to 10.0, with differences at 0.5 pH intervals. Suspensions were prepared of bacteria cultivated on such substrates and AP and AcP activity levels were determined for each suspension.

At the same time suspensions of bacteria cultivated on pH 7.0 substrates were exposed to the following buffers: pH range from 4.5 to 7.0 — 0.2 M citric buffers, from 7.5 to 8.5 — 0.2 M Tris buffers, from 9.0 to 10.0 — 0.2 M veronal buffers, 10.5 — 0.2 M carbonate buffer, taking 1 part of suspension per 1 part of the respective buffer substance in each case. After the buffer was added, the mixtures have been incubated for 30 minutes at 20°C. After incubation, pH of each sample, as well as AP and AcP activity was determined.

PREPARATION OF VARIOUS SUBSTRATE CONDENSATIONS

Natrium beta glicerophosphate in 0.048 M veronal buffer at pH 8.6 in condensations from 5.0 mM to 500.0 mM was used as substrate. Bacteria suspended in physiological salt solution were exposed to substrates of various condensations and AP activity was then determined.

3. RESULTS

In order to define the systematic identity of the experimental strain D_2 a number of routine identity tests were performed, namely cell and colony morphology was determined and certain basic biochemical tests were carried out.

Cell morphology: bacilli 3–4 μ long, gram-negative, forming long threads. In fixed preparation the visible threads are homogeneous and divided into sections. Luminous structures visible in preparation hued for survival. Non-sporing rods with coats.

Colony morphology: 1–2 mm in diameter, round, very slightly convex in profile, grained structure, waved surface with irregular edges. Different colonies on nutrient agar and on beta glicerophosphate morphologically.

Culture characteristics: poor growth on broth which becomes slightly opaque, with sediment on bottom. On nutrient agar the culture grows in form of a concave, luminous and non-transparent scratch. On a potato it does not cause substrate change and grows in form of a diffused scratch.

In Table 1 the most remarkable results of physiological tests of strain D_2 are presented.

According with the systematics accepted by Bergey (Breed et al. 1957) the strain was classified as *Azotobacter agilis*. It was found out by methods described above that it produces phosphatase and for that reason it was used in further investigations.

Table I. The most remarkable results of physiological tests carried out on strain D_2

Type of test	Result
Absorption of free nitrogen	absorbs nitrogen from air
Test for catalase presence	positive
Fluidation of gelatine	makes gelatine fluid
Growth on milk	ferments and congeals
Indole production	does not produce
Sugar fermentation	ferments lactose and mannitol; glucose and sacharose not vulnerable
Production of sulfuretted hydrogen	does not produce
Production of ammonium	produces in small amounts
Production of asotines	does not produce
Production of pigment	from sightly yellow to brown, intensity depending on age

Figure 1 presents the influence of culture age upon AP and AcP activity in *Azotobacter agilis*. It was found that AP activity was greatest during the first two days and decreased by about 30% during the 3rd, 4th and 5th day; thus, only two days old cultures were used in further research works.

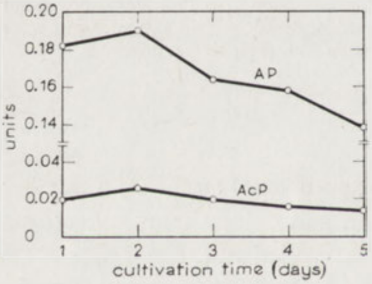

Fig. 1. Influence of culture age on the activity of alcalic phosphatase (AP) and of acidic phosphatase (AcP) of *Azotobacter agilis*

Figure 2 presents the influence of pH upon AP activity. It was greatest within pH range from 8.4 to 8.7. If pH is lowered to 6.1, activity decreases by about 89%; if pH is pushed to 9.5, activity decreases by 55%. Further increase of pH level caused a sudden fall of activity which at pH 10.4 is only 2% of that in optimal pH conditions.

Figure 3 presents the influence of various substrate pH levels on AP activity of bacteria. It was found that bacteria grown on substrate with pH 7.5 were the most AP active. Substrate pH level decrease to 5.0 decreased AP activity of bacteria by about 40% and pH increase to 10.0 brought about activity fall by about 80%.

Figure 4 presents the influence of pH on AcP activity. It was found to be the greatest at pH 5.5. At pH 4.0 activity was lowered by about 60%. At pH raised to 6.5 activity was lowered by about 40% and further pH decrease brought about a gradual deterioration of activity which at pH 11.0 was about 20% of that observed in optimum pH conditions.

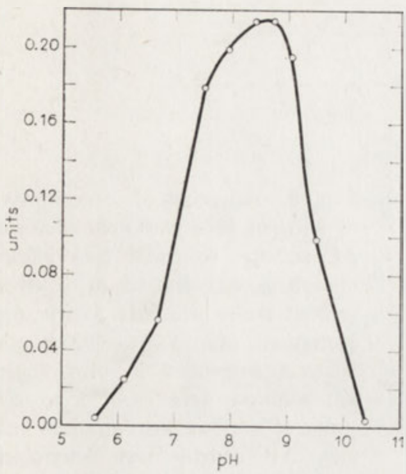

Fig. 2. Influence of pH on AP activity (Contact with buffers within the range from 4 to 11 pH lasted for 30 minutes at 20°C; after that time pH level was corrected by pH-meter and AP activity was then determined. Protein concentration, determined by spectrophotometric method, was 8.6 mg/ml in average)

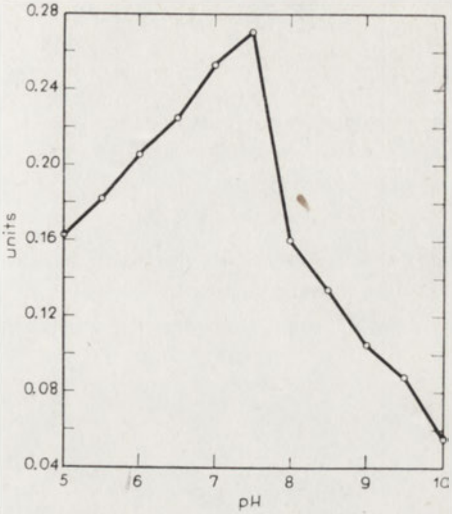

Fig. 3. Influence of various pH levels of substrate on AP activity of bacteria. (Suspension of bacteria in physiological salt solution after 2 day of cultivation on substrates with pH from 4.5 to 10.0 was found to have pH from 6.4 to 7.4. Protein content was from 5.0 to 6.4 mg/ml)

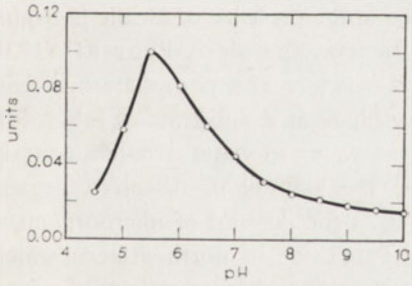

Fig. 4. Influence of pH on AcP activity. (Contact with buffers within the range of 4 to 11 pH lasted for 30 minutes at 20°C; after that time pH was corrected and AcP activity was then determined. Protein concentration, determined by spectrophotometric method, was 5.4 mg/ml)

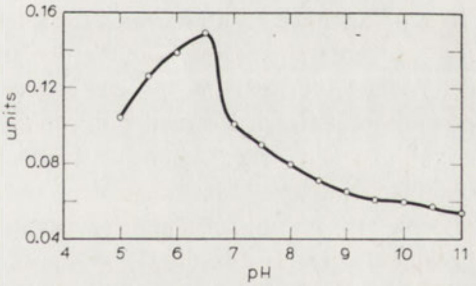

Fig. 5. Influence of various substrates on AcP activity of *Azotobacter agilis*. (Suspension of bacteria in physiological salt after 2 days of cultivation on substrates with pH from 4.5 to 10.0 was found to have pH 6.5. Protein content was 7.0 mg/ml)

Figure 5 presents the influence of various substrates on AcP activity of bacteria of the species *Azotobacter agilis*. It was highest in bacteria cultivated on a substrate with pH 6.0. Activity dropped by 40% when pH was either lowered down to 4.5 or raised to 6.5. Further raising of pH level caused only gradual fall of activity as compared with that in optimal pH conditions.

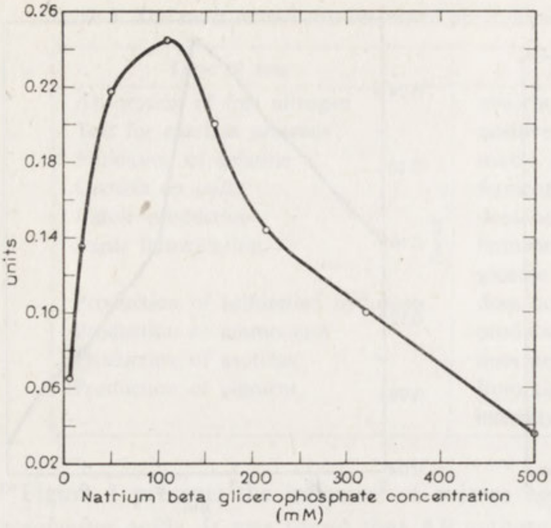

Fig. 6. Influence of concentration of natrium beta glicerophosphate on AP activity. (Natrium beta glicerophosphate was applied in 0.048 M veronal buffer with pH 8.6, in concentrations from 5.0 to 5000.0 mM. Bacteria suspended in physiological salt solution were exposed to substrate of various concentrations, and then AP activity was determined)

Figure 6 presents the influence of concentration of natrium beta glicerophosphate on AP activity. The activity was highest at 106 mM of natrium beta glicerophosphate and only slightly less intensive in 50 mM and 160 mM. Concentration level of 16 mM inhibits the activity by about 35% and 500 mM by 84%.

4. DISCUSSION

Scholars have been interested for a long time in the presence of alcalic phosphatase in water microorganisms, particularly in bacteria and algae. Pearsall (1921) determined the total phosphorus concentration in water and phosphatase activity of microorganisms. He used p-nitrophenol phosphate as a substrate in laboratory conditions and organic phosphates dissolved in water in natural conditions; he obtained similar results, dependent only upon the amount of dissolved organic phosphorus. Phosphatase activity was increasing as the amount of microorganisms became larger. Jones (1971, 1972) studied AP activity in north-western waters of England and he found a positive relation between phosphatase activity of microorganisms in water and the degree of eutrophication of the investigated lakes. Overbeck (1967, 1968), Stewart, Brown (1969) and Shilo (1970) believe that certain groups of bacteria which carry out the lysis of cell membrane of algae are responsible for more rapid transfer of their cell contents to the water environment. Thus, according to those authors, an increase of the amount of bacteria and algae leads to higher enzymatic activities found in water.

In the present work we tried to disclose the influence of culture age, pH and substrate concentration on AP and AcP activity of a selected strain. Both alcalic and acidic phosphatase was found to be present in bacteria of the species *Azotobacter agilis*. It must be remarked that AP activity was much higher than AcP activity and e.g. it was 0.19 units in a two days old culture, while AcP activity in the same culture was only 0.03 unit.

Our results concerning the influence of pH on phosphatase activity in Azotobacter seem to be interesting. During two days incubation of bacteria on substrates with different pH their growth was equally good, but their AP activity was different. It was found to be highest in bacteria that had grown on agar with pH close to neutral. Short-term exposition of bacterial suspension to various buffers proved that the highest AP activity occurred at pH ranging from 8.4 to 8.7, i.e. at pH similar to those at which AP is active in higher organisms.

Similarly, the highest AcP was found in bacteria that had grown on a pH 6.5 substrate, rather than in those growing on substrate with other pH values, and short-term exposition of bacterial suspension to various buffers proved AcP activity to be highest at pH level 5.5.

The highest AP activity was observed in concentration of 106 mM of natrium beta glicerophosphate, i.e. more than six time greater than the concentration applied in Bodansky method.

5. SUMMARY

Alcalic phosphatase (AP) and acidic phosphatase (AcP) activity in saptrophytic bacteria of the species *Azotobacter agilis* was investigated. The influence of population age, pH level and various substrate concentration was determined.

The highest AP and AcP activities were observed during the first two days of cultivation, i.e. during logarithmic growth. Optimum pH for AP is in the range 8.4 to 8.7 and optimal substrate was found to have pH level 7.5. For AcP the optimal pH was found to be 5.5 and substrate pH 6.5. The highest AP activity was observed at the concentration of 106 mM of natrium beta glicerophosphate.

6. STRESZCZENIE

Zbadano aktywność fosfatazy alkalicznej (AP) i fosfatazy kwaśnej (AcP) u bakterii saprofitycznych z gatunku *Azotobacter agilis*. Określono wpływ wieku populacji pH i różnych stężeń substratu.

Najwyższą aktywność AP i AcP stwierdzono w pierwszych dwóch dobach tj. w okresie logarytmicznej fazy wzrostu. Optimum pH dla AP waha się w granicach 8,4–8,7, a podłoże o pH 7,5 okazało się optymalne. Optimum pH dla AcP wynosi 5,5, a podłoże o pH 6,5 jest optymalne. Najwyższą aktywność AP stwierdzono w 106 mM stężeniu beta glicerofosforanu sodu.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	343–353	1974

NORBERT WOLNOMIEJSKI AND ELŻBIETA PAPIS

"PSEUDOLITTORAL" OF THE JEZIORAK LAKE — A SEPARATE BOTTOM ZONE OF A STRONGLY EUTROPHIED WATER BODY

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ABSTRACT

The pseudolittoral zone has developed in result of the accumulation of mud and the shallowing of the lake to such an extent that mud sediments have entered within the range of the photic zone. The prevailing ecological conditions are modified significantly by the effect of light. Some biotopic properties of this zone have been analized and characteristics of the bottom fauna based on the findings of the two-year studies are presented. The *Einfeldia* e.g. *carbonaria* larvae, proved to be the essential and indicatory element of the benthos; they constitute 50% of the total population of the benthic fauna.

1. INTRODUCTION

The shallowing of lakes is one of the outward signs of the uninterruptedly progressing process of natural eutrophication. Simultaneously with changes in the complex limnological features such as "the getting old of lakes" there are also visible changes in the character of the bottom zonality. In the shallowed eutrophic water bodies the sublittoral zone has practically disappeared (Lundbeck 1926) and the vertical range of the littoral zone extent diminishes.

In the highly eutrophied lakes the excessive accumulation of the layers of mud sediments accretes to such a degree (in particular in smaller bays) that the primarily profundalic bottom begins to approach the photic zone. In spite of the fact that the consistency of the sediments and partially also other features remain alike those in the profundal nevertheless the presence of the light conditioning the photic character of sediments metabolism, the development of photosynthetizing algae, another thermic rhythm, and other similar agents form a specific type of biotope.

Already during preliminary and still fragmentary studies on the benthic fauna of this specific kind of biota (Wolnomiejski unpublished) it has been supposed that it must be a specific, new-forming, in a category by itself, lake zone. The author has proposed to use the name "pseudolittoral" for the muddy regions of the bottom lying within the range of the photic zone and formed in result of the shallowing of the extralittoral parts of the lake basin. Since the assigned term has been previously used in the limnological terminology for other meanings, quotations marks are used herein.

The purpose of the present study was to investigate the structure of the benthic fauna groupings in the "pseudolittoral" of the Jeziorak Lake as a faunal indicator of the specificity of the whole zone and the evaluation of the character of some ecological factors with the aim in view to define more precisely the conception of the "pseudolittoral".

On account of the continuous and intensively progressing process of the shallowing of the examined lake it is also a contribution towards prognostication of the future trends in the development of the benthic fauna communities.

2. METHODS AND DESCRIPTION OF THE TESTING GROUND

Samples were collected by means of the Ekman-Birge dredge. For washing the sediments a 0.5 mesh strainer was used. Usually, one sample consisted of the contents of four samplers, in cases of a great density of benthos only of two samplers.

In the course of the investigations in 1969 every month from April to November samples of a few milliliters of the surface layer of mud from the "pseudolittoral" at the Site No. 2 and from a selected site in the shallow profundal have been collected. In these samples, fixed in formalin, the composition and the frequency of different types of algae were determined. For each test preparates were stained with methylene blue and Lugol solution.

All the algae were determined and counted under the microscope in the subsequent fields of view so as to make the number of the determined individuals or colonies not less than 100 in each test. In similar manner the composition of algae in the preparates of the alimentary tracts of Chironomidae larvae was elaborated. Preparates in this case were smears of alimentary canals. Results concerning nutritive selectivity of *Einfeldia* and *Chironomus* larvae are based on conversion in terms of the Ivlev indicator (1955) after Fischer (1968).

During the studies in experimental grounds in 1969 measurements of thermic conditions of the surface of the "pseudolittoral" sediments and the water layer at the bottom of the lake were carried out, every month. For measurements a thermometer with temperature-sensitive resistor was used.

The material of benthic fauna was sampled at four permanent stations located in the inlet called Moty. Samples were collected once a month in the period from April to November 1969 and once a fortnight in the period from May 1970 to January 1971. In the summer months of 1969, once, samples were collected additionally from 16 sites located all over the lake.

The Moty Bay constitutes a southwest basin of the Jeziorak Lake and is separated from the main gully of the lake by a long island. This bay is distinguished by a particularly high degree of eutrophication. This is evidenced by a series of its characteristic features:

Turbid water, with transparency always lower than in the other parts of the lake (visibility of the Secchi's disc is never more than about 1 meter).

Strong sliming and shallowing (maximal depths of the bay is but 2.5 m and thickness of muddy sediments is up to 7 m or even more).

Highly eutrophic character of vascular plants (Bohr 1965) and in particular predominance of *Typha angustifolia* over *Phragmites communis* and the abundant development of Potamogetonaceae.

Greatly advanced and spreading all over process of marshing and overgrowing of the banks.

Samples were collected in four permanent stations, at varied depths: site No. 1 approx. 0.5 m deep; Nos. 2 and 4 approx. 1 m deep (site No. 4 was located on the opposite bank as compared with the other three) and No. 3 approx. 1.5 m deep. Sediments consisted in grey-brown mud with bluish-green surface layer stained by algae. After the washing of the mud there was usually a lot of green, greenish-yellow, and yellowish-orange clumps of algae colonies left over on the strain. There were also quite often clusters of whole or crumbled *Valvata piscinalis* shells to be seen. At the sites No. 2 and 4 a considerable admixture of detritus was found in the mud.

For the most part during the sampling of the sediments characteristic odor of H_2S was perceivable.

The additional sampling sites situated in the central and northern part of the lake (depth of 0.5–1 m) were of a similar character.

3. RESULTS

Altogether 3231 specimens of macrofauna have been collected. Out of this number 80.7% were Chironomidae larvae, 11.6% — Oligochaeta, 7.7% — the remaining groups of fauna. Results from the quantitative analysis of the frequency of various taxons are shown in Table I.

The following forms have occurred in greater numbers (* indicates these forms which have a high proportional share only locally): *Einfeldia* e.g. *carbonaria*, *Tanytarsus* e.g. *gregarius*, *Tubifex tubifex*, *Procladius Scuse*, *Polypedilum* e.g. *nubeculosum*, *Ceratopogonidae n. d.*, * *Endochironomus* e.g. *tendens*, * *Chaoborus* e.g. *flavicans*, *Chironomus f.l. plumosus*.

As results from the data in Table I the density of the "pseudolittoral" benthos decreases progressively with the increasing depth (from site No. 1 to site No. 3). The low mean value of the density of bottom fauna in the additional sampling sites results from the fact that samples were collected there in summer months when a strong depression of the quantitative occurrence of benthos was noted at all the

Table I. Mean density of population of the benthic fauna in the "pseudolittoral" of the Jeziorak Lake (indiv./m^2)

Taxons	Sampling stations and their approx. depths; permanent sampling stations at the Moty Bay				Average	Additional stations 16 samples collected in summer 1969
	No. 1 0.5 m	No. 2 1 m	No. 3 1.5 m	No. 4 1 m		
Chironomidae						
Einfeldia e.g. *carbonaria* Mg.	1054	428	181	321	496	48
Tanytarsus e.g. *gregarius* Kieff.	322	23	13	133	123	27
Procladius Scuse	114	39	41	50	61	21
Polypedilum e.g. *nubeculosum* Mg.	40	19	23	74	39	13
Endochironomus e.g. *tendens* F.	1	1		110	28	
Chironomus f.l. plumosus L.	13	12	20	8	11	23
Tanytarsus e.g. *mancus v.d.* Wulp	19			3	5	10
Glyptotendipes e.g. *gripekoveni* Kieff.	1	2	7	3	3	
Cryptochironomus e.g. *viridulus* F.		2	5	2	2	2
Polypedilum e.g. *convictum* Walk.	9				2	
Cricotopus e.g. *silvestris* F.		1	1	4	2	
Chironomus f.l. semireductus Lenz	1	1	1		1	
Pelopia vilipennis Kieff.		2	1		1	21
Pelopia kraatzi Kieff.	2			1	1	
Microtendipes e.g. *chloris* Mg.			2	3	1	
Chironomus f.l. anthracinus Zett.	2				1	
Limnochironomus e.g. *tritomus* Kieff.			1	1	1	
Paratendipes e.g. *albimanus* Mg.				2	1	
Endochironomus e.g. *dispar* Mg.		1			1	
Chironomus f.l. salinarius		1			1	
Cryptochironomus e.g. *defectus* Kieff.				1	1	
Pelopia punctipennis Mg.						6
Tanytarsus e.g. *lobatifrons* Kieff.						6
Pupae	7	5	12	4	7	4
Orthocladiinae n.d.	27				7	13
Pelopiinae n.d.	2				1	
Chironomidae n.d.	8	2	5	13	7	
Oligochaeta						
Tubifex tubifex Müll.	98	126	64	36	79	4
Stylaria lacustris L.				27	7	
Ilyodrilus hammoniensis Mich.		7	7		3	
Limnodrilus hoffmeisteri Clap.		1		2	1	2
Tubificidae n.d.	59	23	12	6	25	
Others						
Ceratopogonidae n.d.	21	47	18	44	33	10
Chaoborus e.g. *flavicans* Meig.	2	10	79	2	23	15
Valvata piscinalis Müll.	13	2		7	6	15
Trichoptera n.d.	15	1			4	
Hydracarina n.d.	2	2		7	3	25
Corixidae n.d.		5		9	3	2
Caenis horaria L.				1	1	2
Piscicola geometria (L.)				1	1	
Nematomorpha n.d.						2
Baetidae n.d.						2
Total	1834	763	493	875	991	275

sampling stations. Data from the additional sites were not taken into account in the quantitative results of conversion presented in the present paper.

In 1969 as well as in 1970 a similar picture of seasonal changes was observed: high density of the benthic fauna in spring, a decrease in numbers in summer, a repeated increase in autumn, and a moderate decrease in winter.

The mean value of the biomass of benthos, calculated on the basis of the measurements of 1969, was 3.27 g/m^2 of the surface area of the bottom. At different sampling stations in the Moty Bay this value was as follows: site No. 1 — 6.18 g/m^2, site No. 2 — 2.26 g/m^2, site No. 3 — 1.59 g/m^2, site No. 4 — 3.05 g/m^2.

The qualitative composition and proportional share of algae in the bottom sediments of the "pseudolittoral" and profundal of the Jeziorak Lake is shown in Table II. The same type of data as regards the contents of the alimentary canals larvae of *Einfeldia* e.g. *carbonaria* and *Chironomus plumosus* is presented in Table III.

Table II. Frequency distribution of algae in the bottom sediments (in %)

Alga species	"Pseudolittoral" mud sediments	Profundal mud sediments
Ulothrix	42.6	26.3
Fragillaria	22.4	13.15
Melosira	9.1	36.8
Cyclotella	3.7	—
Scenedesmus	2.6	5.25
Nitzchia	2.5	1.75
Pediastrum	2.2	4.4
Tetraëdron	2.2	3.5
Lyngbya	1.8	—
Navicula	1.7	1.75
Straurastrum	1.5	4.4
Epithemia	1.4	1.75
Gyrosigma	0.95	—
Synedra	0.8	—
Aphanizomenon	0.5	—
Cocconeis	0.3	—
Actinastrum	0.2	—
Cymbella	0.2	0.8
Phacotus	0.1	—
Surirella	0.1	—
Rhopalodia	0.1	—

Results from the measurements of thermic conditions of the surface sediments and the bottom water layer were very fragmentary and do not allow yet a proper and adequately documented determination of the thermic phenomena, occurring in the "pseudolittoral" zone, considered in their seasonal and daily aspects. On the basis of eight-times repeated measurements it was possible to draw only general conclusions, which were anyway concordant with the observations of the thermic conditions in the shallow waters of the Gopło Lake (Grześ 1973), as follows:

Table III. Proportional content of algae in alimentary canals of Chironomidae larvae. Nutritive selectivity acc. to Ivlev's coefficient (in %)

Alga species	Chironomidae larvae			
	Einfeldia e.g. *carbonaria*		*Chironomus f.l. plumosus*	
	Consumed food (%)	Ivlev's coefficient	Consumed food (%)	Ivlev's coefficient
Ulothrix	61.7	+0.183	67.5	+0.226
Fragillaria	34.9	+0.218	28.3	+0.116
Melosira	1.5	−0.717	1.9	−0.654
Cyclotella	1.05	−0.558	0.85	−0.626
Tetraëdron	0.3	−0.760	0.25	−0.796
Nitzchia	0.21	−0.845	0.3	−0.786
Pediastrum	0.2	−0.833	0.7	−0.517
Synedra	0.4	−0.333	—	—

the direct effect of the insolation upon the temperature of the "pseudolittoral" surface sediments was well-marked;

in late summer and autumn at the depth of 0.5 m the phenomenon of a higher temperature (difference of 1°C) was noted at the surface of the bottom sediments as compared with the water layers ±10 cm above the bottom of the lake.

4. DISCUSSION

As results from the data presented in Table I the presence of 40 different taxons of benthos (in this number 25 of Chironomidae) has been observed in the "pseudolittoral". As compared with 170 taxons of benthos found by Wolnomiejski (unpublished) in the littoral of the same lake this is an indication of a considerable qualitative impoverishment of the biota in the "pseudolittoral" — except as concerns the Chironomidae. This is doubtlessly caused by the slimy substratum precluding the existence of such forms as Hirudinea, Gastropoda, and numerous crawling arvae of insects. The only one permanent, though occurring in small numbers, representant of Gastropoda was *Valvata piscinalis*, well known for its predisposition for existence in the slime at the bottom of a water body.

The absence of many representants of the hydrofauna may be due to a certain monotony of the substratum and lack of hidings. Thus, the forms that are not able to dig themselves in, are easy prey to all sorts of invertebrate and fish predators. The abundance of the fish fry observed usually near the sampling sites may also play a role in the formation of the Chironomidae larvae communities.

In the specific conditions of the "pseudolittoral", where the light reaches down to the bottom, larvae of smaller sizes have, no doubt, greater chances of survival. Thus, it may be one of the possible reasons of a relatively low frequency of *Chironomus f.l. plumosus* larvae in the benthos. The strong nutritive pressure of fish, and of fry in particular, may be evidenced indirectly by the small quantity of epiphytic forms found at the bottom though considerable amounts of them are usually washed out from the surface of the plants by the water motion. The site No. 4. situated near a wide and dense *Stratiotes aloides* belt is an exception to that. Quite a large amount of epiphytic forms (*Endochironomus* e.g. *tendens* and *Stylaria lacustris*) was encountered in there. It occurred however mainly in the autumn when many epiphytic forms leave the macrophytes and may be carried away by water movements to another biota.

Chaoborus e.g. *flavicans* larvae occurred in fairly large quantity only at the deepest sampling station (site No. 3) and had the second position as regards the density of population of other forms in the benthos at this site.

The most representative group of the benthic fauna in the "pseudolittoral" were Chironomidae larvae. Among them *Einfeldia* e.g. *carbonaria* played the most important part making up 44.77% of the total amount of the collected benthos. Many authors have observed the presence of this benthic form in the lakes but its occurrence was usually sporadic and scanty (Błażniak 1959, Klimek 1960, Smoleńska 1963, Sikorowa 1965, Leszczyński 1968, Prejs 1969). Those larvae were also noted in small amount in river inlets and old river-beds (Kajak 1959) and riverine water bodies as well (Kajak 1968 b). According to some authors the occurrence of *Einfeldia* e.g. *carbonaria* is associated with shallow-water biota (Giziński, Mikulski 1965, Leszczyński 1968, Giziński, Toczek-Boruchowa 1972), according to others (Błażniak 1959, Sikorowa 1965) this form occurs mainly in sublittoral. In the Gopło Lake a relatively high density of Einfeldia larvae population was noted at the depths smaller than 5.5 m deep (Giziński, Kadulski 1972). In the Jeziorak Lake, according to the data given by Giziński et al. (1967) and Wolnomiejski (unpublished) the larvae of this taxon have occurred either in the slimy parts of the shallow bottom or in a zone corresponding to sublittoral.

The relation between the occurrence of *Einfeldia* e.g. *carbonaria* larvae and the slimy shallow-water biota was indicated likewise by the data of Kolosova, Lyakhov (1957) based on investigations in shallow riparian water bodies at the Wolga River terraces. On the other hand, it is worth mentioning that in the Drużno Lake (Klimek 1960) an excessively shallowed and slimed water body, *Einfeldia* larvae were very scarce. In small, newly formed water bodies in the woodland the presence of those larvae was noted but also in small numbers — as a matter of fact they were markedly less numerous than *Einfeldia* e.g. *pagana* (Meig.), according to Giziński, Paliwoda (1972).

Certainly, a great variety of data in respect of the character and ecology of *Einfeldia* larvae may be provided by the investigations of the shallowed and slimed lake bays where the prevailing conditions are similar to those in the "pseudolittoral" zone of the Jeziorak Lake as described in the present study.

Out of the remaining forms of the Chironomidae larvae *Tanytarsus* e.g. *gregarius* is noted at a high position as regards the density of its population. This is an ubiquitous form in the entire littoral zone though in the Jeziorak Lake it did not occur in the benthos in great numbers. The frequency of the occurrence of this taxon as well as of the other Chironomidae noted in greater numbers in the communities determined by dominancy of *Einfeldia carbonaria* in the slimy bottom zone of the "pseudolittoral" cannot be yet defined by the term "co-occurrence" due to the insufficient data. It is worth mentioning however that a similar composition of the Chironomidae groupings was observed also by Prejs (1969).

The taxonomic composition of the benthic fauna in the "pseudolittoral" and particularly of Chironomidae larvae is characterized by a much larger number of taxons as compared with profundal zone. In the shallow profundal of the Jeziorak Lake, according to Giziński, Wiśniewski (1971), only 8 taxons have been observed and out of this number three taxons were Chironomidae larvae.

On the average in the whole "pseudolittoral" zone, as calculated from the measurements at the permanent sampling stations in the Moty Bay, the density of fauna populations was 994 individuals per 1 m^2 of the bottom surface area. The average value of the benthos biomass in the "pseudolittoral" (without Mollusca) amounting to 3.27 g/m^2 does not differ significantly from the value given by Giziński, Wiśniewski (1971) for the profundal zone — 2.84 g/m^2. It may be supposed, hawever, that with the mentioned above postulation of a great nutritive pressure of fish in respect of benthonic communities in the shallow, insolated parts of the slimy bottom — the "pseudolittoral" zone has in fact a much greater abundance of the benthic fauna than it appears from the presented data.

In the course of the present studies a series of other observations was also undertaken as regards ecological conditions prevailing in the investigated zone. However, in the preliminary stage of those observations it is difficult to interrelate them closely with the actual occurrence of the benthic fauna.

The character of the occurrence of algae in the mud of the "pseudolittoral" and profundal zone appears to differ from each other. It concerns in particular the degree of the proportional share of the main components of the algae communities in the sediments of those two zones (Table II).

As results from the Table III, where the values of the Ivlev's coefficient are also included, the phenomenon of the nutritive selectivity in the examined Chironomidae larvae concerned merely the two dominant algae genera i.e. *Ulothrix* and *Fragillaria* (positive values of the coefficient).

The composition of the content of algae in the larvae alimentary canals found in that type of examinations is, however, conditioned not solely by nutritive selectivity but also by other phenomena. Since, the preparates of the contents of the larvae alimentary canals contain material of a varied degree of maceration and destruction therefore it is not possible to determinate some of the present forms. This fact may have a significant effect upon the changes in the values of the proportional share of the other determined forms. Compatibly with the suggestions of Kajak (1968 a) differences in the evaluation of the quality of food in the sediments and in the alimentary canals of larvae may also result from the fact that larvae are preying in another layer of mud as well as in that one where the composition of algae has been examined. From the available data it is not yet possible to draw final conclusions about the trophic usefulness of the "pseudolittoral" sediments for benthos feeding. In the process of feeding the microflora connected with algae plays certainly an important part.

The access of light down to the bottom besides the direct photoactinic effect it may produce on the fauna also stimulates a whole range of phenomena modifying the investigated biota. Under the specific photic conditions the type of the metabolism of the sediments is certainly different being conditioned not only by the specific composition of the benthic microorganisms and algae but also by another mode of oxygenation of the water layers at the bottom of the lake and another character of the thermic conditions in the sediments.

It can be assumed that the bottom layers of water in the "pseudolittoral" zone are relatively well-oxygenated due to the photosynthetic activity of algae in the surface layer of the sediments and the motion of water. The latter phenomenon may be dependent not only on the effect of the water undulation but on the convection currents as well. Those movements of water are associated with the direct heating of the mud and the contiguous water layers produced by a strong insolation.

Kolosova, Lyakhov (1957) likewise have reported good oxygenation of water in the shallow water bodies with abundant occurrence of *Einfeldia* e.g. *carbonaria*. It seems that *Tanytarsus* e.g. *gregarius* larvae occurring in fairly great numbers (Table I) may ne considered as indicatory form proving good oxygenation conditions in water. Giziński (unpublished) in his experiments with "incubation" of the mud from the Tynwałd Lake in laboratory conditions has observed a mass appearance of *T. gregarius* in cases of good artificial aeration.

It can be assumed, nevertheless, that at night or at the time of winter stagnation there may occur a considerable oxygen deficit. This is not always manifested by the perceptible H_2S odor as this characteristic stink may emanate from the deeper layers of the sediments stirred with the sampler. Gas bubbles accumulated during decomposition processes often escape to the water surface in result of an artificially produced bubbling of the sediments. This phenomenon is caused most frequently by the motorboats passing over the shallow, slimy parts of the bottom of the "pseudolittoral" zone. This certainly has some effect upon the biological economy of oxygen of the "pseudolittoral" zone in the water body under investigation.

In the specific conditions when the radiation of the sun reaches directly to the bottom of the lake the phenomenon of the heating of the sediments surface is produced by the radiant energy. This phenomenon is reflected not only in specific character of the temperature at the surface layers of the "pseudolittoral" sediments during the whole season but in a day-cycle as well. In comparison with the conditions prevailing in the profundal zone the conditions in the surface layer of the "pseudolittoral" sediments are distinguished by far-advanced thermic astaticism.

The thermic phenomena, to be sure, play an important role in the biology, differentiation and the processes of hydrofauna production as well as in the character of a whole range of physico-chemical and microbiological phenomena occurring in the "pseudolittoral" zone.

Data presented in this paper show that some of the characteristic ecological features of the "pseudolittoral" are similar to those in the profundal zone others

Table IV. Ecological conditions and benthic fauna characteristics in "pseudolittoral" compared with littoral and profundal zones

Characteristics	Zone		
	Profundal	Littoral	"Pseudolittoral"
Photic conditions	bottom in aphotic zone	bottom in euphotic zone	bottom in euphotic or disphotic zone
Physical character of sediments	soft (slimy)	consistent (various types)	soft (slimy)
Photosynthetizing algae in sediments	absent	present	present
Vascular plants	absent	all belts of macrophytes	only nymphaeidae
Oxygen deficit at the surface of sediments	in long periods	seldom (in winter or by night)	rare (in winter beneath ice)
Direct effect of insolation upon sediments	none	distinct	distinct
Benthos utility for fish	limited (especially for fry)	high	high
Benthic fauna quality	very poor	rich	moderately poor
Benthic fauna quantity	very scanty	abundant	scanty
Characteristic representants of benthos	*Chaoborus* sp., *Tubificidae*, *Chironomus plumosus* (Mollusca absent)	all groups of fauna (Mollusca abundant)	mainly Chironomidae (*Einfeldia carbonaria*, *Tanytarsus gregarius*), Mollusca single

to those in the littoral. Also some specific features typical for the investigated zone have been demonstrated which together with the characteristic picture of differentiation of the benthic fauna gives evidence of the ecological specificity of this particular zone. Ecological and taxonomic characteristics of the benthic fauna in the "pseudolittoral" are presented in Table IV in comparison with the littoral and profundal zones.

"Pseudolittoral" in the Jeziorak Lake, according to calculative estimation, covers approx. 1.5% of the total surface area of the lake which amounts to about 48 ha. With continuously progressing extensive processes of sliming and shallowing of the lake the range of the "pseudolittoral" zone extends gradually especially in smaller, isolated bays. Therefore, the present studies may be of great value for prognostication of the future changes in the character of the benthic fauna communities and their potential productivity.

5. SUMMARY

The term "pseudolittoral" was used to define a specific zone in the investigated lake formed due to accumulation of mud and the shallowing of the primarily profundal parts of the bottom — where the slimy sediments are already within the range of the photic zone (for the most part — euphotic). The access of light and especially the direct radiation upon the surface of the sediments have a significant effect on the ecological conditions of the biota of the "pseudolittoral".

Differentiation in the character of the benthic communities indicates the separate and specific character of the "pseudolittoral" zone. The most frequently occurring *Einfeldia* e.g. *carbonaria* larvae constituting 50% of the total amount of benthos appear to be the indicatory element of the benthic fauna. Beyond the range of "pseudolittoral" biota zone the larvae of this taxon occurred only sporadically and in small quantities in other parts of the lake.

Chironomidae larvae were decidedly predominant among the investigated benthic fauna and constituted 80.7% of the collected material. Oligochatea — 11.6% and the remaining groups of the hydrofauna — 7.7%. In the qualitative composition of the collected benthos absence of many characteristic taxons occurring frequently in the benthos of the littoral zone — was recorded.

The average density of the benthic fauna population and its biomass in the "pseudolittoral" zone were 994 individuals/m^2 and 3.27 g/m^2 of the surface area of the bottom, respectively.

On the basis of the biotic factors and the characteristic picture of the differentiation of the benthos the distinct ecological character of this zone has been evidenced.

6. STRESZCZENIE

Terminem "pseudolitoral" określono strefę badanego jeziora powstałą na skutek nagromadzenia się mułu i wypłycenia pierwotnie profundalowych części dna, gdzie muliste osady znalazły się już w zasięgu strefy świetlnej (głównie eufotycznej). Dostęp światła a zwłaszcza promieniowania bezpośredniego do powierzchni osadów ma istotny wpływ na warunki ekologiczne tego siedliska.

Zróżnicowanie charakteru zgrupowań bentosu wskazuje na odrębny i swoisty charakter tej strefy. Najliczniej występującym i wskaźnikowym elementem faunistycznym okazały się larwy *Einfeldia* e.g. *carbonaria* stanowiące blisko 50% całości bentosu. Poza siedliskami "pseudolitoralu" larwy tego taksonu w badanym jeziorze występowały jedynie sporadycznie i w niewielkich ilościach.

Wśród ogółu badanej fauny zdecydowanie dominowały larwy Chironomidae — 80,7% całości materiału. Oligochaeta stanowiły 11,6%, a pozostałe grupy hydrofauny 7,7%. W składzie jakościowym zanotowano brak wielu taksonów charakterystycznych i licznych w bentosie litoralu Jezioraka.

Średnia liczebność fauny dennej i jej biomasa w strefie "pseudolitoralu" wynosiły odpowiednio 991 osobników i 3,27 g/m^2 powierzchni dna.

Na tle rozpatrywanych czynników siedliskowych i w oparciu o charakterystyczny obraz zróżnicowania bentosu uznano za udokumentowaną odrębność ekologiczną tej strefy.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	355–368	1974

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SEASONAL CHANGES OF *PHRAGMITES COMMUNIS* TRIN. PART I. GROWTH, MORPHOMETRICS, DENSITY AND BIOMASS

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ABSTRACT

During two vegetation seasons the growth rates, morphometrics, density and biomass of reed in 5 reedbeds on lakes near Mikołajki were compared. Besides the differences between the reedbeds, large differences were also found between reed growing on the shore, in the middle and on the edge of a reedbed. In this connection the reedbeds were classified into three types: undifferentiated, differentiated in one direction, irregularly differentiated. It was found that the reed growing in shore parts of reedbeds, only periodically inundated with water, was characterized by the lowest values of the studied variables.

1. INTRODUCTION

Phragmites communis Trin., accounting for a large proportion of the emergent flora of lake littorals, is an important link in the circulation of organic matter and mineral salts in a reservoir.

There are several monographs on that species (Bykov et al. 1964, Rudescu et al. 1965, Björk 1967, Haslam 1970) containing phenological, morphological, genetical and environmental data, as well as data on mineral composition of reed. They point to extensive differentiation in its condition (length and diameter of stem, length and weight of panicles), biomass, density and mineral elements content. There are differences both between geographical varieties of the species and between specimens within a single reedbed.

The aim of the work has been to study the growth rates and the regularities of the changes in mineral contents of reed growing in a few lakes in the Mazurian Lakes Region.

The work has been carried out in the Applied Limnology Section of the Institute of Ecology of the Polish Academy of Sciences at Mikołajki, Olsztyn province, in the years 1966–1967 and it consists of two parts, concerning respectively: morphometric characteristics, density and biomass of reed (Part I, here presented) and its mineral contents (Mochnacka-Ławacz 1974).

2. DESCRIPTION OF THE REEDBEDS

The study was carried out of five reedbeds on lakes near Mikołajki, differing by trophics and morphometrics. The characteristics of the investigated reedbeds are listed in Table I. Compact reedbeds were selected, with the maximum amount of plant species other than reed not exceeding 10% of the total biomass. The reedbeds on the lakes: Mikołajskie, Tałtowisko and Skonał, were typical for small lake littoral zones (Bernatowicz, Zachwieja 1966), in which emergent plants settle on the shore and reach to the limit of the littoral because of the lack of strong waving of water. The reedbed on the Śniardwy Lake was placed 200 m off the shore, beyond the streak of wave breaking, and thus it approached the great lake type of the littoral, marked by two stretches of emergent plants, within and beyond the reach of high waves (Bernatowicz, Zachwieja 1966). Reed on the Kotek Lake was not rooted in the bottom. The reedbed was of a floating mat type, about 50 m off the shore, and according to the classification of Bernatowicz, Zachwieja (1966) it could be best classified among the vanishing littoral, with shallowed and strongly mudded bottom. There was also an additional, sixth studied reedbed, reaching 10 m into the land from the shore line of the Mikołajskie Lake, flooded only in spring and with more than 10% of non-reed biomass.

Table I. Description of the investigated sampling spots

Lake	Trophy	Surface (ha)	Shore	Bottom in reedbed zones		Proportion in bottom soil (%)			Depth of water (cm)
				Kind	pH	earthen parts	skeleton parts	detritus parts	
Śniardwy	eutrophic	10,660	flat	sand, gravel	7.5	90.5	8.5	1.0	150
Mikołajskie	eutrophic	460	flat	I. mud, sand	7.5	88.9	11.1	—	0
				II. mud, sand	7.0	87.5	2.0	10.5	20
				III. clay, sand	8.0	98.5	0.9	0.6	60
Tałtowisko	mesotrophic	326	flat	I. mud, sand	7.5	89.6	0.5	9.9	20
				II. mud, sand	8.0	96.2	3.7	0.1	25
				III. clay, sand	7.5	96.4	3.5	0.1	100
Kotek	eutrophic	42	flat	II. mud	7.0	80.0	—	20.0	0
				III. mud	7.0	100.0	—	—	50
Skonał	eutrophic	9	high	I. sand, mud	6.5	99.0	0.5	0.5	0
				II. sand, clay	6.5	100.0	—	—	30
				III. sand, clay	7.0	100.0	—	—	70
Extra sampling spot on the land	—	—	flat	sand, clay	8.0	88.0	12.0	—	0

I — shore, II — middle, III — edge of reedbed.

3. METHODS

INVESTIGATIONS OF REED

The investigations have been carried out from May to October, during two consecutive vegetation seasons. Only the aboveground parts of reed were studied. Three zones were distinguished in each reedbed, depending on their distance from the shore line: the shore zone — on the frontier between water and land, coexstensive with the eulittoral (Pieczyńska 1972); the middle zone; the edge zone — bordering on the open water. Each zone was represented by four squares 0.25 m^2, arranged in a square pattern at 5 m distances from each other. Individual sprouts of reed were marked in the squares. Measurements of stem length and diameter, of the amount of leaves and of the length of panicles were taken every week, always on the same marked individuals. The diameter was measured at the base of a stem, by means of a slide gauge, up to 0.5 mm.

Materials for measurements of the weight of stems, leaves and panicles and of the density and biomass per 1 m^2 were sampled from each zone of each reedbed every month; the reed was cut at the bottom, randomly, 5 individuals at 2 m intervals, along reedbed sections perpendicular to the shore. Each zone of a reedbed was represented in each sample by 50 to 80 individuals. Dry weight of stems leaves and panicles was determined after drying the material to a constant weight at 150°C. The total weight of the particular parts of reed allowed to calculate an average weight of an individual and, multiplying it by the average density of reed, its biomass per 1 m^2 could also be determined.

INVESTIGATIONS OF THE GROUND

At the period when reed biomass was at its maximum (August) some physical and chemical features of the ground were investigated. This was done only once. At each sampling spot 3 holes 1 m deep were bored with a ground drill and the following variables were determined: type of ground, pH (with a field pH-meter), the weights of earthen and skeleton parts and morphic detritus. The material was divided on soil sieves 0.5 mm of mesh diameter.

4. RESULTS

GROWTH RATES OF REED

The growth rates of reed in both seasons were similar on all the studied sampling spots (Fig. 1). The emergence of reed above water level has been observed since the beginning of May. By the half of May the length of reed on various sampling spots ranged from 20 to 100 cm, its diameter was from 0.5 to 0.8 cm and the number of leaves from 2 to 4. The dry weight of an individual ranged by that time from 2.5 to 8.0 g and the biomass per 1 m^2 was from 50 to 500 g/m^2 (dry weight).

Average daily increases of length were, at the beginning of the vegetation season (from May to the half of June), from 0.4 to 3.0 cm; increases of weight of individuals were from 0.03 to 0.4 g d.w., and biomass per 1 m^2 increased from 1 to 20 g.

Maximum growth rate of reed was observed, in the two seasons of investigations, on the break of June and July. During that time daily increases of stem lengths reached the values from 1 to 4 cm, increases of weight per individual were from 0.08 to 0.65 g d.w., and biomass increases per 1 m^2 ranged from 4 to 41 g d.w. The maximum increase of the amount of leaves also occurred in that period.

In the second half of July the growth rate of reed decreased and in most sampling spots it was the period of appearance of panicles, which were growing intensively from 15th July to 15th August (Fig. 2).

At the break of August and September, length increases came to an end and reed attained its maximum biomass. On this period the following value ranges of the investigated variables were recorded: stem length from 120 to 300 cm; stem

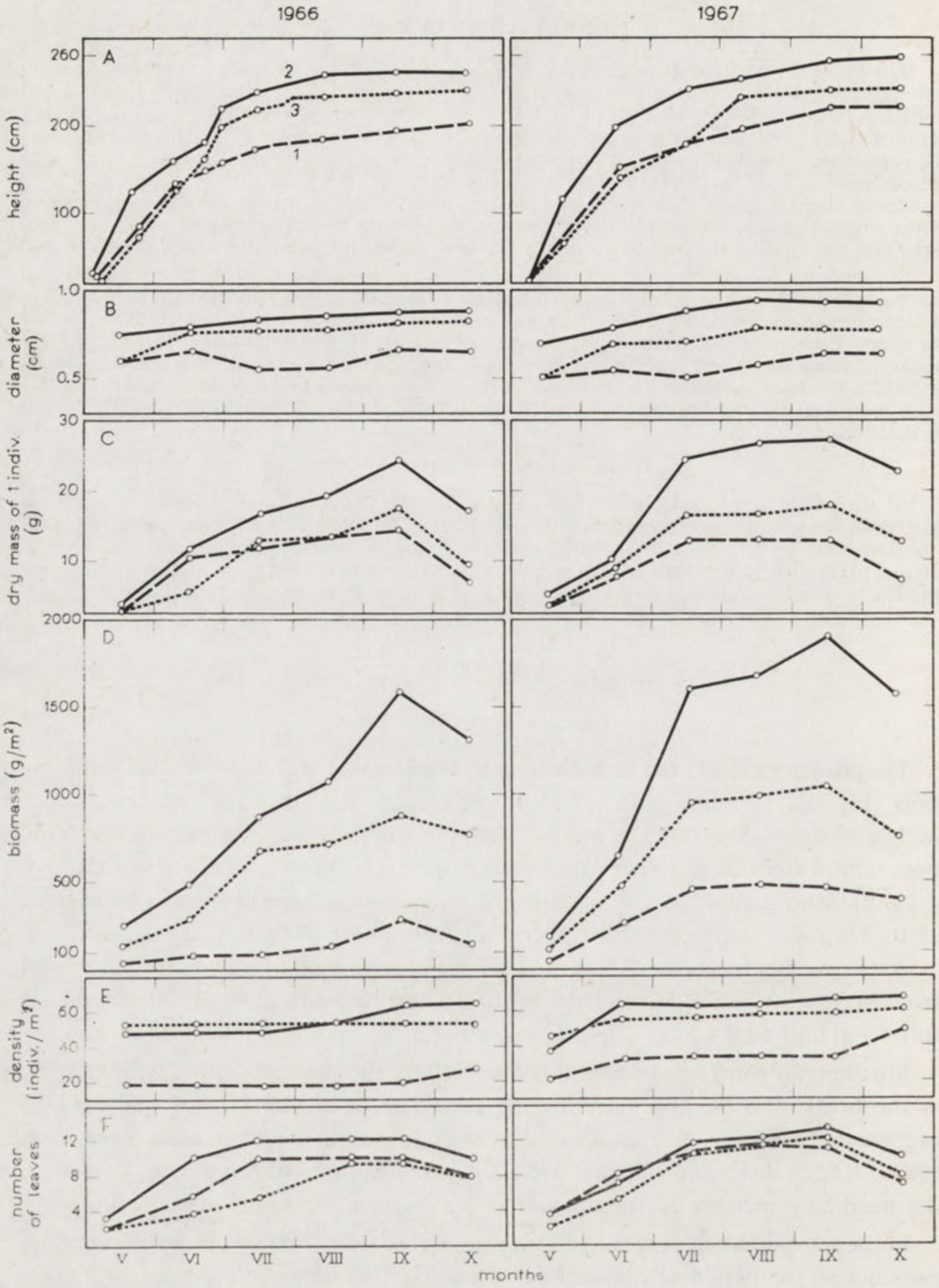

Fig. 1. Growth of reed in three reedbed zones on the Mikołajskie Lake in 1966 and 1967. A — length, B — diameter, C — individual weight, D — biomass, E — density, F — mean number of leaves per 1 indiv., 1 — shore, 2 — middle, 3 — edge of reedbed

diameter 0.4 to 1.0 cm; individual weight from 6.7. to 30.5 g d.w.; biomass per 1 m² from 298 to 2055 g d.w.

The lengths of panicles in the period of maximum biomass ranged from 5 to 30 cm and their weights from 0.15 to 1.7 g. The amount of leaves in this period was 4.5 to 6 times greater than at the beginning of the vegetation season and it was 10 to 17 per stalk. After the maximum biomass periods, in the half of October, the losses of leaves as compared with their highest amounts attained in average 20%. This accounted for the decrease in reed biomass which amounted by that time to about 15%.

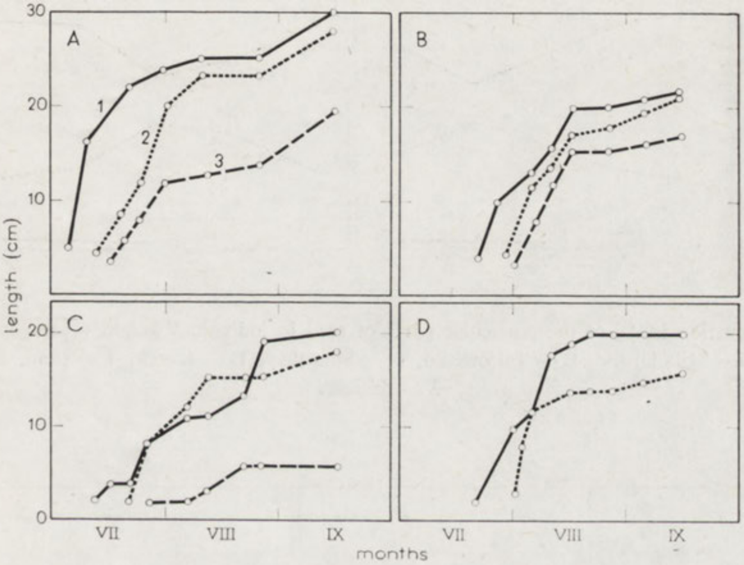

Fig. 2. Growth of panicles of reed in three reedbed zones on four lakes. A — Mikołajskie, B — Tałtowisko, C — Skonał, D — Kotek, 1, 2, 3 as in Fig. 1

The relative share of each part of the plant in the weight of a whole individual indicated that it was the stem which accounted for most of the increases during the whole period of growth, but less and less so as the leaves were becoming heavier. At the maximum of biomass, the stems accounted in average for 73.6% of weight, leaves for 23.4% and panicles for 3% of dry weight of an individual.

A correlation was found between the amount of leaves and the length of an individual (Fig. 4A), as well as between the length and diameter of a stem on one hand and the weight of a plant on the other (Fig. 4 DE). No correlation was found between the length and weight of panicles as compared with the length of stem (Fig. 4 BC).

The greatest increases in the density of reed occurred in both seasons in spring. Young individuals have also been appearing during the whole summer period. Autumn increases in the density of reed were observed mainly in the shore zones

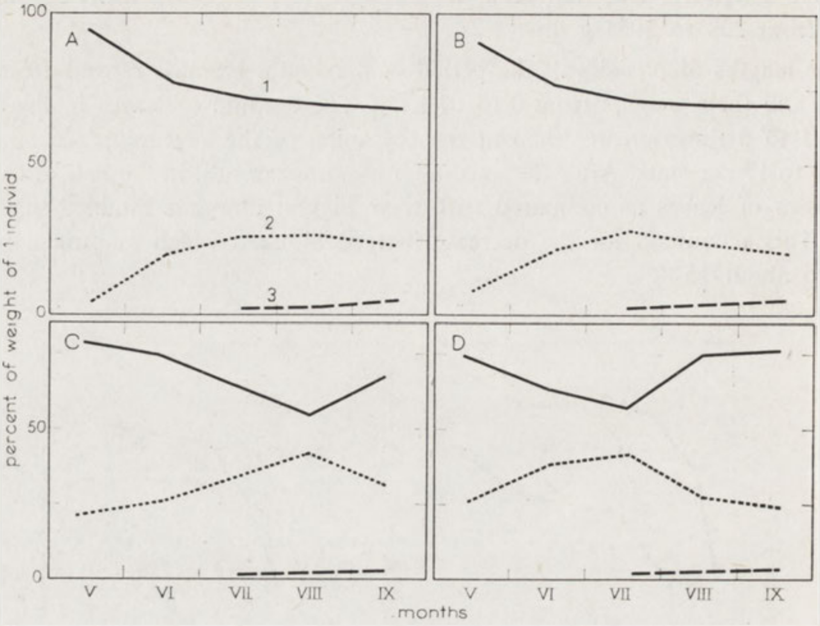

Fig. 3. Proportion in % of the particular parts of reed in individual weight (averages from two seasons). A — Mikołajskie, B — Tałtowisko, C — Śniardwy, D — Kotek, 1 — stem, 2 — leaves, 3 — panicles

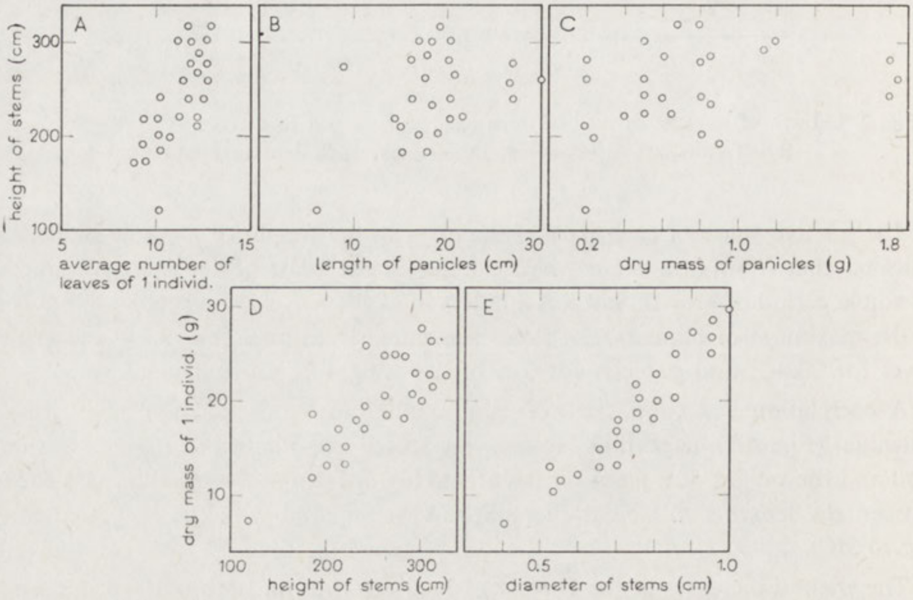

Fig. 4. Relationships between values of selected variables of reed. Average values from the several reedbed zones during biomass maximum are indicated (August–September 1966 and 1967)

of the investigated reedbeds. The density per 1 m^2 at the beginning of the season ranged from 20 to 50 individuals on different sampling spots, from 35 to 70 individuals at the maximum of biomass and from 38 to 80 individuals in autumn.

COMPARISON OF MORPHOMETRIC CHARACTERISTICS OF REED FROM VARIOUS REEDBEDS

In both seasons the reed from the additional, sixth reedbed on land was the shortest (180 cm); among the littoral reedbeds, the reed from the Skonał Lake was characterized by the smallest length (average length from its three zones was 200 cm). Reed grew heighest (300 cm in average) on the Śniardwy Lake (Fig. 5 A).

In the 1966 season the greatest weight and diameter of an individual and the greatest biomass per 1 m^2 was recorded for the reed from the Tałtowisko Lake, (24 g, 0.85 cm, 1500 g, respectively) and in 1967 for that from the Kotek Lake (25 g, 1.0 cm, 1500 g). The lowest values of those variables in both seasons were recorded for the Skonał Lake (12 g, 0.85 cm, 850 g) and for the extra reedbed (7.0, 0.5 cm, 300 g), see Fig. 5 BCE.

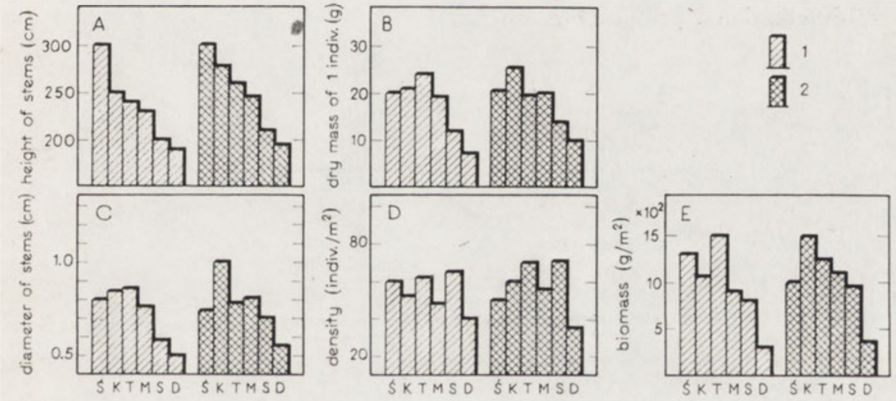

Fig. 5. Description of reed from various lakes at the maximum of biomass (August–September), in 1966 (1) and 1967 (2). A — length, B — individual dry weight, C — diameter, D — density, E — Biomass, Ś — Śniardwy, K — Kotek, T — Tałtowisko, M — Mikołajskie, S — Skonał, D — extra sampling spot

In both seasons, the greatest lengths and weights of panicles were found on the reedbed on the Mikołajskie Lake (average length at the biomass maximum in 1966 was 23.7 cm and weight was 6.9 g). The smallest panicles in both seasons were remarkable for the reed from the Skonał Lake (15.5 cm; 1.9 g), while at the extra sampling spot no panicles were observed at all.

With respect to the densities of reed, the investigated reedbeds can be classified among the group with medium density (according to the classification proposed by Bernatowicz 1960 a). The reed from the Skonał Lake, in spite of its low ratings on the other scales, was marked by maximum densities (60 to 70 indiv./m^2) in both seasons, while the reed in the extra reedbed was the least numerous (38 to 40 indiv./m^2), see Fig. 5D.

If we compare the data for the two investigated vegetation seasons (Fig. 5), it appears that the biomass per 1 m^2 was determined to a greater extent by length, weight and diameter of reed than by its density. With respect to all the investigated characteristics it was the extra reedbed, placed on the shore of the Mikołajskie Lake which had the poorest ratings. It was marked by the lack of direct contact with open water and by the highest proportion of plants other than reed.

COMPARISON OF MORPHOMETRIC CHARACTERISTICS OF REED BETWEEN THE SEVERAL ZONES OF THE REEDBEDS

It can be seen from the results of measurements that the investigated reedbeds differed, with respect to the condition, biomass and amount of reed, not only among each other, but there were also marked differences as to the discussed characteristics of reed among the several zones of particular reedbeds. Those differences permitted to distinguish three types of reedbed profiles, mainly with reference to length and biomass measurements:

I — uniform profile, II — one-directionally differentiated profile; III — irregularly differentiated profile (Fig. 6).

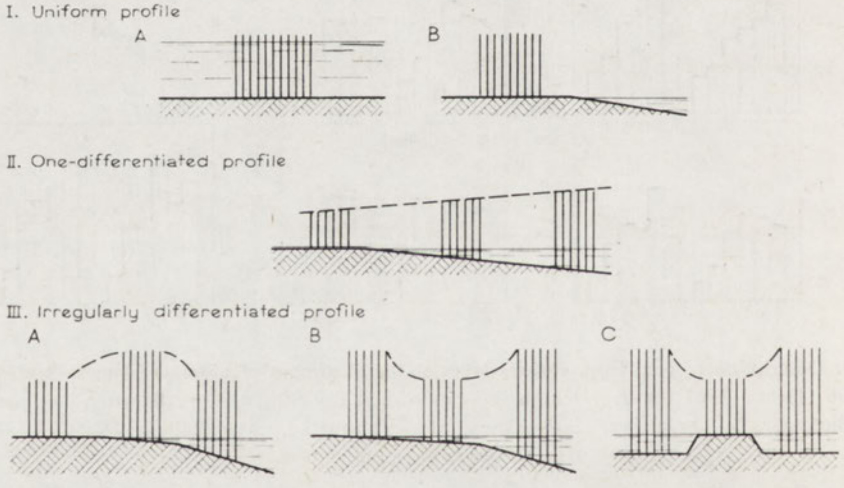

Fig. 6. Types of profiles of the investigated reedbeds. IA — Śniardwy, IB — extra sampling spot, II — Skonał, IIIA — Mikołajskie, IIIB — Tałtowisko, IIIC — Kotek

In opposition to the reedbeds with differentiated profiles, that on the Śniardwy Lake (Fig. 6, I, A) and the extra land reedbed (Fig. 6, I, B) had no zones differing with respect to the condition, biomass and amount of reed (a uniform profile). Soil analyses on those sampling spots revealed that the ground was uniform throughout as to all its considered characteristics (Table I).

A uniform improvement of the condition and biomass of reed from shore to edge of a reedbed was observed only on the Lake Skonał (a one-directionally differentiated profile; Fig. 6, II). On the Mikołajskie reedbed (an irregularly differentiated profile; Fig. 6, III, A) the middle zone scored best in all the investigated characteristics of reed, while the shore zone scored poorest. On the reedbeds on the lakes Tałtowisko and Kotek, also irregularly profiled, the edge zones were marked by the greatest stem lengths, and also by higher biomasses and densities than those observed in the other two zones. However, in opposition to the Mikołajskie reedbed, it was the middle zones which ranked lowest with respect to condition and biomass of reed on both lakes now discussed (Fig. 6, III, BC). On all the irregularly profiled reedbeds differences were also observed as to the types of ground in the several zones (Table I). Thus, e.g., the middle zone of the Mikołajskie reedbed and the shore zone of the Tałtowisko reedbed had bottoms thickly covered with detritus, accounting for as much as 10% of the bottom soil at a depth of 0.5 m. The other zones of those reedbeds had hard bottoms, without a layer of organic remnants. Reed growing in detritus zones was marked by high ratings in condition, biomass and amount (on the Mikołajskie reedbed it was the best zone and in that of Tałtowisko it was better than in the middle zone).

It appears from the evidence that the presence or lack of a constant water surface in a reedbed also influences the characteristics of reed, besides the type of ground. Thus, e.g., the shore zones of the reedbeds on the Lakes Mikołajskie and Skonał, the poorest as to the condition of reed, were washed with water in summer only when there were waves on the water. The necessity of a constant presence of water in a reedbed was also confirmed by the poor condition and low biomass of reed on the extra sampling ground on the land. However, the depth of submergence had no influence on the investigated characteristics of reed. E.g., two zones differing with respect to the condition of reed on the Tałtowisko Lake, the shore and the middle zone, had similar levels of water during the whole vegetation season (Table I). The height of the water column had also no influence on the moment of the emergence of reed at the beginning of the vegetation season. Within the investigated reedbeds, reed appeared at the same time on all the sections of the littorals. However, there were marked differences as to the time of appearance of panicles and as to their condition. Reed in the middle zones flowered earliest and attained the greatest lengths and weights of panicles (Fig. 2). Shore reed blossomed in average a week later than middle zone reed and the lengths and weights of its panicles were the lowest among the three investigated zones. Thus, the condition of panicles was not related to the condition of the whole plants.

Differentiations within reedbeds were of multiple nature and the variability of the investigated characteristics of reed was influenced by a number of environmental factors; only some of them have been considered in the present work.

A COMPARISON OF MORPHOMETRIC CHARACTERISTICS OF REED IN THE TWO VEGETATION SEASONS

In the 1967 season reed appeared in average a week earlier than in the preceding year; this probably accounted for the slight increase of its length and biomass in comparison with 1966 (Fig. 1 AD), as well as for an increase in the number and weight of leaves (Fig. 7 AC).

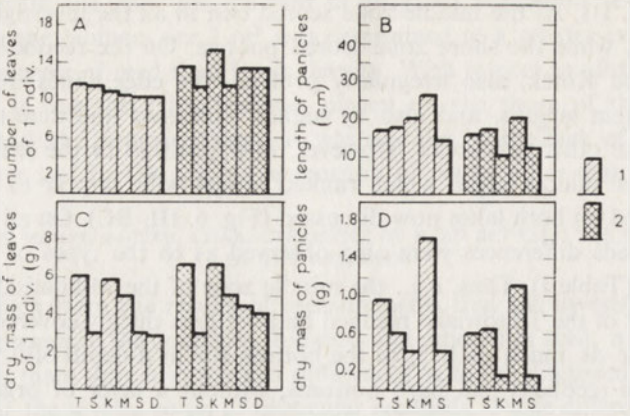

Fig. 7. Leaves and panicles of reed during two periods of biomass maximum; (1966 — I, 1967 — II). A — number of leaves, B — lenght of panicles, C — dry weight of leaves, D — dry weight of panicles. T — Tałtowisko, Ś — Śniardwy, K — Kotek, M — Mikołajskie, S — Skonał

The earlier beginning of the 1967 vegetation season was perhaps also related to the one-week-earlier term of blossoming of reed, but the panicles were in general shorter and lighter than in 1966 (Fig. 7, B, D). Panicles of the reed from the Kotek Lake deteriorated most (their length and weight decreased by about 50%). At the same time, it was on this reedbed that the greatest increase of the number of leaves in comparison with the preceding season was observed (by about 37%), as well as of their weight (by about 14%). Only the reed from the Śniardwy Lake had similar numbers and weights of leaves, as well as similar conditions of panicles during both seasons (Fig. 7). The growth rates and the differences in condition, density and biomass in the respective zones of reedbeds were similar in 1966 and in 1967 (Fig. 1).

5. DISCUSSION

The large range of differences in the studied characteristics of reed from various lakes, and even within a single reedbed, is consistent with data by many authors (Borutskij 1950, Bernatowicz, Radziej 1964, Björk 1967, Dykyjová 1970, 1971 a, b, and others), who most often explain the differences in terms of environmental variability of reed from grounds with different fertility.

However, the differences in condition, biomass and amount of reed presented here have not always been strictly related to the differences in the studied characteristics of ground. Thus, e.g., the highest reed from the Śniardwy Lake (the greatest lake, eutrophic, with strong waves and hard mineral bottom) did not differ much as to the weight of an individual and the density of reed from plants from the Kotek Lake (the smallest lake, also eutrophic, but with little waving and soft, strongly mudded bottom; see Table I). This example does not corroborate the view, frequent in literature, that it is mainly the physical features of the bottom that influence the condition of reed (Bernatowicz 1960 a,b, Björk 1967).

However, the results of measurements of reed growing on grounds covered with a layer of detritus, from the middle and shore zones of the reedbeds on the lakes Mikołajskie and Tałtowisko, are consistent with the opinion by Björk (1967) that reed is apt to attain high values of metrical variables in environments with high proportions of organic remnants in the ground.

Apparently, the narrow scope of the present work does not permit to offer an ultimate solution to the problem, but the results seem to suggest that among the set of environmental factors, a constant presence of water in a reedbed plays a major positive role.

Again, the view that the length of reed is proportional to the depth of its submergence (Rudescu et al. 1965) finds no support in our material. Among the investigated reedbeds, only the reed from the several zones in the littoral of the Skonał Lake can be an example of a positive influence of the depth of submergence upon the length of reed (Fig. 6, II).

However, the depth of submergence seems to determine the weight of reed, e.g., the weight of the highest reed stalk from the Śniardwy Lake was in both seasons smaller than the weight of such a reed from the Kotek Lake. The reed from Śniardwy Lake grew on the depth of 1.5 m throughout the whole section of the reedbed, while it grew on only 50 cm on the Kotek Lake. The above examples confirm the data by Nikolajevskij (1971) that a higher level of water in a reedbed is related to a lesser amount of constructive and conductive tissue in a stem, and to a greater space filled by air; the reed is thereby lighter.

The differences in the investigated characteristics of reed from the several reedbeds found out in the present work did not indicate any definite direction in the changes of condition, biomass and amount. Many authors, however, report the existence of such directional changes related to the increasing distance from the shore line. According to Borutskij (1950) and Buttery, Lambert (1965), the density of reed decreases, while its condition and biomass increases in proportion to the distance from shore. Similar findings are reported by Bernatowicz, Pieczyńska (1965) on the grounds of measurements of reed from the Tałtowisko Lake. In the investigated material, reed marked by higher biomasses was also in better condition, so that biomass per 1 m^2 did not depend on density; this finding is consistent with data by Bernatowicz, Pieczyńska (1965). However, the distinguished types of profiles indicate the irregular character of the reed biomass changes on the cross-sections of the reedbeds.

The lack of relationship between the term of appearance of reed in spring and its distance from the shore line is inconsistent with results reported by Borutskij (1950), which imply that young reeds appear in deeper places and farther off the shore first, and that they grow faster than the shore reed.

The regularity found out for the panicles that they appear earliest and have the best condition in the middle zones of the investigated reedbeds, suggest the possible influence of differences in the microclimate of the particular zones, but it does not seem to confirm the opinion of Björk (1967) that the size of panicles is mainly determined by genetical factors.

Table. II. Comparison of results of measurements of reed from three reedbed zones on the lakes Mikołajskie and Tałtowisko

Type	Mikołajskie Lake			Tałtowisko Lake		
	Biomass (g/m^2)	Density (ind./m^2)	Ind. weight (g)	Biomass (g/m^2)	Density (ind./m^2)	Ind. weight (g)
	Present findings					
Shore	133	18	7.4	1692	72	23.5
Middle	890	53	16.7	1608	80	80.0
Edge	693	53	13.0	1896	80	23.7
Average	572	34	12.4	1372	77	22.4
	Data from literature					
	Kowalczewski, Wasilewski (1966)			Bernatowicz, Pieczyńska (1965)		
Shore	820	93	8.8	901	71	11.0
Middle	690	69	10.0	915	70	14.0
Edge	502	55	9.1	970	52	20.0
Average	670	72	9.3	915	62	15.0

The average results of measurements of length and biomass of reed during two seasons are similar to those reported by other authors for the same lakes studied in earlier years (Kosicka 1958, Bernatowicz, Pieczyńska 1965, Kowalczewski, Wasilewski 1966, Bernatowicz et al. 1968). Thus, the data, though they refer only to sections of reedbeds on the investigated lakes, seem to be representative for the appreciation of average production and condition of reed on those lakes. From comparisons of the detailed data with those reported by the above authors concerning the production and condition of reed in the several zones (Table II) it can be seen, however, that each of the discussed lakes is represented by more than one type of reedbed profiles, which seems to indicate, among other things, a great differentiation of the character of the littoral bottom, even within a single lake.

6. SUMMARY

Subject to investigation were the growth rates, the values of some morphometric characteristics of reed (length, diameter, number and weight of leaves, length and weight of panicles), as well as its density and biomass per 1 m^2 during two vegetation seasons, from May to October. The study was carried out on six reedbeds, on lakes near Mikołajki, Olsztyn province differing by size and type of bottom. Each reedbed was divided into three zones with reference to their distance from the shore line and from the open water (shore, middle, edge). Each zone was represented by four spaces 0.4 m^2 each, upon which individual reed stalks were marked for continuous metrical measurements. Each measurement in a zone was represented by about 50 individuals.

Besides the differences in the investigated reed characteristics among the reedbeds, their large variability within the reedbeds was also observed. On this ground the reedbeds were classified into three types, mainly with respect to the differences in the length and biomass of reed: I, reedbeds with undifferentiated profiles, uniform throughout their whole cross-sections as to the selected characteristics of reed; II, reedbeds with profiles differentiated in one direction (increase of the relevant values from shore to edge); III, reedbeds with irregularly differentiated profiles. It was found that the factors responsible for the character of differences within reedbeds were the variety of ground and the lack or presence of a constant water column. Reed from shore zones, only periodically inundated with water, was in general shorter and poorer in biomass per 1 m^2. It was found that the biomass was more strongly determined by individual weight than by density of reed per 1 m^2. The length and weight of panicles was found to be independent from the length and weight of stalk. Panicles from the middle zones of reedbeds were the longest and heaviest.

The growth rates of reed observed during two seasons were similar in all the three distinguished reedbed zones; also, no differences as to the growth rates were found between the sampling spots representing trophically and morphometrically different lakes.

The most rapid growth of reed was observed from the half of June to the half of July, and the period of maximum biomass occurred at the break of August and September.

7. STRESZCZENIE

Badano tempo wzrostu i analizowano wartości niektórych cech morfometrycznych trzciny (wysokość, średnica, liczba i ciężar liści, długość i ciężar kwiatostanów), oraz jej liczebności i biomasy z 1 m^2 w kolejnych miesiącach dwóch sezonów wegetacyjnych, od maja do października. Badania prowadzono na sześciu trzcinowiskach jezior okolic Mikołajek, woj. olsztyńskie, różniących się wielkością oraz charakterem dna. Każde trzcinowisko podzielono na strefy, w zależności od odległości od linii brzegowej i otwartego lustra wody jeziornej (brzeg, środek i skraj). Każdą strefę reprezentowały cztery powierzchnie po 0,4 m^2, na których oznakowano poszczególne osobniki trzciny do stałych pomiarów metrycznych. Każdy jednorazowy pomiar w strefie reprezentowany był przez około 50 osobników.

Obok różnic w wartości badanych cech trzciny pomiędzy trzcinowiskami stwierdzono także dużą jej zmienność w obrębie poszczególnych trzcinowisk. Na tej podstawie dokonano podziału trzcinowisk na trzy typy, bazując głównie na różnicach w wysokości i biomasie trzciny: I typ trzcinowiska, o profilu niezróżnicowanym (jednorodnym na całym przekroju pod względem wyróżnionych cech trzciny), II typ — o jednokierunkowo zróżnicowanym profilu (wzrost wartości badanych cech trzciny od brzegu do skraju), II typ — o nieregularnie zróżnicowanym profilu.

Stwierdzono, że na taki charakter zróżnicowań w trzcinowiskach mają wpływ: niejednorodność podłoża, oraz obecność lub brak stałego lustra wody. Trzcina stanowisk brzegowych, jedynie okresowo zalewanych, charakteryzowała się na ogół najmniejszą wysokością i biomasą z 1 m^2. Stwierdzono, że o biomasie decyduje bardziej średni ciężar osobnika niż liczebność trzciny z 1 m^2.

Długość i ciężar kwiatostanów nie były zależne od wysokości i ciężaru źdźbła. Największe długości i ciężar miały kwiatostany stref środkowych trzcinowisk.

Badane w ciągu dwóch sezonów tempo wzrostu trzciny było podobne we wszystkich trzech wyróżnionych strefach trzcinowisk; także nie strwiedzono różnic w tempie wzrostu pomiędzy stanowiskami, reprezentującymi różne troficznie i morfometrycznie jeziora.

Najszybszy wzrost trzciny obserwowano od połowy czerwca do połowy lipca, a okres maksimum biomasy następował na przełomie sierpnia i września.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	369–380	1974

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SEASONAL CHANGES OF *PHRAGMITES COMMUNIS* TRIN. PART II. MINERAL CONTENTS

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ABSTRACT

Subject to analysis was the mineral contents of particular parts of reed (ash, SiO_2, Ca, K, P, Fe, general N) in successive phases of its growth on 6 reedbeds on lakes near Mikołajki, during two vegetation seasons. Differences in contents of the particular mineral elements were found; there were phenological differences, as well as between reed from various sampling spots, and between its morphological parts. There were also quantitative differences in mineral contents of reed correlated with its distance from the shore line. The greatest proportion of ash was recorded in reed from shore zones, inundated temporarily. Greater amounts of phosphorus were in general found in stems and leaves of taller reed, with greater biomass, independent of its distance from the shore.

1. INTRODUCTION

Mineral contents of reed was studied by many authors: Tóth, Szabó (1963) analyzed the chemistry of reed from the Balaton Lake; Allen, Pearsall (1963) studied chemical contents of reed leaves from various regions of England and Ireland; Bykov et al. (1964) investigated the dynamics of mineral elements in various phases of growth of reed from lakes and marshes in Kazakhstan; Rudescu et al. (1965) analyzed the mineral contents of reed from the Danube delta; Björk (1967) studied the mineral contents of the particular morphological parts of reed from Swedish lakes; Květ (1973) and Úlehlová et al. (1973) analyzed the mineral contents of reed from various settings in southern Moravia. Because of the large differences between the data by those authors, perhaps reflecting regional differences in biotopes and climate, it seemed desirable to undertake a study concerning the mineral contents of reed from a few lakes in northern Poland for comparative purposes. The work also aimed to compare the mineral contents of reed in its successive phases of growth, as well as of reed from reedbeds on lakes with various trophic and morphometrical characteristics.

An analysis of the relationships between chemical contents and condition of plants was undertaken with reference to results from Part I of the study, concerning seasonal changes of morphometrical features, density and biomass of reed from the same sampling spots (Mochnacka-Ławacz 1974).

2. TERRAIN AND METHODS

In the Introduction to Part I of the study (Mochnacka-Ławacz, 1974) a detailed characteristic of the 6 investigated reedbeds on lakes near Mikołajki was offered, as well as the principle underlying the division of each surface into 3 zones: shore middle and edge. The studied reedbeds were compact, with an amount of other plant species associated with reed not exceeding by its biomass 10% of the total biomass. The reedbeds on the lakes Mikołajskie, Tałtowisko and Skonał represented the type of littoral with slight waving, with the emergent plants settling the shores and reaching to the border of the littoral on the open water side. The reedbed on the Śniardwy Lake was about 200 m off the shore occupying the littoral zone beyond the wave-breaking streak. The reedbed on the Kotek Lake, also placed by about 50 m off the shore, had the character of a floating plot, with reed not rooted in the bottom. There was also a sixth, additional reedbed, about 100 m^2 of surface, placed about 10 m from the littoral of the Mikołajskie Lake towards the land, beyond the reach of continuous flood.

The studies were carried out during two vegetation seasons 1966 and 1967, from May to October. Materials for chemical analysis (stems, leaves and panicles separately) were sampled at one month intervals. Reed was cut at the bottom level, 5 randomly picked each specimens 2 m, along reedbed cross-sections, perpendicularly to the shore. Each zone of ar eedbed was represented in each sample by 50 individual reeds in average. Besides, every month a rhizome was sampled from the middle zone of the Mikołajskie reedbed, from the depth of about 30 cm above the bottom level. The material was cut, dried to a constant weight at 105°C and combusted in a muffle stove at 550°C. Each sample was analyzed in three experiments. The average proportion of ash in a sample was determined, and then ash was hot dissolved in HCl and seeped. The remainder after the seeping was assumed to be silica and the mineral elements in the solution were determined by the following methods: Ca, K, Na — photometrically (with a flame photometer); Fe — colorimetrically (with o-phenantroline; Jackson 1960); P — colorimetrically (by the ammonium-molybdate method; Struszyński 1957). The general nitrogen was determined by the method of Kjeldahl. In elaborating the seasonal dynamics of the mineral contents in the particular parts of reed the average values from the two seasons and from all zones of the investigated reedbeds were used which rendered in effect 28 tests, each repeated three times for each morphological part and for each month of investigations. The term "average annual content" of the particular mineral elements refers to arithmetical means from the values of successive monthly measurements, calculated as above, for each morphological part of reed.

3. RESULTS

PHENOLOGICAL DATA

The quantitative mineral content of the particular parts of reed was subject to extensive changes during a vegetation season (Fig. 1). The amount of ash ranged from 5% in stem in spring to 14% in leaves in autumn (Fig. 1 B). Silica constituted the basic ingredient of ash in all parts of reed and during the whole vegetation season, from 11.5% in stem ash in spring to 90% in leave ash in autumn (Fig. 1 C). Reduced to dry mass, the amounts of silica were 2 and 11.5% respectively (Fig. 1 D).

Potassium appeared in reed in amounts about 10 times lesser than silica (annual averages). Its greatest and smallest amounts were found in stem (0.02% d.m. in autumn and 2% d.m. in spring; Fig. 1 E).

The annual average of calcium was much smaller than that of potassium; in panicles and rhizomes it amounted to about 40% and in stem to about 13% of potassium content. Extreme values for calcium were found in autumn samples, viz. 0.04% d.m. in stems and 0.5% d.m. in leaves (Fig. 1 F). The average annual amounts of phosphorus in stems and panicles were about two times greater, and in leaves about two times smaller than those of calcium. The smallest amount of phosphorus, 0.01% d.m., appeared in stem in autumn, and the greatest, 0.5% d.m., in spring leaves and summer panicles (Fig. 1 G).

The sodium amount ranged from 0.02% d.m. in autumn leaves to 0.2% d.m. in spring stems (Fig. 1 H).

Among the investigated elements, iron appeared in reed in smallest amounts: from 0.005% d.m. in stems and leaves in autumn to 0.08% d.m. in rhizomes in summer (Fig. 1 I).

General nitrogen appeared in amounts from 0.01% d.m. in stem in autumn to 0.25% d.m. in leaves in spring (Fig. 1 J).

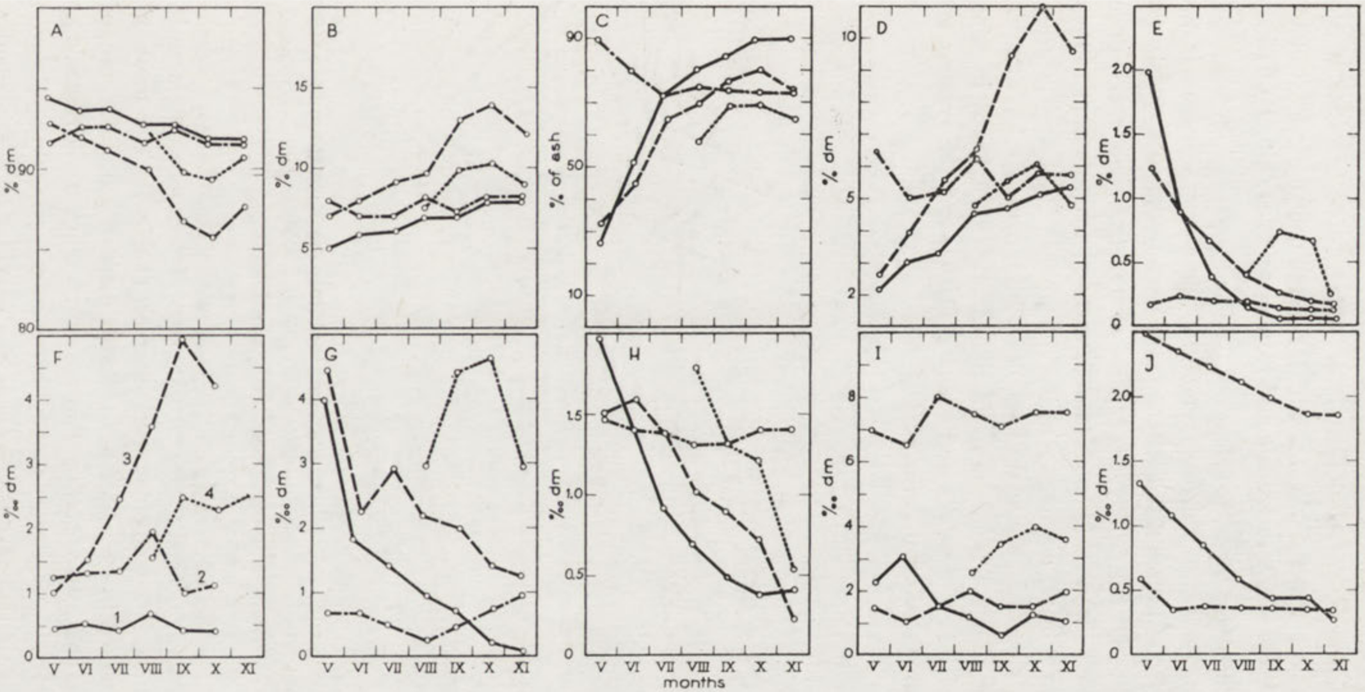

Fig. 1. Seasonal changes in mineral contents of reed (mean data for two vegetation seasons and for all the sampling grounds). A — organic matter, B — ash, C — silica in ash, D — silica in dry mass, E — potassium, F — calcium, G — phosphorus, H — sodium, I — iron, J — general nitrogen, 1 — stems, 2 — rhizomes, 3 — leaves, 4 — panicles

Percentage proportions of most investigated elements in dry mass of the particular morphological parts of reed decreased from spring to autumn. The least changing was the organic matter content (Fig. 1 A). The amounts of general ash and of silica in all parts of reed (Fig. 1 BCD) and of calcium in leaves and panicles (Fig. 1 F), as well as of phosphorus and iron in panicles (Fig. 1 GI) increased in the course of the vegetation season.

Quantitative changes in rhizomes were much less extensive than in the aboveground parts of reed; among the latter, leaves were found to change most widely.

An analysis of all the underground parts of reed (rhizome, roots, underground stems and winter buds; Fig. 2) revealed differences between them as to the amounts of the particular mineral elements. During the whole period of vegetation, underground parts of stems had the least of ash (Fig. 2 A), namely 4.2% d.m. in average. The greatest amounts of potassium and phosphorus were found in winter buds, 0.62% d.m. and 0.11% d.m. respectively (Fig. 2 BD) and the greatest amount of calcium in roots, 0.43% d.m. (Fig. 2 C).

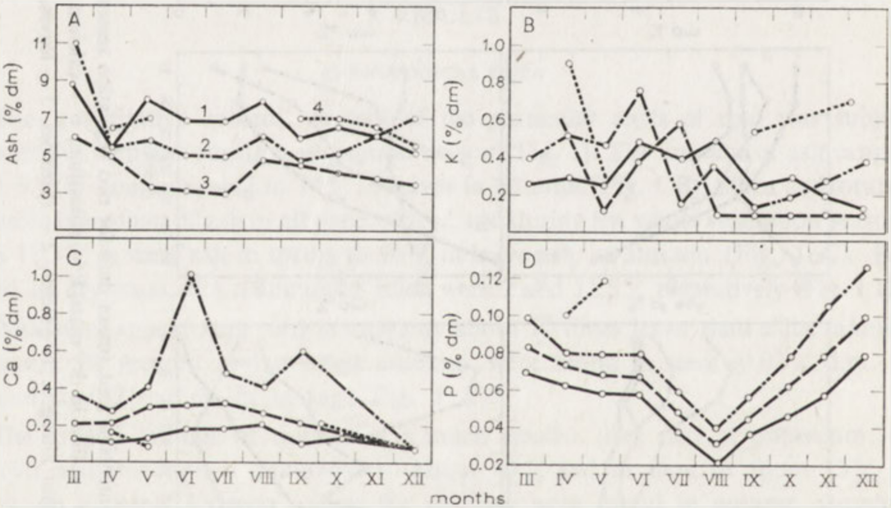

Fig. 2. Seasonal changes in underground organs of reed from the Mikołajskie Lake (1966 season). A — ash, B — potassium, C — calcium, D — phosphorus. 1 — rhizomes, 2 — roots, 3 — underground stems, 4 — winter buds

Large differences between the particular parts of reed as to the annual average amounts of the studied elements were observed (Table I). The most of organic matter was contained in stem; leaves contained most of ash, silica, potassium and calcium; sodium and iron were found in maximum amounts in rhizome and phosphorus in panicles.

No proportional relationships between the contents of the particular mineral elements in reed were noted. The only exception was silica; its proportion to ash in dry mass of stem and leaves was in average 0.73 and in panicles 0.68 (Fig. 3).

Table I. Average annual contents of the studied elements in the separate parts of reed (% of d.m.)

Element	Stems		Leaves		Rhizomes		Panicles	
	1966	1967	1966	1967	1966	1967	1966	1967
Organic matter	94.3	94.0	90.4	90.9	91.9	93.7	91.5	94.3
Total nitrogen	—	0.047	—	0.31	—	0.078	—	—
Ash	5.7	6.0	9.6	9.1	8.1	6.3	8.5	5.7
SiO_2	4.7	3.9	6.0	5.9	6.0	4.0	5.7	3.5
K^+	0.30	0.65	0.60	0.67	0.30	0.38	0.40	0.30
Ca^+	0.063	0.054	0.49	0.32	0.16	0.10	0.23	0.11
P^{---}	0.050	0.080	0.22	0.40	0.082	0.090	0.44	0.39
Na^+	0.077	0.080	0.11	0.12	0.14	0.15	0.10	0.12
Fe^{++}	0.027	0.015	0.014	0.014	0.076	0.080	0.029	0.053

— not investigated.

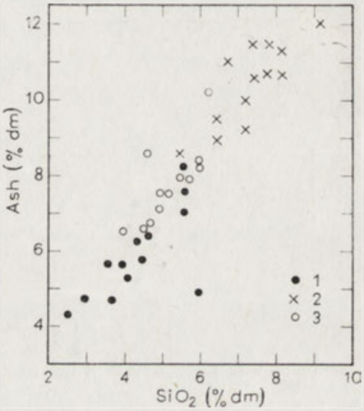

Fig. 3. Ash and silica in above-ground organs of reed (annual averages for the separate reedbed zones; 1967 season). 1 — stem, 2 — leaves, 3 — panicles

Comparing the average annual amounts of the particular mineral elements in different parts of reed with their amounts during the maximum of biomass (August–September) it was found out that the amounts of ash, silica and calcium during the maximum of biomass corresponded to their average annual amounts in all the above-ground parts of reed (Fig. 4 and 5). The same regularity was observed for phosphorus in panicles and leaves (Fig. 6) and for potassium in panicles (Fig. 7). The above regularities seem to be important for the choice of terms of sampling reed for analyses of its mineral contents and suggest that the determinations of the particular elements in reed during its biomass maximum are representative for the whole vegetation season.

It was found that the percentage share of stem in the organic matter content in reed was in average three times as high as that of leaves (Table II A). Because of the higher ash content in leaves than in stem, the share of leaves in the general ash content of reed was only by a half lower in comparison with the share of stem (Table II B) though leaves constitute only 23% of total weight (Mochnacka-Ławacz 1974).

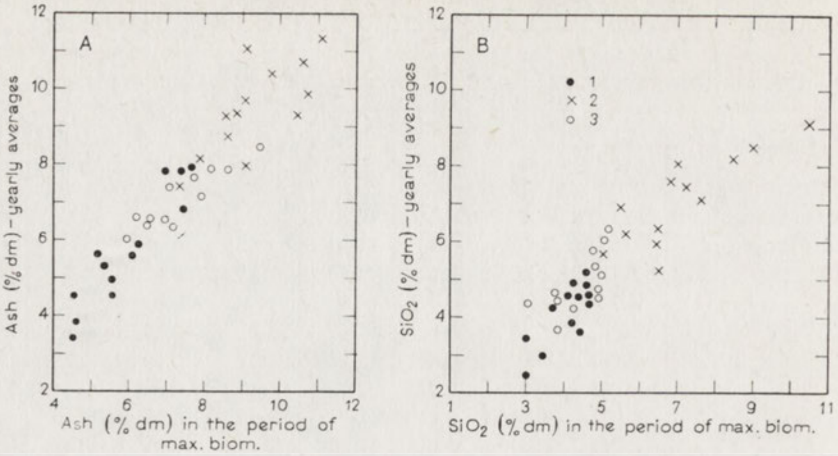

Fig. 4. Amounts of ash and silica in reed during biomass maximum as against annual averages (several zones of reedbeds; 1967 season). A — ash, B — silica, 1 — stem, 2 — leaves, 3 — panicles

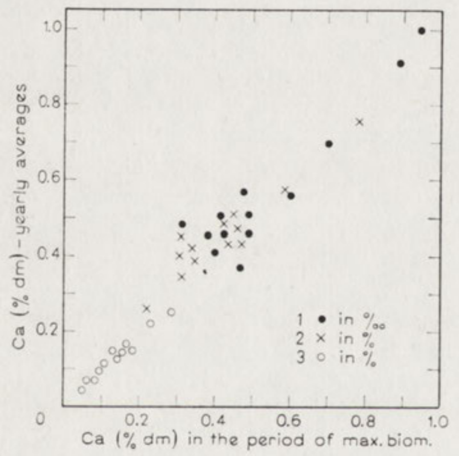

Fig. 5. Amounts of calcium in reed during biomass maximum as against annual averages (several reedbed zones, 1967 season). 1 — stem 2 — leaves, 3 — panicles

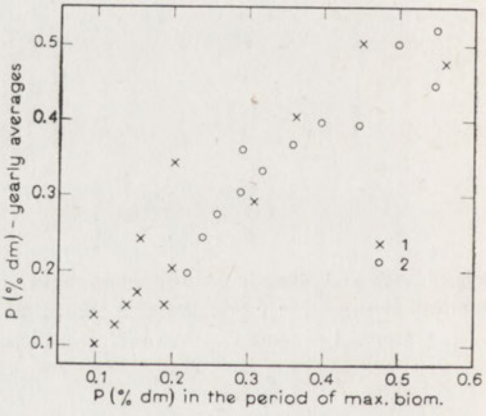

Fig. 6. Amounts of phosphorus in reed during biomass maximum as against annual averages (several zones of reedbeds, 1967 season). 1 — leaves, 2 — panicles

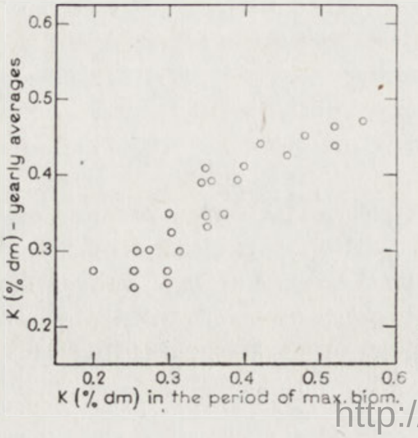

Fig. 7. Amounts of potassium in panicles during biomass maximum as against annual averages (several reedbed zones, two vegetation seasons)

Table II. Proportional share of stems, leaves and panicles in organic matter and ash contents of reed during biomass maximum in 1966 and 1967

	Stems	Leaves	Panicles
A. Organic matter			
1966	74.3	21.5	4.3
1967	74.5	23.4	2.1
B. Ash			
1966	60.5	36.7	2.8
1967	63.7	35.7	0.6

The amount of ash from 1 m^2 of the particular reedbeds at the maximum of biomass (August–September) ranged from one to a few dozens of grams, depending on the reed biomass.

The two seasons differed as to their annual average amounts of the particular mineral elements of reed (Table I), while the patterns of quantitative changes of the investigated elements during the course of each season were similar in both years (Fig. 8).

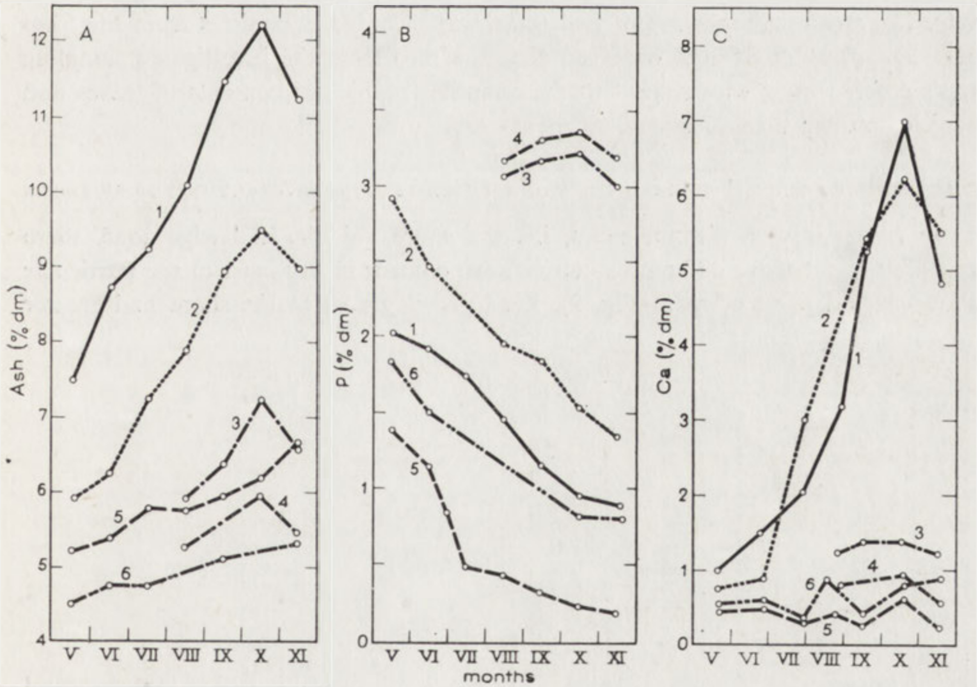

Fig. 8. Comparison of the contents of ash (A), phosphorus (B), and calcium (C) in reed from the Tałtowisko Lake in 1966 and 1967, 1 — leaves 1966, 2 — leaves 1967, 3 — panicles 1966, 4 — panicles 1967, 5 — stem 1966, 6 — stem 1967

COMPARISON OF THE MINERAL CONTENTS OF REED BETWEEN THE INVESTIGATED REEDBEDS

The seasonal changes in the mineral contents of reed were similar for all the reedbeds, but differences were apparent between the reedbeds as to the average annual contents of the studied elements. On account of the correspondence, established earlier, between the amounts of most elements during the biomass maximum and their annual averages, the latter values were chosen for the comparison of the reedbeds.

The greatest amounts of ash, silica and potassium were found in stem and leaves of reed from the Skonał Lake. In 1966 season they were, respectively, for the stem 6.5, 4.5, 0.6% and for leaves 10.8, 7.9 and 0.62%. The lowest contents of ash and silica were found in the above-ground parts of reed from the Śniardwy Lake in both seasons. In 1966, the stem contained 4.5% of ash and 2.4% of SiO_2; the leaves had, respectively, 8.8, 5.8% and the panicles 5.5 and 4.3%.

In the stem and leaves from this sampling spot the greatest amounts of calcium and phosphorus were found (stem: 0.1% Ca, 0.2% P; leaves: 0.5% Ca and 0.32% P in 1966). There was the least of calcium in all the above-ground parts of reed from the Kotek Lake (stem: 0.048%, leaves: 0.21%, panicles: 0.05%) and of phosphorus in stem and leaves of reed from the Tałtowisko Lake (stem: 0.05%, leaves: 0.12%). The greatest proportions of ash, calcium and phosphorus in panicles were found in both seasons in reed from the Mikołajskie Lake, i.e. 8.5, 0.21 and 0.41%, respectively. The greatest proportion of potassium was recorded in panicles from the Skonał Lake (0.48%). It was observed that the reed from the investigated sampling spots differed most with respect to the quantitative mineral contents of leaves and panicles, rather than of stems.

COMPARISON OF THE MINERAL CONTENTS OF REED BETWEEN THE SEVERAL ZONES OF REEDBEDS

In reedbeds with distinct zones, i.e. the shore, middle, and edge zone, there appeared quantitative differences between ash content in dry mass of the particular above-ground parts of reed (Fig. 9). Reed growing next to the shore had greater

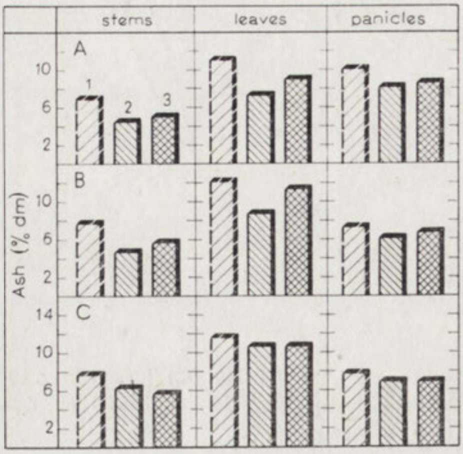

Fig. 9. Ash content in reed of the investigated reedbed zones (annual averages, 1966 season). A — Mikołajskie Lake, B — Tałtowisko Lake, C — Skonał Lake. 1 — shore, 2 — middle, 3 — edge

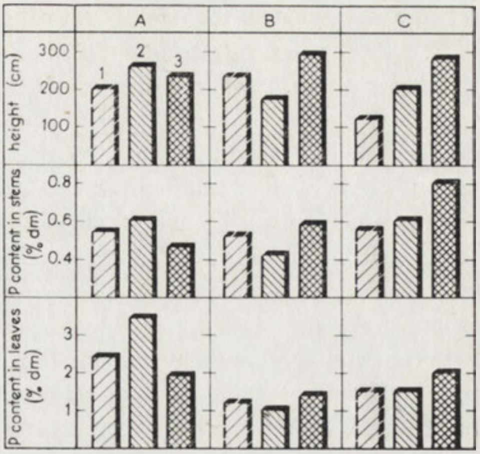

Fig. 10. Length of reed, phosphorus contents in stem and leaves in the several reedbed zones (biomass maximum period in 1966). A — Mikołajskie Lake, B — Tałtowisko Lake, C — Skonał Lake. 1 — shore, 2 — middle, 3 — edge

ash content in stem, leaves and panicles in comparison with reed from the other two zones. The least of ash was found in all the above-ground parts of reed from the middle zones.

Correlations were also discovered between phosphorus content in dry mass of stem and leaves of reed and its length (detailed results of metrical measurements of reed from the several zones of the reedbeds are reported in Part I of this study, Mochnacka-Ławacz 1974). The greatest phosphorus content in dry mass of stem and leaves during the maximum of biomass was observed in the tallest reed (Fig. 1), from zones with the greatest biomass.

4. DISCUSSION

The main cause of the decrease of the relative contents of many mineral elements in dry mass of reed in the course of a vegetation season is the increase of dry mass of tissues with the impoverishment of the soil associated with it (Curtis, Clark 1958).

Květ (1973) studied the seasonal pattern of changes in the mineral contents of reed and he also observed a rapid decrease of amounts of all the elements except calcium and silica. A similar pattern of changes is reported by Solski (1962) in a study about the mineral contents of *Typha latifolia*. The processes of accumulation of silica in cell membranes of old plants can be the direct cause of the differences between zones with respect to the general ash content in above-ground parts of reed during the maximum of biomass. Reed from the shore zones of the studied lakes, only periodically inundated and thus growing in conditions most similar to those prevailing on dry land, probably produces a stronger protective system than reed from the two remaining zones with constant inundation. The obtained evidence indicates the most intensive accumulation of ash, and thus also of silica which constitutes its large part, in shore reed. Buttery, Lambert (1965) have found a higher phosphorus and potassium content in shore reed as compared with reed growing next to the open water range. As one of the causes of such a differentiation those authors mention the smaller amount of living roots in edge reed, as it takes roots in deeper layers of the bottom. It seems, however, in accordance with the view by Haslam (1973), that the roots of reed in less humid places reach deeper than in constantly inundated places. If so, then shore reed would take advantage not only of the surface-water mineral store, but also of that of ground waters. Such a situation can be one of the causes of the greater ash content in reed from shore zones. Besides, easier access to mineral salts by shore reed can be interpreted in terms of the peculiarity of the lake eulittoral zone (Pieczyńska 1972), which is under the strongest impact of the surrounding drainage bassain, enriching the biotope in mineral salts of allochtonic origin. Planter (1970) found out higher concentrations of many mineral elements in water of shore zones of reedbeds in comparison with the open water zones.

The obtained results which point to the greater percentage contents of phosphorus in stems and leaves of higher plants, confirm the suggestion of Buttery, Lambert (1965) and Haslam (1973), who consider this element to be most limiting. Boyd, Hess (1970) also emphasized positive influence of abounding phosphorus in a biotope on height and biomass of *Typha latifolia.*

In the studied material mineral composition of soil failed to be determined: it seems, however, that in case of phosphorus its role is greater as an element intensely circulating in biotopes constantly inundated than as one dissolved in the soil (Oświt 1968). The higher reed plants with greater phosphorus content in their dry mass grew in the middle and edge reedbed zones thus in more eudynamical biotopes (Patalas 1960) due to more intense water movements occurring there than in the shore zones.

To disclose the relationships between the condition of reed and the contents in its dry mass of the other studied mineral components it should be perhaps necessary to consider in investigations its more biotopically differentiated determinants of growth.

In the present work only a narrow scope of biotopes accessible to reed was studied. Comparing the obtained results with those by other authors (Dykyjová, Hradecká 1973, Úlehlová et al. 1973, Květ 1973) it appears that reed from pond biotopes analysed in those works is marked by greater amounts of the particular mineral elements in its dry mass.

5. SUMMARY

Mineral contents of the particular parts of reed were analysed (stems, leaves, panicles and rhizomes) on 6 lake reedbeds near Mikołajki, during two vegetation seasons (from May to October, 1966 and 1967). Each reedbed was divided into zones relative to their distances from the shore line and from the open water range of the lake (shore, middle, edge).

Phenological differences in the contents of particular elements were found out, as well as differences between reed from various sampling grounds and between its morphological parts. In the course of a vegetation season the contents of K, Na, P, Fe and N were decreasing while the amounts of Ca, SiO_2 and general ash in dry mass of leaves were increasing. During the biomass maximum of reed (August–September), leaves contained most of ash, SiO_2, K and Ca, rhizomes most of Na and Fe and panicles most of P. Quantitative changes of amounts of elements in rhizomes were

less extensive than in above-ground parts, among which the broadest scope of change was observed in leaves.

Quantitative differences in mineral contents of reed from various sampling grounds were more obvious in leaves and panicles than in stems.

No proportional relationships were found between the contents of the particular elements, except for SiO_2, which remained in a constant proportion of 0.73 to the general ash in dry mass of stems and leaves and 0.63 in dry mass of panicles (average annual values).

It was established that the differences in ash content in dry mass of above-ground parts of reed depended on its distance from a shore line. The greatest amounts of ash were typical for shore zone reed. It seems that one of the causes of such differentiation is the different availability of mineral salts of allochtonic origin and different degrees of development of protective tissue, which is probably related to the level of water in various parts of reedbeds.

In general, higher concentrations of phosphorus were found in dry mass of stem and leaves of tall reed as compared with lower plants. Because of the fact that tall reed usually inhabits middle and edge zones of reedbeds, it seems that the phosphorus content in its dry mass could be influenced by the more intensive waving of water in those zones than in the shore zone.

6. STRESZCZENIE

Na sześciu trzcinowiskach jezior okolic Mikołajek analizowano w ciągu dwóch sezonów wegetacyjnych (od maja do października 1966 i 1967 r.) skład mineralny poszczególnych części trzciny (źdźbła, liści, kwiatostanów i kłącza). Każde trzcinowisko podzielono na strefy, zależnie od odległości od linii brzegowej i otwartego lustra wody jeziornej (brzeg, środek i skraj).

Stwierdzono różnice fenologiczne w zawartości poszczególnych elementów, a także pomiędzy trzciną z różnych stanowisk, oraz w obrębie jej części morfologicznych. Wraz z upływem sezonu wegetacyjnego zawartości K, Na, P, Fe i N obniżały się, natomiast wzrastały ilości Ca, SiO_2 i popiołu ogólnego w suchej masie jej liści. W okresie maksimum biomasy trzciny (sierpień–wrzesień) liście zawierały najwięcej popiołu, SiO_2, K i Ca, kłącza najwięcej Na i Fe, a kwiatostany — P. Zmiany ilościowe pierwiastków w kłączu były mniejsze niż w częściach nadziemnych, spośród których liście wykazały największy zakres zmienności.

Zróżnicowania ilościowe w składzie mineralnym trzciny z różnych stanowisk dotyczyły bardziej ilości i kwiatostanów niż źdźbła.

Nie stwierdzono proporcjonalnych zależności pomiędzy zawartością poszczególnych elementów, za wyjątkiem SiO_2, której stosunek do ilości popiołu ogólnego wynosił w suchej masie źdźbła i liści 0,73, a kwiatostanach 0,68 (średnie roczne wartości).

Stwierdzono, że różnice w zawartości popiołu w suchej masie części nadziemnych trzciny zależne były od jej odległości od linii brzegowej. Największe ilości popiołu miała trzcina stref brzegowych. Wydaje się, że jedną z przyczyn takiego zróżnicowania jest różna dostępność soli mineralnych pochodzenia allochtonicznego, oraz niejednakowy stopień wykształcania tkanki wzmacniającej, co prawdopodobnie pozostaje w związku z poziomem wody w różnych częściach trzcinowiska.

Stwierdzono na ogół wyższe koncentracje fosforu w suchej masie źdźbła i liści trzciny wysokiej, w porównaniu z niską. Ze względu na fakt, iż trzcina wysoka zasiedlała najczęściej środkowe i skrajne strefy trzcinowisk, wydaje się, iż na zawartość fosforu w jej suchej masie mogły mieć decydujący wpływ bardziej intensywne ruchy wody w tych strefach, w porównaniu ze strefą brzegową.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	381–386	1974

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THE EFFECTS OF MOWING ON THE DYNAMICS OF QUANTITY, BIOMASS AND MINERAL CONTENTS OF REED (*PHRAGMITES COMMUNIS* TRIN.)

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ABSTRACT

Mowing of reed repeated three times during a vegetation season significantly lowered its biomass, quantity and percentage of leaves in biomass of sprouting plants. The pattern of seasonal changes of the amounts of particular mineral ingredients in mown and unmown reed was similar, but the amounts of the accumulated substances were different. Elements typical of old plants (ash, silica, calcium) were more abundant in unmown than in mown reed. In the latter, a large proportion of elements typical of young plants was observed (potassium, phosphorus, nitrogen).

1. INTRODUCTION

Common reed (*Phragmites communis* Trin.) constitutes about 80% of the biomass of emergent plants (mactophytes) in lake littorals (Bernatowicz, Pieczyńska 1965) and thus its participation in the circulation of mineral salts in a reservoir seems to be significant for the overall salt balance. It was shown by Haslam (1970, 1973) that reed, like most vascular aquatic plants (Sculthorpe 1967), draws mineral salts both from the bottom and from water around it. Since the production of reed is rather high (Westlake 1963, Wetzel 1964, Wetzel, Hugh 1973) and its exploitation for industrial purposes is steadily growing, the amount of mineral salts taken out from the littoral per each square metre can be one of the factors preventing excessive eutrophication of the environment. As there can be many possible economic uses of reed (e.g., as silage for farm animals; cf. Sakowicz, Kocół 1952), it is apt to be mown in different phenological phases; this probably influences the amount of extracted mineral salts and of the produced biomass of reed.

2. TERRAIN AND METHODS

Experiments were made from June to September 1971 in the middle zone of a reed bed on the Mikołajskie Lake.

From an area of about 30 m^2 reed was mown three times at one month's intervals starting from mid June. Material for determination of biomass from 1 m^2 and of the chemical composition of reed, mown and unmown separately, was drawn at mowing days in eight random areas of sampling, 0.25 m^2 each. The samples were also used for appreciation of the number of reed specimens per 1 m^2. The weight of reed was determined after drying at 105°C to a constant weight level; the dried material was combusted at 550°C to determine its mineral composition. General ash and silica content was determined by weighing; ash was hot-dissolved in 1:1 HCl solution to determine potassium and calcium by photometric method (flame photometer) and phosphorus by colorimetric method (with ammonium molybdate). The elements selected for determinations were those that seemed to be crucial for both the mineral metabolic processes in plants (Curtis, Clark 1968) and for the productivity of water reservoirs (Patalas 1960). Mineral contents of stems and leaves were analysed separately. In the half of September, when the biomass of reed is at its maximum (Mochnacka-Ławacz 1974) the sum of biomasses from three mowings per 1m^2 was compared with the biomass of unmown reed.

3. RESULTS

BIOMASS, QUANTITY AND MORPHOMETRIC CHARACTERISTICS OF REED

The medium values of the recorded measurements of reed in the initial material from the first mowing (mid June) were: number per 1 m^2 — 52 indiv.; biomass per 1 m^2 — 468 g; height — 168.3 cm; diameter — 0.68 cm; number of leaves per stem — 6.4. Leaves accounted for 5% of the biomass.

In the material from the second mowing (mid July) the number of young sprouting specimens was only 48% of the initial amount and it was 60% lower than the actual amount of unmown reed (Table I). The biomass of mown reed was only 10.7% of the initial biomass and 5% of the actual biomass; the height was 55% of the initial value and 37% of the actual value for unmown ren (Table I).

Table I. Comparison of the investigated morphometric features, amounts and biomasses of mowed and unmown reed

Date	Height (cm)	Diameter (cm)	Biomass (g/m^2)	Density (indiv./m^2)	Mean number of leaves per indiv.
1st mowing 15.VI.1971	168.3	0.68	468.0	52.0	6.4
2nd mowing 15.VII.1971	93.3	0.35	50.0	25.2	5.6
Unmown 15.VII.1971	251.0	0.75	1000.0	64.0	12.2
3rd mowing 15.IX.1971	87.4	0.34	52.0	20.0	8.9
Unmown 15.IX.1971	229.5	0.75	1406.0	64.0	12.5

In the material from the third mowing (mid September) unmown reed reached the amount of 64 indiv. per 1 m^2, its biomass was 1406 g/m^2; height — 230 cm; diameter — 0.75 cm. In comparison with the initial values this was, respectively, 123, 300, 136 and 110% (Table I). By that time leaves accounted for 28% of the biomass of unmown reed per 1 m^2. The relevant values for mown reed were: its number was 38% of the initial; the biomass was 11% of the initial and 3.8% of the actual for unmown reed; the length was 51% of the initial and 38% of the actual value. The proportion of leaves in the biomass of mown reed per 1 m^2 was constant during the whole experiment and it was maintained at the 5% level. At the same time the number of leaves in mown reed increased by about 39% in comparison with the mid-June level and it was about 74% of the amount of leaves in unmown reed in the half of September (Table I).

The sum of biomasses of mown reed was 570 g/m^2 in the half of September and it was thus about three times lower than the biomass of unmown reed (1406 g/m^2). It can be seen that the mowing impaired both the amount and biomass of newly sprouting reed.

MINERAL CONTENTS OF REED

The seasonal changes in the amounts of particular mineral elements and of general ash in dry weight of stems and leaves were similar in mown and in freely growing reed (Fig. 1 and 2). The amounts of general ash and silica in dry weight were increasing from spring to autumn; in stems, concentrations of those elements were similar for mown and unmown reed (Fig. 1A, B), while in leaves they were greater for unmown reed (Fig. 2A, B).

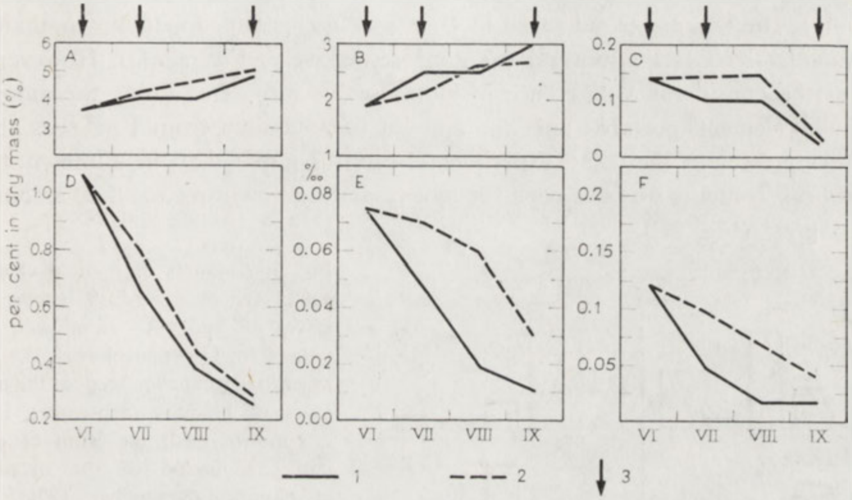

Fig. 1. Comparison of mineral contents of reed mown three times during a season and unmown (stems). A — total ash, B — silica. C — calcium, D — potassium, E — phosphorus, F — nitrogen. 1 — unmown reed, 2 — mown reed, 3 — dates of mowing

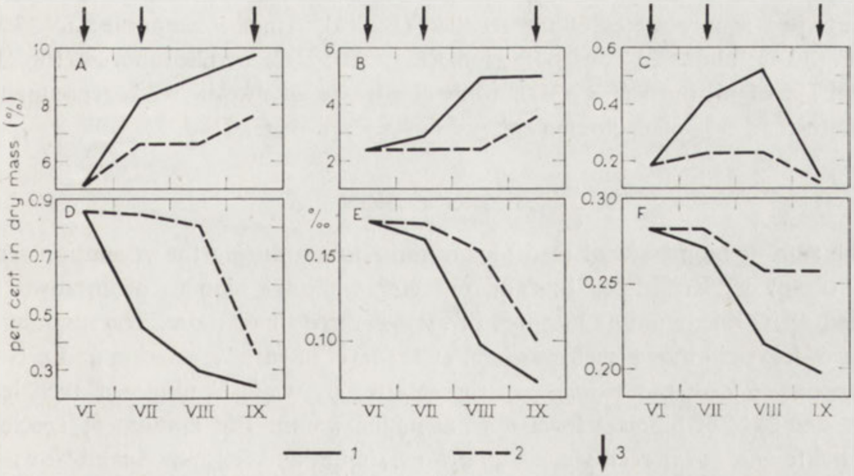

Fig. 2. Comparison of mineral contents of reed mown three times during a season and unmown (leaves). The same symbols as in Fig. 1

Concentrations of calcium were also similar and decreasing in stems of mown and unmown reed within the same ranges (Fig. 1C), while the amounts of calcium in leaves of unmown reed were higher than in mown one (Fig. 2C).

However, the amounts of potassium, phosphorus and general nitrogen in dry weight of stems and leaves of mown reed were higher than in freely growing plants during the whole vegetation season (Fig. 1 and 2D,E,F).

Amounts of mineral elements taken out from 1 m² of a reedbed during a vegetation season were assessed (Fig. 3). It was found that there was much more of general ash (80 g/m²) in unmown reed than in the total crop of experimental reed (20 g/m²). The amount of nitrogen in 1 m² was also greater for unmown than for experimental reed (1.1 g/m² and 0.7 g/m² respectively; Fig. 3A,D). However, in spite of the considerable difference in biomasses usually determining the amounts of mineral elements per area unit, the amount of potassium from 1 m² was about 3.5 times greater in the total experimental crop (7 g/m²) than in unmown reed. Phosphorus contents was the same for mown and unmown reed and it amounted to 0.04 g/m² (Fig. 3C).

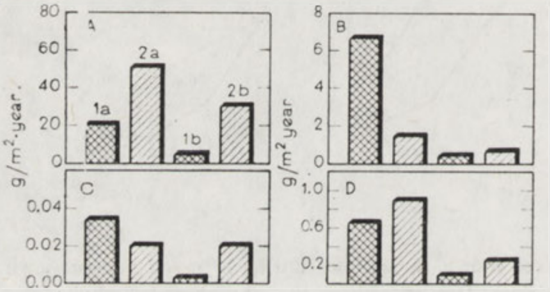

Fig. 3. Amounts of mineral elements per 1 m² of a reedbed (stems and leaves of reed). A — total ash, B — potassium, C — phosphorus, D — nitrogen. 1 — unmown reed at the maximum of biomass (September 1971), 2 — mown reed, the sum of crops until the period of the maximum of biomass (September 1971). a — contents of an element in leaves, b — contents of an elements in stems

Leaves constituted a much greater proportion of biomass in unmown reed than in mown plants and so was their participation in the total amount of mineral elements per square meter of the reedbed (Fig. 3). Thus, it amounted to 37% of ash (Fig. 3A); about 22% of potassium (Fig. 3B); 50% of phosphorus (Fig. 3D); about 27% of nitrogen (Fig. 3D), while the leaves of mown reed accounted for not more than 5% of each element per one square meter (Fig. 3).

4. CONCLUSION

The sum of biomasses of reed mown three times during the vegetation season was 570 g of dry weight per one square meter, while the biomass of unmown reed attained at its maximum 1406 g/m², or almost three times more. The amount and weight of leaves in mown reed was kept at the level of early vegetation and accounted for only 5% of the biomass of the total crop, while in unmown reed leaves constituted 28% of biomass from 1 m² at its maximum. The amount of specimens per 1 m² for unmown reed was 64 at the maximum and for mown reed it was 20.

Thus, mowing impairs the biomass and amount of reed. In mown reed the direction of changes in the mineral content was the same as in freely growing reed (maturing and ageing), but condensations of the particular elements were modified by mowing. The elements which are accumulated in tissues of old plants during the whole vegetation season (Curtis, Clark 1958), i.e. silica in stems and leaves, calcium in leaves, general ash in leaves, appeared in greater amounts in unmown reed. On the other hand, the elements whose contents in plant tissues decreases as the plants grow and mature (potassium, phosphorus, nitrogen in stem and leaves) maintained higher condensations in mown reed which remained young in each period of the vegetation season. Thus, the levels of mineral elements in mown reed were mainly determined by the artificial conditions of its vegetation, i.e. by its age, while the general trend of modifications of the mineral contents of mown reed remained consistent with the general seasonal regularities.

Apparently, the higher condensations of potassium, nitrogen and phosphorus in stem and leaves of mown reed indicate the abundance of those elements in the environment as relative to the demand of reed.

In comparison with aggregates of meadow plants intensively exploited during a vegetation season (Falkowski 1965), the crop of reed yielded by three mowings was several times lower than expected. As the mineral elements attained high condensations in mown reed, it seemed that the factor limiting more effective biomass production was not mineral starvation, but rather the low proportion of leaves as the main assimilatory organs.

This is probably directly related both to the biology of the discussed species (Bykov 1964) and to climatic factors which slow down the assimilatory processes in plants as the vegetation season continues, mainly by the shortening of the day from sunrise to sunset occurring between June and September. It is by those factors that Whitehead (1970) explains the fact that the crops of many grass species are lowered if the intensity of mowing is increased between spring and autumn; he stresses that the sum of new growths often fails to attain the biomass of unmown plants. Besides, it is implied by the data of Bernatowicz, Pieczyńska, (1965) that physical features of the bottom largely influence the rate of growth of new reed after mowing.

Thus, if we consider the final balance of the biomass of newly growing reed and the balance of mineral elements in 1 m^2 of repeatedly mown and unmown reedbeds, it seems that it is enough to do the mowing only once in a season, when the biomass of reed is at its maximum, as one of measures against the eutrophication of lakes.

5. SUMMARY

Unmown reed was more numerous and its biomass was three times greater than the sum total of biomasses of reed mown thrice during a season: there were, respectively, 64 and 20 individuals per 1 m^2 and 1406 against 570 g/m^2. At the same time, the proportion of leaves in the biomass of unmown reed was 28%, or over five times more than in mown reed (only 5% in the sum of biomasses from all mowings). Thus, the mowing impaired the production of reed biomass, in opposition to most exploited grass communities (Falkowski 1965, Nowiński 1967 and others). It was also found that in mown reed there was less of general ash, silica and calcium, but more of potassium, phosphorus and general nitrogen in dry weight of stem and leaves as compared with unmown reed. Thus, the elements which are usually accumulated in tissues of old plants (Curtis, Clark 1958) were found in greater condensations in freely growing reed, while those which tend

to condense in tissues of young plants were more abundant in mown reed, artificially kept in early phases of growth.

Hence, in spite of the different biomasses, the amount of phosphorus from 1 m^2 of a reedbed was the same for mown and for freely growing reed and the amount of potassium was even a few times greater in mown reed (Fig. 3C,B). This seems to indicate sufficient abundance of those elements in the environment as relative to the demand of reed, and at the same time to some significance of mowing in the circulation of those elements in the littoral zone.

6. STRESZCZENIE

Trzcina niekoszona miała wyższą liczebność, oraz trzykrotnie wyższą biomasę, w porównaniu z sumą biomas trzciny koszonej trzykrotnie w ciągu sezonu: odpowiednio 64 i 20 szt./m^2, oraz 1406 i 570 g sm/m^2. Jednocześnie procentowy udział liści w biomasie trzciny niekoszonej, stanowiący 28%, był ponad pięciokrotnie wyższy od udziału liści trzciny koszonej (zaledwie 5% w biomasie z sumy koszeń). Tak więc koszenie wywarło ujemny wpływ na ilość wyprodukowanej biomasy trzciny, w przeciwieństwie do większości zbiorowisk trawiastych użytkowanych gospodarczo (Falkowski 1965, Nowiński 1967 i inni). Jednocześnie stwierdzono, że trzcina koszona zawierła mniej popiołu ogólnego, krzemionki i wapnia, oraz więcej potasu, forsforu i azotu ogólnego w suchej masie źdźbła i liści, w porównaniu z niekoszoną. Tak więc te składniki, które na ogół są kumulowane w tkankach roślin starych (Curtis, Clark 1958) wystąpiły w większych stężeniach w trzcinie nie poddawanej zabiegowi koszenia, te natomiast, których wyższe stężenia występują w tkankach roślin młodych, znajdowano w większych stężeniach w trzcinie koszonej (sztucznie utrzymywanej na etapie wczesnego wzrostu).

Stąd, mimo różnic w biomasach trzciny, ilość fosforu z 1 m^2 trzcinowiska była jednakowa w trzcinie koszonej i niekoszonej, a ilość potasu nawet kilkukrotnie wyższa w trżcinie koszonej (Fig. 3B,B). Wskazywałoby to na wystarczającą zasobność środowiska w te elementy w stosunku do zapotrzebowania na nie trzciny, a jednocześnie na pewną rolę zabiegów wykaszania w procesach krążenia tych elementów w strefie litoralu.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	387–390	1974

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LARVAE OF THE GENUS HYDROPSYCHE (INSECTA: TRICHOPTERA) FROM POLAND

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ABSTRACT

A modified and simplified version of a key to Hydropsyche larvae in Poland is presented. The key includes 10 species found up to the present in Poland and 4 species which occurrence in this area is probable. The most important taxonomic features of larvae of 12 species are presented and supplemented by photographs. The larvae of *H. bulbifera* and probably *H. exocellata* are described and figured for the first time. A temporary taxonomical status is defined for larvae of *H. borealis* and *H. fulvipes* which were unknown in science up to the present.

For a number of years the genus Hydropsyche was considered, in relation to European species, to be one of the most difficult taxa within the order Trichoptera. Subtle differences in the morphology of the genitals created many difficulties for trichopterologists in correct identification of species and were the cause of numerous erroneous items of information about the distribution of individual species. Only thanks to elaborations of recent years by Botosaneanu, Marinković-Gospodnetić (1966), and especially to the splendid monograph of imagines of the genus Hydropsyche (Tobias 1972a, b), it may be said that the taxonomical problems of the genus Hydropsyche have been solved what concerns the imagines of European species.

More difficult is the matter of acquiring knowledge about the juvenile stages in species of Hydropsyche. Older elaborations concerning descriptions of individual species, as well as comprehensive works on the genus generally, are either outdated and incomplete, or erroneous. Doubtless the best and most complete is the newest paper by Sedlák (1971). It deserves attention, amongst other reasons, because the author used here successfully a method of photography in presenting individual elements of larvae and restricted the descriptive form to the minimum, thus avoiding superfluous verbal ballast.

30 caddisfly species of the genus Hydropsyche are known from Europe now (Tobias 1972a). There are 10 species known in Poland from literature at present. They are: *angustipennis* (Curt.), *bulbifera* McLach., *contubernalis* McLach., *exocellata* Duf., *fulvipes* (Curt.), *guttata* Pict., *instabilis* (Curt.), *ornatula* McLach., *pellucidula* (Curt.) and *saxonica* McLach. Considering the distribution of the remaining 20 European species not mentioned from Poland one may expect here still 4 other species: *borealis* Mart. (Sudetes Mts and northern Poland), *nevae* Kol. (mainly in the north of the country), *silfvenii* Ulm. and *siltalai* Döhl.

Larval forms were known from the following species of the total of 14 presented above: *angustipennis*, *contubernalis*, *guttata*, *instabilis*, *nevae*, *ornatula*, *pellucidula*, *saxonica*, *silfvenii* and *siltalai*. Larval stages of the remaining 4 species: *borealis*, *bulbifera*, *exocellata* and *fulvipes* were unknown. From amongst the latter the author was able to distinguish larvae of *H. bulbifera* during work on Trichoptera of the Raba River. The species was identified on hand of imagines caught near the river and imagines from chrysalis cocons; first erroneously as *H. subguttata* Mart., but then correctly after the Tobias (1972b) elaboration. Also from the Raba River originate specimens of this species collected by Dziędzielewicz identified as *H. guttata* (ex coll. Mus. Inst. Zool. Polish Acad. of Sciences, Kraków). The author distinguished also larvae belonging probably to the species *H. exocellata* from the rivers Vistula and San, but there is no absolute proof that this assumption is correct, as he did not find any pupae of this species. Larvae of *H. fulvipes* and *H. borealis remain* unknown.

A KEY TO LARVAE OF THE POLISH HYDROPSYCHE

1 (6). Anterior edge of the apotome* convex (Fig. 1A and 2B).

2 (3). Anterior edge of the apotome symmetrical (Fig. 2B) — *H. exocellata.*

3 (2). Anterior edge of the apotome asymmetrical (Fig. 1A, 3B and 5B).

4 (5). No figure or patches in the central area of the apotome; only dark terminal bands (Fig. 3B and 4B) — *H. ornatula* (also *H. borealis*?).

5 (4). Dark figure and patches in the central area of the apotome — *H. guttata* (Fig. 5B), *H. contubernalis* (Fig. 1A and 6BC).

6 (1). Anterior edge of the apotome straight or slightly concave (Fig. 1B–E).

7 (10). Lateral parts of the submentum long and narrow (Fig. 7); proportion of a : b less than 0.18.

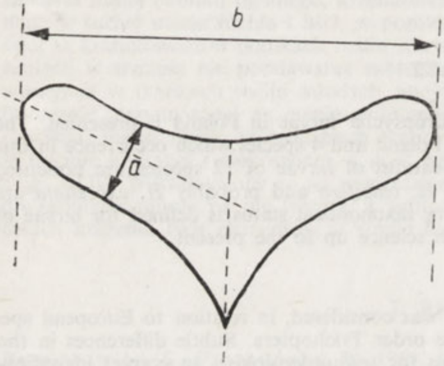

Fig. 7. Submentum of Hydropsyche larvae

8 (9). The head almost as long as broad (Fig. 8) — *H. angustipennis.*

9 (8). The head distinctly longer than broader (Fig. 9) — *H. pellucidula.*

10 (7). Lateral parts of the submentum shorter and broader; proportion of a:b exceed 0.2 (about 0.22–0.23).

11 (18). Gills on 7th abdominal segment.

12 (13). Arrangement of the apotome pattern of pigmentation very characteristic (Fig. 10); there is a bright patch in the central area of the apotome, just behind the cross-line of the anterior tentotrial pits. The apotome markedly longer than broader (Fig. 11), (proportion of k:l in *H. silfvenii* 0.54) — *H. silfvenii* and *H. nevae.*

13 (12). Arrangement of the apotome pattern of pigmentation different; there is a dark patch in the central area of the apotome. The apotome not so markedly longer as broad (proportion of k:l in *H. saxonica* 0.64–0.71, in *H. instabilis* 0.66–0.69, in *H. bulbifera* 0.58–0.62).

14 (15). Anterior area of the apotome (between the submarginal clypeal ridge and the epistomal sulcus) bright, without dark Y-shaped figure; in the more chitinised specimens two indistinct spots are found (Fig. 1C–E and 12 C) — *H. bulbifera.*

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* Terminology of the head after Badcock (1961).

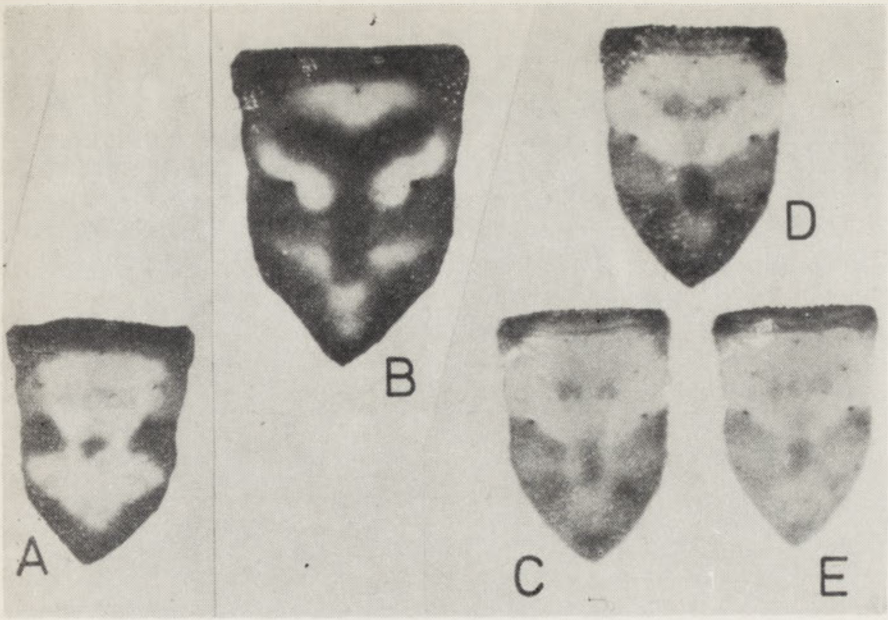

Fig. 1. Examples of different apotomes of several Hydropsyche larvae. A — *H. contubernalis*, B — *H. pellucidula*, C–E — *H. bulbifera*

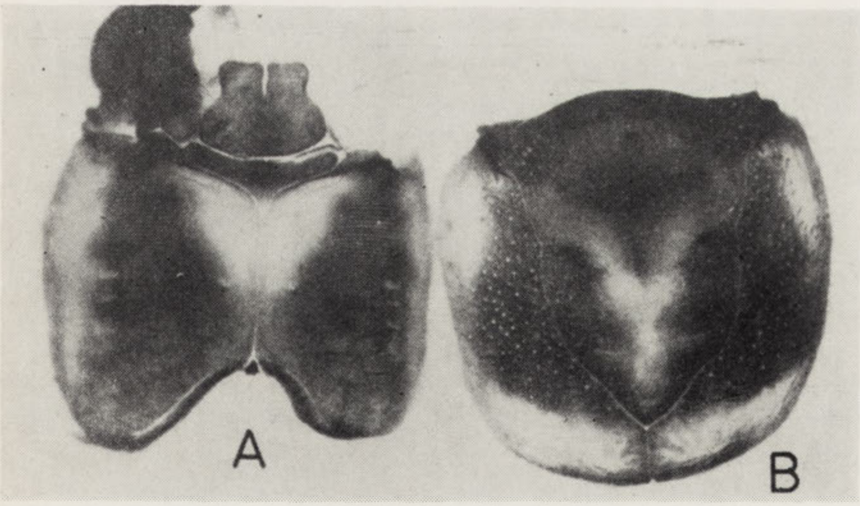

Fig. 2. Larval head capsule of *H. exocellata* (from River San, near Stalowa Wola). A — ventral view, B — dorsal view

Fig. 3. Larval head capsule of *H. ornatula* (from Bug River, near Wyszków, col. R. Sowa). A — ventral view, B — dorsal view

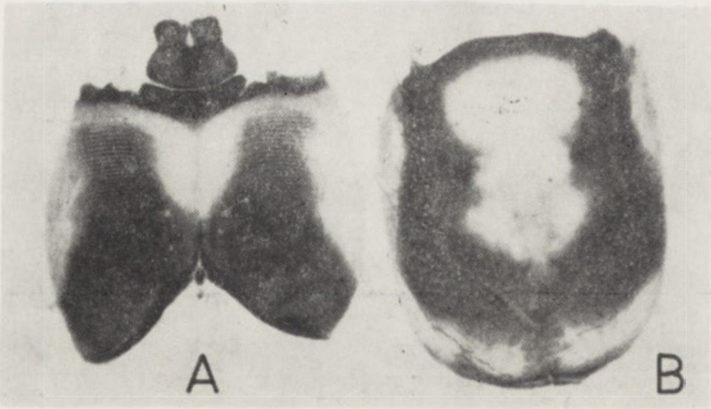

Fig. 4. Larval head capsule of *H. oxnatula* (other specimen from Bug River). A — dorsal view, B — ventral view

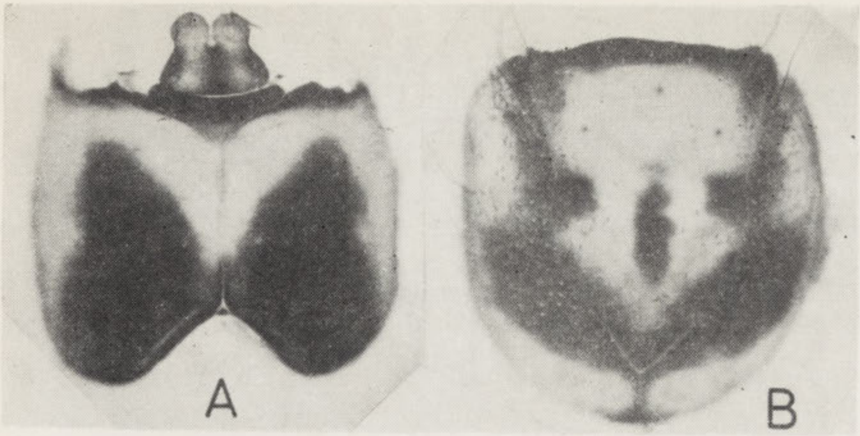

Fig. 5. Larval head capsule of *H. guttata* (from Vistula River, near Annopol). A — ventral view, B — dorsal view

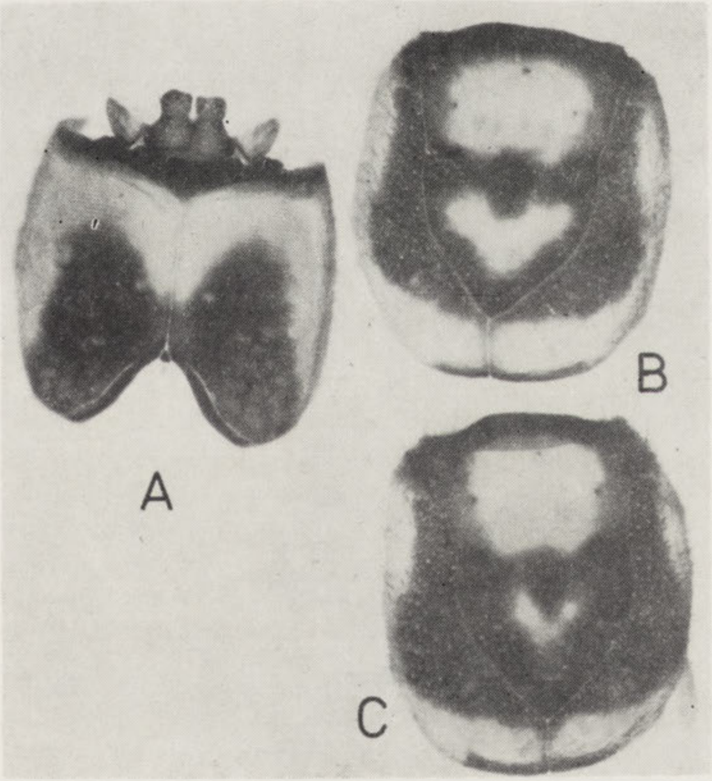

Fig. 6. Larval head capsules of *H. contubernalis* (from Raba River, near Bochnia). A — ventral view, B — dorsal view, C — dorsal view (other specimen)

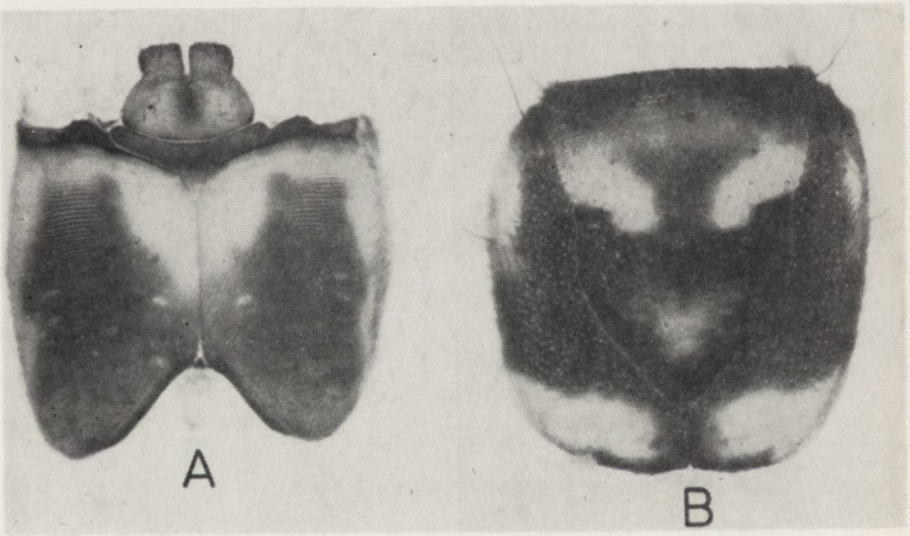

Fig. 8. Larval head capsule of *H. angustipennis* (from small stream, near Bochnia). A — ventral view, B — dorsal view

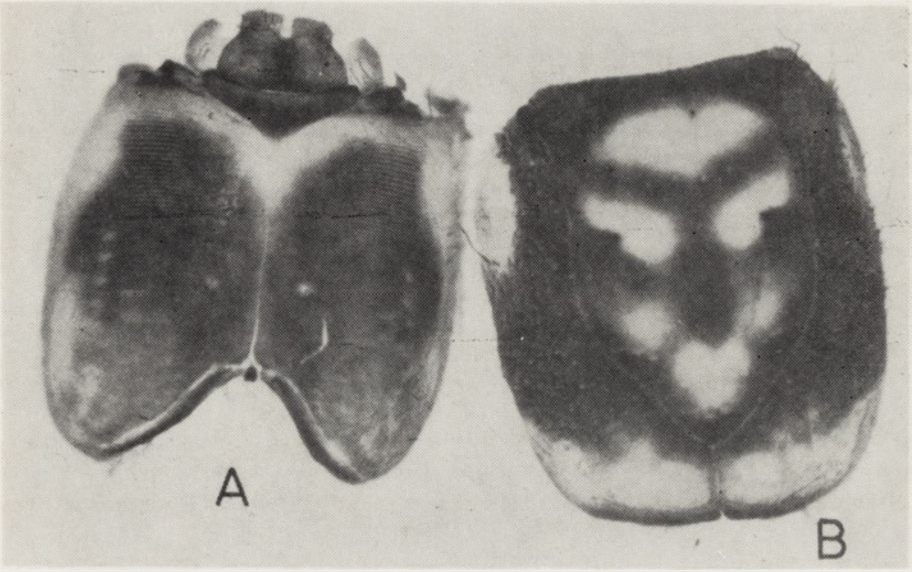

Fig. 9. Larval head capsule of *H. pellucidula* (from Raba River, near Myślenice). A — ventral view, B — dorsal view

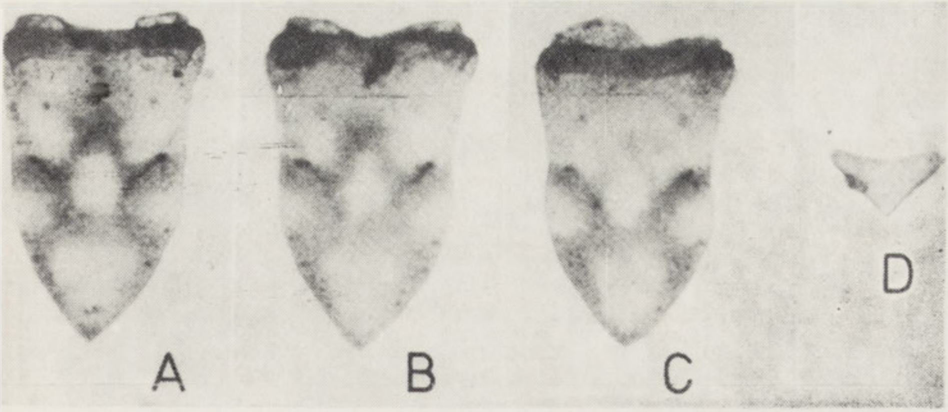

Fig. 10. *H. silfvenii*. A–C — apotome, D — submentum. Reproduced from Sedlák (1972)

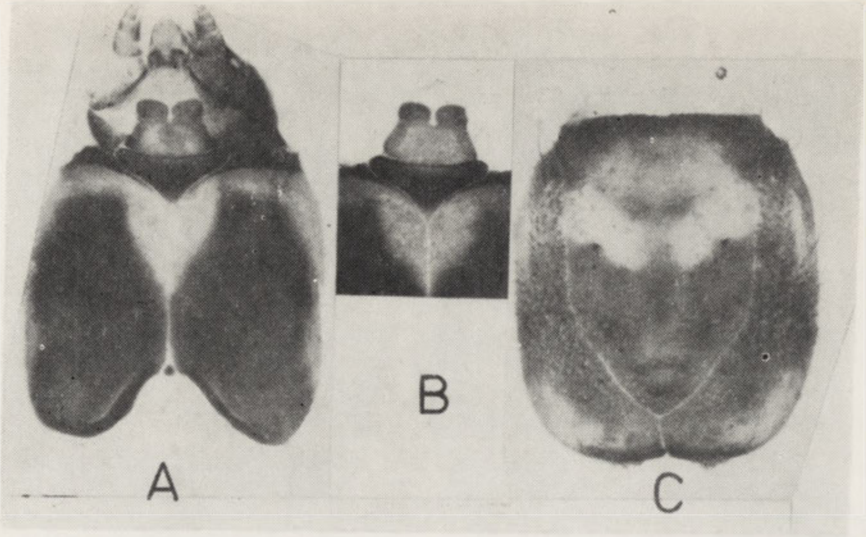

Fig. 12. Larval head capsule of *H. bulbifera* (from River Raba, near Bochnia). A–B — ventral view, C — dorsal view

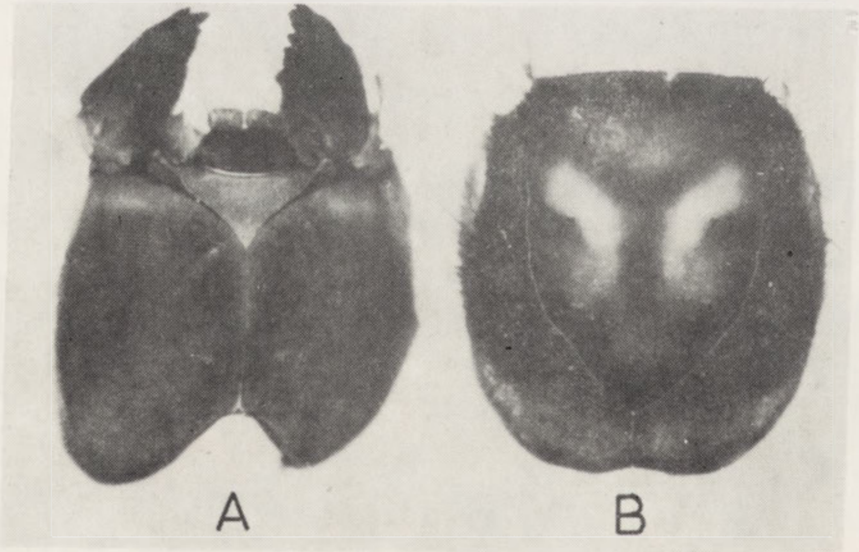

Fig. 13. Larval head capsule of *H. saxonica* (from small stream, near Myślenice). A — ventral view, B — dorsal view

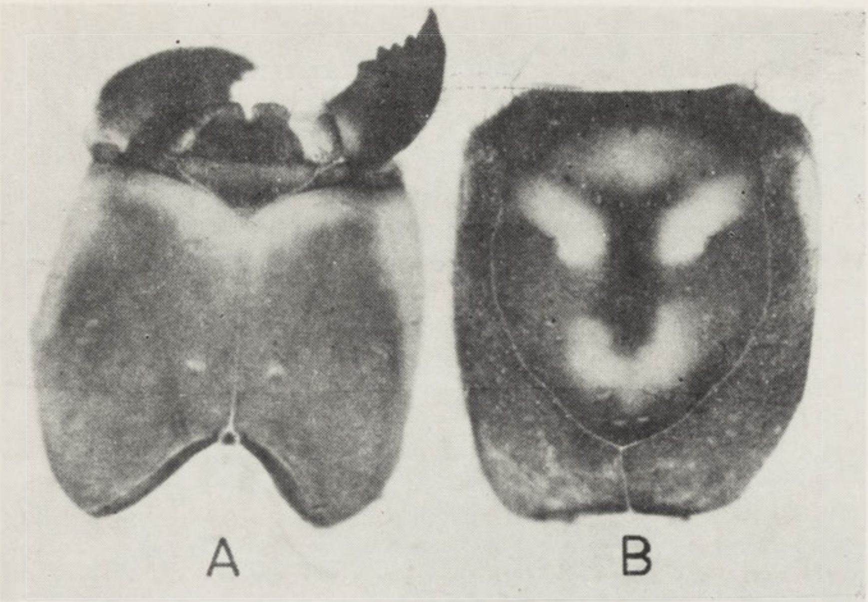

Fig. 14. Larval head capsule of *H. instabilis* (from stream Sąspówka, in Ojców National Park). A — ventral view. B — dorsal view

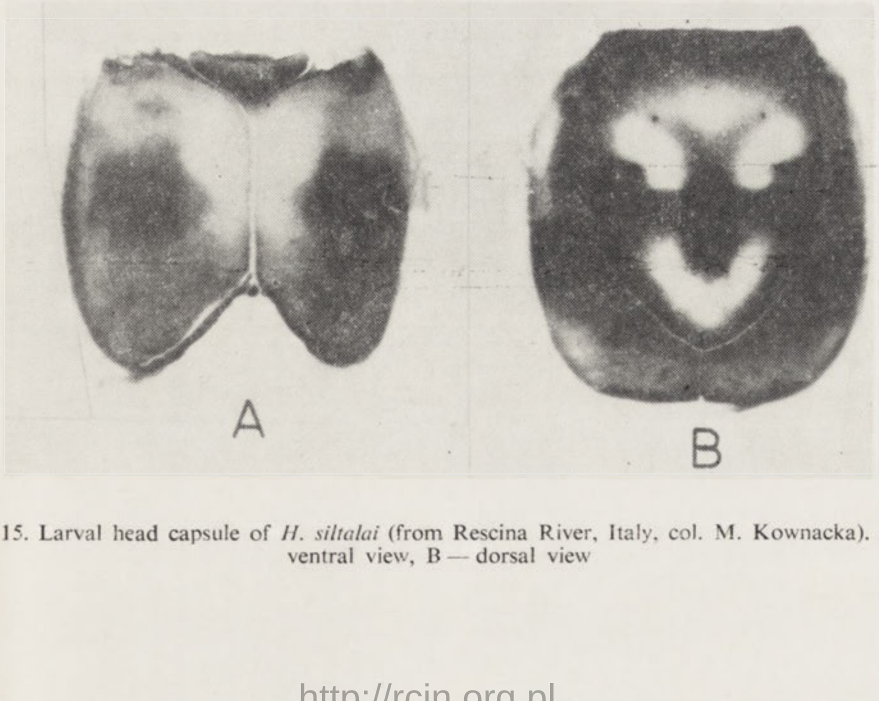

Fig. 15. Larval head capsule of *H. siltalai* (from Rescina River, Italy, col. M. Kownacka). A — ventral view, B — dorsal view

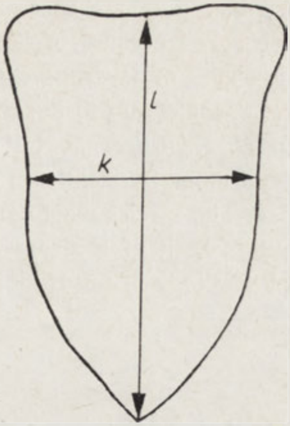

Fig. 11. Apotome of Hydropsyche larvae

15 (14). Anterior area of the apotome equally dark, or most frequently with a dark Y-shaped figure.

16 (17). The apotome is widest in the anterior part (posterior edge of the submarginal clypeal ridge) — *H. saxonica* (Fig. 13B).

17 (16). The apotome is widest or equal in the middle part, compared to the anterior part (Fig. 14B) — *H. instabilis* (also *H. fulvipes*?).

18 (11). Gills on 7th abdominal segment absent — *H. siltalai* (Fig. 15).

The set of characteristic features presented in the key provides easy distinguishing of several species groups within the genus Hydropsyche. The most compact group consists of the species: *ornatula*, *contubernalis* and *guttata*. The above species are distinguishable through their asymmetry of the anterior edge of the apotome, a very interesting feature, as in *trichopterous* larvae was known only in the family of Philopotamidae up to now. Close to this group is the species *H. exocellata* thanks to the bulging edge of the apotome. Considering the characteristic morphology of genitals in imagines, probably also *H. borealis* belongs to this group.

A separate group is made up by the species *silfvenii* and *nevae*, distinguished by the characteristic shape of patches on apotome and its shape itself; other species, such as *instabilis*, *saxonica* and *siltalai* differ in the shape of the submentum and shape of spots on the apotome.

Explanation is still needed in the problem of systematical status of larval form described by Lepneva (1964) under the term *H. ornatula*; either the figure shows a larva of *H. borealis* Mart. (Tobias 1972a), or the *H. ornatula* is not very accurately drawn, as on Lepneva's drawing the anterior edge of the apotome is symmetric.

According to Tobias (1972a,b) *H. nevae* inhabits northern parts of Europe, mainly Scandinavia and the Baltic Lowlands, whereas *H. contubernalis* occurs rahter in West and South-West Europe. The remaining species are found in all Europe. There is little known about the occurrence of *H. borealis* at this time, as only recently the species was acknowledged as a separate taxonomical unit. Data on the distribution of the species: *bulbifera*, *exocellata*, *ornatula* and *guttata* are still to be checked because of the many cases of mistaken identity.

Juvenile stages of Hydropsyche (larvae and pupae) live is water courses from the smallest brooklets up to big rivers. In lowland rivers and brooks one may find most often *H. angustipennis*, but less frequently *H. pellucidula*. The remaining species occur more frequently in swift rivers and mountain torrents and in the foothill zone. The species most common in these areas in *H. pellucidula*. The vertical distribution limit of Hydropsyche larvae in Poland seems to be about 700 m above sea level; only in the High Tatra this limit may be slightly shifted upwards.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.) | 21 | 3/4 | 391–400 | 1974

LECH SZLAUER

USE OF STEELON-NET VEILS FOR PROTECTION OF THE HYDRO-ENGINEERING WORKS AGAINST *DREISSENA POLYMORPHA* PALL.

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ABSTRACT

Serviceability of the veils used for safeguarding of hydro-engineering installations against *Dreissena polymorpha* invasion has been studied. Mass settling down of young individuals of this mussel species has been observed on the veil hung in the headrace channel furnishing water to the hydro-engineering installation. The veil of 26 m^2 in surface was able to withhold 5 milions young mussels. The large-scale of the settling of *D. polymorpha* on the veil allows to believe that a range of veils could protect the hydro-engineering installations effectively against the harmful mussels.

1. INTRODUCTION

Problems created by a fresh-water mollusc *Dreissena polymorpha* (zebra mussel) become a matter of great importance, in the last few years, all over Europe. This mollusc is the cause of serious troubles and often involves considerable damage to the hydroelectric power plants, waterworks, power generating stations with an open water-cooling system, and other works. *D. polymorpha* in the larvae stage penetrates into various works (pipes, channels, etc.) and grows there; and after a few years is about 3 cm long.

The flow of water in the hydrotechnical installations promotes the development of *D. polymorpha*. It can subsist even at the rate of flow as high as 2–2.5 m/sec (Lyakhov 1962). Dreissena reduces the efficiency of waterworks as well during its life as after its death. The dead individuals and empty shells of *D. polymorpha* carried along by a current of water pile up inside the pipes and block them up.

No fully efficacious protective methods against *D. polymorpha* invasion have been found hitherto. All the methods used to this purpose: chlorination of water, treatment with metal ions, hyperthermia, mulluscocides, destruction of larvae with UHF sound waves or by means of electric field, are for greater part very complicated and quite often falling short of expectations. Moreover, some of them are likely to cause the danger of the poisoning of water environment. Therefore, in the face of this situation, there is an urgent need for some new methods of fight against *Dreissena polymorpha*.

The aim of the present study was to investigate the possibilities of mechanical protection of the hydro-engineering works against *D. polymorpha* invasion. It has been decided to utilize special veils made from steelon fishing-nets. It has been expected that Dreissena larvae, characterized by an inherent tendency to settle down on a stable substratum, may likewise live and grow on the veils and consequently a series of screens installed upstream upwards the hydrotechnical plants will prevent the inflow of the larvae of Dreissena into the waterworks.

This concept of using preventive barriers against *D. polymorpha* is not a new idea. Wilhelmi (1922), cited after Liebmann (1960), proposed to sink fascine in the water intakes to serve as a substratum to hold down Dreissena. This proposition repeated by Lüdemann (1954) was not utilized on a larger scale in practice. The author of the present study has introduced some novelty to this method by utilization of the steelon mesh waste material lying uselessly in the stores of great fishing companies. The advantage of using this kind of substratum is that, besides being cheap, it is resistant to putrefaction. Thus, those steelon nets may be of practicable good use and many years' standing service as protective veils against *Dreissena polymorpha* larvae.

3. METHODS

Investigations were carried out in 1973, in the open channel conveying water from the Odra River's mouth region to the Chemical Works near the town of Szczecin. This water is used mainly for and cooling systems of various chemical apparatuses in the factory. The channel is about 2 km long, 13 m wide, and up to 3 m deep. In the spring, in the period between May 3 and 29, five veils have been installed accross the channel, interspaced from one another at a distance of a few up to several scores meters. Only one veil out of the five has outlasted till the end of the experiment, October 20. It was hung on May 29, on a hoisting rope spanned over the water-level of the channel at about 80 m from the outset of the pipeline. The upper edge of the veil did not stick out over the water surface and its lower edge reached the bottom of the channel. The greater part of the veil was made of 3 cm-mesh network of 1.8 mm double-twine steelon cord. The total surface area of the veil was 26 m^2. The veil has been under continuous control. The process of the Dreissena and other organisms settling down on the veil was observed regularly and individuals of Dreissena settled upon the veil were counted and measured.

On september 10 a supplementary experiment was made i.e. on the already overgrown with Dreissena veil a new piece of network was hung. This was done with the view to determine whether there is a possibibity of young Dreissena penetrating onto the new substratum. During the course of the summer, water temperature in the channel was measured and quantitative samples of plankton were collected in which Dreissena larvae and more numerous organisms were counted. The frequency of *D. polymorpha* and other organisms occurrence was also observed in the channel furnishing water to the hydrotechnical installations and inside those installations as well. The experimental veil overgrown with mussels was pulled out of water on October 20 and exposed to normal weather conditions on the land.

3. RESULTS

In the above-mentioned Chemical Works *Dreissena polymorpha* was present in the cooling and fire-control systems, and moreover, also in the concrete chambers in the pumping station from which water (conveyed by the open channel) is pumped into the pipelines. The thickness of the Dreissena layer on the surface of the inside walls of the pipes was in the autumn 1972 up to 5 cm thick and the density of its population in there averaged 10,500 individuals per 1 m^2. These data show the situation after three years of the functioning of the pipeline. This was the initial stage of Dreissena invasion; at that time its population consisted of representants of only three generations.

Contrary to the situation in the pipelines and pumping station chambers the open channel conveying water from the Odra River was practically free of Dreissena. More numerous *D. polymorpha* colonies were found on the concrete constructions of a road bridge. Moreover, some single, alive individuals of Dreissena were encountered in one place in the littoral zone of the channel. The central part of the channel with a thick layer of mud on its bottom was devoid of this mollusc.

The presence of the *Dreissena polymorpha* larvae in the water of the channel was for the first time noted on May 28, at the 21°C water temperature (Table I). At that time the maximum of the density of their population was recorded (405 larvae in 1 liter of water). A very high density of larvae was also observed at the beginning of June (June 6 — 282 larvae per 1 liter of water). Some time later (June 23) almost complete absence of larvae was noted. They appeared anew on July 2. And from that day on till September 11 the number of larvae was decreasing gradually. On October 20, at the water temperature of 9°C, Dreissena larvae were not present in the plankton, any more (Table I).

The data of larvae measurements are given in Table II. The length of the larvae in plankton was in the range from 70 to 200 microns. Among the larvae trochophore as well as the larvae of veliger type were present. The former were encountered only on May 28. The trochophora larvae were in the range of 70 to 90 microns long. Veliger in the earliest stage measured 90 microns, likewise. All the larvae measuring 100 microns were already in the veliger stage. The number of the largest larvae was strikingly scarce (Table I and II). The period of their occurrence in plankton, from the beginning of June till the beginning of August, is much shorter as compared with the occurrence of the larvae on the whole. The maximum of the density of larvae ranging from 170 to 200 microns long was recorded on August 10 (12.6 individuals per 1 liter of water).

The density of Dreissena larvae population (Table I) is presented in comparison to the number of occurrence of some species predominant in the channel plankton the diatoms of the Asterionella genus (*A. formosa*, *A. gracilima*) *Melosira granu-*

Table I. Seasonal changes in the density of population of the *Dreissena polymorpha* larvae and other plankton organisms in the channel furnishing water to the cooling system (1973)

Date	Temp. (°C)	Number of individuals/l			Number of colonies	
		Total larvae	Larvae 170–200 µ long	Brachionus	Asterionella	Melosira
7.V	14.5	0.0	0.0	285	6300	475
28.V	21.0	405.0	0.0	201.6	2764	950
6.VI	19.8	282	9.3	930	1990	650
23.VI	23.0	1.0	0.0	7.2	172	13,000
2.VII	26.0	106	2.6	3.6	432	6300
10.VIII	20.0	87	12.6	3.6	950	12,400
11.IX	18.0	4	0.0	0.0	864	12,180
20.X	9.0	0.0	0.0	86.4	259	518

Table II. Per cent of various length larvae in the channel conveying water to cooling systems (1973)

Larval length (µ)	28.V	6.VI	23.VI	2.VII	10.VIII	11.IX
70	2	—	—	—	—	—
80	10	—	—	—	—	—
90	36	1	—	—	1	—
100	30	22	+	36	44	+
110	12	42	+	33	23	—
120	5	11	—	7	10	—
130	3	10	—	8	3	+
140	2	7	—	6	1	+
150	—	2.5	—	5.5	1.4	—
160	—	1.2	—	2	2.1	+
170	—	0.8	—	2	4.1	—
180	—	0.8	—	—	2.1	—
190	—	0.4	—	0.5	4.1	—
200	—	1.3	—	—	4.2	—
Total	100	100		100	100	

lata, and *Brachionus calyciflorus amphiceros* (Rotatoria). The comparison between the occurrence of larvae and the other planktonic organisms shows that June 23 was, more or less, the turning point for the occurrence of those organisms. On this day a violent increase in the number of Melosira population was observed and contrariwise a dramatic decrease in the number of Asterionella and Brachionus c.a. Another turning point was October 20 when the number of Melosira decreased and the number of Brachionus increased, strikingly.

The *Dreissena polymorpha* larvae floating in the canal since May did not settle down on the bottom or the veil until July 2. Only after that data they have started to pass into a sedentary mode of life. On August 10 a mass appearance of Dreissena young individuals was observed settling on the veil and on the old colonies of that species overgrowing the cement structures of the bridge over the channel. It has not been possible to determine more exactly the end of the process of Dreissena settling down on the veil. Yet, it is quite certain that they did not settle down any more after September 13. Two, new veils installed in the channel on that day have not been occupied by Dreissena at all.

The results from the measurements of the Dreissena individuals settled on veil are shown in Table III. On August 10, the group of individuals, 1–2 mm long, was decidedly predominant, the second significant group in respect of numbers consisted of individuals 2–3 mm long. A month later (September 10) individuals 4–5 mm long predominated over all others. On October 20, the 6-8 mm individuals were predominant. In all the three columns under the dates given in Table III the structure of the measurements of the individuals of Dreissena population has a single-apex character and in the course of the progressing time the apex became gradually less and less sharp.

Table III. Per cent of the *Dreissena polymorpha* individuals of various length settled on the veil (1973)

Individual length (mm)	10.VIII	10.IX	20.X
>1	3	—	—
1–2	60	7	0.5
2–3	33	5	3
3–4	4	12.5	3.5
4–5	—	21.5	10.5
5–6	—	16.0	11
6–7	—	15.0	17.5
7–8	—	9.5	16.0
8–9	—	4.0	13.5
9–10	—	7.0	10.5
10–11	—	0.5	6.5
11–12	—	1.0	4.5
12–13	—	0.5	2.5
13–14	—	0.5	0.5
Total	100	100	100

The net veils proved to be a very convenient framework for counting the density of the Dreissena population. On August 10, September 10, and October 20, samples of the network from the veil, most densely overgrown by Dreissena, were duly collected. Then, out of those fragments of the net 5 mesh openings were selected at random to count the molluscs settled in there. The average results were calculated in respect of 1 m^2 of the net. The maximal density of Dreissena population (216,920 individualas/1 m^2) was recorded on August 10, i.e. at the beginning of the settling down period. Later on, the number of the molluscs has decreased. On September 10, the number of individuals on 1 m^2 of the net was 120,360, on October 20 only 82,280. Despites the decrease of the density of the Dreissena population on the veil the value of the biomass was increasing rapidly due to the growth of individuals. The weight of the veil overgrown with mussels has increased to such a degree that at the end of the experiment on October it was very difficult to lift it up from the channel.

The densely packed and quickly growing Dreissena on the veil caused a considerable reduction of the size of the open spaces in the network (Fig. 1). This phenomenon is of great consequence as the veil extending across the channel in the course of the passing time holds the water bark more and more. The distribution of the Dreissena population over the veil is not uniform. The molluscs did not show up on the lower border of the veil, resting on the bottom mud or plunged in it. They occurred also in less numerous numbers in those places where the network has formed thicker layers.

The experiment of the hanging of a new piece of net over the veil overgrown with Dreissena has shown that in the period of one month quite a large number

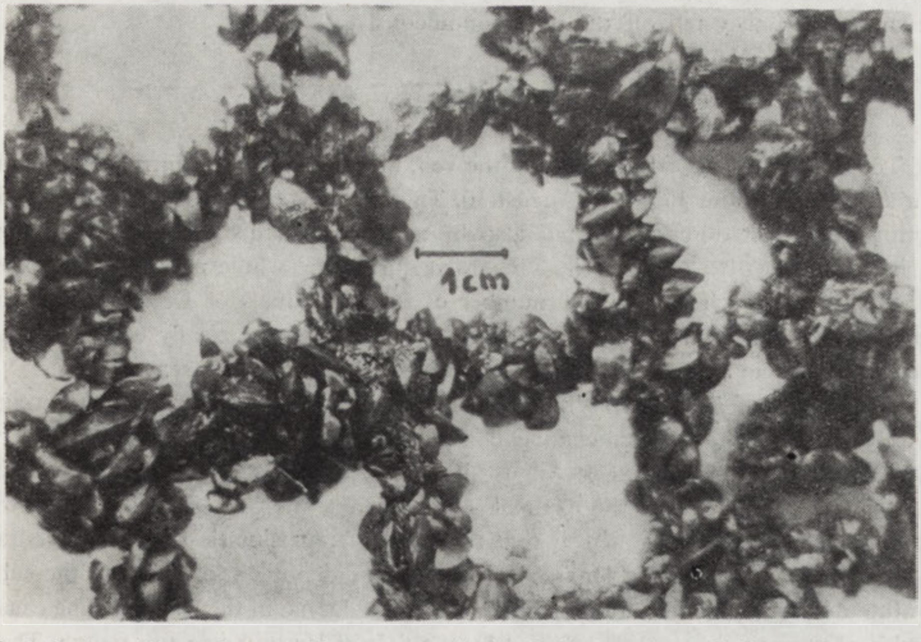

Fig. 1. Section of the steelon-net veil overgrown with the *Dreissena polymorpha* (September 11, 1973)

of the Dreissens individuals, measuring from 1.5 to 10.5 mm, had crossed over and settled down on the new substratum. A greater inclination for migration was observed in smaller individuals. The class of 5–6 mm size predominated on the new substratum whereas in the same period of time individuals 6–7 mm long were predominant on the veil. The smallest individuals (2–4 mm long) constituted 12% of the total number of Dreissena on the new substratum whereas on the veil only 6.5%. Nevertheless, even the 10.5 mm long individuals were passing over onto the new substratum.

Dreissena polymorpha was not the only species observed on the veil. A few days after the hanging of the veil it was already overgrown with periphyton and covered all over with diatomic colonies of Melosira. Out of other larger organisms the predatory Oligochaeta of the Chaetogaster and Hydra genus have appeared pretty soon on the veil. Slightly later a mass development of *Cordylophora caspia* (Coelenterata) has been noted. The freshwater Spongia were also encountered on the veil. However, their colonies were very scarce and scanty. Simultaneously with the settling down of the Dreissena also the crayfish (*Orconectes limosus*) have appeared on the veil and remained there until the time of the lifting up of the veil from the channel (October 20). And then it become evident that they were present in a great abundance. Adult and young individuals about 3 cm long were recorded. In the crayfish alimentary canals fragments of Dreissena shells were found. It is worth mentioning as a curiosity that the veil has the utilized by a green frog (*Rana esculenta*) as a place for wintering.

The veil was dragged to the shore (October 20) and taken under observation. On November 19, after having been left for a month in the open, all mussels were dead and undergoing the process of putrefaction. Due to the highly advanced decomposition they fell off the net spontaneously.

4. DISCUSSION

The settling down of Dreissena on the veil, in 1973, was an occurrence of short duration lasting from July 2 to August 10. This fact was evidenced not only by the findings of direct observations but also by a single-apex distribution of the sizes of individuals settled on the veil — recorded during the whole period of the experiment (Table III). Therefore the number of the individuals of Dreissena population found on the veil on August 10 may be treated as practically equipollent to the number of individuals settled on the veil during the whole season.

The settling of *Dreissena polymorpha* on the veil was associated with the occurrence of its larvae in the open channel conveying water from the Odra River. Before May 28, on June 23, and after August 10, the Dreissena larvae did not occur in great numbers (Table I) in the channel. Consequently the Dreissena settling down on the veil has not been noted at that time. A different situation was observed at the end of May — then the Dreissena individuals did not settle down on the substratum despite the fact of a mass occurrence of the larvae in the channel. The cause of this phenomenon may be explained by the fact of larvae being too young. They were at that time 70–140 microns long (Table II). There were present among those larvae Trochophora as well as stages of development, which according to the nomenclature of Kirpiczenko (1964) are determined as the floating veliger. Whereas, it is taken as a matter of fact that only the largest larvae, encountered already in the plankton, are in that stage of development when they are apt to pass into the sedentary mode of life. Those larvae, caught in the water of the open channel, measured 200 microns (Table II).

The largest veliger larvae observed by Hillbricht-Ilkowska, Stańczykowska (1969) in the plankton of the Tałtowisko and Śniardwy lakes measured 170 microns. The length of the largest larvae, as recorded by Breitig (1961) and Kirpiczenko (1964), was 220 and 250 microns, respectively. Basing on the data obtained from various lakes it has been determined that veliger larvae are passing into the sedentary mode of life only after they had grown and reached the length of about 170 microns. The accepted lower limit of the size of larvae able to settle down on the substratum is not always constant. It depends on varying conditions and especially on the density of water (Wiktor 1969).

The growing of larvae to the proper size and the presence of an accessible substratum are not the only conditions necessary for the settling down of Dreissena. Doubtlessly, there are some other prerequisites conditioning that process. Thus, it seems that due to those unknown factors the settling down of *D. polymorpha* on the substratum was made impossible on June 6 and July 2 though the larvae, and among them the largest of the veliger stage, were present in the plankton.

The unknown and known factors preventing the settling down of the larvae are of a great practical importance. Owing to those factors out of those hundreds of thousands of larvae flowing in the water through the cooling systems of the Chemical Works during a period of nearly three months only a minimal percentage of the larvae carried along by the water of the channel between July 2 and August 10 has settled down on the veil. This does not mean, nonetheless, that the absolute, actual number of the settled down individuals was small.

The density of the larvae population in the open channel as compared with the Szczecin Bay showed approximate values. The maximal density of larvae in the Szczecin Bay between 1961 and 1969, as recorded by Wiktor (1969), was in the range from 200 to 700 individuals per 1 liter of water.

The origin of larvae drifting in the water of the investigated channel may be referred to the shoals of the adult individuals of Dreissena population present in the Szczecin Bay and the Odra River and also to the individuals existing in the channel itself. A complicated hydrological situation in the Odra River's mouth region makes it possible that the inflow of the water into the channel may come either from the Odra River or the Szczecin Bay. The rise and piling up of the surface of the Bay caused by the wind results in turn in a backward current in the Odra River. Then the larvae from the bay may penetrate into the open channel. At the normal flow of the Odra waters the larvae from the river are carried along into the channel. These pecularities were encountered during the present experiment. On June 23 all the larvae have practically left the plankton. It has occurred in the period of the maximum of the density of larvae population in the water bodies e.g. in the Szczecin Bay the peaks of the larvae density in the years of 1961–1964 were observed in mid-June and the beginning of July (Wiktor 1969). It is difficult to explain those phenomena otherwise than by the inflow into the channel of the masses of waters without larvae deriving from another source than hitherto. This interpretation seems to be supported by the abrupt changes, occurring on the same day (June 23), in the numbers of population of the other components of the plankton (Table I).

Taking into account those hydrological pecularities within the water-intake sector of the Odra River it is possible to foresee a very irregular incidence of the Dreissena larvae in the channel conveying water into the cooling systems of the Chemical Works. The settling down of Dreissena in the hydrotechnical installations, depending likewise upon the incidence of the larvae, may be of a different and very variable intensity in subsequent years.

The Dreissena population existing in the open channel (e.g. on the bridge-pillars) due to its scant occurrence can be left out of the account as the source of the larvae derivation. Moreover, the larvae hatching from the eggs of the *D. polymorpha* individuals living in the channel do not have a sufficiently long time to be able to reach the adequate stage of development. Drifting rapidly with the water current the larvae penetrating into the hydrotechnical installations are too young and consequently incapable of settling down. According to Wiktor (1969) larvae are capable of settling down on the substratum not sooner than after 10 days of life at the temperature of 18°C.

The course of life of the individuals of Dreissena settled down on the veil runs in a similar way like that of the population existing on the natural substratum. With the passing time the density of the Dreissena population was decreasing progressively. In relation to the initial state (216,920 individuals/m^2) observed on August 10 in October an over 2.5-fold decrease in the density of the Dreissena population was recorded on the veil. This might have been caused by the shifting of the *D. polymorpha* individuals to those parts of the veil that were less densely occupied or by death on a large scale. The first of the probable causes, as suggested above, has been confirmed by the findings of the experiment indicating possibility of the displacement of even relatively large specimens of the Dreissena on the veil. Doubtlessly, the absolute reduction of the population was also occurring due to the eating-up of the young generation of the Dreissena by the crayfish (*Orconectes limosus*) permanently present in large numbers on the veil. The Dreissena population may have been likewise eaten up by fish living in the channel and its larvae by the Coelenterates — Cordylophora existing on the veil in great abundance. The mass eating-up of the Dreissena larvae in the Bay of Szczecin by Cordylophora in particular has been described by Wiktor (1969). In the light of these facts the role of the veil appears as an element that makes possible not only the catching up of the Dreissena but also a biological warfare against it. Another cause of the decrease in the number of Dreissena on the veil may involve the falling off of the individuals from the veil and wasting-away in the slime at the bottom of the channel.

However, the mud samples collected directly behind the veil did not confirm this hypothesis.

The results of the investigations dispose to the acceptance of the usefulness of the network veils serving as protective barriers against invasion of the hydrotechnical installations by *Dreissena polymorpha.* This is supported by the evidence of the mass-character of the settling down of the younger generations of Dreissena on the veil. The veil has retained 216,920 individuals on 1 m^2 area which in conversion to its total surface area amounts to 5640,000 individuals of *D. polymorpha.* Obviously, these numbers represent rather the upper limits of the veil capacity for the retainment of the larvae since the calculations were based on the sections of the network with the thickest layers of the settled on Dreissena. For better visualization of the magnitude of those numbers they should be compared with the density of 10,500 individuals per 1 m^2 recorded in the autumn 1972 within the pipes of the waterworks. The number of Dreissena individuals retained on 1 m^2 of the veil corresponds therefore to the number of the *D. polymorpha* individuals which have contrived during three years to settle down and occupy 20.6 m^2 of the surface of the pipes. About 5 million individuals which were retained on a single veil correspond to the number of molluscs settled on 535 m^2 of the inside surface of the pipes, in the autumn 1972.

The presented calculations allow to presume that by installation of an adequate number of veils across the channel to the hydrotechnical works it will be possible to safeguard them against the mass invasion of the *Dreissena polymorpha.* The accurate determination of the exact number of the required veils calls for further studies. Moreover, one cannot disregard the fact that even a very great number of veils put across the channel cannot hold back all the larvae living in the channel.

The use of the veils in the warfare against Dreissena is a preventive method. It can be used for protection of the water engineering works and installations still unoccupied by Dreissena as well as those the molluscs have already invaded. In the latter case, the veils preventing further settling down of Dreissena may lead to the improvement of the situation. The installations protected by the veils against the inflow of new generations of Dreissena may in course of time undergo spontaneous purification in result of gradual, natural death of the settled in Dreissena.

The use of the veils is a bio-mechanical method of the warfare against Dreissena which should not cause any negative side-effects in the water environment. This method has also the virtue of being cheap and simple. The low cost of the veils is due to the fact that they are made from useless waste material and are not labour-consuming as regards their installation and servicing. As a matter of fact, the attendance to the veils is reduced to hanging them in the water at the end of the spring and pulling them out to the shore in the autumn. In the open air the veils undergo a spontaneous clean-up.

5. SUMMARY

Dreissena polymorpha is a mollusc which invades and settles down in the hydrotechnical installations in various industrial plants and quite often causes serious damage to the equipment, pipelines, waterworks, etc. Useability of the steelon veils as protection against *D. polymorpha* has been under investigation.

Mass occurrence of young individuals of *D. polymorpha* has been observed settling down on the veil hung across the open channel conveying water to the hydrotechnical installations. The 26 m² veil was capable to retain about 5 million young Dreissena. The great abundance of the Dreissena population settled down on the veil allows to believe that a series of veils hung in the head race channel will protect the hydrotechnical installations against that species. The pulling out of the veil to the shore and leaving it in the open air was followed by a rapid decay and putrefaction of the mussles. The decayed Dreissena fell off the veil spontaneously.

The *Dreissena polymorpha* larvae were observed in the open channel in the experimental period from May 28 to September 11, 1973, The maximum of their density (405 indiv./l) was recorded on May 28. The larvae were in the range of 70–200 microns long. Huge amounts of larvae carried along with the water of the channel, before and after that period of time, were not able to settle down in the investigated installations.

At the end of October the 6–8 mm long individuals of Dreissena were predominant on the veil; the largest specimens measured 14–15 mm long. The veil became also a good substratum for the settling down *Cordylophora caspia* and other animals. The crayfish (*Orconectes limosus*) preying on Dreissena was present in great abundance on the veil.

6. STRESZCZENIE

Dreissena polymorpha jest małżem masowo osiedlającym się w urządzeniach hydrotechnicznych zakładów przemysłowych i częstą przyczyną ich awarii. Badano przydatność kotar z sieci jako ochrony tych urządzeń przed inwazją *D. polymorpha*. Stwierdzono masowe osiedlanie młodocianych osobników *D. polymorpha* na kotarze, zawieszonej w kanale doprowadzającym wodę, powyżej urządzeń hydrotechnicznych. Kotara o pow. 26 m² była w stanie zatrzymać około 5 milionów młodocianych małży. Masowość osiedlania się Dreissena pozwala sądzić, że szereg kotar zawieszonych przed urządzeniami hydrotechnicznvmi, będzie w stanie ochronić je przed tym małżem. Po wyciągnięciu na ląd kotary porośniętej Dreissena następował szybki, gnilny rozpad małży. W tym stanie Dreissena samorzutnie odpadała od sieci.

Larwy *Dreissena polymorpha* występowały w badanym kanale od 28.V do 11.IX.1973 r. Ich maksimum (405 osobn./l) stwierdzono 28.V. Długości larw mieściły się w granicach 70–200 mikronów. Osiedlanie się larw na kotarze nastąpiło pomiędzy 2.VII i 10.VIII. Kolosalne ilości larw, niesione przez wodę przed i po tym okresie, nie były zdolne do osiedlania się w badanych urządzeniach. U końcu października dominowały na kotarze osobniki Dreissena o długości 6–8 mm, a największe okazy posiadały dł. 14–15 mm. Kotara stała się również miejscem bytowania *Cordylophora caspia* i innych zwierząt. Licznie występował na niej rak (*Orconectes limosus*), który odżywiał się Dreissena.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	401–410	1974

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THE ROLE OF THE CRAYFISH *ORCONECTES LIMOSUS* (RAF.) IN EXTINCTION OF *DREISSENA POLYMORPHA* (PALL.) SUBSISTING ON STEELON-NET

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ABSTRACT

The object of study was the role of the crayfish *Orconectes limosus* as a natural enemy of Dreissena subsisting on network curtains protecting hydrotechnical equipment against invasion by that bivalve. Adult crayfish 90 mm long ate in average 93 to 114 individuals of Dreissena 1 to 15 mm long daily, and during the whole summer season they ate from 5580 to 6600 bivalves. The amount and size of the devoured Dreissena depended on the size of crayfish. Even the biggest crayfish could not eat Dreissena bigger than 12 mm. Lowering of temperature decreased the intensity of feeding by crayfish.

1. INTRODUCTION

Dreissena polymorpha is a bivalve which appears in mass amounts on various hydrotechnical equipment, causing disturbances in its functioning or damages. For that reason methods leading to its extinction have been sought for. One measure protecting equipment against the bivalve have been steelon networks used as barriers (Szlauer 1974). Such curtains, hanged for summer periods in a channel feeding pipelines with water, were found to be settled by huge amounts of *Dreissena polymorpha*. On a single curtain 26 m^2 of surface about 5 millions of molluscs were found. It was early observed (Szlauer 1974) that Dreissena were eaten up by crayfish (*Orconectes limosus*), which were very numerous on the curtain, too. It seemed useful to evaluate the role of crayfish in extinction of Dreissena on this peculiar substrate. As it was difficult to perform the task on the curtain, a laboratory procedure was elaborated.

2. MATERIAL AND METHODS

Dreissena and crayfish for the study were sampled from a water supply channel of the chemical works at Police near Szczecin, on the River Odra. The channel, 13 m wide and up to 3 m deep, has a rather muddy bottom without macrophyts. Curtains were hanging vertically in the channel and the animals for study were sampled from them. Crayfish were drawn from September 14 to October 24, 1973, and bivalves were sampled during the whole period of study, from September 14 to February 23, 1974. Animals were adapted for a few days to room temperature (17 to 20°C) at which most investigations were carried out. Experiments were performed in glass vessels 23 cm in diameter, filled with tap water up to 3.5 cm. One crayfish was placed in each vessel. Crayfish were given counted portions of Dreissena and a 5 cm section of *Elodea canadensis* each day during six days. Dreissena not eaten by crayfish were counted every day. The average number of Dreissena eaten up by the given crayfish per day was then calculated. Results obtained on the first day were not included into the calculations and thus the average values were based on 5 repetitions. Each series of experiments had been preceded by feeding crayfish with Dreissena to satiation. Water was exchanged in the vessel twice every day which permitted to keep oxygen concentration in water always above 4 mg O_2/l, and usually above 7 mg O_2/l. The group of crayfish used for study was approximately representative for the Orconectes population in the channel. There were males and females, as well as young crayfish 24 mm long, of undetermined sex. *D. polymorpha* used for feeding were up to 7 months old. They were mainly small bivalves, 1 to 8 mm long, such as were the most numerous on the curtain. The amounts of Dreissena individuals given to crayfish in each experiment, their sizes, as well as the sizes and amounts of the studied Orconectes are presented in Table I. In all, seven experiments were carried out.

Experiment 1. Aimed at studying the feeding of *O. limosus* when food was available in excess in form of Dreissenas not attached to their ground.

Experiment 2. Aimed at studying the feeding of *O. limosus* when Dreissena were scarce.

Experiment 3. Aimed at studying the feeding of *O. limosus* at a temperature about 10°C below room temperature.

Experiment 4. Carried out to determine the amount of Dreissena eaten by crayfish fed exclusively with small bivalves, 1 to 3 mm in length. Bivalves were available in excess.

Experiment 5. It differed from experiment 4 by that the crayfish were fed with bivalves 4 to 5 mm long.

Experiment 6. Aimed to reveal, to what extent the fact that Dreissena were attached to the stilon-net made the feeding more difficult for crayfish. The crayfish were given intermittently, at one day intervals, 2 or 3 meshes of the net grown by Dreissena and loose bivalves.

Experiment 7. Aimed at studying the availability for crayfish of individual Dreissena attached to the net. A single mesh cut from a curtain, 3 by 3 cm in size and densely grown by Dreissena, was administered to each crayfish. Amounts of Dreissena eaten by a crayfish during the first and during the second day of feeding on the mesh were counted.

3. RESULTS

Data on feeding of crayfish fed in excess with Dreissena of various sizes are shown in Fig. 1 (Table I, experiment 1). Amounts of Dreissena eaten by this group of crayfish depended on their size and sex. Males could eat markedly less bivalves, i.e. from 9 to 18 per day, than females (23 to 72 bivalves per day). Females also ate the pieces of Elodea given to them, while males did not do it. A clear relationship between the amounts of eaten Dreissena and the size of crayfish was observed in females only. Individuals of that sex measuring 34, 47, 67 and 90 mm ate 23, 42, 48 and 72 Dreissena per day respectively. The smallest studied crayfish (21 and 24 mm of length) ate only 1 to 3 molluscs per day. A clear relationship was also found out between the size of female crayfish and the length range of molluscs eaten by them (Fig. 1). The biggest females ate molluscs of all the four length classes. Smaller females ate only three classes of length and the smallest crayfish of undetermined sex ate only bivalves of the first class, from 1 to 3 mm of length. Males ate bivalves of three smaller length classes only. Observations and tests preceding the discussed experiment had proved that crayfish fed on Dreissena above 8 mm of length only very rarely or not at all. This finding is confirmed by data from Fig. 1 which show that the biggest bivalves (class IV) were practically eaten only by the biggest female, 90 mm long. From this class of molluscs she ate mainly individuals from 8 to 10 mm of length and only occasionally bigger ones (6 individuals during 5 days). This female strongly preferred bivalves of class III (6–7 mm of length).

The position of male and female crayfish fed with reduced amounts of Dreissena (32 indiv., Table I, experiment 2) was different. It can be seen from Fig. 2 that the dose was too small for females, while it covered the needs for food of males. Females fed with insufficient amounts of bivalves ate 100% of those from 1 to 7 mm of length, but they left most of those above 8 mm. The biggest of the observed females ate most bivalves of class IV. Moreover, this female, as the only one in the experi-

Table I. Plan of experiments on feeding *O. limosus* with Dreissena loose (L) or attached (A) to ground

No. of experiment	Date	Temp. (°C)	Orconectes used in experiments (No. of ind. and their body length in mm)			Dreissena given as a food (No. of indiv.)				Loose L or attached A
			Juveniles	Males	Females	1–3 mm	4–5 mm	6–7 mm	8 mm	
1	7–12.XI.1973	19–20	2 21, 24	4 30, 48, 60, 80	4 34, 47, 67, 90	40	40	40	15	L
2	1–6.X.1973	17–19	—	3 50, 60, 80	3 60, 70, 80	8	8	8	8	L
3	17–23.XII.1973	6–10	2 21, 24	4 30, 48, 60, 80	4 34, 47, 67, 90	40	40	40	13	L
4	23.XI.–3.XII.1973	16–18	2 21, 24	4 30, 48, 60, 80	4 34, 47, 67, 90	100	—	—	—	L
5	12–18.XII.1973	17–20	2 21, 24	4 30, 48, 60, 80	4 34, 47, 67, 90	—	120	—	—	L
6	14–25.II.1974	18–20	—	3 80, 85, 90	3 80, 90, 95	2–17 mm		82–137 70		A L
7	16–27.I.1974	18–20	—	3 60, 70, 80	3 65, 75, 90	2–17 mm		22–53		A

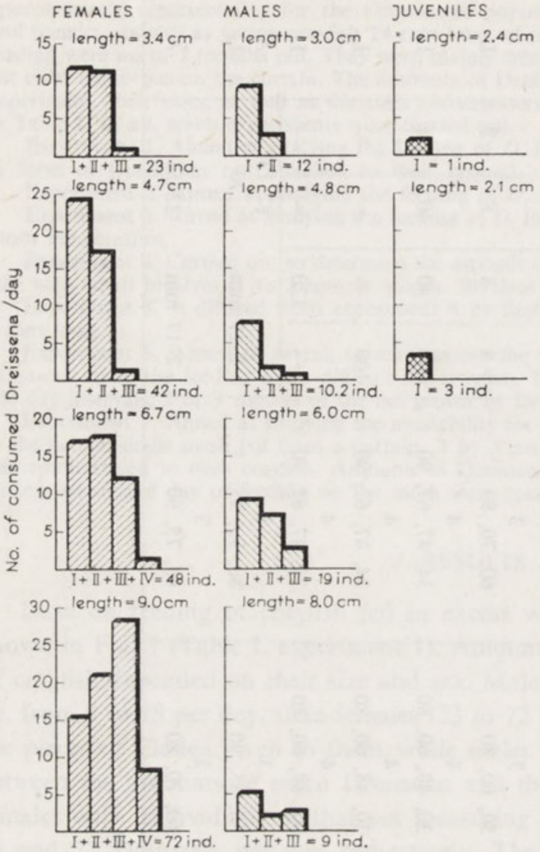

Fig. 1. Amounts of *Dreissena polymorpha* (classes of size I to IV) eaten during one day by *Orconectes limosus* of different size and sex. Dreissena supplied in excess (detailed data in Table I, experiment 1)

mental group, managed to eat two large bivalves (during 5 days), exceeding 12 mm of length. In distinction to females, male crayfish left the biggest as well as small bivalves uneaten. The smallest male (50 mm) ate almost exclusively Dreissena 1 to 3 mm long. 65 and 80 mm long males ate similar amounts of bivalves in class I, II and III and only occasionally individuals above 8 mm of length.

A lowering of water temperature (Table I, experiment 3) radically influenced the feeding behaviour of crayfish. They ate very few Dreissena in water cooled down to 6–10°C (7°C in average, Fig. 3). Females ate from 2 to 28 individuals per day and males ate much less, with the maximum of 6 bivalves per day, and sometimes they failed to feed at all. The smallest crayfish did not feed. Also in this case it was only in females that a marked relationship between the size of crayfish and the amount of bivalves eaten by them was observed. A relationship between the size of crayfish and length range of eaten bivalves was also found out. In low temperature crayfish ate small bivalves, 1 to 3 mm long.

Table II presents daily consumption by crayfish fed with small bivalves only which are most eagerly eaten by them. The experiment permitted to determine the amount of Dreissena in the size group predominating in their populations that can be eaten by crayfish. This is particularly relevant for the population of Dreis-

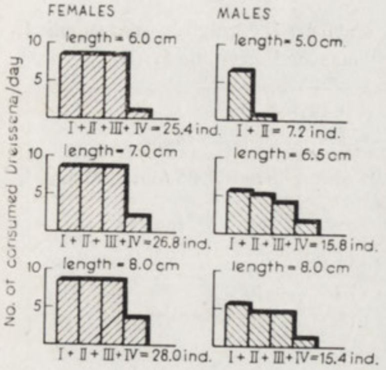

Fig. 2. Amounts of *Dreissena polymorpha* (classes of size I to IV) eaten during one day by *Orconectes limosus* of different size and sex. Dreissena supplied in insufficient amounts (detailed data in Table I, experiment 2)

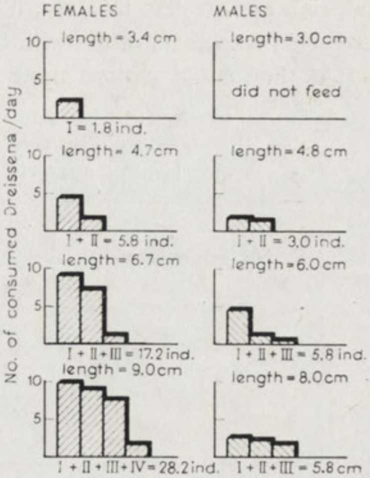

Fig. 3. Amounts of *Dreissena polymorpha* (classes of size I to IV) eaten during one day by *Orconectes limosus* of different size and sex at the temperature of 6 to 10°C. Dreissena supplied in excess (detailed data in Table I, experiment 3)

sena on curtains, consisting almost exclusively of small individuals in the summer season (Szlauer 1974). Amounts of such Dreissena eaten by crayfish depended on sex and size of latter. The biggest female (90 mm) could eat 93 bivalves 1 to 3 mm long in average per day. The same female ate 110 molluscs 4 to 5 mm long in average per day. Smaller females could eat relatively less (Table II). In general, young crayfish refused to eat bivalves of size classes I and II given to them. Remarkably, females 34 and 47 mm long ate but very small amounts of bivalves 4 to 5 mm long; the same can be said about males of similar sizes (Table II). Males could eat less bivalves of size classes I and II than females. Most Dreissena 1 to 3 mm long were eaten by a 48 mm long male (maximum 29 individuals, 24 in average per day). Most Dreissena 4 to 5 mm long were eaten by an 80 mm long male (maximum 29, average per day 22 individuals).

In natural conditions Dreissena are attached to their ground by byssus threads. Experiment 6 (Table I) was carried out to show, to what extent this fact influences the feeding of crayfish. The results are presented in Table III. The observed females feeding on loose and attached Dreissena ate similar amounts in both situations.

Table II. Amounts of Dreissena eaten by crayfish during one day, if fed with loose bivales of equal size

Dreissena	Crayfish									
	Females				Males				Juveniles	
	34 mm	47 mm	67 mm	90 mm	30 mm	48 mm	60 mm	80 mm	24 mm	21 mm
1–3 mm long	11	47	73	93	8	24	10	12	0.6	0
4–5 mm long	1	6	49	110	0.2	8	19	22	0	0

Males always ate less bivalves, if the latter were attached to ground. The crayfish were given fragments of net with large amounts of Dreissena, up to 137 individuals, so that they could choose those that were more easily available.

Table III. Amounts of Dreissena eaten daily by crayfish. The crayfish were supplied with bivalves attached to nets (A) or detached from them (B)

No. of Dreissena		Crayfish					
		Females			Males		
		80 mm	90 mm	95 mm	80 mm	85 mm	90 mm
A	supplied	93	112	92	93	103	104
	consumed	25	40	14	11	27	18
B	supplied	70	70	70	70	70	70
	consumed	37	38	13	25	57	35

Table IV presents the consumption of Dreissena by crayfish feeding on the same piece of net grown with molluscs during two consecutive days. They ate many times less Dreissena on the second day of feeding than on the first one, although many bivalves were still left on the net after the first day. The bivalves left by crayfish were mostly the biggest ones. Besides, crayfish did not eat Dreissena situated in corners of the net meshes and thus less easily accessible. Data collected in Table IV confirm the relationship, disclosed earlier, between the size of crayfish and the amount of bivalves eaten by them. In the discussed experiment (Table IV) however, no significant differences were found between the amounts of bivalves eaten by males and females.

Table IV. Amounts of Dreissena eaten by crayfish during the first two days of feeding on the same fragments of nets inhabited by the bivalve

No. of Dreissena	Crayfish					
	Females			Males		
	67 mm	75 mm	90 mm	60 mm	70 mm	80 mm
Supplied	30	41	45	30	43	53
Consumed during the first day of feeding	11	26	28	8	23	25
Consumed during the second day of feeding	4	6	7	3	4	8

The feeding of crayfish on a vertically hanged fragment of a net grown with Dreissena was also observed in an aquarium. The observed female, 90 mm long, remained on the net almost all the time and fed intensively. During 6 days it ate up 100% of Dreissena 1 to 3 mm long from a piece of net consisting of 7 meshes 2 by 3 cm large. A female of the same size ate up only 90% of Dreissena when the net was grown mainly by molluscs 4 to 5 mm long. Even after a prolonged period of feeding not all Dreissena were eaten.

It was observed that small bivalves very weakly attached to the ground, were easily torn away by crayfish by means of chelipeds of 2nd and 3rd walking legs, and then led to the mouth. Bigger, more strongly attached bivalves were grasped directly with mandibles. To do it, a crayfish made a "sit-down" movement, raised up on its legs and tore away a bivalve held in its mandibles. The bivalves were usually crunched only after they had been torn away from gound. Vain attempts to crunch Dreissena too big in proportion to crayfish were observed many times. In effect such bivalves were rejected. Observing a group of crayfish feeding on bivalves attached to a net in aquarium it was noted that the smallest crayfish, unable to crunch them by themselves, fed on remnants of Dreissena left by big crayfish.

Crayfish survived on the curtain hanged in the reservoir not only in summer, but they were found there even in January. In winter they did not eat Dreissena; their stomachs were completely empty.

The period of laboratory observations covered in its part the season of copulation of *Orconectes limosus*. Copulating crayfish were observed in September and October both in natural and laboratory conditions.

4. DISCUSSION

Results of experiments can serve as a basis for appreciation of the role of *Orconectes limosus* in the extinction of *Dreissena polymorpha* on a curtain used in summer to protect hydrotechnical equipment against this bivalve. Some explanations are necessary before we offer such appreciation. One of them refers to the feeding of male *O. limosus*. In a part of experiments they ate much less Dreissena than females, and besides they were more likely to eat smaller bivalves. We should not conclude, however, that males fed in this manner in summer season. The cause of poor feeding by males in experiments was probably their physiological state, related to the autumn copulation period of the species. This state was certainly a temporary phenomenon. This may be confirmed by results of experiments 6 and 7 (Table III and IV), carried out in January and February, i.e., after the copulation period of crayfish. It was at this time that similar or even greater amounts of Dreissena eaten by males were observed (Table III). In opposition to males, the period of copulation did not affect the feeding of females which are inseminated in autumn and lay eggs only in spring. For this reason data on feeding of males were not considered in the appreciation of the impact of feeding of 0. Limosus on the Dreissena population, but it was based on data for females only. Results of experiments seem to indicate that in summer, at the temperature about 20°C, female crayfish subsisting of the curtain grown by Dreissena have been eating mainly the smallest bivalve. The impact of crayfish on Dreissena can be defined by the numbers of bivalve eaten daily by females of different sizes (Table II). Those data can be related to the whole period of feeding by crayfish which lasted for about 60 days in case of the curtain analysed by Szlauer (1974). The settling of bivalves on the curtain (August 10, 1973) was accepted as the beginning of this period and the first days of October were recognized as its end, as the fall of water temperature limited the feeding of crayfish. After calculation it turned out that a single 90 mm long female could eat from 5580 to 6600 of small Dreissena (1 to 5 mm long) from the curtain during the summer season, and a 34 mm female could eat only 60 to 600 bivalves. It can be supposed that the contribution of males to the extinction of Dreissena was on a similar level. The contribution of the smallest crayfish, below 30 mm of length, can be described as negligible. However, it is highly probable that those crayfish ate up larvae of Dreissena at the moment of their settling on the curtain. In appreciating the role of crayfish in extinction of Dreissena on the basis of experimental data, the attachment of bivalves to the curtain as a possible limitation was not accounted for. Such approach is validated by data from Table III, indicating that this fact was not an essential obstacle in the feeding of females. Much more important factors making the feeding on the curtain difficult for crayfish were the location of bivalves in places difficult to reach, e.g. in corners of net meshes, and above all the size of bivalves. Crayfish could eat only Dreissena not longer than a certain maximum. The limit size of bivalves was strictly related to the size of crayfish. Bigger crayfish included respectively bigger molluscs to their diet. The biggest crayfish could eat bivalves up to 8 or 10 mm long. It can be assumed that individuals of *Dreissena polymorpha* above 10 mm of length were unaccessible to *O. limosus*. Even when food was to some extent scarce, crayfish did not use this group of bivalves in any significant degree (Fig. 2). As the limit size is attained by Dreissena after a few months of life, it can be said that crayfish eat mostly bivalves younger than one year. Larger specimen of Dreissena probably cannot be eaten by crayfish because of their limited ability to open up their mandibles or the limited strength of the muscles of mandibles with which they crunch the bivalves.

Factors that must be also taken into account in appreciating the extinction of Dreissena by crayfish are seasonal changes of environmental conditions, physiological rhythms, and individual characteristics of crayfish. Certainly temperature influenced the feeding of crayfish in a very important manner. It can be supposed that in low winter temperatures crayfish fail to eat Dreissena. This is caused by the fall of activity observed in crayfish at low temperatures (Dauscher, Flindt 1969, Westman 1973) The sexual rhythm can also influence the feeding of crayfish. Results of experiments seem to indicate that the poor feeding observed in males transported during the copulation period (autumn) to room temperature was brought about by their increased sexual activity. The factors described above could not significantly influence the feeding of crayfish in summer season. The crayfish were not sexually active then. It seems, however, that individual characteristics of crayfish could affect their feeding in important ways, as crayfish grow by leaps related to molting.

The described research was carried out on crayfish coming from a specific environment provided by a channel devoid of macrophyts, with very muddy bottom and very poor bottom fauna. In such a situation Dreissena growing on the curtains and concrete elements of hydrotechnical equipment were practically the only food available to crayfish. Thus it can be supposed that the population of *O. limosus* existing in the channel was specialized in feeding on Dreissena. For those reasons the results of the present study cannot be related to other environments in which Orconectes and Dreissena coexist. Crayfish living in lakes or rivers with well developed plant and animal benthos are certainly apt to feed differently, as it is indicated by data in Pieplow (1938). However, there are water reservoirs in which *O. limosus* must feed mainly on Dreissena. Among them there are some artificial channels and reservoirs, e.g. of the kind represented by the lower section of the river Odra and the Szczecin Bay. Large stretches of such reservoirs, covered with beds of Dreissena, can be settled by O. limosus feeding mainly on this bivalve. The role of crayfish in extinction of Dreissena in such environments is certainly very large.

The eating of large amounts of Dreissena by *O. limosus* can have a practical importance. In case of the stilon curtains mentioned here the role of crayfish is certainly positive. By eating Dreissena they clean the curtains and thus provide space for newly settling larvae of the bivalve. The role of Orconectes in extinction of Dreissena depends on its amount. In order to enhance it, curtains should be kept in a reservoir for a possibly long time, so as to provide a relatively steady food supply as well as living space for crayfish, the latter factor being important in waters with muddy bottom.

The feeding of crayfish can reduce Dreissena populations on various hydrotechnical equipment.

No detailed data on the devouring of Dreissena by *Orconectes limosus* have been found in literature. There is, however, a number of reports on food content of *O. limosus* in various types of reservoirs (Pieplow 1938, Sakowicz, Kompowski 1962 and others). It can be seen from the data that this crayfish is an omnivorous species, eating also Dreissena. According to Pieplow's study (1938), Dreissena is particularly frequently eaten by crayfish in the summer period. This finding can be explained by the results of the present work. In summer young Dreissena appear in mass amounts; they are readily available for crayfish on account of their small size and thus they are frequently used as food. Data on food content of related American crayfish of the genus Orconectes (*O. rusticus rusticus* Griard, *O. viridis* Hagen) are offered by Prins (1968), Momot (1967) and others. According to them, those crayfish feed mainly on plants.

A certain failing in method has been committed in this work. The study concerning the feeding of crayfish on the curtain during the summer season was carried out in autumn and winter. However, the results could be related to summer conditions, as the experiments were performed at the temperature of about 20°C. Still, it would have been better to make the study in summer.

Acknowledgments

I am greatly indebted to Prof. L. Szlauer for his help and comments during my work on this study.

5. SUMMARY

The role of the crayfish *Orconectes limosus* (Raf.) in extinction of the mollusc *Dreissena polymorpha* (Pall.) subsisting on net curtains used for protection of hydrotechnical equipment against its invasion during the summer season was appreciated by laboratory experiments carried out at the temperature of 17 to 20°C during autumn and winter. Adult crayfish 90 mm of length could eat 93 to 114 individuals of Dreissena of the size 1 to 5 mm in average per day. Smaller crayfish ate relatively less, and crayfish below 30 mm of length ate only single specimen of bivalves. The sizes of eaten Dreissena were also related to the sizes of crayfish. Dreissena 8 to 10 mm long were eaten in small amounts only by large crayfish. Molluscs exceeding 12 mm were eaten only sporadically. Lowering of water temperature to 7°C markedly limited the feeding of crayfish. The fact that Dreissena were attached to their ground hardly, if at all, impeded the process.

On the ground of laboratory findings it can be assessed that a single adult crayfish of the length of 90 mm could eat from 5580 to 6600 of small Dreissena (1 to 5 mm) from the curtain during the whole summer season. The feeding crayfish cleaned the Dreissena from the curtain and thus provided space for newly settling individuals. It can be supposed that O. *limosus* eats Dreissena in some other types of reservoirs, thereby limiting the amount of the bivalve. A similar role can also be performed by crayfish on hydrotechnical equipment grown by Dreissena.

6. STRESZCZENIE

Drogą badań laboratoryjnych próbowano ocenić rolę raka *Orconectes limosus* (Raf.) w niszczeniu małża *Dreissena polymorpha* (Pall.), zasiedlającego kotary z sieci. Kotary te służyły w okresie letnim do ochrony urządzeń hydrotechnicznych przed inwazfą Dreissena. Badania przeprowadzono w temp. 17–20°C w okresie jesienno-zimowym. Dorosłe raki o dł. 90 mm zjadały w ciągu doby średnio 93–114 osobników Dreissena o dł. 1–5 mm. Mniejsze raki zjadały odpowiednio mniej, a raki o dł. poniżej 30mm — tylko pojedyncze okazy małży. Również wymiary zjedzonych Dreissena zależały od wielkości raków. Dreissena o dł. 8–10 mm była zjadana w niewielkich ilościach tylko przez duże raki. Zjadanie przez raki małży o dł. przekraczającej 12 mm, stwierdzono tylko sporadycznie. Obniżenie pokojowej temperatury do 7°C, w wydatny sposób ograniczyło żerowanie raków. Przyrośnięcie Dreissena do podłoża tylko w niewielkim stopniu, utrudniało żerowanie rakom.

W oparciu o badania laboratoryjne można sądzić, że pojedynczy dorosły rak o dł. 90 mm był w stanie zjeść z kotary, podczas całego sezonu letniego, od 5580 do 6600 małych Dreissena (1–5 mm dł.). Żerujące raki oczyszczały kotarę z Dreissena i w ten sposób robiły miejsce dla nowoosiedlających się osobników. Można przypuszczać, że *O. limosus* również zjada Dreissena w niektórych typach zbiorników wodnych i przez to ogranicza tam liczebność małża. Podobną rolę mogą też spełniać raki w urządzeniach hydrotechnicznych, porośniętych przez Dreissena.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	411–422	1974

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DEVELOPMENT CYCLE OF *TUBIFEX TUBIFEX* MÜLL. IN EXPERIMENTAL CULTURE

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ABSTRACT

Among four differently fed groups of *Tubifex tubifex* the highest rate of growth and fertility was observed in those fed with tainted lettuce. Increasing density of population from 1500 to 18,000 inivd./m² did not influence the mortality rates, while the fertility of animals notably deteriorated. The life cycle of *T. tubifex*, observed from egg to egg, takes 50 to 57 days at 24°C. The cumulative production of cocoons by one individual during 100 days of fertility was calculated to be 4.4 mg of wet weight. The regression formula describing the relationship between the weight and length of animals is: $W = 6.42\ L^{1.76}$.

1. INTRODUCTION

The oligochaet, *Tubifex tubifex* (Müller), is an eurybiotic and cosmopolitan species. It is most common in the littoral and profundal zones of lakes and in rivers; 1420 indiv./m² (Zoloznyj 1972) and even 9000 ind./m² (Miroshnichenko 1972) were found. Its most proper environment is slowly flowing water with slushy bottom abounding in plants, but it is also frequent in different settings. Brinkhurst (1964) reports *T. tubifex* to be predominant species in mountain lakes of Alps and Tatra; Dembitskij (1972) reports to have found it in sources and streams. On the other hand, *T. tubifex* is rather common in polysaprobic waters. It is often found near the outlets of organic sewage (Brinkhurst, Kennedy 1965). It is most abundant in sewage of breweries, sugar mills and starch processing factories (Liebmann 1962).

Thus it can be seen that the species can survive in various habitats and it is tolerant to extreme conditions. It can stay alive and even lay cocoons in a wide range of temperatures (from 0 to 30°C), but its optimum is rather high, 20 to 25°C (Timm 1972).

T. tubifex plays a double role in the trophic chain. It mineralizes unassimilable bottom sediments, thereby introducing valuable mineral substances into biological circulation, and it is itself a link in the trophic chain as a very good food for fish.

Taxonomy and ecology of Tubificidae has been fairly well studied (Szczepański 1953, Poddubnaya 1959, Liebmann 1962, Chekanovskaya 1962, Brinkhurst 1964, Cook 1971). Their physiology, however, has been less elaborately investigated. Data on the time necessary for the eggs to mature in cocoons and for theii further development are insuffictent. According to Soloviev (1922; in: Chekanovskaya 1962) eggs of *T. tubifex* hatch from cocoons within 20 days under optimal conditions. After a month or a little longer the animals attain sexual maturity. It is difficult to observe the whole cycle of development in natural conditions as the youngest stages are so small and delicate that sampling fails to reflect the actual quantitative relations. Adult forms often reject the rear part of the body under stress, making it impossible to trace the relationship between the weight and length of animals through the whole cycle (Poddubnaya 1963). Such a study can only be carried out in experimental environment similar to optimal field conditions.

The aim of the present work was to study the full life cycle of *T. tubifex* in experimental culture. Optimal conditions of cultivation had to be catered for so as to obtain a large amount of material. The best kind of food and the optimal density of the population had to be found. A food was sought warranting full development, quick weight increases and high fertility of animals. The effects of four kinds of food on the rate of growth, time of development and fertility of young animals were investigated. The same four kinds of food were offered to mature animals from a natural reservoir. We known that in natural reservoirs, when food is abundant, Tubificidae live in very large groupings. On the other hand, in very dense populations of water animals the "group effect" sets in, inhibiting their growth and fertility (Styczyńska-Jurewicz 1967). The problem of density could not be neglected in the present work, too. The optimal density with lowest mortality rates and high fertility was sought.

2. MATERIAL AND METHODS

The animals used to start the culture were bought in a zoological shop. Probably they had been taken from the sedimentation tank of Warsaw waterworks. The material was sorted and then identified according to the field guide by Chekanovskaya (1962). Individuals belonging to the species *T. tubifex* (Müller) with developed clitella were selected.

In all the experiments here described the animals were kept in all-glass vessels, with a 5-cm layer of calcined sand in each (Lehmann 1941). The vessels were filled with tap water kept for at least three days in a container with activated carbon and aerated. The vessels were submerged in a water thermostat with the temperature fixed at 24°C±0.2°C. The thermostat was coated in black paper to protect the animals from light as reportedly (Ivlev 1939) harmful to them.

FOOD EXPERIMENTS

These were carried out in vessels 8×8×8 cm. Two series of experiments were made.

I. In the first series, 10 days old animals with initial weight of 0.452 mg were used, 60 individuals in each vessel.

II. In the second series, mature individuals with conspicuous clitella were investigated, 10 individuals in each vessel.

Food in excess was put into the sand layer. The kinds of food used in both series were:
vessel No. 1 — yeast formed into balls and dried up (Lehmann 1941),
vessel No. 2 — standard pressed mixture for mice,
vessel No. 3 — tainted lettuce,
vessel No. 4 — colonies of bacteria on agar. The bacteria had been cultivated from the mud of a natural reservoir,
vessel No. 5 — no food.

Every 3 or 4 days water was exchanged and fresh food was put. Sand was exchanged completely every 16 to 20 days. The animals were counted every 7 days. Sand was stirred briskly so that the worms got into water from which they were picked by a pipette. After the change of water they were put back into the vessels. It was difficult to pick the cocoons because they looked in size and colour like grains of sand. However, they were found to be much lighter than sand so that by brisk stirring they could be made to flow on top of it and within the reach of a pipette. The cocoons were counted and the eggs in each of them were then counted under a binocular microscope. The average number of eggs laid by one animal was determined. If between the readings a part of animals died, the number of those which survived plus a half of the number of those which had died was taken as the basis of calculations.

To determine the growth rate of young animals (series I) cultivated on the four kinds of food they were weighed with the accuracy up to 0.02 mg and the average weight of an individual was calculated.

POPULATION DENSITY EXPERIMENTS

The influence of population density was studied on mature animals with visible clitella. They were cultivated in all-glass vessels, 10×20×10 cm, each filled with a 5-cm layer of calcined sand on the bottom and conditioned tap water. Sand was changed every 9 days and water every 4 days. All animals were fed with tainted lettuce in excess, put into sand. The experiment lasted for 16 days. The vessels contained:
vessel No. 1 — 30 individuals, equivalent to 1500 indiv./m^2,
vessel No. 2 — 120 individuals, equivalent to 6000 indiv./m^2,
vessel No. 3 — 240 individuals, equivalent to 12,000 indiv./m^2,
vessel No. 4 — 360 individuals, equivalent to 18,000 indiv./m^2.

OPTIMAL CONDITIONS FOR THE CULTURE

The next phase of the study was a cultivation of *T. tubifex* from egg to egg. To obtain newly laid cocoons it was necessary to initiate a culture of adult animals. Experiments on effects of food and population density on physiology of the worms suggested the following conditions for the culture.

In each of 10 vessels 200 cm² of surface, filled with sand and water, were put 100 individuals with well developed clitella. They were fed in abundance with tainted lettuce. Sand was exchanged every 16 days, water was changed and fresh food was given every 3 or 4 days. At the same intervals cocoons were collected by the method described above.

The cocoons developed in separate vessels, 8 × 8 × 8 cm. As in a natural environment cocoons are laid some centimeters deep in the mud, in the present experiment they were pressed into the sand by a pipette. It was necessary because the cocoons placed in water were covered with mildew. The vessels were examined and the hatched worms were collected for the first time after 5 days. The procedure was then repeated every two days. Individuals of known age were thus obtained. They were then placed in similar vessels as described above, 100 in each, and in similar conditions. After they attained sexual maturity the cocoons laid by them were collected again.

THE METHODS OF DETERMINING THE LENGTH AND WEIGHT OF ANIMALS

Changes in the length and weight of animals were determined at 5 and then at 10 days intervals. 10 animals in the respective age group were sampled randomly, weighed and measured. They were not put into the culture again. They were weighed individually on a Sartorius balance with the accuracy up to 0.02 mg. The container for weighing had itself a weight of 7 to 8 mg. A worm was placed on a circular piece of aluminum foil laid on filter paper. The foil was punctured a few times with a surgical needle so that water was absorbed by the filter paper. After the drying, the foil was shaped into a closed container in which the animal was weighed. In this manner excessive drying of animals was avoided. The error of this method of drying was estimated; the coefficient of variation was found to be 18.43%.

Measuring of the length of live worms was impossible because of their agility. Common fixatives like formalin or ethyl alcohol caused permanent coiling and shortening of the worms, preventing exact measurements of their length. In effect, the animals were treated with ethyl ether. The narcosis took place in a tightly closed jar. The worms so treated retained elasticity of their bodies while becoming immobile. They were measured on graph paper up to 0.1 mm by means of a magnifying glass. As the measurements could not be continued on the same individuals, worms from identical conditions were sampled for this purpose. The initial density of cultures was about 100 of newly hatched animals. During the experiment the losses were not made up for. The mortality rates in vessels with animals of the same age were similar. Each age group is characterized by results from two cultures started at different moments. No differences between animals cultivated in different vessels were noted.

3. RESULTS

PRODUCTION AND FERTILITY OF *T. TUBIFEX* AS DEPENDENT ON THE TYPE OF FOOD

Figure 1 presents the cumulative production of body weight of animals kept on various kinds of food from their 10th day of life (series I). Individuals fed with tainted lettuce had the greatest wet weight. Their increase of wet weight cumulated for 100 days of cultivation was 4468 mg. The worms fed with the standard mice mixture were gaining weight most slowly and their cumulative increase of wet weight for 100 days was 0.388 mg. The starved animals were loosing weight and after 56 days they were all dead. During that time the animals in all food groups except the mice mixture group (curve 2 in Fig. 1) have attained sexual maturity and started to lay egg cocoons. In Fig. 2 (series I) is presented the number of cocoons and eggs laid by one individual between the 45th and 59th day of life. The animals fed with tainted lettuce have laid more cocoons than others. Animals fed with yeast and bacteria have laid less cocoons and those fed with the mice mixture have laid none at all by that time. In the second experimental series (Fig. 3) the average number of cocoons and eggs laid by a single worm during 14 days of observation was also highest for the group fed with lettuce. Tainted lettuce has thus proved to be the best food (Fig. 1, 2, 3) and it has been used exclusively in the further experiments.

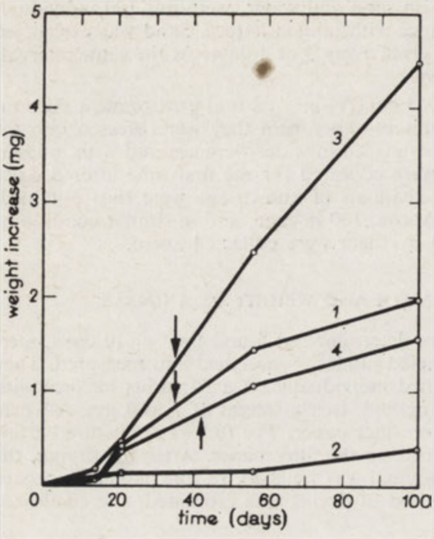

Fig. 1. Increase of wet weight of young animals (series I) fed with: 1 — yeast; 2 — standard food mixture for mice; 3 — tainted lettuce; 4 — bacteria growing on agar. Vertical arrows mark the begining of laying cocoons

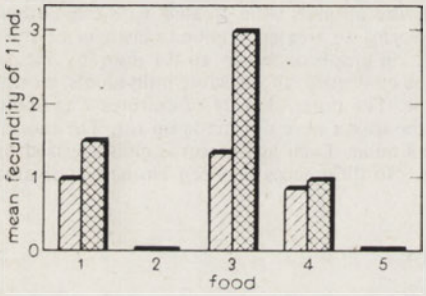

Fig. 2. Average number of cocoons (hatched bars) and eggs (chequered bars) laid by animals between 45th and 59th day of life (series I)-kept of different foods: 1 — yeast; 2 — standard mixture for mice; 3 — tainted lettuce; 4 — bacteria on agar; 5 — starved animals

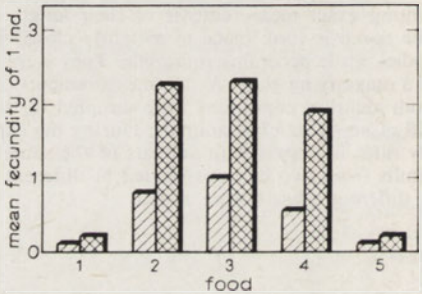

Fig. 3. Average number of cocoons (hatched bars) and eggs (chequered bars) laid by animals during 14 days (series II); animals with developed clitella, fed with different foods. Food groups as in Fig. 2

Survival of animals fed with tainted lettuce and starved has been observed for 135 days (series I; Fig. 4). 11.6% of lettuce fed worms have survived till that date. They have laid 660 eggs in 159 cocoons during that time, with the average of 13.9 cocoons with 61 eggs per individual. The average number of eggs per cocoon was 4.15. Wet weight of cocoons produced by a single individual was 4.408 mg (Fig. 5).

There is a known relationship between the weight of a cocoon and the number of eggs which helped to calculate the mass of the laid cocoons. The relevant formula is $W=aN^b$, where W stands for wet weight of one cocoon (mg); N represents the number of eggs in a cocoon; a and b are coefficients. For 2265 cocoons the formula reads: $W=0.1236N^{0.5556}$; the logarithmic formula accounting for the 95% confidence interval for a and b reads:

$$\log W=(\bar{1}.0921\pm0.0056)+(0.5556\pm0.0171)\log N.$$

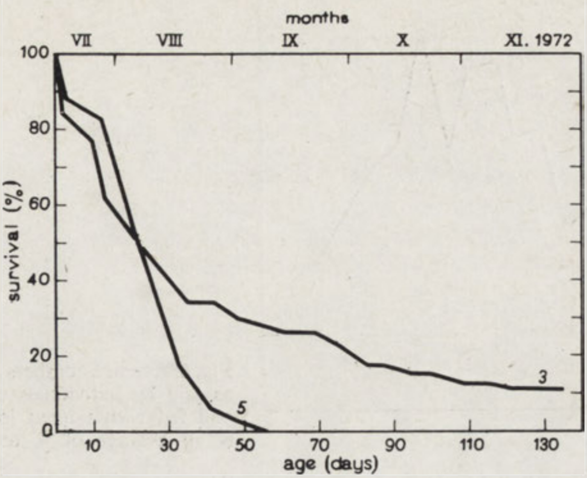

Fig. 4. Survival of young animals (series I) fed with: 3 — tainted lettuce; 5 — starved

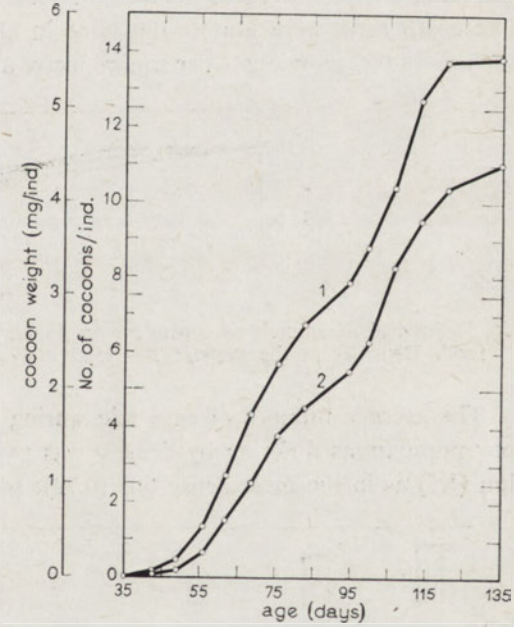

Fig. 5. Cumulative production of cocoons and eggs. 1 — number of cocoons; 2 — cumulative wet weight of cocoons. Temp. 24°C; tainted lettuce as food

It should be noted that the 135th day of experiment was the last day of observation and not the last day of cocoon laying by the worms. The average number of eggs in a cocoon is represented in Fig. 6; it varies from 2 to 6. Out of 100 days of cocoon laying during as many as 50 days (from 82nd to 132nd day) this number was greater than four.

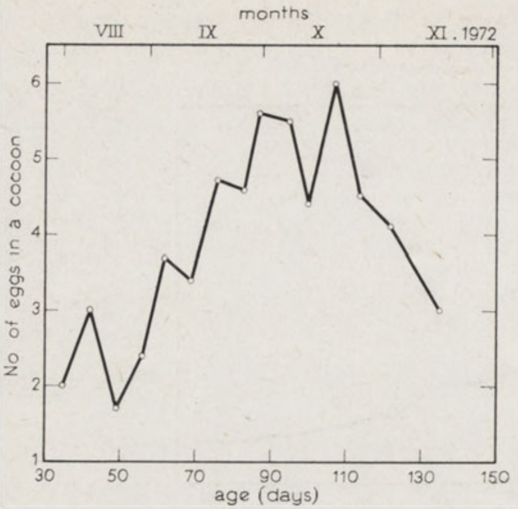

Fig. 6. Average numbers of eggs in a cocoon as laid by individuals cultivated at 24°C and fed with tainted lettuce. Each point is an average of 4 to 24 observations

SURVIVAL AND FERTILITY OF ANIMALS IN POPULATIONS OF DIFFERENT DENSITIES

In those experiments the animals were fed with tainted lettuce; only mature individuals were observed. Survival in such conditions was observed for 16 days. The death rates were almost the same in all the populations with densities from 1500 to 18,000 individuals per square metre and rather low, i.e. about 15% (Fig. 7).

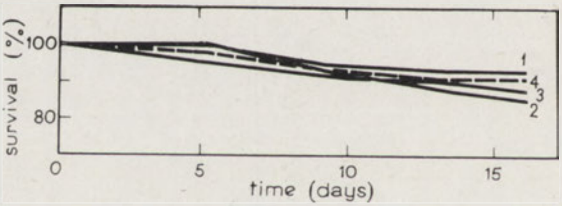

Fig. 7. Survival of animals as dependent on population density. Temp. 24°C, tainted lettuce as food. Densities in the vessels: 1 — 1500 indiv./m²; 2 — 6000; 3 — 12,000; 4 — 18,000

The average number of eggs laid during 16 days by a single individual in the four populations differing by density was twice as high in the least dense population (1.5) as in the most dense one (0.72); see Fig. 8.

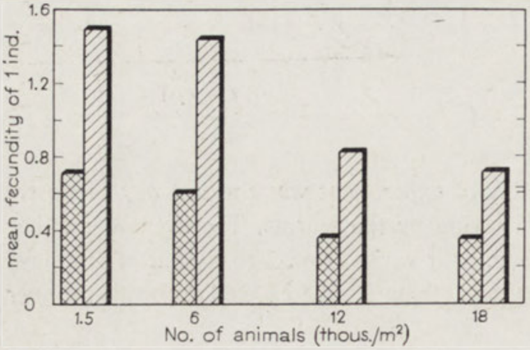

Fig. 8. Fertility of animals as dependent on population density. Temp. 24°C, tainted lettuce as food. Chequered bars — cocoons; hatched bars — eggs

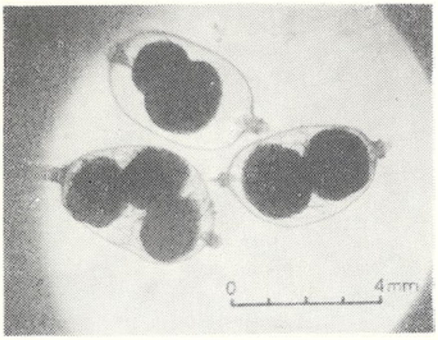

Fig. 9. Newly laid cocoons of *T. tubifex*. Not yet dividing eggs can be seen through the tunicle. The cocoon is closed by "plugs"

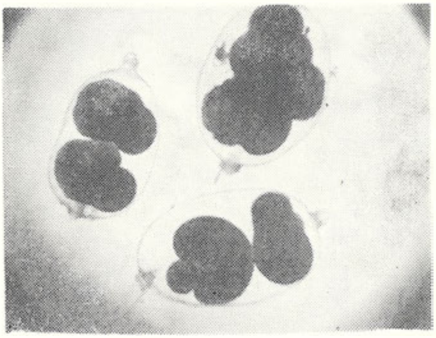

Fig. 10. Cocoons of *T. tubifex* during the first divisions

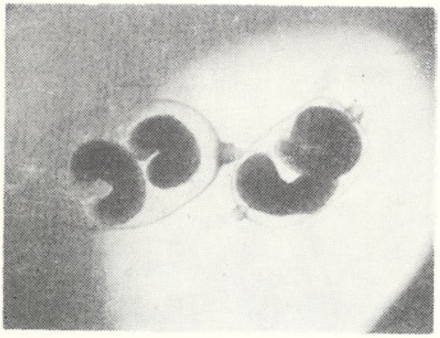

Fig. 11. Cocoon of *T. tubifex*; the falciform stage

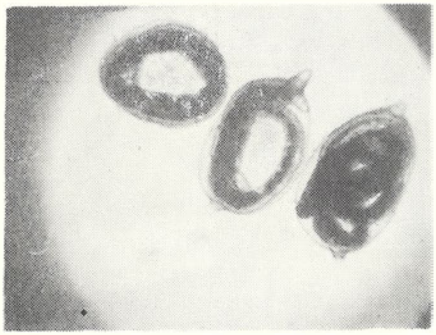

Fig. 12. Cocoons of *T. tubifex* with smoving animals inside

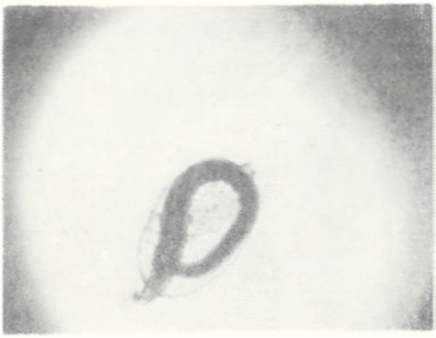

Fig. 13. A young individual vileans out the front part of its body from the cocoon

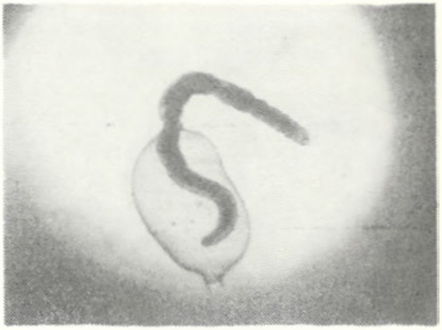

Fig. 14. A young *T. tubifex* leaving the cocoon through the hole that had been pugged

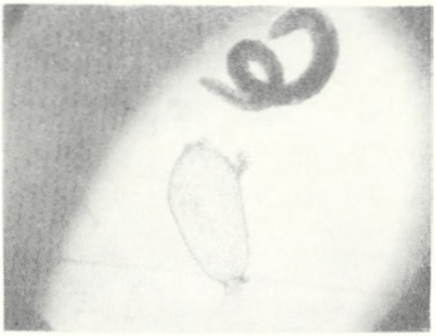

Fig. 15. A young *T. tubifex* beside the abandoned cocoon

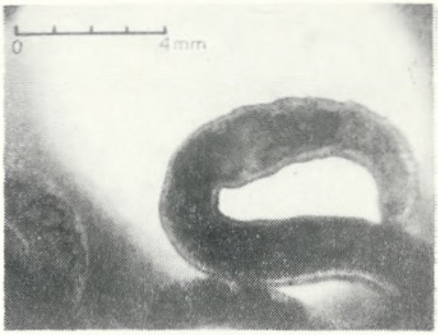

Fig. 16. An adult *T. Tubifex* (Müller)

THE LIFE CYCLE OF *T. TUBIFEX* IN EXPERIMENTAL CULTURE

The cocoons of *T. tubifex* are similar to those of other Tubificidae. The outer tunicle is an oval membrane, with elongated ends closed by the characteristic "plugs" (Fig. 9). It is about 1 mm long and 0.7 mm broad. Inside it the eggs are floating in perivitelline fluid. Figure 10 presents the stages of morula. The spherical embryo begins to stretch out and the first three segments of the body are formed. The embryo is falciform in shape (Fig. 11). Later on the other segments of the body develop. After a few days there is a fully developed worm in the cocoon; it remains there for some time (Fig. 12). It gets out through the elongated end. The "plug" is probably enzymatically dissolved. The animal can lean out from its cocoon partially (Fig. 13). Figure 14 shows a young *T. tubifex* leaving the cocoon; in Fig. 15 it starts its independent life.

At 24°C, the full embryonal development, from an egg being laid until the young animal leaves it, takes 10 to 12 days. The whole cycle of development lasts for 40 to 45 days from the hatching until the mature animal layes its own first cocoons. Figure 16 portraits an adult individual.

CHANGES OF BODY LENGTH AND WEIGHT IN TIME

From the curve illustrating the changes in body length and weight during 70 days (Fig. 17) it can be seen that *T. tubifex* grows intensively until sexual maturity and then its length remains constant.

Figure 18 illustrates the rate of the growth of weight of *T. tubifex*. Until sexual maturity the worms assume weight rather rapidly; from the first laying of cocoons there can be observed a slight decrease of their weight. The losses are related to the egg production.

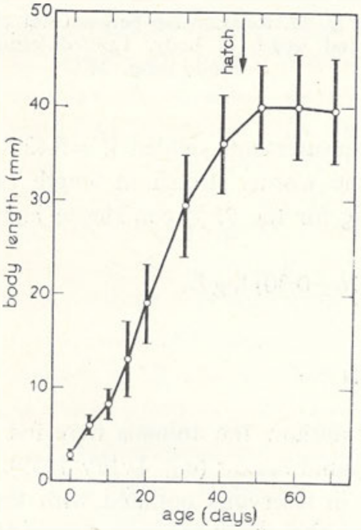

Fig. 17. Average length of animals. Tainted lettuce as food; temp. 24°C. Vertical lines for standard deviation. Vertical arrow marks the beginning of laying cocoons

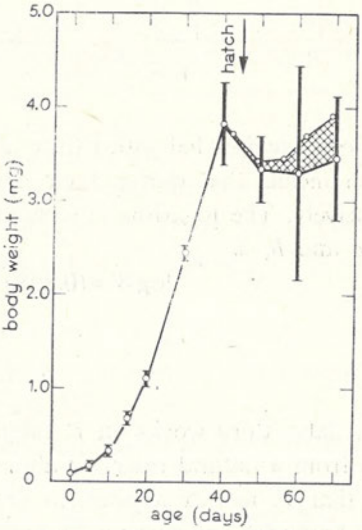

Fig. 18. Average wet weight of animals. Tainted lettuce as food; temp. 24°C. Vertical lines for standard deviations. The hatched area denotes cocoons production. Vertical arrow marks the beginning of laying cocoons

RELATIONSHIP BETWEEN LENGTH AND WEIGHT OF *T. TUBIFEX* UNDER EXPERIMENTAL CONDITIONS

The relationship between the length (L, mm) and wet weight (W, μg) of *T. Tubifex* is presented in Fig. 19. It can be described by the regresion formula

$$W = aL^b$$

where a stands for the weight of an individual 1 mm long and b is the regression coefficient.

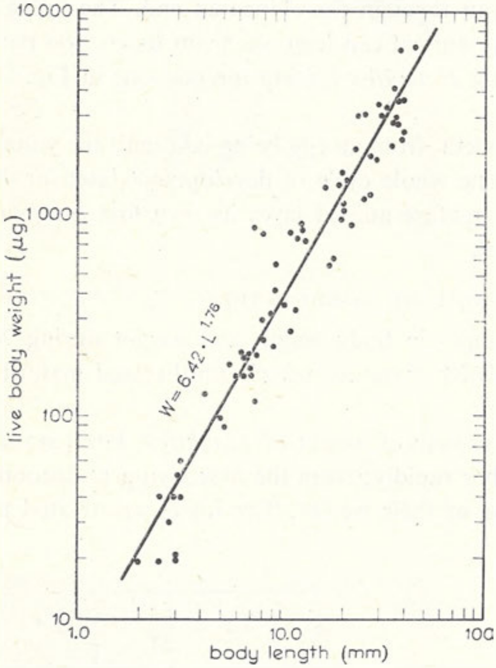

Fig. 19. Relationship between wet weight and length of body. Tainted lettuce as food; temp. 24°C

For *T. tubifex* fed with lettuce at 24°C, 70 measurements yielded $W = 6.42\,L^{1\cdot 76}$, which means that during their development the worms stretch in length rather intensively. The logarithmic formula, accounting for the 95% confidence intervals for a and b, is

$$\log W = (0.8980 \pm 0.25) + (1.76 \pm 0.30) \log L.$$

4. DISCUSSION

In laboratory works on *T. tubifex* by other authors the animals were fed with mud from a natural reservoir abounding in organic matter (e.g. Ivlev 1939). The fact that *T. tubifex* appears in great numbers in reservoirs polluted with sewage from breweries and sugar mills suggested an idea to cultivate them on yeast (Lehmann 1941). Poddubnaya (1958) kept *T. ablicola* in vessels filled with mud mixed with yeast and dried *Elodea canadensis*. Birnkhurst, Kennedy (1965) cultivated Tubificidae in jars with a layer of sand, some autumn leaves of elm, a layer of sterilized mud, a small amount of the green alga *Cladophora glomerata* and a few *Daphnia obtusa*. Kajak (1965) tried to enrich the bottom sediments of a natural Lake Lisunie with boiled mashed potatoes.

In the present work four types of food were tried. Their common characteristic was that in water they provided favourable conditions for the development and increase of bacterial flora. Observations of weight increase and fertility of animals fed with the four kinds of food proved that tainted lettuce was the best of them (Fig. 1). Indeed, very rich bacterial flora develops on tainted lettuce and it also contains valuable substances which can be easily assimilated by animals. *T. tubifex* probably needs much of easily assimilable iron, necessary, e.g., for haemoglobin formation. Iron content in *T. tubifex* is 228.76 mg% (Malikova 1971). In a closely related species *Enchytraus albidus* iron content is the scant 25.22 mg%. Lettuce is rich in iron as it contains 0.5 mg% of it. It also contains fairly much of vitamin A (1.1 mg% — Boruchowski 1966). In *T. tubifex* body vitamin A was found in the amount of 0.22 mg% and vitamins B_1 and B_2 from 0.03 to 0.08 mg% (Malikova 1971).

The quality of lettuce as a food is also confirmed by the high fertility of experimental animals. The average for a single individual during 100 days of observation was 61 eggs. This is a high value, as in a natural reservoir in optimal food and temperature conditions (without the cool break) one animal can lay from 100 to 200 eggs during the whole year (Timm 1972). In the present work the animals were laying eggs under experimental conditions irrespective of the season. Their fertility did not decrease in late autumn, though in field conditions they lay cocoons in July and August only (Poddubnaya 1959, Liebmann 1962). Poddubnaya (1971) described a cyclical, season related resorption and regeneration of the sexual apparatus in Tubificidae living in natural conditions. She also observed (1958) the fertility of another Tubificidae, *T. albicola*, in laboratory conditions. Cocoons were laid during 4 to 7 days at 19°C. The average for one animal was 1.2 cocoons with 7 to 10 eggs in each. In another case (a culture with *Elodea canadensis* and yeast but without mud) the average per animal was one cocoon with 1 to 4 eggs. In my culture with tainted lettuce as food the average number of eggs in one cocoon was 4.15 (Fig. 6). According to Poddubnaya (1973), the average number of eggs in one cocoon in natural conditions is ten. The greatest observed number in my culture was eight eggs in a cocoon.

Embryo development of *T. tubifex* eggs at 24°C takes 10 to 12 days. In natural conditions the time required for the cocoon development is similar and, according to Poddubnaya (1973) it is 240 days-degrees.

It was observed that a large per cent of cocoons placed in water or on the surface of sand failed to develop. They were opaque and looked like attacked by fungi or some other parasites. Cocoons placed inside the sand layer were clean on surface and wholly transparent. In light protected vessels algae did not develop; in those not protected from light I observed cocoons overgrown with algae. Those matters must be taken care of in order to have good results of cultivation.

In natural reservoirs Tubificidae appear in great amounts. Their densities in rivers and lakes are estimated to be several thousands per square meter. In reservoirs with sewage inlets their order of density attains hundreds of thousands and even near a million per square meter. The maximum number of animals in the experiments was 18,000 per square meter. In such conditions the death rate did not increase, but the fertility was observed to decrease. The inhibiting influence of population density was only partial. Frequent exchange of water is suggested to prevent negative effects of too dense population (Styczyńska-Jurewicz 1967). I noticed that in case of *T. tubifex* it was important for a good development of the culture to change completely not only water but also the whole supply of sand from time to time.

After attaining sexual maturity (the moment of laying the first cocoons) *T. tubifex* hardly grows in length any more. The average body length during 70 days, or 30 days after the beginning of propagation, remains almost unchanged. According to Poddubnaya (1971), *L. newaenensis* continues to grow after attaining sexual maturity, but less intensely than before.

The weight of *T. tubifex* decreased a little after the animals began to lay cocoons. It can be accounted for by losses caused by cocoon laying.

The coefficient *b* calculated from the formula describing the relationship between weight and length of body characterizes the type of growth of the studied organisms. For most animals the weight increases almost as the cube of their length, the coefficient *b* approaching 3. E.g., for *Tribolium castaneum* (Klekowski et al. 1967) it is 2.88. Similarly, for *Asellus aquaticus* (Prus 1972) the regression coefficient *b* is almost 3. The relationship of weight and length for *T. tubifex* can be described by the formula $W=6.42\,L^{1.76}$. Such a low coefficient *b* indicates very intensive increase of length. A similarly low coefficient *b* 2.10 is reported for *Stylaria lacustris* (Naididae, Oligochaeta) cultivated in laboratory (Kamlyuk, Koval'chuk 1972); this result was calculated from 17 measurements. The type of growth even more similar to that of *T. tubifex* was found by Poddubnaya (1972) in *Isochaetides newaenensis* (Tubificidae, Oligochaeta). Their coefficient *b* was 1.8. The animals were sampled from a natural reservoir; 700 measurements were made.

5. SUMMARY

A number of experiments was carried out to determine the optimal conditions of cultivation, possibly similar to those in natural reservoirs. Four kinds of food were tried out; the most rapid growth and highest fertility were observed in animals fed with tainted lettuce. The cumulative increase of body weight of an individual fed with lettuce was 4.468 mg during 100 days. From the studied densities, 5000 individuals per square meter was selected, as the fertility in such population was still high.

In the experimental culture the full development cycle of *T. tubifex* could be observed. Embryonal development of eggs from laying to hatching was 10 to 12 days at 24°C. The whole cycle from egg to egg took from 50 to 57 days.

The animals were cultivated for 135 days; the period of fertility was 100 days. During that time the average per an individual was 13.9 cocoons with 61 eggs in it (with due account for the death rate). There was thus 4.15 eggs in average in one cocoon. Actually the cocoons contained from 1 to 8 eggs. The weight of cocoons laid by an individual during 100 days was 4.4 mg.

Curves of growth of animals during 70 days were drawn. The cultivated animals weighed about 4 mg at the moment of their first cocoon laying; their length was about 40 mm. The relationship between length and weight of animals cultivated at 24°C was found to be $W=6.42\,L^{1.76}$.

6. STRESZCZENIE

Przeprowadzono szereg doświadczeń mających na celu określenie optymalnych warunków hodowli zbliżonych do warunków w naturalnydh zbiornikach. Okazało się, że z czterech proponowanych pokarmów najszybsze tempo wzrostu, najwyższą płodność wykazują zwierzęta karmione nadgniłą sałatą. Skumulowane przyrosty ciała jednego osobnika hodowanego na tym pokarmie w ciągu 100 dni wynoszą 4,468 mg. Z badanych zagęszczeń wybrano zagęszczenie 5000 osobników na metr kwadratowy, przy którym płodność zwierząt była jeszcze wysoka.

W warunkach hodowli doświadczalnej uzyskano pełny cykl rozwojowy *T. tubifex*. Rozwój embrionalny jaj od momentu złożenia do wyjścia młodego organizmu w temp. 24°C wynosi 10 do 12 dni. Cały cykl od jaja do jaja zamyka się w ciągu 50–57 dni.

Zwierzęta hodowano 135 dni, z czego na okres płodności przypada 100 dni. W tym czasie (po uwzględnieniu śmiertelności) średnio na jedno zwierzę przypadło 13,9 kokona, a w nich 61 jaj. Czyli średnio w jednym kokonie było 4,15 jaja. W poszczególnych kokonach znajdywano od 1 do 8 jaj. Skumulowana masa kokonów złożonych przez jednego osobnika w czasie 100 dni wynosiła 4,408 mg.

Wykreślono krzywe wzrostu zwierząt za okres 70 dni. Zwierzęta hodowlane w momencie składania pierwszych kokonów osiągają wagę około 4 mg, a długość ciała waha się w granicach 40 mm. Zbadano zależność długości od ciężaru ciała zwierząt hodowanych w temp. 24°C. Zależność tę opisuje wzór: $W = 6{,}42\ L^{1.76}$.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	423–429	1974

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STANDARD, ROUTINE, AND ACTIVE METABOLISM OF THE ANTARCTIC AMPHIPOD — *PARAMOERA WALKERI* STEBBING

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ABSTRACT

The marine amphipod *Paramoera walkeri* routine and active metabolism, at the temperature of -1.9°C, is 1.36 and 2.57μl O_2/indiv.·hr, respectively. The standard, routine, and active metabolism, at the temperature of 2.0°C, is 1.83, 2.61 and 3.02 μl O_2/indiv.·hr, respectively. The Q_{10} coefficient, in the range of temperatures from -1.9 up to 2.0°C, is 5.4 for the *Paramoera walkeri* routine metabolism and 1.5 for its active metabolism. Together with an increase in the motor activity of the animals there was noted a concomitant increase in the quantity of the consumed oxygen.

1. INTRODUCTION

Metabolism in animals is dependent in great measure upon their actual motor activity (Fry 1957, Ivlev 1963, Smit 1965). The motor activity in poikilothermic animals is conditioned by a series of various factors, such as hunger (Marsden et al. 1973, Bayne 1973, Newell 1973), environmental temperature (Halcrow, Boyd 1967, Percy 1972, 1973) and others.

Three types of the metabolism in animals are distinguished in relation with their motor activity and its intensity, i.e. standard metabolism, routine metabolism and active metabolism. Standard metabolism concerns the metabolism in animals which do not show any other motor activities except respiratory movements (Winberg 1950, Newell, Bayne 1973). Routine metabolism refers to the metabolism in animals showing spontaneous motor movements typical for them under natural conditions (Smit 1965, Fry 1957). Active metabolism defines the metabolism in animals moving actively but not being stimulated artificially to an increased motor activity (Halcrow, Boyd 1967, Newell, Bayne 1973).

The same animals, at each of these three metabolic levels, are reacting in a different manner to some exterior factors such e.g. environmental temperature (Newell, Pye 1971, Widdows 1973).

The aim of the present study is to determine the standard, routine, and active metabolism in the Antarctic marine amphipod *Paramoera walkeri* Stebbing (Pontogeneidea). This is a circumpolar species inhabiting the coastal zone of the Antarctic seas.

2. MATERIAL AND METHODS

Studies on the dependence of the metabolism on the activity of the Antarctic marine amphipod *Paramoera walkeri* Stebbing were carried out by the First Polish scientific Group in Antarctica working within the Project of the XIV Soviet Antarctic Expedition, 1968–1970, in the Soviet Antarctic station Molodezhnaya (67°40′S, 45°50′E, Enderby Land, Eastern Antarctica).

Paramoera walkeri is a dominant component of the sub-fast-ice communities of the fauna living on the lower surface of the marine fast-ice in the coastal zones of the Antarctic seas (cf. Bunt 1966, Andriashev 1967). It has been stated on the basis of the observations of *P. walkeri* behaviour that this animal under normal conditions is creeping on the underneath surface of the ice and is rarely seen swimming in the depths of water.

The investigations were carried out in October, 1969, during the Antarctic winter. By then the ice-cover was ca, 1.5 m and water temperature under the ice was −1.8°C, mean salinity was 34°/₀₀.

Measurements were performed on the mature *P. walkeri* females of live weight ranging from 32 to 70 mg, on average 43.7 mg. The results from the measurements of oxygen consumption were calculated in terms of one individual of average weight.

The oxygen consumption was measured in the constant-pressure volumetric respirometers modified by Klekowski (1968). Respiration chambers of 35 ml capacity were used. Two animals were placed into 10 ml of sea water taken from the sampling place. Reading of oxygen consumption were recorded every 10 minutes during the experiment lasting 3 hours. The experiments were made at the temperature of −1.9°C and 2.0°C. The temperature in the thermostat was kept with an accuracy of 0.05°C. The collected animals were acclimated for 24 hrs to the thermic conditions of the experiment. During the period of acclimation and the duration of experiment no food was administered to animals.

Measurements of the routine metabolism in *P. walkeri* were made in the following way. A small flock of cotton wool was put into the respiratory chamber so as to make a substratum similar to that of the natural rough lower surface of the underside of the ice-cover. This made possible the creeping movement of the animals just as on the substratum under normal conditions. Fifteen measurements of this type has been carried out.

For the measurements of the active metabolism the animals were placed in the respiratory chambers without using the cotton wool flocks. This forced the animals to swim continuously since *P. walkeri* are not able to move or even stay in place on the smooth surface of the glass-walls of the respiratory chamber.

During those experiments the animals were not stimulated artificially to an intensified locomotor activity. There were observed short intervals when the animals fell down to the bottom of the chamber. Ten measurements of this type has been carried out.

The second type of the experiments consisted in measurements of oxygen consumption with simultaneous recording of the locomotor activity of the animals. The locomotor activity of the animals was determined by means of a four-grade discontinuous scale: grade 1 — a motionless animal on the bottom of the chamber (corresponds to the standard metabolism in the animal); grade 2 — the animal stays put or moves slightly on the flock of cotton wool; grade 3 — the animal creeps on the flock of cotton wool or swims up from the bottom of the vessel onto the cotton wool; grade 4 — the animal swims round the walls of the chamber, this corresponds to the active metabolism in the animal. Seventy five measurements of this type has been carried out.

The results from all measurements of *P. walkeri* respiratory metabolism are given in µl O_2/indiv.·hr of an average animal with live weight of 43.7 mg.

3. RESULTS

On the basis of measurements made at the temperature of −1.9°C the routine metabolism in *P. walkeri* females of average weight of 43.7 mg was 1.36 µl O_2/indiv.·hr whereas their active metabolism measured at the same temperature was 89% higher, 2.57 µl O_2/indiv.·hr.

On the basis of measurements of oxygen consumption with the simultaneous recording of the motor activity of the animals it has been calculated that at the temperature of 2.0°C the standard metabolism of *P. walkeri* females of average weight of 43.7 mg is 1.83 µl O_2/indiv.·hr, the routine metabolism 2.61 µl O_2/indiv.·hr (the mean value for the second and third grade of their locomotor activity) and the active metabolism 3.02 µl O_2/indiv.·hr (Fig. 1).

As results from the measurements of the active and routine metabolism at the two given temperatures the *P. walkeri* routine metabolism is dependent upon the temperature to a much higher degree than its active metabolism (Fig. 2). This fact is illustrated more distinctly by the value of the Q_{10} coefficient in the range of temperatures from −1.9 to 2.0°C. The Q_{10} coefficient for the routine metabolism is 5.4 and for the active metabolism it is 1.5.

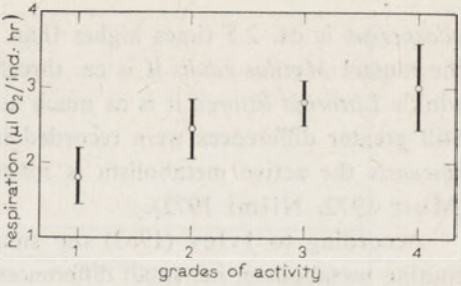

Fig. 1. Dependence of respiration on locomotor activity of the mature females of *Paramoera walkeri* at 2.0°C. Vertical lines indicate 95% confidence limits. For explanation of the scale of activity see Methods

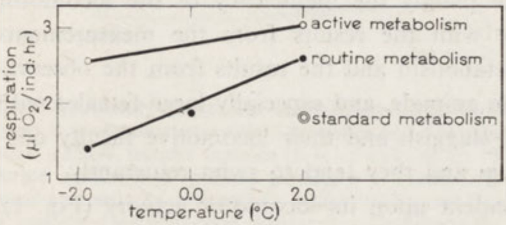

Fig. 2. Respiratory metabolism of mature females of *Paramoera walkeri* at −1.9°C and 2.0°C (data for 0.0°C from Klekowski et al. 1973)

The increase in the locomotor activity of the animals is followed by an increase in the quantity of the consumed oxygen (Fig. 1). However, due to the discontinuous character of the accepted scale for the locomotor activity of the animals it is not possible to determine whether this dependency is of a straight line or an exponential character.

5. DISCUSSION

The results from the experiments have shown significant differences between the standard, routine, and active metabolism in the *Paramoera walkeri* females. The relative differences obtained for the *P. walkeri* standard, routine, and active metabolism approximate the analogical values obtained for other Amphipoda, e.g. *Gammarus lacustris* (Winberg 1950) and *Gammarus oceanicus* (Halcrow, Boyd 1967); see Fig. 3.

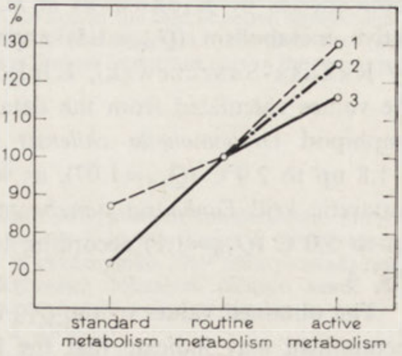

Fig. 3. Comparison of standard, routine and active metabolisms in some amphipod species: 1 — *Gammarus lacustris* at 15°C (Winberg 1950), 2 — *Gammarus oceanicus* at 5°C (Halcrow, Boyd 1967), 3 — mature females of *Paramoera walkeri* at 2.0°C (present paper). Routine metabolism was assumed as 100%

The differences between the standard, routine, and active metabolism values in *P. walkeri* and other amphipods are small in comparison with the differences observed in other invertebrates, e.g. the active metabolism in the shrimp *Leander* *adaspersus* is ca. 2.5 times higher than the standard metabolism (Ivlev 1963); in the mussel *Mytilus edulis* it is ca. threefold higher (Widdows 1973); while in the winkle *Littorina littorea* it is as much as fivefold higher (Newell 1971, Pye 1971). Still greater differences were recorded in vertebrates, e.g. in the fish *Kuhlia sandvincensis* the active metabolism is ninefold higher than the standard metabolism (Muir 1972, Niimi 1972).

According to Ivlev (1963) the small differences between the active and the routine metabolism, i.e. small differences in the metabolism values at varying locomotor activity of the animals, indicate plainly the inefficiency of the locomotor system. This conclusion is compatible with the results from the measurements of the standard, routine, and active metabolism and the results from the observations of the *P. walkeri* behaviour. These animals, and especially large females with eggs in their marsupium, are relatively sluggish and their locomotive faculty consists mainly in creeping on a substratum and they tend to swim reluctantly.

The *P. walkeri* metabolism is dependent upon its locomotor activity (Fig. 1). Two types of the dependence of the metabolism upon the locomotor activity of animals are described in the literature: the straight line dependence observed in e.g. the amphipod *Gammarus oceanicus* (Halcrow, Boyd 1967), the mussel *Mytilus edulis* (Thompson, Bayne 1972) and the fish *Kuhlia sandvincensis* Muir, Niimi 1971) and the exponential dependence observed in the shrimp *Leander adaspersus* (Ivlev 1963) and in the mussel *Mytilus edulis* (Bayne et al. 1973).

The effect of the temperature on the active and the routine metabolism in *P. walkeri* varies in its intensity (Fig. 2). Changes in the external temperature have a much greater effect on the routine metabolism than on the active metabolism. Halcrow, Boyd (1967), Widdows (1973), Newell, Northcroft (1967), and Newell (1966) likewise have stated that the change in temperature has a greater effect on the routine metabolism than on the standard and active metabolism.

The obtained value of the Q_{10} coefficient, amounting to 5.4 for the *P. walkeri* routine metabolism, approximates the Q_{10} values, $Q_{10}=6.0$, obtained for the same species by Klekowski et al. (1973). Whereas, the value of the *P. walkeri* active metabolism ($Q_{10}=1.5$) approximates the values obtained for *P. walkeri* by Rakusa-Suszczewski, Klekowski (1973) $Q_{10}=2.05$. It approximates also the values calculated from the data given by Armitage (1962) for the Antarctic amphipod *Orchomonella chilensis* measured at the temperatures ranging from -1.8 up to 2.0°C ($Q_{10}=1.07$), as well as the values of the Q_{10} coefficient for the Antarctic krill *Euphausia superba* measured at the temperatures ranging from 0.0 up to 5.0°C ($Q_{10}=1.1$) according to the data given by McWhinnie (1964). See Fig. 4.

The obtained values of the Q_{10} coefficient for the *P. walkeri* routine and active metabolism may indicate that the low values of the Q_{10} coefficient obtained for animals living at low temperatures may express not only the adaptive feature of those animals to low temperatures (Rao, Bullock 1954) but may also result from the changes in the locomotor activity of those animals due to the changes in the temperature (McWhinnie 1964).

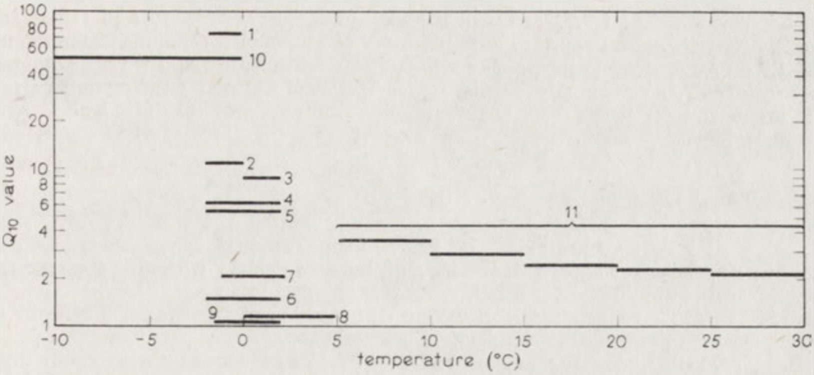

Fig. 4. Q_{10} values in the low temperature range (horizontal lines indicate the studied temperature range). 1 — *Paramoera walkeri*, juveniles and adolescens (Klekowski et al. 1973); 2, 3, 4, — *Paramoera walkeri*, mature females (Klekowski et al. 1973); 5 — *Paramoera walkeri*, mature females, routine metabolism (present paper); 6 — *Paramoera walkeri*, mature females, active metabolism (present paper); 7 — *Paramoera walkeri* (Rakusa-Suszczewski, Klekowski 1973); 8 — *Euphausia superba* (McWhinnie 1964); 9 — *Orchomonella chilensis* (Armitage 1962); 10 — *Littorina littorea* (Kanwisher 1959) and Chironomidae larvae (Scholander et al. 1953); 11 — "Krogh's normal curve" (according to Winberg 1956, 1968)

5. SUMMARY

Paramoera walkeri Stebbing (Amphipoda, Pontogeneidea) is the main component of the fauna of the so-called sub-fast-ice community living on the lower surface of the marine fast-ice in the coastal zone of the Antarctic seas. The measurements of the standard, routine, and active metabolism in the mature *P. walkeri* females were carried out during the Antarctic winter in October 1969. Measurements were made in the constant-pressure volumetric respirometers modified by Klekowski (1968), at the temperatures of -1.9 and 2.0°C.

The term standard metabolism defines the metabolism in animals which do not show any locomotor activities except respiratory movements; the routine metabolism refers to the metabolism in animals showing spontaneous locomotor movements — the creeping movement on the substratum; the active metabolism refers to the metabolism in animals swimming in the depths of waters.

At the temperature of -1.9°C the *P. walkeri* routine metabolism is 1.36 μl O_2/indiv.·hr and its active metabolism is 2.57 μl O_2/indiv.·hr. At the temperature of 2.0°C analogical values are 2.61 and 3.02 μl O_2/indiv.·hr, respectively, and the standard metabolism is 1.83 μl O_2 per average individual per one hour. The Q_{10} coefficient, calculated from these data for the temperatures ranging from -1.9 up to 2.0°C, is in the case of routine metabolism 5.4 and in the case of active metabolism 1.5. These results show that besides the effect of the adaptation of the organism to the given thermic conditions the motor activity of the investigated animals plays also an important part in the decrease of the values of the Q_{10} coefficient.

6. STRESZCZENIE

Paramoera walkeri Stebbing (Amphipoda, Pontogeneidea) jest głównym komponentem fauny tzw. zespołu podlodowego rozwijającego się na spodniej stronie lodu morskiego w przybrzeżnej strefie mórz antarktycznych. W czasie antarktycznej zimy w październiku 1969 roku prowadzono pomiary metabolizmu standardowego, rutynowego i aktywnego dojrzałych płciowo samic *P. walkeri*. Pomiary prowadzono w stałociśnieniowych respirometrach wolumetrycznych w modyfikacji Klekowskiego (1968), w temperaturach $-1{,}9$ i 2,0°C.

Za metabolizm standardowy przyjęto metabolizm zwierząt nie wykonujących ruchów lokomotorycznych oprócz ruchów oddechowych; za metabolizm rutynowy przyjęto metabolizm zwierząt wykonujących spontaniczne ruchy lokomotoryczne — chodzenie po podłożu; za metabolizm aktywny przyjęto metabolizm zwierząt aktywnie pływających w toni wodnej.

W temperaturze -1.9°C metabolizm rutynowy *P. walkeri* wynosi 1,36 μl O_2 na osobnika na godzinę, a metabolizm aktywny — 2.57 μl O_2. W temperaturze 2,0°C analogiczne wartości

wynoszą odpowiednio 2,61 i 3,02 µl O_2, a metabolizm standardowy — 1,83 µl O_2 na średniego osobnika na godzinę. Obliczony z tych danych współczynnik Q_{10} dla zakresu temperatur od — 1,9 do 2,0°C wynosi w przypadku metabolizmu rutynowego 5,4, a w przypadku metabolizmu aktywnego — 1,5. Wyniki te wskazują, że na obniżenie wartości współczynnika Q_{10} oprócz adaptacji organizmu do danych warunków termicznych ma również duży wpływ aktywność motoryczna badanych zwierząt.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	431–454	1974

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THE EFFECT OF THREE SPECIES OF PHYTOPHAGOUS FISH ON ALGAE DEVELOPMENT

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ABSTRACT

Studies were carried out on the effect of three species of phytophagous fish, namely: bighead carp (*Aristichthys nobilis* Rich.), silver carp (*Hypophthalmichthys molitrix* Val.), grass carp (*Ctenopharyngodon idella* Val.) on the biomass, density, and structure of species composition of algae population. It has been found that at the stock of 1500–3000 of silver carp and bighead carp individuals/ha the biomass of algae had increased. The number of algae decreased inasmuch as the small forms gave way to the larger ones. In the presence of bighead carp Cyanophyta were particularly abundant and in the presence of silver carp — Bacillariophyceae. Seasonal changes in the structure of algae composition were favourable for fish development which was evidenced by a considerable increase in their growth. The stock of grass carp numbering 1500 individuals/ha did not show any effect upon algae.

1. INTRODUCTION

In the experiment carp was cultured with mixed stock of one-year-old phytophagous fish, namely: bighead carp, silver carp, and grass carp. As it is well known the two former species are seston filterable. The later one, grass carp, in the natural conditions feeds mainly on macrophytes. Phytophagous fish utilizes another trophic level of water biocenosis than carp and in consequence they do not outvie one another for food. On the basis of that premise fish cultures were started with miscellaneous stock consisting of those species. In this way food resources of the environment are better utilized. Therefore, in the final effect it should lead to an increase of fish production.

The purpose of the present study was to investigate the effect of phytophagous fish upon the biomass, density, and structure of species composition of algae.

2. MATERIAL AND METHODS

Experiments were carried in 26 ponds belonging to the Department of Fish Culture of the Inland Fisheries Institute, at Żabieniec.

The 26 experimental ponds constituted a complex whole where each of the factors under examination was observed in three repeating ponds — in one case only in two ponds. Groups of the repeat ponds were called "variants". There were nine variants (Table I) and combinations between one another made it possible to obtain six arrangements. Each of the arrangements comprised two variants and one of them was the control for another.

Arrangement I — analizes the effect of bighead carp on algae (variant 1 was compared with variant 2);

Arrangement II — as above (variant 3 compared with variant 4);

Arrangement III — the effect of grass carp (compared var. 5 and 6);

Arrangement IV — the effect of silver carp (comp. var. 5 and 7);

Arrangement V — as above (compared variant 5 and 8);

Arrangement VI — the effect of the three species of phytophagous fish together (compared variants 5 and 9).

Table I. Fish stocks in the ponds

Variants No.	Ponds No.	Type of fish species (indiv./ha)				Fertilizing	Feeding
		carp	bighead carp	grass carp	silver carp		
1	20, 40, 44	2000	—	—	—	—	—
2	26, 32, 48	2000	1500	—	—	—	—
3	18, 36, 45	2000	—	—	—	+	—
4	28, 50	2000	1500	—	—	+	—
5	29, 51, 46	2000	—	—	—	+	+
6	25, 31, 43	2000	—	1500	—	+	+
7	22, 39, 55	2000	—	—	1500	+	+
8	21, 37, 17	2000	—	—	3000	+	+
9	42, 33, 56	2000	1500	1500	1500	+	+

All the ponds had the same area of 0.2 ha and a collective water supply from the Jeziorka River. They were stocked with fish in the second half of April 1968 and were used till mid-October. At the same time studies on fish production were carried out in those ponds (**Wolny** 1970 a, b), simultaneously. Fertilizing with 200 kg N/ha of urea was dosed in 10 portions, and with 40 kg P_2O_5/ha of superphosphate in 6 portions. The operation was carried on from May 15 until August 31. Feeding with sorghum was started on May 3 and finished on September 27. Food was given every day, ad libitum.

Differentiation of the ponds was analized in respect of the variety of algae species living in there. To this purpose an index of species similarities by **Kulczyński** (1939) has been used:

$$P=\frac{2c\cdot 100}{a+b}$$

where: P — index of similarities expressed in per cent, c — number of the species common to both of the compared ponds, a — number of species in the first pond, b — number of species in the second pond.

Likeness in species predomination was also analized as regards density of population and biomass using the index of dominance similarities by **Renkonen** (1938). With the help of that index lower precentage values of common dominant species in the two comparative areas (ponds) are summed up. The higher is the obtained value the higher is the dominance similarity. .

Water samples for the examination of algae were collected once a week from April 18 till October 10, 1968. Altogether 24 samples were collected from every pond. To collect a single sample 10 l of water were taken diagonally from the pond by means of the Patalas apparatus; out of this 5 l were drawn from beneath the surface, the other 5 l from just above the bottom of the pond. All the collected water was poured off into one bucket and thoroughly stirred then 10 ml of it was taken out to be used as a sample of natural plankton for microscopy studies. A part of the sample was examined in vivo by means of the continuous drip method the other part was fixed with Lugol for exact qualitative determinations. The density of organisms was calculated per 1 cm^3 of water. Algae appearing in one-celled form, cenobia, and single cells of algae living in colonies were used for calculation units. Biomass was calculated from the cell volume assuming that specific weight of phytoplankton equals 1, thus 1 $\mu m^3=10^{-9}$ mg. The value of the biomass weight is given in g/m^3.

3. RESULTS

ARRANGEMENT I WITH BIGHEAD CARP

Variant 1 (control) was compared with variant 2 (Table I). The mean seasonal values of density and biomass of algae are presented in Table II. The mean seasonal value of the biomass in variant 1 was 32 g/m^3 and in variant 2 — 67 g/m^3, so there was an increase of 109%. Fish production in the former was 194 kg/ha, in the latter — 419 kg/ha, including 213 kg of carp and 206 kg of bighead carp.

Differences between those two variants as regards biomass and density of population of particular types of algae are presented in Table II. Chlorophyta — the mean seasonal biomass value has increased insignificantly in var. No. 2., while the density of its population showed a decrease of about one third of the value, which proves that in the ponds stocked with bighead carp the Chlorophyta species were of bigger size. Bacillariophyceae — the increase of the biomass was of 50%, on the average, in var. 2. There was also more of Euglenophyta. Chrysophyta, and Pyrrophyta. The most noteworthy, however, were Cyanophyta on account of their decisive predominance in all the repeats of the variant 2, which gave on the average more than a fourfold increase in their biomass.

The highest density of algae population and their biomass occurred in both variants alike in July and August. The peaks of density and biomass were higher and in general longer lasting in variant 2 than in the control ponds (cf. variants 1 and 2 in Fig. 1). In the spring time the percentage share of various types of algae was similar to one another. A radical change in the structure of algae composition in the two variants occurred by in July and the difference between them lasted till the end of the season. In that period of time, in the control ponds Bacillariophyceae predominated, while in the ponds with bighead carp there was a mass development of Cyanophyta (cf. variants 1 and 2 in Fig. 2).

In both variants there was a very high 86—91% similarity in the composition of the algae species. Similarity of the species with predominant density of population was characterized by a fairly great variability, in all the 15 comparative combinations, ranging from 36 to 95%. In the ponds stocked with bighead carp the values are the highest, this gives evidence of a greater homogeneity of the species with predominating density of population living in those ponds. On the other hand the indices for biomass were in all the ponds slightly lower.

Greater variability and smaller similarity of the species with predominant biomass gets more comprehensible in the light of the fact that those algae are, as a rule, the largest in the pond environment and even the smallest fluctuations in their population density may change radically their dominance in biomass.

In the control ponds *Chlorella minutissima* and *Anabaena flos aquae* had predominant density of population, while *Anabaena flos aquae* and *Melosira granulata* dominated in biomass. In the ponds stocked with bighead carp *Anabaena flos aquae* had everywhere predominance in density of its population, while *Melosira granulata* and *Anabaena flos aquae* or *Anabaena solitaria* predominated in biomass.

ARRANGEMENT II WITH BIGHEAD CARP

In this arrangement the control variant No. 3 was compared with variant 4 (Table I).

The mean seasonal biomass value in var. 3 was 27 g/m^3 and in var. 4 with bighead carp — 66 g/m^3, so there was an increase of more than twice as much. Fishing production in var. 3 was 310 kg/ha and in var. 4 — 464 kg/ha, this includes 215 kg of carp and 249 kg of bighead carp.

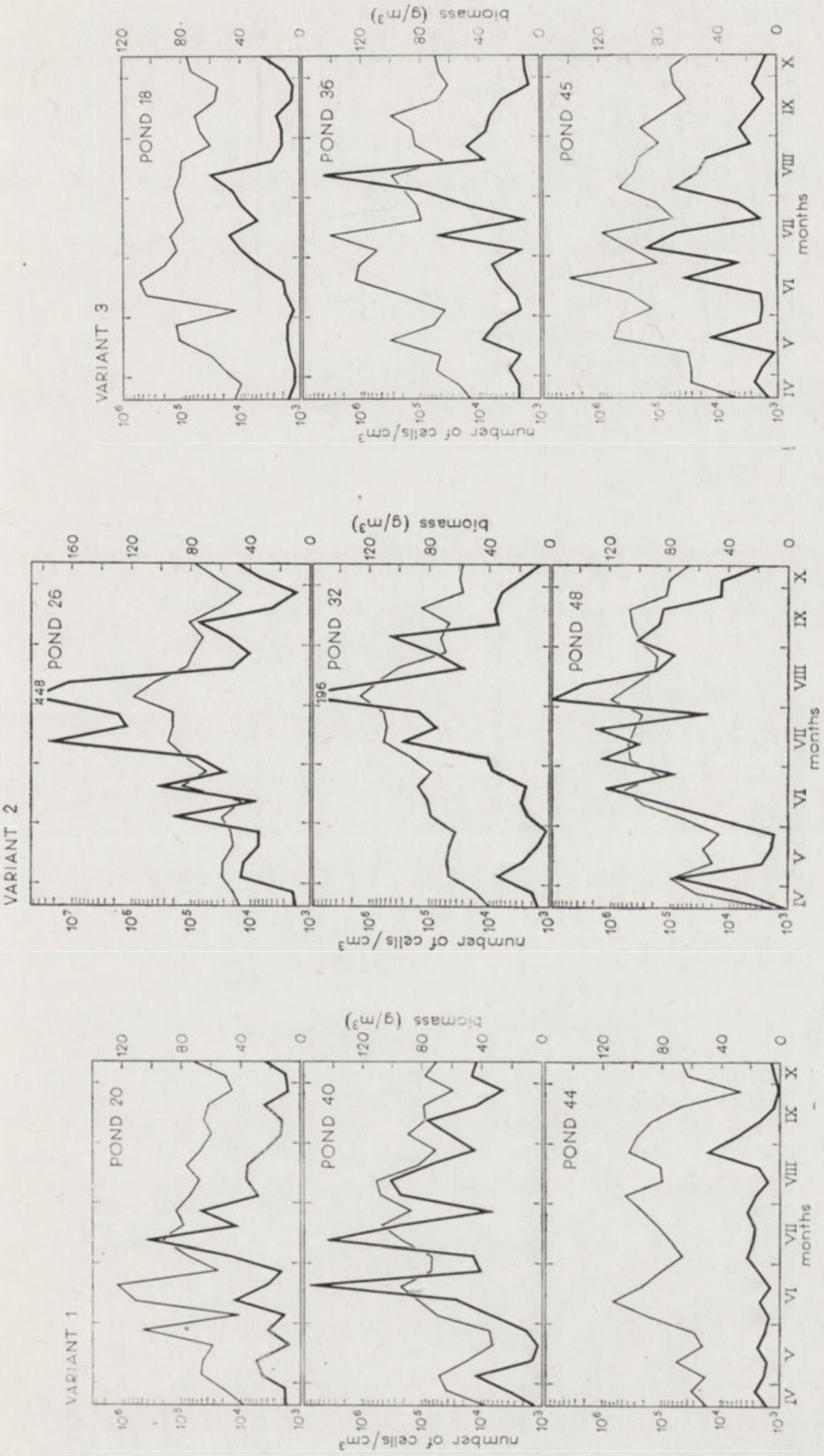
Table II. Mean seasonal density D in $1000/cm^3$ and biomass B in g/m^3

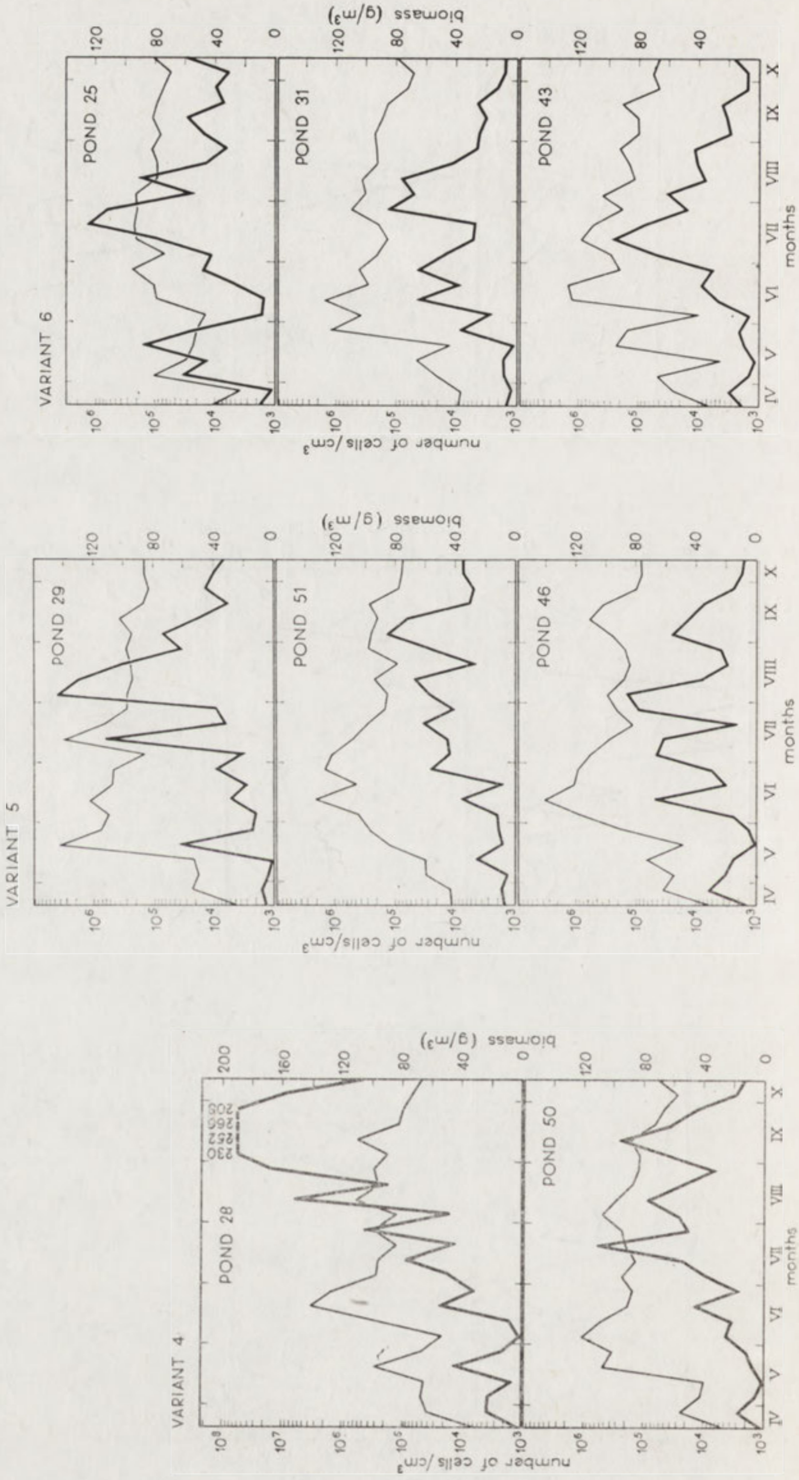
Variants No.	Type	Mean seasonal		Mean seasonal		Mean seasonal		Mean seasonal from three ponds	
		D	*B*	*D*	*B*	*D*	*B*	*D*	*B*
		Pond No. 20		Pond No. 40		Pond No. 44		Average	
1	Chlorophyta	129.8	6.7	44.3	5.7	123.1	5.7	99.0	6.0
	Bacillariophyta	114.0	17.0	19.1	35.4	5.3	4.3	12.8	19.0
	Euglenophyta	0.3	1.5	0.4	2.0	0.2	1.3	0.3	1.6
	Chrysophyta	0.4	0.7	0.3	0.5	0.1	0.2	0.3	0.5
	Cyanophyta	5.7	2.0	90.0	10.8	26.2	3.0	40.7	5.2
	Pyrrophyta	0.1	0.1	—	0.1	—	0.1	—	0.1
	Total	150.3	28.0	154.1	54.5	154.9	14.6	153.1	32.4
		Pond. No. 26		Pond No. 32		Pond No. 48		Average	
2	Chlorophyta	38.4	11.4	64.9	5.2	73.8	6.7	59.0	7.8
	Bacillariophyceae	21.4	39.9	9.3	9.2	17.1	33.1	15.9	27.4
	Euglenophyta	0.4	2.2	1.2	6.7	0.5	2.8	0.7	3.9
	Chrysophyta	0.1	0.1	0.3	0.5	0.4	0.7	0.3	0.4
	Cyanophyta	78.6	26.8	178.5	26.7	192.4	29.1	149.9	27.5
	Pyrrophyta	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.2
	Total	139.0	80.7	254.3	48.4	284.3	72.5	225.9	67.2
		Pond No. 18		Pond No. 36		Pond No. 45		Average	
3	Chlorophyta	109.1	10.4	381.0	13.0	356.4	19.6	282.2	14.4
	Bacillariophyceae	5.8	6.5	11.1	7.5	7.8	4.0	8.3	6.0
	Euglenophyta	0.4	2.2	2.2	11.3	0.8	4.1	1.1	5.9
	Chrysophyta	0.1	0.2	0.4	0.6	0.2	0.4	0.2	0.3
	Cyanophyta	0.5	0.4	1.6	0.8	1.0	0.3	1.0	0.5
	Pyrrophyta	—	—	—	—	—	—	—	—
	Total	115.9	19.7	396.3	33.2	366.2	28.4	292.8	27.1

		Pond No. 28		Pond No. 50				Average	
4	Chlorophyta	335.4	9.1	186.8	14.5			261.1	11.8
	Bacillariophyceae	15.7	9.7	7.5	8.7			11.6	9.1
	Euglenophyta	2.2	11.3	1.3	7.4			1.8	9.4
	Chrysophyta	0.4	0.7	0.2	0.2			0.3	0.5
	Cyanophyta	50.2	58.9	44.2	10.4			47.2	34.7
	Pyrrophyta	0.1	0.2	0.1	0.3			0.1	0.2
	Total	404.0	89.9	240.1	41.5			322.1	65.7
		Pond No. 29		Pond No. 51		Pond No. 46		Average	
5	Chlorophyta	626.5	23.3	391.7	20.9	447.5	20.5	488.7	21.6
	Bacillariophyceae	4.4	5.1	5.5	7.4	9.8	8.4	6.6	7.0
	Euglenophyta	3.3	17.5	0.6	3.1	0.3	1.9	1.4	7.4
	Chrysophyta	4.2	0.1	0.7	1.1	0.5	0.8	0.2	0.7
	Cyanophyta	—	0.6	8.9	3.8	5.7	0.8	6.3	1.7
	Pyrrophyta	—	—	—	0.2	—	—	—	0.1
	Total	638.4	46.6	407.4	36.5	463.8	32.4	503.2	38.5
		Pond No. 25		Pond No. 31		Pond No. 43		Average	
6	Chlorophyta	84.0	21.9	317.2	24.5	299.3	18.6	233.5	21.6
	Bacillariophyceae	16.1	25.2	7.3	3.3	6.4	8.4	10.0	12.3
	Euglenophyta	0.3	1.6	0.7	3.8	0.5	2.7	0.5	2.7
	Chrysophyta	0.1	0.1	0.1	0.2	0.2	0.3	0.1	0.2
	Cyanophyta	5.6	1.4	0.4	0.2	4.7	0.7	3.5	0.8
	Pyrrophyta	—	—	—	—	—	—	—	—
	Total	106.1	50.2	325.7	32.0	311.1	30.7	247.6	37.6

Continued Table II

Variants No.	Type	Mean seasonal D	Mean seasonal B	Mean seasonal D	Mean seasonal B	Mean seasonal D	Mean seasonal B	Mean seasonal from three ponds D	Mean seasonal from three ponds B
		Pond No. 22		Pond No. 39		Pond No. 55		Average	
7	Chlorophyta	80.5	8.6	276.5	18.9	191.0	12.3	182.7	13.3
	Bacillariophyceae	21.3	37.9	9.2	12.7	10.0	11.9	13.6	20.8
	Euglenophyta	0.2	1.2	0.8	4.2	0.9	4.5	0.6	3.3
	Chrysophyta	0.4	0.6	0.3	0.4	0.4	0.6	0.3	0.5
	Cyanophyta	62.4	7.6	36.1	4.5	0.9	0.3	33.1	4.1
	Pyrrophyta	—	0.1	—	0.1	—	—	—	0.1
	Total	164.8	56.0	322.9	40.8	203.2	29.6	230.3	42.1
		Pond No. 21		Pond No. 37		Pond No. 17		Average	
8	Chlorophyta	43.8	8.8	144.9	12.1	165.7	9.8	118.2	10.3
	Bacillariophyceae	20.6	27.5	8.2	7.6	6.0	11.7	11.6	15.6
	Euglenophyta	0.4	2.1	1.6	7.8	1.0	5.2	1.0	5.0
	Chrysophyta	0.1	0.2	0.3	0.5	0.4	1.3	0.3	0.6
	Cyanophyta	37.0	6.7	4.5	1.0	0.6	0.3	14.0	2.7
	Pyrrophyta	0.1	0.2	—	—	—	—	—	—
	Total	102.0	45.5	159.5	29.0	173.7	28.3	145.1	34.5
		Pond No. 42		Pond No. 33		Pond No. 56		Average	
9	Chlorophyta	154.7	18.4	100.6	13.7	137.9	14.2	131.0	15.4
	Bacillariophyceae	14.7	25.6	22.8	22.4	46.1	41.1	27.9	29.7
	Euglenophyta	0.9	4.9	1.4	7.2	0.6	3.0	0.9	5.0
	Chrysophyta	0.3	0.5	0.3	0.4	0.5	0.8	0.3	0.6
	Cyanophyta	17.9	2.7	27.0	6.2	46.2	6.1	30.4	5.0
	Pyrrophyta	—	—	—	0.3	0.1	0.3	0.1	0.2
	Total	188.5	52.1	152.1	50.2	231.4	65.5	190.6	55.9


VARIANT 1
POND 20
POND 40
POND 44
VARIANT 2
POND 26
POND 32
POND 48
448
196
VARIANT 3
POND 18
POND 36
POND 45
number of cells/cm^3
10^3
10^4
10^5
10^6
10^7
biomass (g/m^3)
0
40
80
120
160
IV
V
VI
VII
VIII
IX
X
months


VARIANT 6
POND 25
POND 31
POND 43
VARIANT 5
POND 29
POND 51
POND 46
VARIANT 4
POND 28
POND 50
230
252
260
205
number of cells/cm³
biomass (g/m³)
months
IV
V
VI
VII
VIII
IX
X
10³
10⁴
10⁵
10⁶
10⁷
10⁸
0
40
80
120
160
200

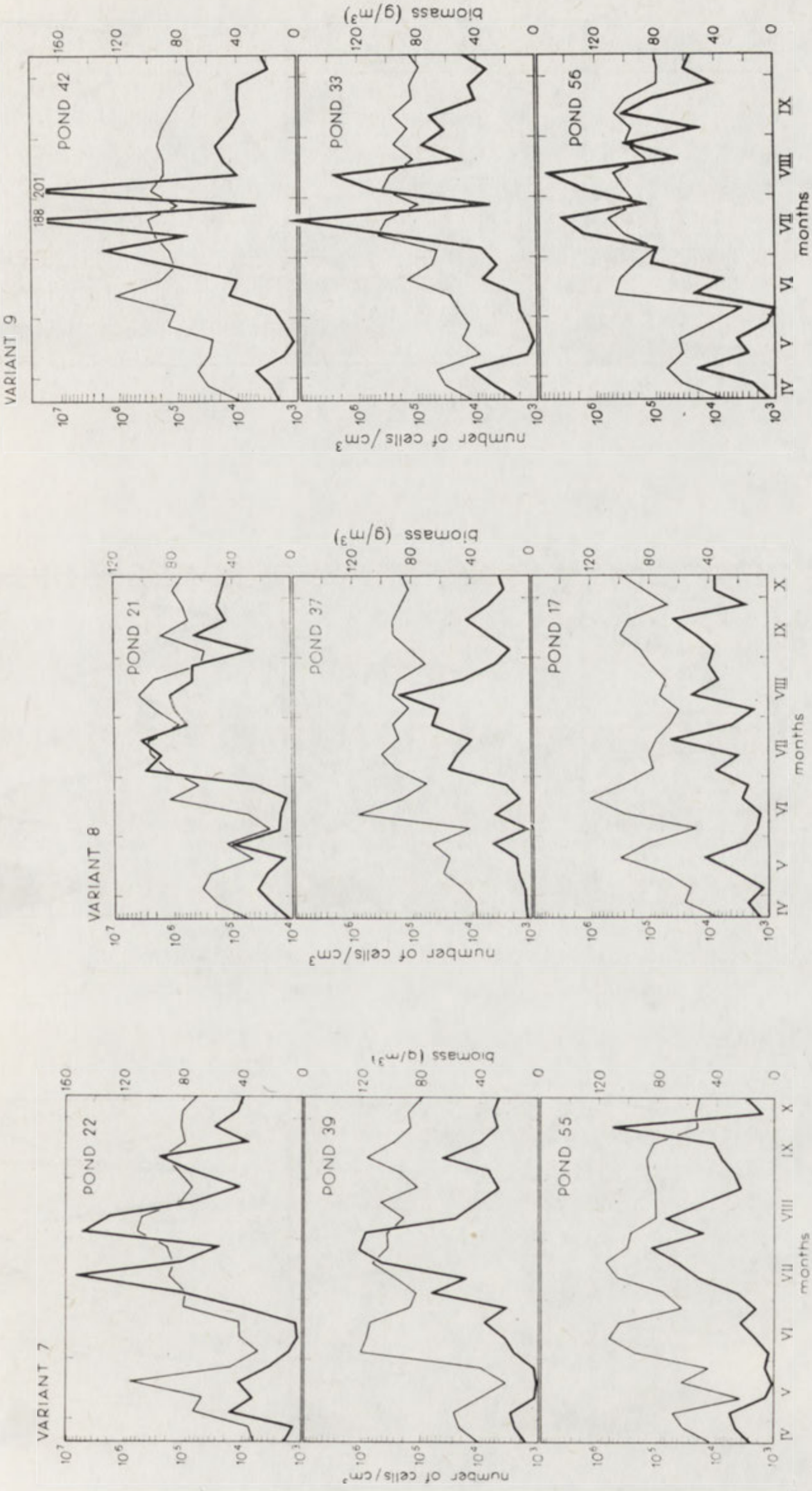
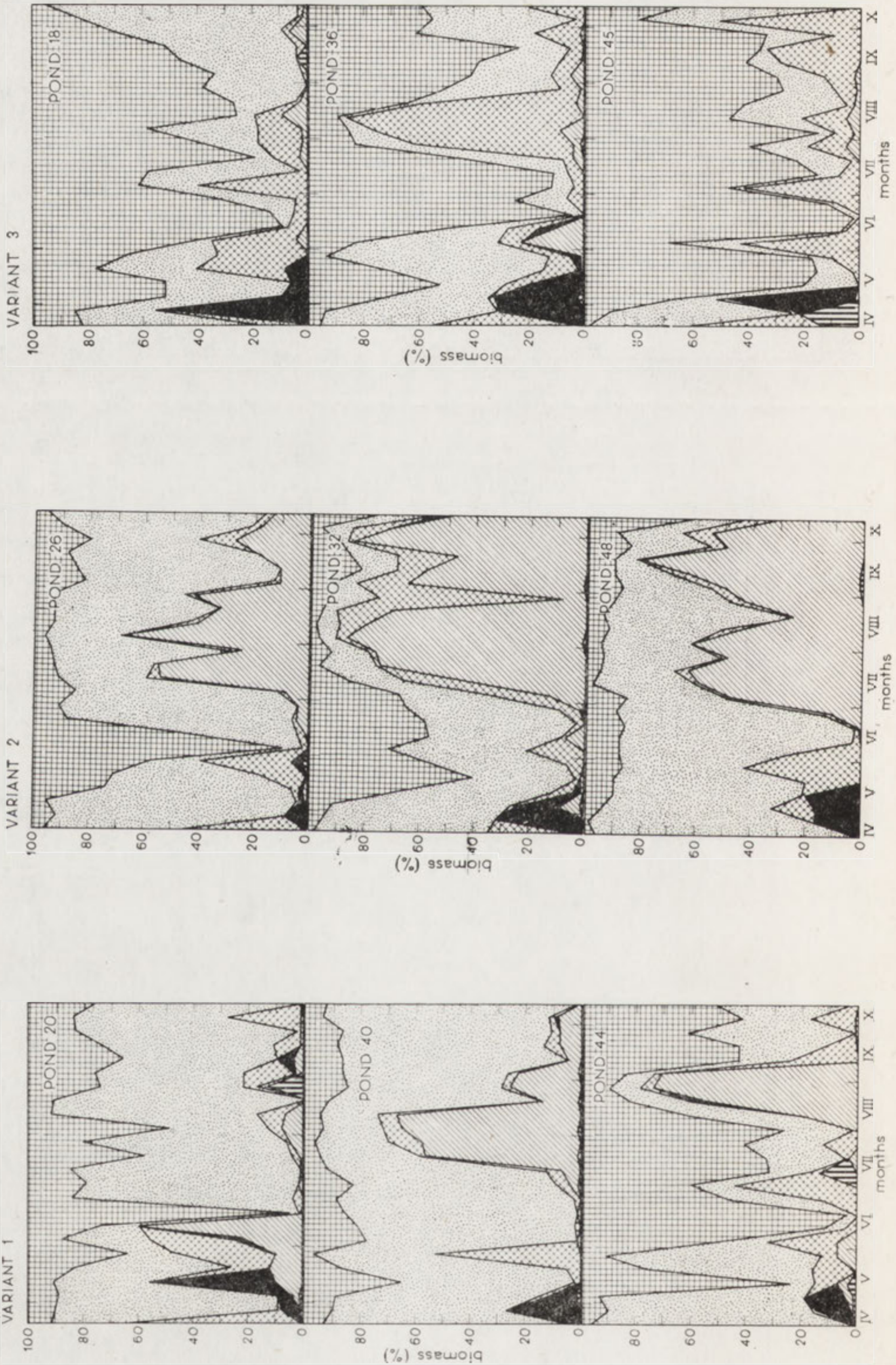
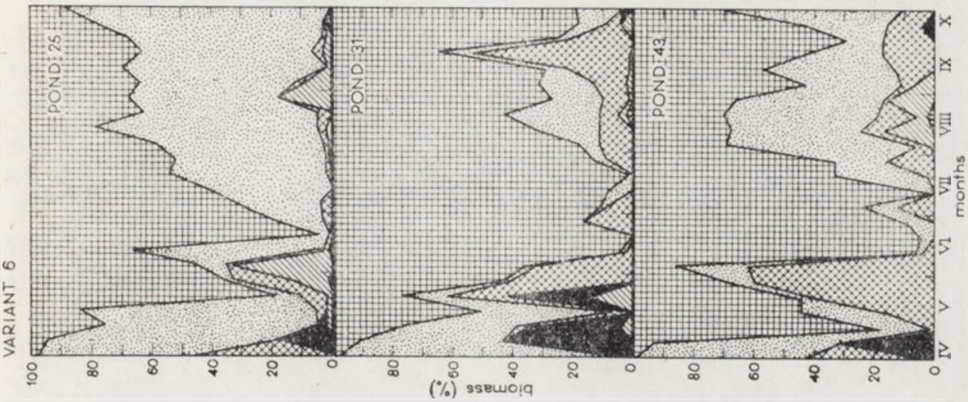
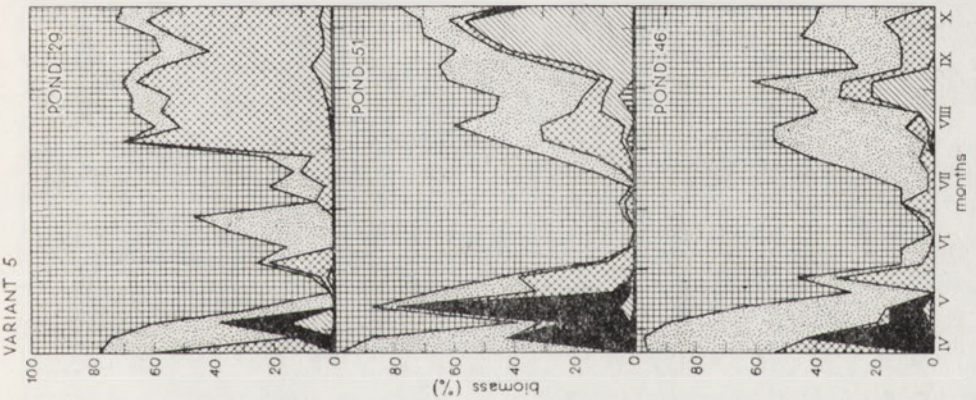
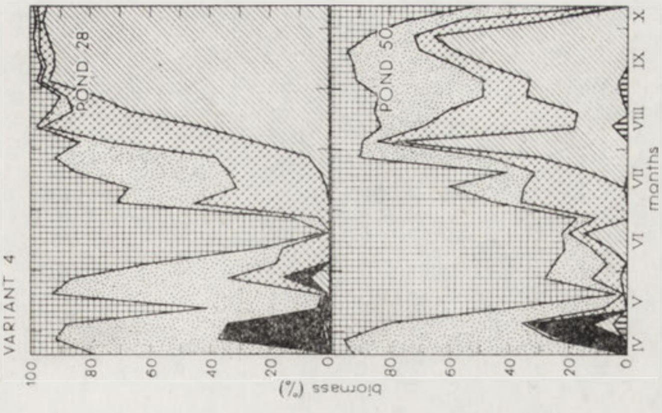


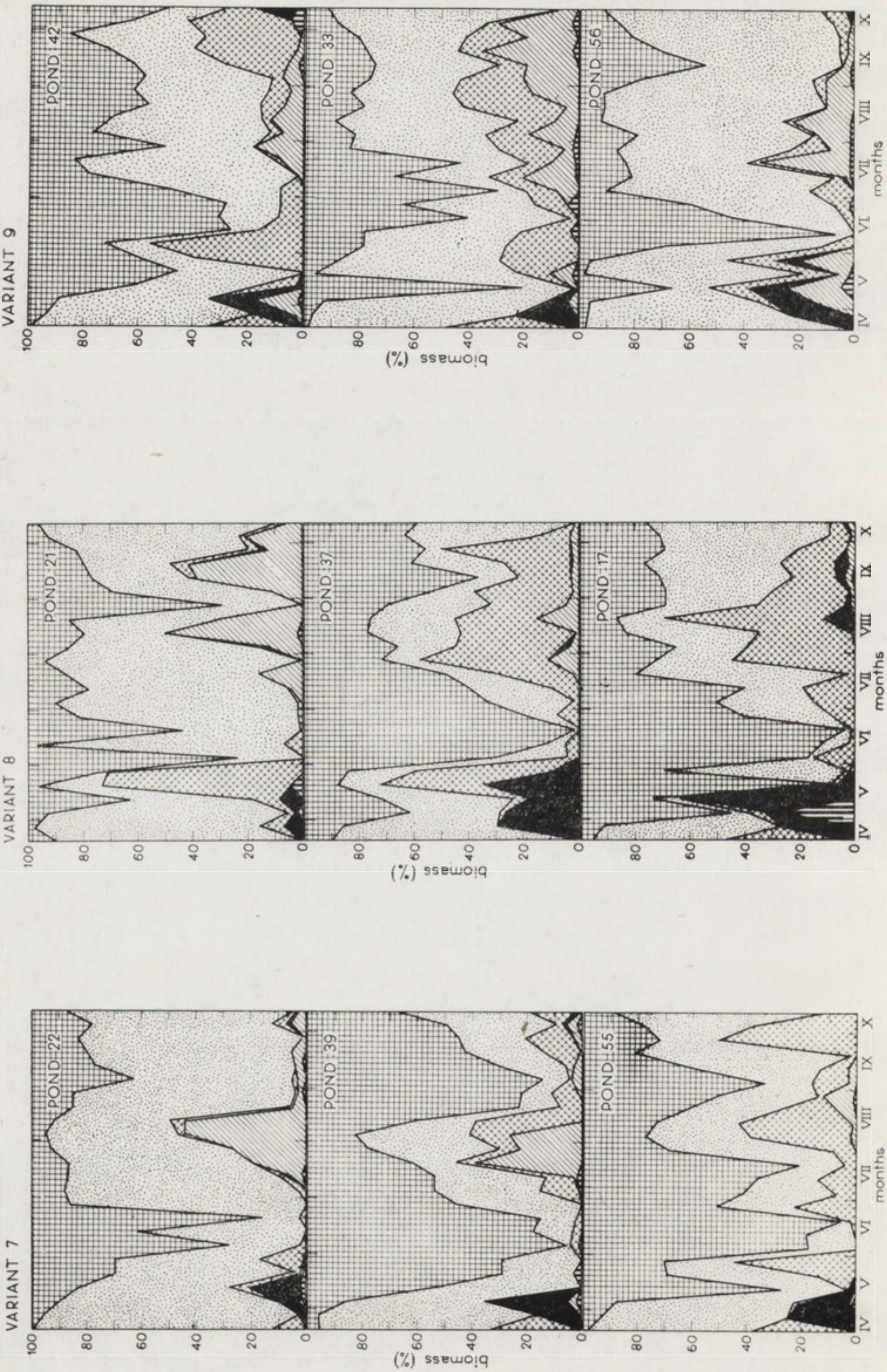
Fig. 1. Seasonal changes in algae density (thin lines) and biomass (thick lines). For explanations of variants 1–9 see Table I


VARIANT 3
POND 18
POND 36
POND 45
VARIANT 2
POND 26
POND 32
POND 48
VARIANT 1
POND 20
POND 40
POND 44
biomass (%)
100
80
60
40
20
0
IV
V
VI
VII
VIII
IX
X
months


VARIANT 6
POND 25
POND 31
POND 43
100
80
60
40
20
0
biomass (%)
IV
V
VI
VII
VIII
IX
X
months


VARIANT 5
POND 29
POND 51
POND 46
100
80
60
40
20
0
biomass (%)
IV
V
VI
VII
VIII
IX
X
months


VARIANT 4
POND 28
POND 50
100
80
60
40
20
0
biomass (%)
IV
V
VI
VII
VIII
IX
X
months


VARIANT 9
POND 42
POND 33
POND 56
VARIANT 8
POND 21
POND 37
POND 17
VARIANT 7
POND 22
POND 39
POND 55
biomass (%)
100
80
60
40
20
0
IV
V
VI
VII
VIII
IX
X
months

In comparison with the above-dicussed arrangement I, we have had here an additional factor of nitro-fertilizers which, as it is known, are stimulating the development of Chlorophyta. And, as a matter of fact, in the ponds of both variants (No. 3 and 4) there was a great density of Chlorophyta population and biomass, indeed. Especially noteworthy, however, is the fact of a huge, about seventyfold increase of the biomass of Cyanophyta in the ponds stocked with bighead carp (Table II). It ought to be reminded that in the unfertilized ponds the additional stock of bighead carp has also caused a considerable increase in the biomass (cf. variants No. 1 and 2).

The algae density and biomass dynamics in the ponds stocked with bighead carp differed from that in the control ponds due to a greater frequency of fluctuations and higher and longerlasting seasonal peaks of production (cf. variants 3 and 4 in Fig. 1). It was possible to observe this distinctly in the biomass curve where, for instance, in the early September in the control ponds the level of biomass was already lower, this being characteristic for the autumn period, whereas in the ponds stocked with bighead carp at the same time there was a very high peak of Cyanophyta development.

In both variants, in the spring and early summer time, the percentage share of algae species illustrated in the biomass spectra was similar to each other (Fig. 2, variants No. 3 and 4). Significant differences in the structure of algae composition began to appear in both variants in the early July. In August, the environment was dominated by Cyanophyta, either entirely as in the Pond 28, or by a very high percentage as in the Pond 50, where there was also a lot of Bacillariophyceae and Euglenophyta. This kind of situation lasted till the end of the season.

The values of the species composition similarities were very high in all the ponds. The most similar dominant species were found in the control ponds (95–87 index values) nonetheless the ponds stocked with bighead carp had, likewise, a rather high dominance index (85%). The indices of the two compared with each other variants were only slightly lower, this confirms the homogeneity of succession. Indices of similarities in density and biomass formed into similar groups though with somewhat lower values, ranging from 77 to 95% for density and 28–60% for biomass.

In both variants *Chlorella minutissima* predominated in density of its population. Predominance in the biomass was shown in the control variant by *Melosira granulata* and *Cryptomonas rostrata* or *Chlorella min.*, but in the variant stocked with bighead carp Cyanophyta — *Oscillatoria Agardhii* was dominant over all others.

Fig. 2. Seasonal changes of the composition of algal biomass.

▦ a ░ b ▩ c ■ d ▨ e ▥ f

a — Chlorophyta, b — Bacillariophyceae, c — Euglenophyta, d — Chrysophyta, e — Cyanophyta, f — Pyrrophyta.

For explanations of variants 1–9 see Table I

ARRANGEMENT III WITH GRASS CARP

The control variant No. 5 was compared with variant No. 6. The mean seasonal biomass of algae was very similar in both variants, i.e. in the range of 38 g/m^3. Fish production in the control ponds was 1101 kg/ha, in the ponds with grass carp — 1269 kg/ha.

In the control ponds Chlorophyta had the greatest biomass. Almost identical results were obtained in the ponds stocked with grass carp. In the control ponds there was also a lot of Bacillariophyceae and Euglenophyta, a little less of Cyanophyta, and scarse Chrysophyta and Pyrrophyta. A similar situation though with some modifications was observed in the ponds stocked with grass carp (Table II). On the ground of the obtained data it is difficult to ascertain the effect of grass carp upon the changes in biomass of the particular types of algae. A decrease in the average density of Chlorophyta population was caused by the fact of its less abundant development in Pond No. 25, where they were borne down extensively by Bacillariophyceae.

The density and biomass dynamics were characterized in both variants by great frequency of fluctuations (Fig. 1, variants 5 and 6). There was some time divergence in the occurrence of the peaks of density and biomass, in the repeats of the control ponds and the other ones stocked with grass carp, as well, in some cases coming up to as much as 3 weeks of difference. In despite of that it was possible to observe in both variants some repetitive regularities i.e. a low level of the occurrence of algae at the beginning of the experiment. Then in the middle of June there was a very sudden increase in the density of algae population and it remained at a high level till the end of the season showing some tendency to a slight downward slope of the curve. The biomass dynamics was subject to very sharp fluctuations due to the occurring changes in the composition of the algae species. The highest peaks of the biomass production were observed in both variants from mid-July to mid-August. Those peaks of production were brought about by Chlorophyta with a considerable participation of Bacillariophyceae (Fig. 2, variants 5 and 6). Ponds No. 29 and 25 were exceptive and were considered to be non-typical. At the end of the season there was a considerable decrease in the biomass in both variants.

Statistical indices have shown a very significant similarity in the species composition in both variants (84–92%). Likewise, there was also a great similarity of the species dominant in the density of population in the control variant (94–96%) and a smaller one in the variant with grass carp (60–87%), but this was caused by a mass development of Bacillariophyceae in one of the three repeat ponds belonging to that variant. A fairly great similarity appeared at the comparison of both variants with each other, as it ranged from 60% upwards. A similar configuration of indices though with lower values has been recorded for the biomass.

In both variants *Chlorella minutissima* predominated in the density of its population. In the biomass, also, *Chlorella minutissima* showed predominance in the control variant, whereas in the variant with grass carp *Melosira granulata* was dominant in two ponds and Chlorella elepsoidea in the third one.

ARRANGEMENT IV WITH SILVER CARP (1500 INDIV./HA)

Here, the control variant No. 5 was compared with variant No. 7. In control ponds the mean seasonal biomass of algae was 38 g/m^3 and in ponds stocked with silver carp — 42 g/m^3, so, there was an increase of 10%. The mean seasonal density of algae population has decreased more than twofold. Fish production in the control ponds was 1101 kg/ha and in those with silver carp — 1233 kg/ha, including 130 kg of that fish. The yield of the carp was the same in both variants with a considerable decrease (700 kg/ha) of food consumption in the pond with the silver carp.

In the control ponds the most numerous were Chlorophyta (Table II). In the ponds with silver carp the share of Chlorophyta decreased by a half in relation to the control ponds and the share of Bacillariophyceae showed a threefold increase.

Curves of the density dynamics in the two ponds were divergent (Fig. 1, variants 5 and 7). In the ponds with silver carp they swerved up more than in the control ponds. There were also significant differences in the course of the biomass curves. They resulted from the fact that the highest peaks of the biomass production occurred earlier in the ponds with silver carp than in the control ones. As it was, the greatest biomass occurred in the control ponds from the first days of August and lasted till the end of that month, and in one pond even a few days longer, while in the ponds with silver carp it occurred a month earlier i.e. in the beginning of July and ended before the end of August.

From the structure of the algae composition presented in the spectra one can see that (Fig. 2, variants 5 and 7) the highest peaks of biomass were brought about by Bacillariophyceae and/or Euglenophyta and the highest peaks of the population density by Chlorophyta. Thus, the abundant development of Bacillariophyceae occurring ahead of time in the ponds with phytophagous fish is doubtlessly connected with the effect of those fish upon that type of algae.

Just as in the other arrangements here also the occurring species were very similar in both variants. The species similarity indices were in the range of 83–92%. The indices of the density predominance were significantly different in both variants, since in the control variant they were rather high (95–96%) and in the variant with silver carp much lower (59–86%). This was caused by the marked predominance of Chlorella in the control variant and besides the percentage value was very similar in all the repeats. On the other hand, Bacillariophyceae did not develop in equal amounts in the ponds with silver carp. There was much more of them in the pond No. 22 than in the other repeats. Consequently, the coefficient values has decreased. In the comparison of the two variants the similarity indices of the density were also fairly high, except in those where Pond No. 22 was involved. The biomass indices for both variants based on similar regularities were of lower values. In the control variant they were in the range of 59–67%, in the other one — 54 to 62%. At the comparison of the two variants with each other, however, a significant and rather chaotic differentiation of their values has occurred.

In all the ponds of the control variant *Chlorella minutissima* predominated in population density and the biomass. In the ponds with silver carp *Chlorella min.*

was, likewise, predominant in the density of its population, however, in the biomass there was nothing but Bacillariophyceae, in one pond *Stephanodiscus Hantzchii* and in the other two *Melosira granulata*.

ARRANGEMENT V WITH SILVER CARP (3000 INDIV./HA)

The control variant was compared with variant No. 8. In the control variant the mean seasonal biomass of algae was 38 g/m^3 and fish production 1101 kg/ha. In the variant No. 8. those values were 34 g/m^3 and 1359 kg/ha, respectively, the latter one included 267 kg/ha of silver carp. The food consumption value was about 600 kg/ha lower.

Thus, in the ponds with silver carp the algae biomass showed a decrease of about 10% (Table II). This is the first case of that kind observed in the presence of phytophagous fish. An interpretation of that incident is difficult on account of great differences in the algae biomass in the ponds 21, 37, and 17. The density of algae in all the ponds of the variant 8 showed a decrease of about 135% in relation to the control ponds. This indicates the withdrawal of small species from the environment and their replacement by the larger ones. Since in the pond No. 21 Melosira was exceptionally abundant the biomass of algae in that pond was, therefore, much higher than the biomass values in the repeat and control ponds. Thus, it is not possible to consider the occurring now and then decrease of the algae biomass, at the 3000 ind./ha stock of silver carp, to be caused by excessive density of its population.

The course of the changes of algae composition in the biomass of the variant 8 was similar to that in the variant 7, i.e. at the density of 1500 ind./ha stock of silver carp. Thus, in the presence of silver carp the biomass of Chlorophyta showed a twofold decrease in relation to the control ponds. The mean seasonal biomass of Bacillariophyceae had an increase of more than 100% and consequently they become predominant Chlorophyta following in the second place.

The density and biomass dynamics in the variant 8 differed from the control by the same features as in the variant 7 (Fig. 1, variants 5 and 8). Those differences consisted in lower values of the density curve, while the maximums of frequency occurred at the same time in both variants, i.e. in the middle of June. The biomass curve showed the highest peaks of production a month earlier than the controls. Thus, they appeared, in general, at the beginning of July and lasted till the end of August, whereas in the control ponds they occurred usually not sooner than in August.

By comparing the two variants (Fig. 1 and 2) it is easy to realise that in both of them the highest peaks of density concerned the development of Chlorophyta, mainly, and only now and then Bacillariophyceae. The highest peaks of the biomass happened then, when there was a mass occurrence of larger species of Bacillariophyceae, Euglenophyta, and/or Cyanophyta. In general, in that case the proportional share of Chlorophyta has significantly decreased.

At the beginning of the season the biomass and the density of algae population was at a low level in both variants. In the early June the environment was almost entirely predominated by Chlorophyta.

In the control ponds Chlorophyta were strikingly predominant till the end of August, i.e. for about two months, and then, till the end of the season their ratio was still quite considerable. In the ponds with silver carp, however, their predominance lasted only from one to four weeks. At the end of the season Bacillariophyceae were predominant.

Those differences in the structure of algae composition were doubtlessly brought about by the effect of phytophagous fish.

The frequency distribution of the various species of algae was very similar in both variants. The dominance similarities index for both variants was in the range of 84–92%.

On the other hand, there were significant differences in the density indices. In the control ponds that index values were in the range of 94–96%, while in the ponds with silver carp they were 42–88%. This was caused by two factors, approximated percentage values of the mass occurrence of one Chlorella species in the variant 5 and a lower percentage share of the dominants in the variant 8. This was connected, doubtlessly, with the lowering of the quantitative priority of one algae species.

The biomass similarity indices were higher (59–67%) in the control ponds than in the ponds with silver carp (39–64%) and this confirms the fact of a wider span in the range of the dominant species fluctuations in the biomass of the latter ponds.

In the control ponds Chlorella minutissima was strikingly predominant in both density and biomass. In the variant ponds stocked with silver carp *Chlorella minutissima* and *Anabaena flos aque* were predominant in the density of population and *Melosira granulata*, *Cryptomonas rostrata*, and *Cymbella lanceolata* — in the biomass.

ARRANGEMENT VI WITH THREE SPECIES OF PHYTOPHAGOUS FISH

In this arrangement the control variant No. 5 was compared with the variant No. 9, where the stock of phytophagous fish was the largest out of all the discussed up-to-now arrangements. In the variant 5 the algae biomass was 38 g/m^3, the density of population 503,000/cm^3, and fish production 1101 kg/ha. In the variant 9 the biomass was 56 g, density 190,000, fish production 1223 kg/ha, including the carp 777 kg/ha, grass carp 140 kg/ha, silver carp 120 kg/ha, and bighead carp 186 kg/ha.

In the variant 9 the algae density had a decrease of 159% in relation to the control, and the biomass an increase of 47%. Fish production showed an increase of 11%. The amount of the consumed food was by 1905 kg/ha lower.

The decrease in the density and increase in the biomass in the ponds with phytophagous fish indicates that the smaller algae organisms were replaced by the larger ones. So, the situation that had been observed in the ponds with silver carp in the arrangements IV and V was repeated.

By analising the mean density of particular types of algae (Table II) it has been found that in the control ponds only Chlorophyta had occurred in mass abundance.

In the variant No. 9 despite of a considerable decrease in the density of Chlorophyta their ratio continued to be high. Nonetheless, Bacillariophyceae had the largest biomass in here, they have increased fourfold in relation to the control. The Cyanophyta biomass showed a threefold increase.

The density and biomass dynamics showed a marked difference between the two variants (Fig. 1, variants 5 and 9). In the variant No. 5 the curves of density run at a much higher level than in the variant 9. Also, the highest summer peak of the density occurred earlier in the variant No. 5, i.e. already in the beginning of June. Then, there was a marked decrease in the density but afterwards this state lasted at almost the same level till the end of the season. Whereas, in the variant No. 9 the peak of the density occurred about 10 days later and was much lower. Thus, it was evident that phytophagous fish had a restrictive effect upon the density of the smaller Chlorophyta population. The time limit of that decrease occurred in the period of the maximal peaks of the density. One can see great differences in their height in both variants. The further run of the curve level of density was similar in both variants.

The biomass curves had in both variants a similar course at the beginning and the end of the season. The differences between them appeared in the middle of the summer when in the period from the beginning of July till mid-August the highest peaks of the biomass occurred in the variant No. 9. In the control ponds the maximum peaks happened about a month later and were much lower.

Under the effect of phytophagous fish the structure of algae composition has changed substantially in relation to the control. This can be seen in Fig. 2, by comparing variants 5 and 9 with each other.

The similarity coefficients of the species dominance were in both variants very high, ranging from 87 to 95%.

The similarity coefficients of the density of population were particularly high (94–96%) in the control ponds, this was caused by the mass occurrence of but one species. In the ponds with phytophagous fish those coefficients were lower i.e. in the range of 60–77%. In the comparison of two variants they ranged from 45 to 78%, thus, they were the most divergent ones but in spite of that not exceedingly low.

A similar situation though with lower values was observed in the comparison of the similarity coefficients for the biomass dominance.

In the variant No. 5 *Chlorella minutissima* predominated in the density and biomass. In the variant No. 9, likewise, the same Chlorella species was predominant in the density but in a lower percentage. Stephanodiscus, Melosira, or Coelastrum predominated in the biomass of the particular repeats.

TYPES OF ALGAE OCCURRING IN THE PONDS UNDER EXAMINATION

In the investigated testing ground 142 algae species were found, belonging to the 5 types, as follows: Chlorophyta, Chrysophyta, Euglenophyta, Cyanophyta, and Pyrrophyta.

From the Chlorophyta type Bacillariophyceae community has been isolated and in all the analyses taken under consideration separately from the type itself. The undertaking of that kind of decision was based on the fact that Bacillariophyceae are a very numerous and plastic group of algae capable of undergoing various changes and easily influenced by biotic and abiotic factors. On the contrary, the other algae in the Chrysophyta type counted only a few species that did not have any significant importance for intensifying processes. That is the reason why it seems that without the isolation Bacillariophyceae from the Chrysophyta type would black out the general picture of the interesting changes within the scope of that group.

Chlorophyta were represented by the largest group of algae consisting of 73 species, next were Bacillariophyceae with 39 algae species, then Cyanophyta — 13, Euglenophyta — 8, Chrysophyta — 7 and Pyrrophyta — 2.

4. DISCUSSION

In the study of the density of fish stocks a distinct effect of the silver carp and bighead carp on the algae has been ascertained. This was revealed by the increase in the biomass and the decrease in the density of algae and it indicates that the smaller algae forms were replaced by the larger ones. In the presence of the bighead carp particularly abundant was the development of the following species of Cyanophyta: *Anabaena flos aquae* Breb. ex Bor. et Flah., *Anabaena solitaria var. planctonica* (Brun.) Kom., *Oscillatoria Aquardhi* Gomont, and in the presence of the silver carp — Bacillariophyceae such as *Melosira granulata* (Ehr.) Ralfs., *Cymbella lanceolata* (Ehr.) Heur., and *Stephanodiscus Hantzschii* Grun. There was a considerable decrease in the ratio of small Chlorophyta and in particular of one of them the mass occurring dominant — *Chlorella minutissima* Fott.

In all likelihood, the change in the structure of algae is connected with the silver and bighead carp way of feeding consisting in filtering off seston together with trypton suspended in the water depths. As noticed by Voropaiev (1968) the gap between filtrating processes in bighead carp, in its second year of life, is 40–60 μ and in silver carp 20–25 μ. Thus, they can filter organisms of that size and larger. Consequently, smaller algae are beyond the limits of their feeding requirements. In the fish alimentary tracts contents small algae constituted only a casual component (Opuszyński verbal report) nonrepresentative for the frequency distribution of those organisms in the environment.

It seems that this kind of food discrimination leads to the pressure of silver carp and bighead carp upon the selected algae species and this, in turn, brings about stimulation of their development and vice versa lack of pressure on the small nannoplankton causes stagnation and a decrease in density of its population. It is difficult, however, to explain stimulation of the development of Cyanophyceae by bighead carp and Bacillariophyceae by silver carp on the ground of the effect of selectivity and pressure, only. Dimensions of various species in those two-size groups of algae are often interrelated closely so, theoretically the ones and the others can be withhold by the filtrating-apparatus of both of those two kinds of fish. The abundant development of the small (10 μ long) Stephanodiscus, neither, does account for this fact, satisfactorily. Therefore, it is possible that some specific environmental conditions arise in the presence of those fish different for each of the two species and favourable for some and not other species of algae. Still another explanation of that phenomenon is given by Grygierek (1973) suggesting that the decrease of small Chlorophyta in the ponds stocked with phytophagous fish is caused by zooplankton eating them up. The author has based that suggestion on her observations showing that in the ponds with polyculture of the carp together with phytophagous fish large forms of zooplankton feeding on small Chlorococcales are more numerous than in the monoculture ponds stocked with the carp, only. In this wise, small algae are eaten up from the environment and the remaining large ones are developing abundantly as they are unassailable by zooplankton. Thus, in all probability, phytophagous fish bear effect on the phytoplankton structure by many interrelated dependences in which each of the above presented hypotheses has its own share.

Panov et al. (1969) quoted Savina that according to her studies Cyanophyceae are only slightly utilized by silver carp and bighead carp and are of a small nutritive value for them. Nikolski, Werygin (1966) have reported that at the blooming time of Cyanophyta the phytophagous fish may feed exclusively on those algae, yet, their growth is considerably slower than when they are feeding on Bacillariophyceae and Cyanophyta, as well.

Those opinions minimize the importance of phytophagous fish for fish farming. Since Cyanophyta blooms occur in various ponds all of them would have to be considered as useless for the breeding of that fish.

Our studies contradict that standpoint, as the effects obtained in the ponds with phytophagous fish were good. In the period of the maximal growth of bighead carp in the middle of July (Wolny 1970 b) there was a mass appearance of Cyanophyta and their bountiful development and dominance in the biomass had started a few days earlier in the environment. (Fig. 2, variation 2). This fact proves that the presence of Cyanophyta in the environment did not interfere with the satisfactory development of bighead carp and served, probably, as a well assimilable food for them.

In the material used in some previous investigations the author of this study has observed the bighead carp alimentary tracts filled almost exclusively with *Oscillatoria Agardhi* at the time when that algae species were present in abundance in the environment.

While studying assimilability of various species of algae by bighead carp and silver carp with C^{14} method Panov et al. (1969) have found that Cyanophyta were consumed willingly and that Anabaena, Aphanisomenon, and Nitzschia were better assimilated than Scenedesmus, Ankistrodesmus, and Chlorella. That kind of situation confirms the results of the present study.

Arrangements IV and V differed from each other by the density of silver carp stocks. At the 1500 indiv./ha stock the biomass of algae has increased whereas at the 3000 indiv./ha it has decreased. The latter was, doubtlessly, caused not by the excessive eating up of algae by silver carp but rather by some other factors in the environment. This is evidenced by the lack of uniformity in the repeats. In one of the repeats of the variant with the greater stock of silver carp the biomass value was much higher than in the control while in the other two it was much lower. In result the outcome of the mean value was negative.

There is probably a critical point at which the algae biomass begins to decrease but within the discussed range of the density of the phytophagous fish population that point, surely, has not been overstepped yet.

In his anisotropic investigations of the ponds with silver carp, Wolny (1970 a,b) has noticed an increase in the quantity of soluble oxygen, in comparison with other ponds stocked with the carp alone. Simultaneously, he has observed an interesting, from the fish-farming viewpoint, phenomenon of a decrease in food consumption in the ponds with the carp cultured together with silver carp, despite of the fact that the carp production was the same or even higher than in the ponds without the presence of silver carp. Thus, production of silver carp gave a supplementary increment of the yield from the ponds. Supposedly, a greater oxygen content in the water facilitated food assimilation and accelerated circulation of mineral compounds in the ponds. The effect of silver carp on the improvement of oxygen conditions was, doubtlessly, connected with its feeding habits consisting in filtering off the suspensions in the pond.

Advantageous effects of the polycultures of the carp together with silver carp and the lack of competition between them for the same food has been also described by Tarasova (1970).

The stimulating effect of phytophagous fish on the development of algae has been observed by Januszko (1972), as well.

No substantial differences were found in the occurrence of algae in the ponds with grass carp and those without it. (arrangement III). On the whole, the mean seasonal biomass had the same value in both variants. The frequency distribution of the phytoplankton species was very similar in both variants and the same species was predominant in the density of population. There were some slight differences, whatsoever, and they have occurred not only in the two variants but also between the corresponding repeats. They consisted in diverse composition of species and nonidentical frequency distribution during the maximums of increase and decrease in the density and the biomass. Therefore those differences were not treated as significant.

The stated lack of the grass carp effect upon the algae development was rather surprising. After all, it is a well-known fact that grass carp feeding on higher water plants fertilizes the ponds additionally with manure. Yet, the fishing results showed that in the total weight of the caught fish, 1269 kg/ha, grass carp was only 164 kg, at the same, high percentage, rate of survival. Such a low growth rate of grass carp could not have any substantial effect on algae development so much more that there were used also two other very effective factors — feed and fertilizers. In the two compared variants the quantity of consumed food and the carp production values were almost the same thus the influence of those factors did not bring about a differentiating effect in the algae development.

From this one can draw an additional conclusion, useful for fisheries, that fish ponds with a small quantity of higher water plants, and such were the investigated ponds, are not a suitable environment for grass carp culturing. Obviously, grass carp cannot utilize the artificial feeding stuff to full extent. Consequently, it is not a serious rival of the carp, and this seems to be a statement of great importance.

Similar studies on the feeding of grass carp were conducted by Prikhodko, Lupacheva (1967) and they have observed that basic food of that species consists in plants growing in water or on land. Concentrated feeding stuffs are for grass carp a sort of imposed unnatural food and they gave in effect a relatively low increment of growth.

Changes in the algae structure were examined in the arrangement VI, as well. The specificity of variant No. 9 consisted in the fact that here the environment was under the effect of, besides the carp, three phytophagous fish species. As it has been stated in the above-mentioned arrangements each of those fish species had another kind of effect, the strongest effects, however, were brought about by the two algaphagous species — silver carp and bighead carp. Their influence showed in the qualitatively and quantitatively differentiated state of algae. On that ground one can assume that in the arrangement VI the decisive role in the changes of algae structure was played by those two species of fish, likewise. Characteristic features of the formation of the environment by silver carp and bighead carp should accumulate, therefore, in the variant 9. In fact, one can observe a considerable increase in Bacillariophyceae and Cyanophyta biomass and a decrease of the smaller forms of Chlorophyta.

The maximal peaks of the biomass in that arrangement occurred in the variant with phytophagous fish a month earlier than in the control ponds. A similar situation was observed with silver carp but it did not occur in the ponds with bighead carp due to the fact that maximal peaks in this case were formed by Cyanophyta and that species occurred in abundance in the autumn.

The algae species found in the examined ponds were very similar to one another. In the whole testing-ground there was a uniform succession of algae showing difference in the predominating species, only, depending on the kind of intensification agents.

5. SUMMARY

One-year-old phytophagous fish were cultured together with one-year-old carp stocks (Table I). It was found that in the ponds stocked with 1500 ind./ha of bighead carp and 2000 ind./ha of common carp there was a more than twofold increase in the biomass of algae in comparison with control ponds stocked with the carp alone. The biomass of Cyanophyta was increased innumerably and the biomass of Bacillariophyceae has doubled. The biomass of Chlorophyta has not changed distinctly, but the number of its small forms has decreased considerably. Fish production in the ponds with phytophagous fish was higher than in the control ponds.

In the ponds with 1500 ind/ha of silver carp there was an increase in the algae biomass and in those with 3000 ind./ha — a decrease. It is suggested, however, the decrease was not caused by fish eating up the algae but by some other factors. In the presence of silver carp the density and the biomas of Bacillariophyceae has increased considerably.

The supplementary grass carp stock of 1500 ind./ha introduced into the common carp ponds stocked with 2000 ind./ha did not have any effect on the biomass and frequency distribution of the algae. Most likely, it was caused by the low yield of grass carp as compared with the carp (total weight of the former 164 kg, of the latter 1105 kg). Most probably, the slow development of grass carp was connected with scarcity of vegetation of higher order and incapability of that fish species to benefit from artificial feeding stuffs.

In the conditions of polyculture of the carp together with three phytophagous fish species (the carp 2000 ind./ha, bighead carp 1500 ind./ha, silver carp 1500 ind./ha, grass carp 1500 ind./ha) there was an increase of 45% in the biomass of algae and a decrease of 60% in the density of their population. The algae biomass value was higher than in other variants with a lower stock of phytophagous fish species. In particular, there was an increase in the biomass of Bacillariophyceae and Cyanophyta and a decrease in the biomass of Chlorophyta.

Thus, in the presence of silver carp and bighead carp the algae density has decreased and their biomass has increased. The smaller forms of algae gave way to the larger ones. Larger forms of algae do not pass through the filtering apparatus of phytophagous fish species.

6. STRESZCZENIE

Jednoroczne ryby roślinożerne hodowano w stawach we wspólnych obsadach z jednorocznym karpiem (Tab. I).

Stwierdzono, że w badanych zagęszczeniach 1500 szt/ha tołpygi pstrej i 2000 szt/ha karpia nastąpił ponad dwukrotny wzrost biomasy glonów w porównaniu ze stawami kontrolnymi z samym karpiem. Biomasa Cyanophyta wzrosła kilkunastokrotnie, a biomasa Bacillariophyceae — dwukrotnie. Biomasa Chlorophyta nie zmieniła się wyraźnie, lecz znacznie zmalała liczebność ich drobnych form. Produkcja ryb w stawach z tołpygą była wyższa, niż w kontrolnych.

Przy obsadzie 1500 szt/ha tołpygi białej nastąpił wzrost biomasy glonów, a przy obsadzie 3000 szt/ha — spadek. Przypuszcza się jednak, że ten spadek nie był spowodowany wyżerowywaniem glonów przez ryby, lecz innymi czynnikami. W obecności tołpygi białej znacznie zwiększyła się liczebność i biomasa Bacillariophyceae.

Dodatkowa obsada amura białego w ilości 1500 szt/ha wprowadzona do stawów karpiowych o obsadzie 2000 szt/ha karpia nie wywarła wpływu na biomasę i skład gatunkowy glonów. Przyczyną tego była zapewne mała masa amura w porównaniu do karpia (ciężar odłowionego karpia 1105 kg, amura — 164 kg). Przypuszczalnie słaby rozwój amura był związany z niedostatkiem roślinności wyższej w badanych stawach i z nieumiejętnością wykorzystywania przez amura paszy sztucznej.

W warunkach polikultury karpia z trzema gatunkami ryb roślinożernych (karp 2000 szt/ha, tołpyga pstra 1500 szt/ha, amur 1500 szt/ha, tołpyga biała 1500 szt/ha) nastąpił wzrost biomasy glonów o 45% i spadek ich liczebności o 60%. Biomasa glonów była wyższa, niż w innych wariantach z niższą obsadą ryb roślinożernych. Szczególnie zwiększyły swą biomasę Bacillariophyceae i Cyanophyta, a zmniejszyły Chlorophyta.

Tak więc w obecności tołpygi białej i pstrej spadła liczebność a wzrosła biomasa glonów. Małe formy glonów ustępowały większym. Większe glony zatrzymują się na aparacie filtracyjnym tołpyg.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	655–466	1974

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THE BIOENERGETICS OF THE GASTEROSTEUS/SCHISTOCEPHALUS HOST-PARASITE SYSTEM

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ABSTRACT

The energetics of a host-parasite system are described for the plerocercoid stage of *Schistocephalus solidus* living in the perivisceral cavity of the fish *Gasterosteus aculeatus*. Respiration rates, growth, ovarian maturation, activity and production results are discussed. The parasite was found to increase the host's metabolism and the growth efficiency of the entire host-parasite complex whilst reducing host growth and fecundity. The energy relationships are used to explain delayed ovarian maturation and discussed as to their significance in the control of the stages in the life cycle of pseudophyllidean tapeworms.

1. INTRODUCTION

It was considered that a quantitative approach to the field of host-parasite relationships was long over due. The literature is chiefly concerned with the effects of parasites on their host and considers that parasites are able to direct the host's food to their own use. This results in a retardation of growth rate often causing actual loss of weight. Such comments are fairly common and are apparently based on little actual experimental data.

In fish the qualitative effects of parasitization by larval pseudophyllideans are well documented with the literature reviewed by Williams (1967) and Arme, Walkey (1970). Attempts to quantity the effects of parasites on their hosts, using the methods of ecological energetics, have been made by Walkey, Meakins (1970) and Kennedy (1972). Work on the effects of parasitism on host growth have been carried out by a number of workers including Lechler (1935) and Miller (1945). Quantitative studies on respiration in host-parasite systems has been carried out in copepods by Klekowski, Guttowa (1968), in tubeficids by Kennedy (1972), and in fish by Walkey, Meakins (1970).

In the present work the energy transformations occurring in a host-parasite system were investigated. The system used was the plerocercoid stage of the pseudophyllidean cestode *Schistocephalus solidus* Müller 1776 living in the perivisceral cavity of the fish, the three spined stickleback, *Gasterosteus aculeatus* L. This system was chosen for the following reasons: Firstly, both uninfected and parasitized fish were easily obtainable. Secondly, the parasite can achieve enormous size accounting for up to 80% of the combined weight of the host and parasite (Hopkins, Smyth 1971). Thirdly, full somatic development of the parasite occurs during this stage with little or no gonadial maturation and the worm in anopolytic (Hopkins, McCaig, 1963). Thus the storage of high energy gonadial products is at a minimum and no losses occur due to shedding of proglottides. Fourthly, it was considered that the parasite inside the perivisceral cavity of the fish, without any direct access to the external environment, offers a more controlled system than would be found in any of the gut-living helminthes. All metabolic requirements and waste products of the parasite must be taken from, or passed into, the host's coelomic fluid. Thus, all of the food utilized by the parasite must have been assimilated by the fish while respiratory and excretory losses are presumed to be mediated or metabolically accomodated by the fish host. Lastly, the life-cycle of this parasite was well documented (Hopkins, Smyth 1961, and Clark 1954).

The principles of ecological energetics have been summarized by Phillipson (1966) and the symbols standardized by Winberg (1971). The energy flow through a population can be expressed by the equation;

$$A=P+T \tag{1}$$

where A — the energy of assimilated food, P — energy of production, or growth, and T — energy metabolically utilized and released in all ways, measured as respiration.

The energy transformation pathways occurring in fish are described by Warren, Davis (1967). They assume that all the energy available to the system must be obtained from the assimilated food. However, not all assimilated energy is available for metabolism as a certain portion is lost during deamination and excreted via the kidneys, and, in the case of fish, through the skin and gills. The remaining absorbed energy is the net available energy which can be used for the maintenance of the processes necessary for life such as respiration, activity and the systems of feeding, digestion, transport and storage of food. It is considered that the energy remaining unused after these needs have been met is stored in the body as tissue and monitored as growth.

In parasitized fish the pathways are complicated by both the host and parasite occupying the same habitat so altering the measurable changes observed. In Fig. 1 the energy transformations described by Warren, Davis (1967) are modified to account for the parasite. It can be seen that the only new measurable energy exchange is considered to be parasite growth. The basic equation for parasitized animals may be derived from equation 1 as follows:

$$A_f + A_p = (P_f + P_p) + (T_f + T_p) \tag{2}$$

and because $R - E = A$, then

$$(R_f + R_p) - (E_f + E_p) = (P_f + P_p) + (T_f + T_p)$$

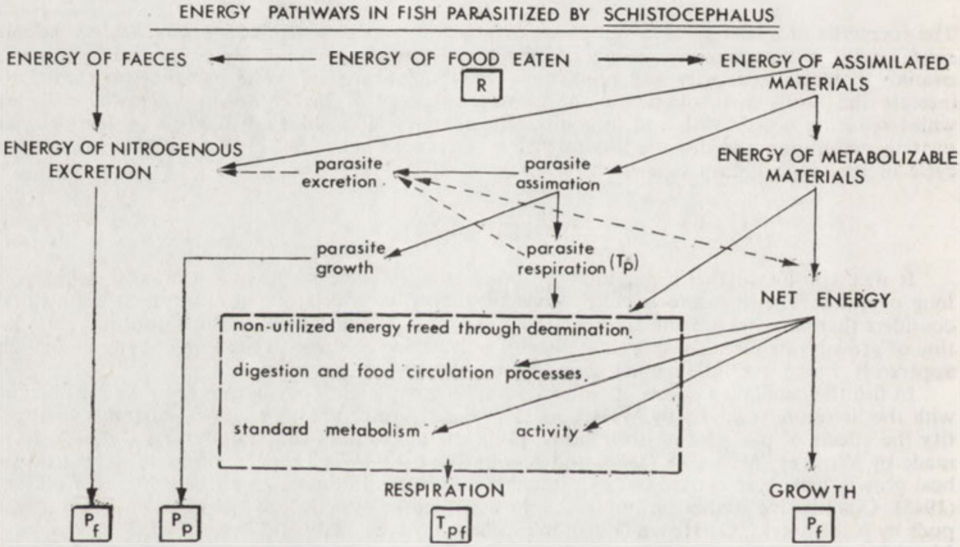

Fig. 1. Energy pathways in fish parasitized by Schistocephalus

where f and p denotes fish and parasite respectively, R — food ration and E — elimination, in this case waste products.

In the present system the assimilation, activity, excretion and metabolism of the parasite are metabolically accomodated by the fish host. So, equation 2 maybe re-written;

$$A = P_f + P_p + T_{pf} \tag{3}$$

where T_{pf} is the respiration of the parasitized fish including parasite metabolism and therefore different from T_f, the respiration of uninfected fish.

The complications involved in dealing with two animals living in close association necessitated adopting the following nomenclature:

u — uninfected fish,
p^- — parasitized fish where effects of parasite are excluded,
p^+ — parasitized fish including the effects of the parasite.

So growth results can be distinguished into those for uninfected fish, for parasitized fish without parasite growth and for the whole host-parasite complex.

2. METHODS

CALORIMETRY

The calorific values of all biological materials used were determined by oxygen bomb calorimetry. An adiabatic bomb calorimeter (Baird and Tatlock Ltd.) was used for samples over 40 mg weight and a Phillipson microbomb calorimeter (Gentry and Weigert Ltd.) for smaller samples. The material was dried in foil for one week at 90°C to give constant weight without losing the majority of the volatile fatty acids present. The results were expressed as calories/gram^{-1} of tissue combusted.

RESPIRATION AND ACTIVITY

Fish respiration rates were determined using a yellow Springs Instrument Company Oxygen Monitor model YSI 53. This consisted of a Clark-type polarographic electrode mounted in a respirometer vessel which was magnetically stirred. The water temperature was maintained at $15°C \pm 0.5$ by using a water jacket connected to a thermostatically controlled circulating water pump. Prior to experimentation the fish were kept for one month at 15°C and fed ad libitum on Tubifex. The fish were not fed for six days prior to experimentation in an attempt to reduce the variations due to different levels and times of feeding.

In the respirometer the fish were left for 15 minutes then allowed to swim without any interference and the oxygen consumptions measured over thirty minutes — this gave the routine respiration rate. The fish were then made to swim against a current in which they could just maintain their station. This initially gave an active or maximum rate which declined as the fish tired until after an hour the respiration levelled off producing the minimum rate. The respiration rates at these three levels of activity were converted into calories using the oxy-calorific coefficient 4.89 cal/ml of oxygen consumed (Winberg 1956).

The activity of the sticklebacks was monitored using photoelectric cells and visual recording. In the former work 9 uninfected and 5 parasitized fish were allowed to swim alone and undisturbed for 24 hours in a glass tank containing 3 cm of water. The sides of the tank were painted blue to prevent visual contact with external stimuli and illuminated overhead by a constant light source. In the bottom of the tank were mounted 11 photoelectric cells covering an area of 26×12 cm and individually connected via a modified Schmidtt trigger (Mullard ORP 12) to a channel of a Rustrac chart recorder.

After each run the fish were killed, weighed and the occurrence of parasites noted. The 24 hour traces were analysed listing the order of firing of the photoelectric cells and, knowing the distances between each cell, the distance travelled was calculated. This was the minimum distance travelled in 24 hours because the fish often swam around a specific cell and so appeared on the recording to be stationary.

GROWTH AND FEEDING

The feeding, growth and behaviour studies were performed in glass aquaria containing 10 litres of water circulated by an air pump. The bottom of each tank was covered to a depth of 1 cm with autoclaved sand. Because of the complicated feeding hierachies found in sticklebacks the fish were kept individually, though with visual contact with one another. In some behavioural studies the fish were kept in groups. All of the fish were fed ad libitum on Tubifex.

The feeding behaviour of Gasterosteus is complicated, the fish undergo a series of runs at the food involving three or four darts at it before successfully ingesting the Tubifex. No reason for this behaviour was apparent nor was the activity consistent. When the fish did feed it often engorged itself so that worms extruded from the already full mouth.

Fish growth and the amounts of food given were recorded weekly and the increases expressed as growth ratios (Ivlev 1945). Initially the weights of the solid waste materials produced were recorded by collecting all of the weekly washings from the tank and filtering the residues present. This showed that faecal waste accounted for 15% of the energy of the food eaten. The soluable matter would not account for more than 5% of the ingested food (Fromm 1963). So, as the results agreed with Ivlev (1960), an assimilation rate of 80% was assumed correct for this system. Any excess food found at the end of the experiment was subtracted from the amount given to give the amount of food consumed.

The growth rates of the plerocercoids were determined in vivo by surgically transplanting parasites of known weight into fish and maintaining the animals alive and healthy for one week at 15°C. This work was carefully controlled and the cuticles of the transplanted worms examined under an electron microscope to check that no damage to the worms had occurred. Details of the procedures used are published elsewhere (Meakins, Walkey 1973).

ENERGY BUDGETS

The energy budgets were calculated for one week at 15°C to reduce the weekly variations. The respiration rates used were the maximum rates for the fish including the parasite, when present, (T_f, T_{pf}) and as T_d the respiration when calculated as the difference between assimilation (A) and production (P), including P_p were relevant.

3. RESULTS

OTHER PARASITES

During the acclimation period before experimentation most ectoparasites were lost. These were *Gyrodactylus sp.* Nordmann 1832, *Trichodina sp.* Ehrenberg 1838 and occassionally *Argulus sp.* Claus 1875. Two endoparasites were found *Diplostomum sp.* Nordmann 1832 and *Schistocephalus solidus* Müller 1776. The former occurred as metacercaria in the eye and were ignored unless the infection was heavy enough to cause partial or total blindness. This left only the parasite which was required for this work.

STARVATION

The effects of starvation on the stickleback were shown by Walkey, Meakins (1970). Two groups of twelve fish, one parasitized the other uninfected, were fed for 24 hours ad libitum on Tubifex and then starved in fast flowing water. Seven of the uninfected fish were alive after 35 days having an LD 50 of 35+days whilst all the parasitized fish died by day 31 having an LD 50 of 15 days. The fish having the highest parasite index died first, though no clear effect of parasite burden on host longevity was found.

CALORIFIC VALUES

The calorific values determined in this work are expressed as calories/gram^{-1}, they are;

Fish$=4732\pm53.7$ ($n=64$) or individuals over 250 mg wet weight,
Faeces$=4373\pm123.6$ ($n=4$).
Tubifex$=5546\pm32.4$ ($n=9$).
S. solidus$=5003\pm61.9$ ($n=28$) for the plerocercoid stage.

RESPIRATION AND ACTIVITY

The presence of Schistocephalus in the fish Gasterosteus increases the host's metabolism significantly at both routine and active rates (Meakins, Walkey in prep.), whilst the differences at the minimum activity level were insignificant.

For the present work the active rates for the respiration of the stickleback are calculated using regression analysis as $Y=a+bX$, where Y and X are $\log y$ and x respectively. y — µl O_2/mg dry weight/hour, and x — dry weight of fish in mg. The regression equations expressing the respiration at 15°C at the maximum (active) level of activity are:

Uninfected fish (u)	$Y=1.5493-0.5126\,X$ $r=0.6897$ $p=0.01$,
Parasitized fish (p$^-$)	$Y=1.4063-0.3829\,X$ $r=0.6557$ $p=0.01$,
Parasitized fish (p$^+$)	$Y=1.1217-0.2839\,X$ $r=0.4440$ $p=0.1$,

where r — correlation coefficient and p — probability.

Table I. Growth of *Gasterosteus aculeatus* at 15°C

Fish. No.	Time (weeks)	Fish (*f*)			Parasite (*p*)		Food	
		Initial d.wt. (mg)	Δd.wt. (mg)	P_f (cal/wk.)	Δd.wt. (mg)	P_p (cal/wk.)	*R* cal/wk.	*A* cal/wk.
Uninfected fish								
1	4	281	473	140	—	—	1674	1338
2	4	252	739	219	—	—	2097	1678
6	3	140	351	138	—	—	1291	1033
8	4	183	253	75	—	—	1451	1161
9	3	198	214	84	—	—	1284	1027
12	5	93	260	62	—	—	1016	813
15	5	168	95	23	—	—	694	555
16	4	289	157	46	—	—	902	721
18	4	190	230	68	—	—	772	618
20	4	137	50	15	—	—	598	479
Parasitized fish								
3	7	114	324	55	34.6	25	1625	1300
4	7	109	503	85	51.1	37	1964	1571
5	4	107	45	13	29.1	36	976	781
7	4	160	169	50	32.7	41	1279	1023
10	4	166	173	51	8.4	11	1102	881
11	2	124	85	50	16.6	42	1004	803
13	3	75	61	24	25.0	42	428	343
14	5	162	71	17	70.3	70	951	761
17	4	240	−119	−35	79,0	99	634	507
19	4	214	308	91	27.0	34	1229	983
21	3	142	−34	−14	15.4	26	697	557

d.wt. — dry weight, Δd.wt. — change in dry weight, cal/wk. — calories per week.

Table II. Growth/food conversion ratios of fish from Table I

Condition of fish	mean K_1	mean K_2
Uninfected fish (u)	6.7 ± 1.75	8.4 ± 2.19
Parasitized fish without parasite	(p^-) 2.7 ± 1.6	3.6 ± 2.12
Parasitized fish including parasite	(p^+) 7.5 ± 1.86	9.6 ± 2.67

Chi-square test results comparing above results

Groups compared	γ^2	probability
$K_1 \cdot u$ v p^-	15.0	0.001
$K_2 \cdot u$ v p^-	15.6	0.001
$K_1 \cdot u$ v p^+	1.5	0.5
$K_2 \cdot u$ v p^+	2.6	0.2

In calculating an energy budget the rates of respiration vary according to the condition of the fish. If the fish has just fed the respiration rate may increase by over 100% (Saunders 1963). Likewise, activity and breeding behaviour account

Table III. Comparison of uninfected and parasitized fish maximum. Respiration rates

Dry weight (mg)	Maximum rate		T (calories/week)	
	u	p^+	u	p^+
50	4.7	4.3	193.1	177.5
100	3.3	3.6	273.6	291.6
200	2.3	2.9	382.8	476.5
300	1.9	2.6	468.3	640.8

Maximum rate — μl O_2/mg dry weight/hour. All dry weights refer to weights of fish plus parasite, where applicable.

Table IV. Energy budgets for matched pairs of fish at 15°C for one week

Number		d.wt. $f, f+p$ (mg)	d.wt. p (mg)	A (cal)	P_f (cal)	P_p (cal)	T_{pf} (cal)	T_d (cal)
u	6	184	—	1033	138	—	370	895
p^+	3	184	30	1300	55	25	450	1220
u	9	225	—	1027	84	—	407	943
p^+	4	227	56	1571	85	37	529	1449
u	12	125	—	813	62	—	306	751
p^+	13	125	43	343	24	42	337	277
u	16	309	—	721	46	—	477	675
p^+	17	321	96	507	—35	99	670	443
p^+	19	299	47	983	91	34	641	858

d.wt. $f, f+p$ — mean dry weight of fish plus parasite when present.
d.wt. p — mean dry weight of parasites.
T_d — respiration calculated as $A - P_{(f, f+p)}$.

for variations in metabolism (Fry 1957). So, in Table III the maximum rates T_f and T_{pf} are compared for differing size of fish and in Table IV the respiration calculated as the difference between assimilation and production is also compared.

The activity experiments showed that in 24 hours all the fish swam similar distances. The mean distances travelled were 3839.2 cm for uninfected and 3870.9 cm for parasitized fish. No significant difference was found between these groups. However, the results do show that without external stimuli Gasterosteus undergoes marked activity over a 24 hour period. This was important as it confirmed visual observations that the fish were highly active and that the maximum respiration rates should be used in the energy budgets.

GROWTH AND FEEDING

In Table II the growth/food conversion ratios are shown from the data in Table I. The efficiency formulae used are based on those produced by Ivlev (1945) and Brody (1945). They are:

$$K_1 = \frac{P}{R} \cdot 100\%; \qquad K_2 = \frac{P}{A} \cdot 100\%.$$

The conversion ratios have been averaged and the means for u, p^- and p^+ categories statistically compared using Chi-square test. The differences between u and p^- give values for chi of 15 and 15.6 for K_1 and K_2 both highly significant. However when u is compared to p^+ the values are 1.5 and 2.6 for K_1 and K_2 giving a probability of only 0.5 and 0.2.

ENERGY BUDGETS

In Table IV energy budgets of matched pairs of parasitized and uninfected fish are compared with all the parameters expressed as calories per week at 15°C. The average values for growth, assimilation, respiration measured and calculated are given below with the 95% confidence limits.

Growth (P):
  Uninfected fish $u = 87.0 \pm 38.6$,
  Parasitized fish $p^- = 35.2 \pm 20.6$,
  Parasite only $= 42.1 \pm 14.1$,
Assimilation (A):
  Uninfected fish $= 942.3 \pm 235.2$,
  Parasitized fish $= 864.6 \pm 134.2$,
Respiration (T) at maximum rate:
  Uninfected fish $u = 404.6 \pm 42.8$,
  Parasitized fish $p^+ = 490.6 \pm 62.3$
Respiration (T_d) calculated as difference between A and P:
  Uninfected fish $u = 855.3 \pm 145.4$,
  Parasitized fish $p^+ = 787.3 \pm 100.5$.

BEHAVIOUR AND OVARIAN MATURATION

Observations on 12 breeding pairs of infected fish during June, July and August showed that no females containing plerocercoids spawned while the uninfected controls bred successfully. In all breeding experiments the male courted the female, built nests and underwent zig-zag display activity. The male then approached the female and rubbed her swolen abdomen with his nose. After a few days when the female had failed to spawn the male either drove her out of this territory or attacked her, often resulting in her death. The gravid female externally appears very similar to a parasitized fish and probably the swollen abdomen is the inate releasing mechanism for the courtship display of the male. Visual observations in the field showed that heavily parasitized fish tended to swim more slowly and nearer the surface of the water than did uninfected animals. This is especially apparent in small fish containing a large number of parasites and maybe due to changes in the specific gravity of the animals or interference in their ability to undergo body flexion while swimming.

A survey of the weights of ovary present in the fish from June to November showed that it accounted for 11.4% in June and 5.6% in September of the total body weight of uninfected individuals. Whilst when *S. solidus* was present these values were reduced to 5.5% in June and 3.7% in September. In October and November the majority of parasitized fish had ovary whilst only a few uninfected fish did. By late November parasitized fish contained 40 mg of gonad compared with 25 mg in the uninfected group.

4. DISCUSSION

The respiration calculated as T_d reflects the effects of feeding, swimming and basal metabolism of the fish during the feeding experiments. Generally uninfected fish were more active during this time. However, at any given state of activity the respiration T_f, T_{f+p} was higher for fish containing *S. solidus* than for uninfected sticklebacks. This is indicative of the parasite either having higher oxygen consumptions or producing gross stress symptoms in the host.

Comparison of respiration rates shows that at 15°C the parasitized fish need at least 2.5 µl O_2/mg dry weight/hour more oxygen than does the uninfected fish. This value is four times the in vitro value for this parasite (Davies, Walkey 1966). The medium, sugar-free Tyrode, used suggests that only the basal metabolism was measured which would be less than the routine in vivo rates especially during this growth phase where the parasite is bathed in the fish's perivisceral fluid being free to move against the walls of the alimentary canal and blood vessels. In this habitat the nutriment levels are optimal for growth which requires high energy uptake and therefore increased metabolism. The oxygen requirement of an 18 mg dry weight worm would be 35 µl O_2/hour which is similar to the levels found in free-living poikilotherms according to Hemmingsen (1960).

In the life cycle of *S. solidus* the plerocercoid grows from about 0.1 mg to over 100 mg inside the fish host by processes involving the storage of glycogen, which may account for 50.9% of the worm's body weight (Hopkins 1950). In its life the parasite occupies the following habitats:

1. the adult lives in the intestine of a bird;
2. the egg and coracidium are free-living in water;
3. the procercoid is in the haemocoel of Cyclops;
4. the plerocercoid is in the perivisceral cavity of a fish.

This system involves marked environmental changes coupled with different parasite development. In the copepod stage, Klekowski, Guttowa (1968) have shown, using the related procercoid of *Diphyllobothrium latum* in the haemocoel of *Eudiaptomus gracilis*, that the parasite reduces the oxygen consumption of the host. This contrasts with the present work. However, the embryos and coracidia of *Triaenophorus nodulosus* and *Diphyllobothrium latum* large changes from aerobic to anearobic metabolism in these early stages (Michajłow et al. 1971).

The transition from plerocercoid to adult in *S. solidus* requires the former to be over 30 mg dry weight and a temperature change to above 34°C for 24 hours. The adult worm will then commence egg laying. This temperature change may control the enzyme systems functioning at any one time (Sinha, Hopkins 1967). Thus it is possible that pseudophyllideans may contain many series of iso-enzymes controlling the development of the animals at each stage of the life cycle. These systems may be functional for only parts of the cycle being controlled by various environmental triggers. Such a system would explain how the parasite manages to hatch, differentiate its organs and mature at different times so making the maximum use of the resources available and successfully completing its life cycle.

The growth efficiencies for the host-parasite system indicate that the parasite considerably reduces fish growth. This has important commercial implications in other systems. However, if parasite growth is included then the host-parasite system has a conversion efficiency just higher than that found in uninfected fish.

The value of energy budgets is limited by the assumptions made in their calculations. These are:

1. All the energy used in growth is uniformly converted into tissues of the same average calorific content of either the parasite or fish tissue as a whole.

2. The respiration rates of an animal under normal feeding conditions are the same or similar to those measured in the respirometer.

3. All efficiencies of conversion are approximately 100% and are accounted for in the system devised.

None of these assumptions are completely correct. Fish store different materials according to the time of year, ranging from high energy materials, such as gonad in spring, to low energy structural materials, such as skin and bone during rapid growth phases. Similarily, the respiration rates of fish vary due to activity, feeding, condition and temperature. In most of these cases the variations can be recognised and accounted for. Finally, no system exists in which energy is not used or gained in the conversion of materials from one form to another. Though the efficiencies of reactions in both chemical and biological processes can approach the 100% level.

The assumption that assimilation was 80% of the ingested food may have been incorrect especially in the case of parasitized fish. There is a distinct possibility that the latter animals had higher assimilation rates than those used and, if this was the case, would increase the values of T_a.

The effects of parasites on the behaviour and physiology of the three-spined stickleback have been recorded for a number of differing habitats (Clark 1954, Vik 1954, Arme, Owen 1967, Walkey, Meakins 1970, Lester 1971; Pennycuick 1971). Vik (1954) showed that the fat layer normally lining the alimentary tract of Gasterosteus during late autumn was lacking in animals parasitized by *S. solidus*. This is an obvious case of loss of host energy reserves due to parasitism. The calorific value of fat exceeds 9000 cal/g^{-1} compared with 4732 cals/g^{-1} for the average tissue content of Gasterosteus. The loss of the fat may explain the disappearance of heavily parasitized fish during the winter months.

The ovarian development of the stickleback is delayed when *S. solidus* is present (Arme, Owen 1967, Pennycuick 1971, Meakins in prep.). Arme, Owen (1967) showed that the parasite does not alter the host's pituitary in any way unlike Ligula which castrates the host changing the latter's pituitary possibly by secreting an antigonadotrophic substance (Arme 1968). The delay in gonad maturation due to *S. solidus* can be explained solely on the loss of energy due to the parasite (Meakins in prep.). The ovary contains 6250 cals/g^{-1} compared with 4732 and 5003 cals/g^{-1} for the fish and parasite respectively. So maturation involves a large energy demand. In uninfected fish in autumn there is very little gonadial material present, as the fish have spawned, and fat reserves are present. In parasitized fish the fat is lacking and gonad is always present. The parasitized fish during the summer contain less ovary and apparently are unable to spawn. It is possible that the distortions of the abdomen due to the worms prevents the female fish from spawning or makes her appear more gravid than she really is.

The energy remaining in late autumn is probably reabsorbed by the fish. In November parasitized fish on average contain 40 mg of gonad compared with 25 mg in uninfected animals. The latter refers only to those fish found to contain gonad, which are mostly those which were attempting a second breeding season. This group consisted of only 15% of the uninfected fish sampled. So the large energy requirements of the plerocercoid brings about the delay in ovarian maturation as well as possibly mechanically preventing spawning. In this way the parasite not only obtains all the energy it requires for growth but also drastically alters the breeding populations of the host fish.

The activity of the fish is altered by the parasite showing reduced swimming speed, changes in bouyancy and inability to breed. So the parasite makes the host easier to catch by the next stage in the life cycle, a bird.

Recently, Kennedy (1972) working on the cestode *Caryophyllaeus laticeps* has attempted to follow the energy flow through the life cycle. He found that the host *Psammoryctes barbatus* over a 6 month period lost 50% of its production when this parasite was present. Work by Michejda, Boczoń (1972) on the bioenergetics of muscle infected with the nematode *Trichinella spiralis* was limited by the difficulties in measuring very small changes. The authors were unable to show any changes in the mitochondria bioenergetics due to this parasite. It is hoped that in future workers will be able to measure the energy transformations occurring at all stages of the life cycles of parasites. This may then indicate the importance of each stage in the ecology and physiology of the host-parasite system which remains one of the most economically important and least understood of the animal associations.

5. SUMMARY

The energetics of the Gasterosteus/Schistocephalus host-parasite complex are discussed together with the pathways concerned. It was found that:

1. the parasite increased the respiration of the fish host;
2. the parasites increased the growth efficiency of the system at the expense of host growth;
3. no significant difference was found between the activities of parasitized and uninfected fish, though the former were noted swimming nearer the surface of the water and more slowly;
4. estimations of parasite respiration in vivo suggested that the plerocercoids had higher oxygen consumptions than previously allowed for being similar to those of free-living poikilotherms;
5. the ovarian development in parasitized fish was markedly reduced possibly due to lack of energy. These fish were observed to be unable to spawn successfully.

Schistocephalus occupies several different habitats during its life cycle and suggestions are made as to how these differing environments may effect their metabolism.

ACKNOWLEDGEMENTS

I wish to thank Prof. K. Simkiss, Queen Mary College, London, for providing facilities in his department and the managers of Mid-Kent Water Company for allowing me the use of Eccles reservoir for the study and collection of Gasterosteus. This work was supported by the Natural Environment Research Council under grant GR3/705 to Dr M. Walkey.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	467–479	1974

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THE EFFECT OF VARIOUS TYPE OF FOOD ON THE GROWTH AND CHEMICAL COMPOSITION OF THE BODY OF PERCH (*PERCA FLUVIATILIS* L.) IN LABORATORY CONDITIONS

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ABSTRACT

The effect of different type of food, such as Tubificidae and *Leucaspius delineatus* L., on the intensity of consumption and growth rate of *Perca fluviatilis* L. has been demonstrated. Differences in body composition and calorific values between perches fed with invertebrates and those fed with fish were also established and confirmed by studies on fish taken from natural environment. Perches fed with fish are characterized by higher consumption intensity, quicker growth rate and also higher content of lipids in the body bringing about higher caloricity.

1. INTRODUCTION

The food base in the natural water environment is of great variety. The kind of the food taken in is dependent on that basis and also on preferences in relation to the available food determined by fish species and age. Karzinkin (1952) stated that together with the growth of fish their food preferences are changing and that change has a strictly defined character. In predatory fish there has been observed a gradual transition from feeding on invertebrates into feeding on food composed of other kinds of fish. However, it does not occur in the same manner in all of them. As stated by Popova (1965), pike (*Esex lucmius* L.) is characterized by a quick growth and early transition into feeding on fish independently from environmental conditions in which it lives. Perch (*Perca fluviatilis* L.), on the other hand, is characterized by a slow growth, dependent on environmental conditions, and a late transition into feeding on fish. Invertebrates, however, always enter into the composition of its food even though in minimal quantities (Terent'ev 1937, Bodnek 1968, Karzinkin 1952, Ivanova 1956, Filuk, Żmudzinski 1965, Chikova 1970, and others). Perch shows a high degree of adaptability to various environments and to feeding on food of great variety. This influences also its outward appearance. Perches living in different environmental conditions differ from one another so much that even some attempts were made to isolate distinct varieties within the species (Żuromska 1961). Quite often one can find in a single water body, especially when there are various feeding ground conditions, a diversified *Perca fluviatilis* L. population (Tolchanov 1952, Dianov 1955, Tesch 1955, Mednikov 1963).

Alm (1954) while studying the growth rate of *Perca fluviatilis* L. on the basis of observations of their operculums has noticed in a single water body the presence of two distinct groups of perch differring from each other by the speed rate of their growth. Popova (1960) has investigated perch from the delta of the Volga River, likewise. Besides the growth rate she was determining also the contents of alimentary canals. She has observed, as well, the occurrence of two types of perches: the quick-growing ones, in which 90% of the alimentary tract contents consisted of fish, and the slow-growing ones, with hardly 23% of fish in the content of their alimentary tracts. This fact denotes differentiation of fish living in the environment where a part of population due to the lack or inaccessibility of food has adapted itself to the intake of food composed mainly of invertebrates.

It seemed to be a matter of interest to find out what kind of changes in physiological processes are produced in perch at the age of transition into feeding on fish food by feeding them with food composed exclusively of either invertebrates or fish. Therefore the purpose of this study was to determine what quantities of food were taken in by perches (*Lerca fluviatilis* L.) and how they are making use of it for the building up of their bodies when they are fed either with Tubificidae invertebrates or *Leucaspius delineatus* H. fish.

2. MATERIAL AND METHODS

EXPERIMENTAL CONDITIONS

Perches used for measurements in laboratory experiments were taken from the ponds of the Institute of Inland Fisheries at Żabieniec, near Warsaw. They were 10–16 cm long fingerlings weighing 20–60 g. With the view to obtain comparative data some measurements were carried out on perches taken directly from the natural environment i.e. from ponds at Żabieniec and from the Mikołajskie Lake.

It was noticed at the transport of the first lot of fish that despite of the usual precautions all the perches has died; the shock resulting from fishing them out and taking over into the laboratory was too violent. The drawing out of the pond water, transport, and new conditions in the laboratory tanks produce a violent reaction in perches manifested by hasty, in-co-ordinate movements and loss of bodily equilibrium. They swim round and round in circles, come up to the water surface, breathe rapidly and heavily, and turn over upside down with their bellies up.

So, the subsequent catching and transporting of perches to be used in experiments were performed with special care and additional precautions. They were transported immediately after they had been caught in pond water continuously aerated. In the laboratory they were put into large screened tanks and after a couple of days transferred into smaller aquariums where they were kept in strictly determined experimental conditions.

Perches kept in laboratory conditions reacted very violently to any movement near their aquarium, to feeding, and especially to weighing. Startled perches bumped against the glass walls of the aquarium and got injured and in consequence quite often they were affected with aphthae which subsequently increased the death rate in the experimental group of fish. Therefore, to avert the accidents the glass of the aquarium has been partly shaded.

Difficulties connected with the culture of perches in laboratory conditions caused a decrease in the number of fish under examination. Consequently, they have brought about also a decrease in the number of the presented results as in the calculations only those measurements were taken into consideration that had been carried out on perches which have survived until the end of the experiment.

Perches in the laboratory were fed on two different kinds of homogenous food, either invertebrates (Tubificidae) or fish (*Leucaspius delineatus* H.). Food was always given in over-abundance, its amount exceeded about three times the value of the average daily ration. Perches were kept singly in 10 l aquariums with water changed every day and constantly aerated (average content of oxygen was about 8.7 mg O_2/l).

In the period of intensive food intake and growth of *Perca fluviatilis* temperature of water in their natural environment approximates 20°C. A number of laboratory studies of perch were carried out at that temperature (Winberg, Belyatskaya-Potaenko 1963, Bogdanov, Strel'tsova 1953). On the basis of those data and also the results obtained by other authors who have studied perches at various temperatures of water (Bily 1938, Stroganov 1962, Mann 1965) in the present study temperature of 20°C was maintained throughout all the versions of experiments.

Before starting the measurements, perches supplied from their natural environment were kept for at least one month in the experimental conditions. It has been proved that after that initial period of time, in principle, perches were eating regularly and growing systematically. Thus, it was assumed that that time was sufficient for acclimation. Measurements were made during a period of about two months. Perches fed on Tubificidae were under examination in the period between September and December, the others fed on *Leucaspius delineatus* H. in the period between May and July.

Nevertheless, since in normal conditions there is a seasonal variability in the growth rate and a particularly distinct one among predatory fish (spring and autumn peak of growth) (Popova 1965, Bodnek 1968), it seemed to be essential to find out whether the time of the year had any influence on the obtained results. With this purpose in view additional experiment was carried out to determine the growth rate of perches fed on Tubificidae in the May–July period. No significant differences were observed as compared with the autumn experiment. In conclusion, it has been admitted that in the given experimental conditions (constant temperature and overabundance of food) the influence of the seasons of the year may be left out of account.

DETERMINATION OF THE FOOD RATION

Perches, in the range 10–16 cm long, used in the experiment can feed on invertebrates and fish, as well (Żuromska 1961, Girsa 1962, Chikova 1970). In the present experiments both types of food were used — invertebrates (Tubificidae) and fish (*Leucaspius delineatus* H.). Tubificidae were present in the intestines of perches living in their natural environment and they are also often used for food of fish in laboratory conditions (Bily 1938, Stroganov 1962).

Daily food ration for perches fed on Tubificidae was determined by weight in terms of the loss of food after 24 hrs exposition (in milligrams of wet weight). In the control portion dry weight and ash contents were determined as well as the calorific value of food. On the basis of the per cent ratio of dry weight contents and the calorific value of 1 mg of dry weight of Tubificidae the daily food ration has been expressed in dry weight and calories. *Leucaspius delineatus* H. used as fish food were taken from Żabieniec, near Warsaw. The daily ration was determined by the number of *Leucaspius delineatus* individuals eaten up by perches during 24 hours. Those specimens were approximate in size and selected in such a way as to be easily caught by perches. Vashchenko (1962) stated that perch fingerlings are able to eat fish 20–50 mm long.

Fortunatova (1961), Girsa (1962), Mantejfel' et al. (1965). Chikova (1970) declare that perches about 16 cm long can catch fish about 40 mm long. Popova (1965) gives a general diagram of dependence between the length of prey and the length of a predator. According to those data perches of the same length as those used in the present experiment are able to catch the specimens of *L. delineatus* of length ranging from 20 mm up to 100 mm long, though they feed mainly on victims about 35 mm long. Therefore, fish individuals selected for food were in the range of 35–45 mm long.

The length of 15 *L. delineatus* specimens was determined in millimetres and their body weight in milligrams of dry weight. On that basis it has been assumed that one fish contains 230 mg of dry weight. From the number of fish eaten up by one perch the daily food ration was determined in mg of dry weight and in calories.

DETERMINATION OF THE GROWTH RATE OF THE BODY WEIGHT OF PERCHES

Perches from experimental cultures were weighed every 10 days with accuracy exact to 50 mg. On the basis of changes in the body weight the mean daily growth was determined. The obtained results were converted into milligrams of dry weight and calories. The relative growth rate in percentage of the body weight was also calculated for each individual according to the formula (Winberg 1956):

$$C_p = \left(10^{\frac{1}{n}\log W_n - \log W_0} - 1\right) \cdot 100$$

where: C_p: — relative daily growth in per cent, W_0 — body weight at the beginning of experiment, W_n — body weight at the end of experiment, n — number of days.

DETERMINATION OF THE CHEMICAL COMPOSITION AND CALORIFIC VALUE

Perches and their food were examined as regards chemical composition (dry weight, ash, total nitrogen and lipids contents) and calorific values.

Material used for measurements was prepared in the following manner: *Perca fluviatilis* taken from their natural environment and divided into groups according to the place where they were caught and their weight. Homogenates were made for each particular group and the dry weight and ash contents and calorific values were determined in them.

Perches from experimental cultures were homogenized one by one, separately, and dry weight and ash content was determined. In the mixture of homogenates of perches fed with uniform food (either Tubificidae or *Leucaspius delineatus*) the content of total oxygen and lipids was determined as well as the calorific value. In order to determine individual variability, calorific values were also measured in homogenates of each individual fish of six specimens taken from each group fed on a different food variant.

All the determinations for *Leucaspius delineatus* were performed in a mixture of homogenates composed of 20 fishes.

In the case of Tubificidae the dry weight content was determined in the control test every time while giving the food ration to perches. All the other determinations were made in the mixture of homogenates of Tubificidae collected from all the control test samples.

The dry weight was determined by drying up the given material to the constant weight in the temperature of 60°C. Then, ash content was determined in the dry weight by burning to a constant weight in a muffle furnace at the temperature of 550°C. The quantity of lipids was determined with the Stern, Shapiro (1953) method and the total oxygen content by means of the Kiejdahl method. Determinations of the caloric values were performed in the Phillipson microbomb (Phillipson 1964).

3. RESULTS

GROWTH RATE OF THE BODY WEIGHT

The growth rate of the body weight of *Perca fluviatilis* fed with various food is shown in Fig. 1, as growth of mean body weight of the examined fish. As can be seen quite clearly the growth rate of perches fed with *Leucaspius delineatus* in higher than of the other ones fed with Tubificidae. Figure 2, showing changes in the body weight calculated in per cent of the initial weight, characterizes that growth more precisely and indicates great individual variability. By comparing the obtained graphs it is easy to ascertain that perches fed on fish are growing quicker and the differences in the speed of their growth are marked distinctly from the very beginning of the experiment. The daily growth rate of each perch was also calculated in per cent of the body weight (after Winberg 1956). It has been found that the relative daily growth of fish fed with Tubificidae is less than 1 %. On the other hand

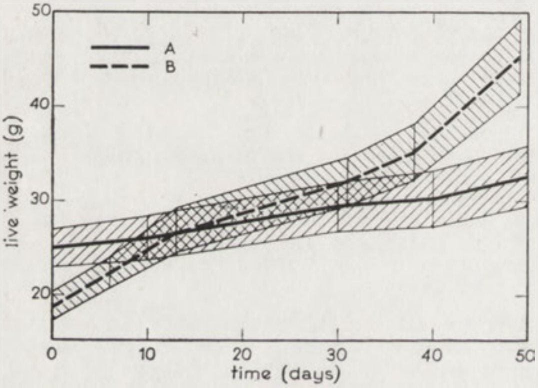

Fig. 1. Mean growth of the body weight of *Perca fluviatilis* L. fed with different food. A — those fed with Tubificidae, B — those fed with *L. delineatus*

Table I. Daily growth of *Perca fluviatilis* L. in the experimental cultures

Food					
Tubificidae			*Leucaspius delineatus*		
Mean wet wt. of perch (g)	Growth *P*		Mean wet wt. of perch (g)	Growth *P*	
	mg dry wt./24 hrs	cal/24 hrs		mg dry wt./24 hrs	cal/24 hrs
16.3	24	110	20.7	78	403
18.9	36	164	25.4	77	400
20.2	39	176	29.0	118	614
23.6	69	310	29.9	130	676
23.8	38	171	35.3	108	566
24.1	37	167	36.3	115	600
25.6	77	346	37.3	93	486
29.1	56	252	47.0	197	1026
29.7	87	391	45.0	157	816
38.0	65	291			

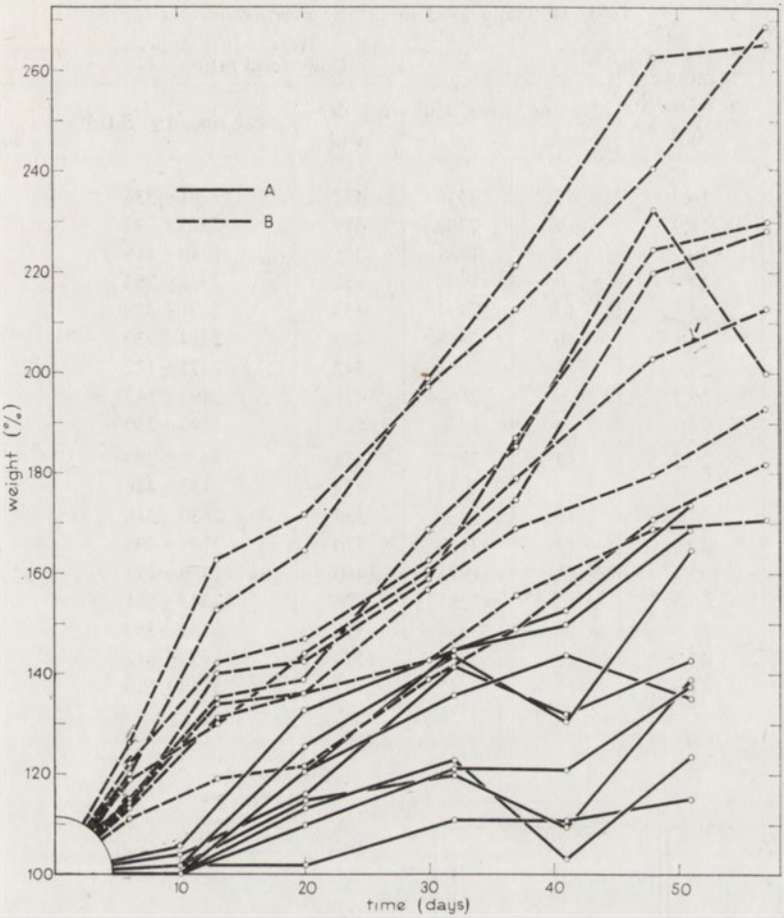

Fig. 2. Growth rate of the body weight of *Perca fluviatilis* L. in the course of the experiment calculated for each particular fish and expressed as percentage of their original weight. A and B as in Fig. 1

perches fed with *Leucaspius delineatus* are characterized by growth rate higher than 1%. Mean daily growth rates characteristic for individual perches fed with varied food are presented in Table I. They confirm the quicker growth of perches fed with fish and likewise they indicate great individual variability and dependence of growth on the body weight of the examined perches.

FOOD RATION

Table II shows mean daily food ration of individual perches fed with different food. It can be seen that perches fed with fish are eating more intensely and their food intake is bigger. The obtained results indicate also great individual variability. However, in spite of that variability there is a marked tendency towards an increase of food intake together with the increase in the body weight. This occurs independently from the type of the consumed food (Fig. 3).

Table II. Daily food intake by *Perca fluviatilis* L.

Food	Mean wet wt. of perch (g)	Daily food ration				
		No. of tests	mg wet wt.	mg dry wt.	cal (mean ± S.E.)	% of body wt.
Tubificidae	16.3	8	2354	432	2291 ± 333	8.81
	18.9	9	2282	419	2223 ± 293	7.37
	20.2	12	1831	335	1780 ± 115	5.53
	23.6	9	3008	552	2927 ± 283	7.78
	23.8	10	2494	453	2401 ± 228	6.33
	24.1	6	2480	455	2414 ± 230	6.28
	25.6	6	2953	542	2877 ± 122	7.04
	29.1	6	2568	471	2497 ± 143	5.39
	29.7	9	2876	527	2796 ± 230	5.91
Leucaspius delineatus	20.7	12	2987	478	2437 ± 280	8.18
	25.4	12	2637	422	2152 ± 448	5.36
	29.0	12	3468	555	2830 ± 516	6.49
	29.9	11	3112	530	2703 ± 495	6.01
	35.3	12	2875	460	2350 ± 275	4.41
	36.3	12	3285	526	2683 ± 581	4.91
	37.3	12	3350	536	2734 ± 352	4.87
	45.0	12	3581	573	2922 ± 612	4.32
	47.0	12	4612	732	3733 ± 510	5.27

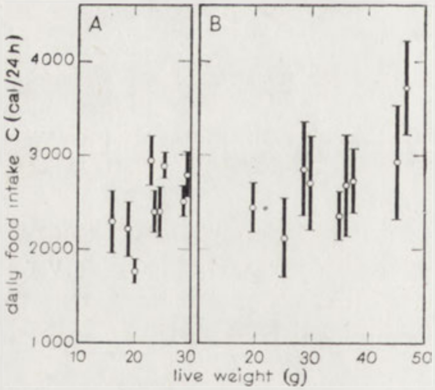

Fig. 3. Dependence of the mean daily food intake by *Perca fluviatilis* L. upon the mean body weight of the examined fish. A and B as in Fig. 1

Table II shows also the so-called relative food ration, its values indicate its dependence on the body weight of the examined perches. One can observe a decrease in the relative food ration value together with the increase in the body weight of fish. This dependence is shown in Fig. 4.

On the basis of the mean daily food ration and mean daily growth rate of various individual perches the corresponding values of the food coefficient were de-

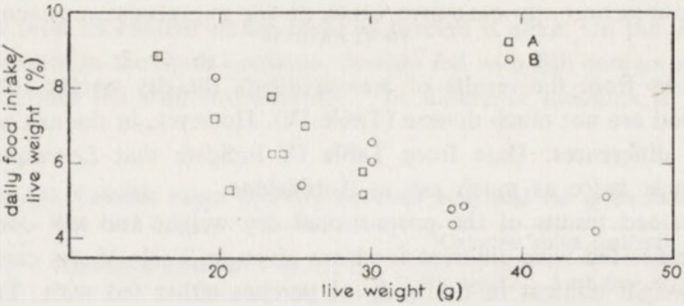

Fig. 4. Dependence of the relative food intake on the body weight of *Perca fluviatilis* (mean values). A and B as in Fig. 1

termined. The food coefficient characteristic for perches fed with Tubificidae is in the range of 4.57–17.8, and for perches fed with *Leucaspius delineatus* 3.6–6.1. Substantiality of the difference in those coefficients was ascertained by criterion *U* of non-parametric statistics at $P=0.01$.

DRY WEIGHT AND ASH CONTENTS AND CALORIFIC VALUE OF THE BODY OF PERCHES TAKEN FROM THE NATURAL ENVIRONMENT

Results from measurements of perches taken from their natural environment are presented in Table III. As can be seen the dry weight content in the fish body does not change much, while the ash content and calorific value are showing some differences. Perches caught in the Mikołajskie Lake in March have more ash and lower calorific value than those caught in September in the fish-ponds at Żabieniec. Since the calorific value of the ash-free dry weight is also lower this would indicate a lower content of lipids of the former fish.

Table III. Dry weight and ash contents and calorific values in perches taken from natural conditions

Area	Body weight of 1 ind. (g)	No. of specimens	Dry wt. (%)	Ash in dry wt. (%)	Calorific value (cal/mg)	
					dry wt.	ash-free dry wt.
Mikołajskie Lake	4.7 (4.0–5.7)	11	28.1	19.1	4.22	5.21
	11.3 (8.1–12.9)	12	26.9	20.9	4.16	5.26
	18.9 (15.2–20.4)	5	26.3	22.5	4.15	5.35
Żabieniec	15.0 (14.2–17.4)	8	28.4	17.2	5.22	6.27

CHEMICAL COMPOSITION AND CALORIFIC VALUE OF THE EXPERIMENTAL PERCHES AND THEIR FOOD RATIONS

As follows from the results of measurements the dry weight contents in the provided food are not much diverse (Table IV). However, in the ash contents there are greater differences. Data from Table IV indicate that *Leucaspius delineatus* contain almost twice as much ash as Tubificidae.

The obtained results of the proportional dry weight and ash contents in the body of perches fed with different food are given in Table V. As can be seen the mean dry weight content in the body of perches either fed with Tubificidae or *Leucaspius delineatus*, is the same and does not diverge much from the values characteristic for perches taken from natural conditions (Table III). Also here, there is a difference in the ash content, a significant one at the 95% confidence limits. Mean proportional ash content in the dry weight of perches fed with invertebrates is higher than in those fed with fish (in consumed food the proportion is inverse).

From Table VI, presenting the lipids and total nitrogen contents in the examined perches and their food, it results that despite the difference in the total nitrogen

Table IV. Dry weight and ash contents in food given to perches in the experimental cultures

Examined material	Dry weight (% of wet wt.)	Ash (% of dry wt.)
Tubificidae	18.0	6.6
Leucaspius delineatus	15.8	11.0

Table V. Dry weight and ash contents in the body of *Perca fluviatilis* L. from the experimental cultures (mean and 95% confidence limits)

Given food	No. of individuals	Dry weight (% of wet wt.)	Ash (% of dry wt.)
Tubificidae	12	29.8 26.3–33.3	19.5 19.0–20.0
Leucaspius delineatus	14	29.5 28.7–30.3	16.8 16.5–17.7

Table VI. Lipids and total nitrogen contents in the dry weight of *Perca fluviatilis* L. and their food ration

Examined material	Lipids (% of dry wt.)	Total nitrogen (% of dry wt.)
Tubificidae	20.0	8.4
Leucaspius delineatus	21.0	10.6
Perca fluviatilis fed with Tubificidae	17.0	9.5
Perca fluviatilis fed with *Leucaspius delineatus*	20.5	9.5

content in the food its content in the body of perches is alike. On the other hand, there is a difference in the lipids contents. Perches fed with fish contain more lipids than the other ones fed with invertebrates. The difference amounts to about 3% of dry weight.

Table VII. Calorific values of *Perca fluviatilis* body and the given food

Examined material	Calorific value (cal/mg)	
	dry weight	ash-free dry wt.
Tubificidae	5.35	5.72
Leucaspius delineatus	5.10	5.73
Perca fluviatilis fed with Tubificidae	4.51	5.60
Perca fluviatilis fed with *Leucaspius delineatus*	5.20	6.25

Table VII shows the calorific values in the perch body and the provided food. Perches fed with *Leucaspius delineatus* have a higher calorific value than those fed with Tubificidae. Those values determined separately for each fish show individual variability. The results varied by ±5% of the calorific value determined in the mixture of homogenates of perches fed with the same food.

4. DISCUSSION

As already mentioned above, perch (*Perca fluviatilis* L.) belongs to the kind of organisms that depending on the environmental conditions can feed on very varied food. Perch is the so-called facultative predator that always, to some extent, preys on invertebrates but when there is not enough fish in its environment then invertebrates constitute a high percentage of its food intake. The observed by Alm (1954) and Popova (1960) differentiation of the *Perca fluviatilis* population, consisting in the presence of perches feeding mainly on fish and besides them also perches feeding above all on invertebrates, both living in the same water body, gives evidence of their capability of a permanent adaptation to various kind of food.

Providing perches with uniform food, either invertebrates (Tubificidae) or fish (*Leucaspius delineatus* H.), at the time of their transition from the food composed of invertebrates into feeding on fry (the most suitable time for that kind of experiments) makes possible the examination of the effect of the supplied food. As indicated by the obtained results it is reflected in the food intake, growth rate, chemical composition and calorific values of the body of perches under examination.

The most marked differences occur in the growth rate of perches fed with different kind of food. Perches fed with *Leucaspius delineatus* are growing quicker and gaining more in weight than those fed with Tubificidae (Fig. 1). The former show the relative daily growth of about 2% of their body weight, while the growth of the latter does not exceed 1%. The present results confirm the data given by other authors, who had fed perches with invertebrates in laboratory conditions. Bily (1938) declared that perches fed with *Tubifex tubifex* have the relative growth equal to 0.9% of their body weight, while Birkett (1969) has determined the growth of perches fed with *Lumbricus sp.* in the range of 0.1–0.6% of their body weight. The effect of the consumed food on the growth of the body weight of fish is stated also by other authors who have examined such predators as pike (*Esex lucius*) and trout (*Salvelius namycush*). Krivobok, Pupurnikova (1950) carried out their studies on pikes fed with either Crustacea or fry. After 100 days of the experiment the former have gained in weight about 3 g and those fed with fry over 8 g. Martin (1966) declared that trout fed with plankton grows slower and reaches maturity later than when fed with fish.

Growth and increase in size are the simplest and most perceptible measure of fish reaction to the environmental conditions (Backiel 1964, Karzinkin 1952) and also the best index of the usefulness of the provided food. Differences in the speed of growth of the body weight of perches fed with different food show that fish is the best food to promote a quick growth of perches. This is confirmed likewise by the values of the food coefficient obtained for perches fed with different food. It points out the dependence of utilization of the consumed food for growth upon the kind of the available food. Karzinkin (1952) declares that food coefficient is a good biological index for utilization of food base and it charcterizes the type of nourishment. He had compiled the data from many other authors and calculated on that basis the food coefficient values, as follows: for predators — about 4, benthophagi — about 12, planktophagi — about 22. The food coefficient for perches fed with Tubificidae oscillates in a rather wide range of 4.5–17.8 but its mean value of about 8 approximates the value characteristic for benthophagi. The food coefficient for perches fed with *Leucaspius delineatus* is in the range of 3.6–6.1 (mean value 4), corresponding to the values characteristic for predators. Similar dependences can be observed in the studies of Krivobok, Pupurnikova (1950). The food coefficient for pike fed with Crustacea is 9.2, when fed with fry 2.7.

Thus, the obtained results indicate that fish was a more suitable food for the examined perches than invertebrates. This is confirmed by greater intensity in feeding shown by perches fed with *Leucaspius delineatus* and higher values of their daily food intake (Table II). The obtained values of the daily food intake are compatible with data of the other authors, who were feeding perches with invertebrates in the laboratory cultures (Bily 1938, Birkett 1969, Chikova 1970). A comparison of those data indicates, also, the tendency to a decrease in the relative food intake following the increase in the body weight of fish, e.g. the food intake of perches weighing 40 g is about 3% of their weight (Chikova 1970), at about 100 g body weight 0.17–1.9 (Birkett 1969). Winberg (1964) states that this is a general dependence applicable to all kinds of fish. That dependence is shown in Fig. 4.

The effects of the consumed food of different kind is also reflected in the chemical composition of the body of perches under the experiment. Their dry weight, ash, lipids and total oxygen values are in the same range as those obtained by other authors (Demoll, Gaschott 1932, Malyarevskaya et al. 1970). As can be seen from the obtained results there are some differences in the ash and lipids contents in the body of perches fed with different kind of food. Thus, perches fed with

Tubificidae contain more ash and less lipids than the other ones fed with *Leucaspius delineatus*. That difference is also to be found in the calorific values of the examined perches. The calorific value of those fed with Tubificidae is lower (4.41 cal/mg) than that of the other ones fed with fish (5.20 cal/mg). Calorific value of the ash-free dry weight (5.60 cal/mg and 6.25 cal/mg, respectively) indicates differences in the lipids content in accordance with chemical determinations.

It seems to be of some interest, the comparison of the chemical composition and calorific values of the body of perches fed with different food in the experimental cultures and those taken from their natural environment. In the present study, dry weight, ash content, and calorific values were determined in perches from two various natural environments: the Mikołajskie Lake and fish-ponds at Żabieniec near Warsaw. Perches from the laboratory cultures fed with Tubificidae and those caught in the Mikołajskie Lake, in March, where especially in the early spring they had a scanty food base, showed a similar ash content and approximating calorific values. Whereas, perches fed with *Leucaspius delineatus* had values similar to those caught in September in the fish-ponds, where after a period of the peak of summer vegetation they had at their disposition a great abundance of food, including fish food as well. Convergence of those data substantiates the conclusion about the effect of various kinds of food upon the chemical composition of the examined perches.

The presented results show what a great influence the kind of the consumed food has on the physiology of fish. In the examined *Perca fluviatilis* that influence was reflected in the feeding intensity, speed of the growth of fish and also in the composition and calorific values of their body.

5. SUMMARY

Perches (*Perca fluviatilis* L.) of size 10–14 cm long, were fed in laboratory cultures with homogeneous food consisting of either invertebrates — Tubificidae, or fish — *Leucaspius delineatus* H. Their rate of growth and daily food intake has been determined during a two-month-lasting experiment preceded by one-month acclimatization under the laboratory culture conditions. After the end of experiments, dry weight, ash, lipids, and total nitrogen contents in the body of the examined fish have been determined. The calorific value of the body of perches fed with different kind of food has been also determined. For comparison, similar determinations were carried out in perches taken from their natural environment.

It has been found that the kind of the given food has an effect upon the intensity of feeding and the growth of *Perca fluviatilis* L. as well as on the composition and calorific value of their body. Perches fed with fish consume food with greater intensity and utilize a greater part of it for their growth; they grow much quicker than perches fed with invertebrates. Their body is more calorific and contains more lipids and less mineral substances (ash). The results obtained in the laboratory confirm the data obtained for perches living under various conditions in their natural environment.

6. STRESZCZENIE

Okonie *Perca fluviatilis* L. o wielkości 10–14 cm żywiono w hodowli laboratoryjnej jednorodnym pokarmem złożonym z bezkręgowców (Tubificidae) lub ryb (*Leucaspius delineatus* H.). Oznaczano przyrosty i dobową rację pokarmową w czasie 2-miesięcznego eksperymentu poprzedzonego miesięczną aklimatyzacją ryb w warunkach hodowli laboratoryjnej. Po zakończeniu doświadczeń w ciele badanych ryb oznaczano zawartość suchej masy, popiołu, lipidów i azotu ogólnego. Oznaczano także kaloryczność ciała okoni żywionych różnym pokarmem. Dla porównania wykonano podobne oznaczenia dla okoni ze środowiska naturalnego.

Stwierdzono, że rodzaj podawanego pokarmu wpływa na intensywność odżywiania i wzrost *Perca fluviatilis*, a także na skład i kaloryczność jego ciała. Okonie żywione rybami intensywniej pobierają pokarm i większą jego część wykorzystują na przyrost, szybciej rosną niż okonie żywione bezkręgowcami. Ciało ich jest bardziej kaloryczne, zawiera więcej lipidów a mniej substancji mineralnych (popiołu). Otrzymane w laboratorium wyniki potwierdzają dane dotyczące okoni pochodzących z różnych warunków środowiska naturalnego.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	3/4	481–502	1974

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FURTHER OBSERVATIONS ON THE EFFECT OF SODIUM SALT OF 2,4-D ON EARLY DEVELOPMENTAL STAGES OF CARP (*CYPRINUS CARPIO* L.)

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ABSTRACT

Continuation, on a larger scale, of the investigations on the effect of herbicide "Pielik" on carps, as reported by Matlak (1972) and Kamler (1972 b), is presented. Acute tests (48 hrs) demonstrated that the herbicidal formulated product "Pielik" is about twice more toxic than the pure sodium salt of 2,4-D. The mortality test proved to be least sensitive of all acute tests applied. Long-term tests were found to be much more sensitive than the acute ones. "Pielik" continuously applied in a concentration of 50 mg active ingredient/l caused delayed hatching, behavioural changes, feeding impairment, severe morphological disturbances, slower growth rate and finally mass mortality of larvae. The observed drop in oxygen consumption, with a simultaneous increase in *RQ* and lactic acid content, indicated that "Pielik" impairs the metabolic aerobic processes in favor of the anaerobic ones.

1. INTRODUCTION

Sodium salt of 2,4-dichlorophenoxyacetic acid is the active ingredient of formulated product "Pielik". In "Pielik" the content of sodium salt of 2,4-D is at least 85% and that of chlorophenols does not exceed 3% (Polish Standard PN-65C-84142). According to Taylor et al. (1972 b), between 1968–1972 "Pielik" and herbicides containing MCPA as active ingredient have been most widely used in the Polish agriculture for the control of cereal culture weeds and maintenance of drainage ditches. Since these herbicides are applied in spring, they entail the potential risk of exposure for the young developmental stages of carp. On these grounds, in 1971 investigations on the effects of exposure to "Pielik" have been undertaken at the Experimental Farm of the Polish Academy of Sciences, in Gołysz, Cieszyn District; the results have been reported by Matlak (1972) and Kamler (1972 b). It has been found (Matlak 1972) that the exposure to "Pielik" resulted in delayed hatching, a decrease in larval body length and body deformation; in the 200th hour of development "Pielik" in a concentration of 50 mg A.I./l brought about mass mortality of larvae. It has also been shown (Kamler 1972 b) that the oxygen consumption was lower in "Pielik"-treated larvae than in controls. Moreover, "Pielik" used in concentrations of 5 and 50 mg A.I./l has been found (Kamler 1972 b) to also cause a rise of the total energy expenditure; namely, the calorific equivalent of endogenously fed larvae decreased more intensely in those treated with "Pielik" than in controls. Consequently, it has been postulated (Kamler 1972 b) that the herbicide studied impairs the metabolic aerobic processes in favor of the anaerobic ones.

On account of the difficulties presented by toxicity studies involving objects as rapidly and radically changing as carp embryos and larvae, it was resolved to repeat in 1972 the investigations performed in 1971, in order to check the correctness of the direction of the observed changes. Moreover, to verify the above-mentioned hypothesis, in addition determinations of RQ and lactic acid content were carried out. These long-term developmental, morphopathologic and metabolic studies are the main subject of the present report.

In most investigations on the mechanism underlying the effect of sodium salt of 2,4-D on the physiological processes in aquatic organisms, pure preparations have been used (Zvirgzds et al. 1971, Klekowski, Zvirgzds 1971, Zimakowska 1973). It is well known, however, that pure preparations are less potent than the formulated products used in agriculture, owing to the high toxicity of supplementary components, as compared with the active ingredient. For example, from the data of Luk'yanenko (1967) it can be inferred that the lethal concentration of chlorophenols for fishes is about 20 mg/l, whereas the median lethal concentration (24 hrs) of pure 2,4-D-Na amounts to 1160 mg/l (Alabaster 1969). However, as there are differences in the composition of various lots of formulated products, it was thought advisable to perform a parallel comparative investigation on the effect of pure sodium salt of 2,4-D and "Pielik" on carp embryos and larvae.

Polish legislation (Decree of the Cabinet, July 18th, 1970, Government Gazette of the Polish People's Republic No. 17, item 144) recommends the acute test for fish mortality (24 hrs) for the purpose of establishing whether the surface water pollution does or does not exceed the admissible values. However, owing to the mechanism of their action, herbicides only uncommonly exhibit acute toxicity to fish and aquatic invertebrates, whereas their noxiousness becomes manifest only after prolonged exposure (Braginskij 1972). Therefore, as postulated by Luk'yanenko (1973), it is necessary to search for another criterion of toxicity, which would be specific for the investigated group of compounds and would manifest itself by way of disturbing the most sensitive function of the organism ("funkciá mishen" — the "target function"). As regards the derivatives of 2,4-D acid, this function involves some metabolic processes.

Consequently, in addition to the above-mentioned long-term studies of the development, morphopathology and metabolism, for the purpose of comparison a series of acute toxicity tests (24 and 48 hrs) was performed. These comparative studies represented an additional aim of the present investigations. Tests were performed on developing embryos, whereupon the whole experimental series was repeated on larvae. Two parallel groups of animals exposed to solutions of either pure sodium salt of 2,4-D or of "Pielik" were compared with a control group. The aim of this experiment was to establish the difference between the action of the well known pure preparation and of the little known formulated product used in agriculture. Parallel application of several acute tests (mortality of embryos and larvae, total number of larvae hatched and percentage of the normal ones, mobility of embryos) permitted evaluation of their sensitivity and comparison with the long-term toxicity tests (development, morphopathology and metabolism).

2. MATERIAL AND METHODS

The investigations were performed in 1972, at the Experimental Farm of the Polish Academy of Sciences, Gołysz, Cieszyn District.

Formulated product "Pielik" used in the present experiments was always taken from the same package. According to its analysis, kindly performed by dr. Stanisław Łakota (Institute of Organic Industry, Pszczyna), the product contained 84.8% of active ingredient, i.e. of sodium salt of 2,4-D, this content was assumed to comply with the Polish Standard. Pure sodium salt of 2,4-D was prepared in accordance with Zvirgzds, Balina (1969). Pure 2,4-dichlorophenoxyacetic acid (produced by May Baker Ltd., England) was dissolved in ethanol and filtered through Schott G3. NAOH (anal. reagent grade) was dissolved in ethanol. Both solutions were combined in stoichiometric ratio and were then filtered through Schott G3; the precipitate was washed with ethanol to constant pH. The product was air-fried to constant weight.

Spawn used in the experiments was obtained from 5-year-old carp spawners (weight: females 4–4.5 kg, males 3.5 kg) from the Experimental Farm in Gołysz. Hypophysis injections were carried out on June 5th, at 7–8 p.m. Eggs were fertilized on June 6th, at 3 p.m., and were freed from jelly. The temperature of the solutions was 24.2–25.0°C.

After swelling of eggs, which then were at the stage of 16 blastomers, on June 6th, at 6 p.m., they were placed in two Weiss glasses (Fig. 1). The flow of water through the glasses was 3 l/min (±0.3 l/min). Use was made of river water of low total hardness, 4.06 German degrees, i.e. 1.45 mval/l, this representing — according to Schäparclaus (1961) — an average value for fish ponds. The total hardness of water used in the earlier experiments (1971) had been closely similar (Matlak 1972, Kamler 1972 b). In addition, to one of the Weiss glasses a concentrated solution of "Pielik" was fed at a flow rate of 1 l/hr (±0.1 l/hr), whereas the other Weiss glass served as control. Fresh concentrated "Pielik" solutions were prepared every 10 hrs. In the experimental Weiss glass the concentration of "Pielik" amounted to 58.9 mg/l (±20%); thus, the concentration of active ingredient was 50 mg/l.

The temperature of water read every hour varied near 23°C (Fig. 2). Oxygen content was measured once a day. In the control glass it averaged 7.64 mg O_2/l, with coefficient of variation amounting to ±4.0%. In the "Pielik"-containing glass the conditions were closely similar: 7.64 mg/l, with c.v. ±4.6%. This corresponded to 93% of air saturation, at mean atmospheric pressure 735 mm Hg.

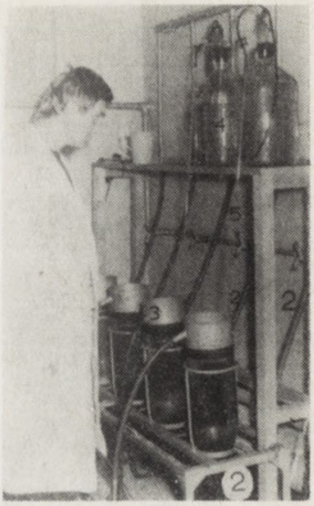

Fig. 1. Culture of embryos. 1 — Weiss glass, 2 — water inlet into Weiss, 3 — outlet from Weiss, 4 — bottle containing concentrated solution of "Pielik", 5 — "Pielik" solution inlet into Weiss. Also one of authors (K. Srokosz) can be seen

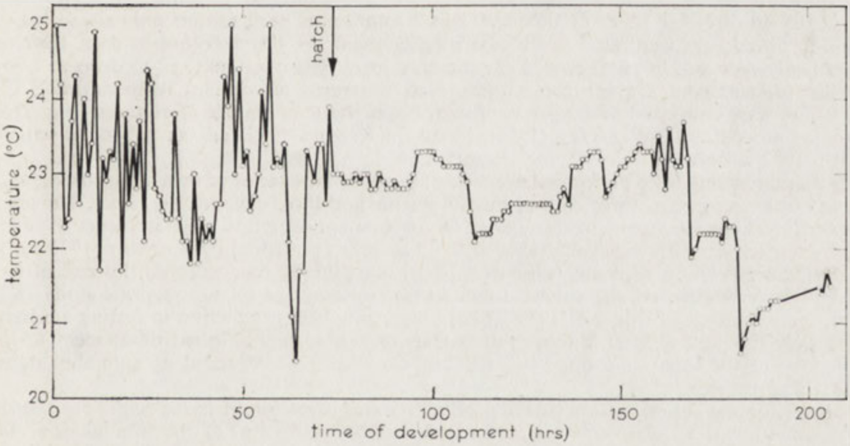

Fig. 2. Temperature of cultures: Weiss glasses with embryos before hatching, aquariums with larvae after hatching

Hatching of larvae took place in the control and in the "Pielik" solution 73.5 and 74 hrs, respectively, after fertilization. The larvae hatched were transferred to aquariums (about 26,000 larvae per 160 l of liquid). Liquids, viz. pure water — in the control aquarium and "Pielik" solution containing 50 mg A.I./l — in the experimental one, were replaced every 24 hours. The solution of "Pielik" was added to the aquarium till the 216th hr of development. The temperature was about 23°C; it somewhat dropped by the end of the experiment (Fig. 2). The aquariums were intensely air-ventilated; oxygen content in the control aquarium was 7.50 mg O_2/l with c.v. ±6.4% and in the experimental one — 7.38 mg O_2/l with c.v. ±10.7%, this corresponding to 91 and 89% of air saturation, respectively. No food was given to the larvae till the 216th hr of development, whereupon they were fed pond plankton.

Three kinds of experiments were performed:

1. Acute tests — comparison of the effect of "Pielik" and pure sodium salt of 2,4-D. For more complete characterization of Polish formulated product "Pielik", permitting its comparison with the well known pure sodium salt of 2,4-D, the survival and hatching of animals exposed to either preparation within a wider concentration range were observed. The material used for study was the same as in the remaining experiments. Spawn was taken from the control Weiss glass in the 3rd hr of development, and larvae — from the control aquarium in the 75th hr of development.

The animals were placed, in groups of 25 animals each, in Petri dishes (diameter 5 cm) in 5 ml of liquid[1]. Concentrations used for both pure sodium salt of 2,4-D and "Pielik" (calculated per active ingredient) were: 0; 25; 50; 100; 200; 400; 800; 1600; 3200 mg/l. Every concentration of each substance was examined, in parallel, in two Petri dishes; thus, a total of 68 Petri dishes were tested (2×2 controls plus 2 developmental stages ×2 substances ×8 concentrations ×2 duplicates). Mortality observations were made every 2 hrs. After 24 hours' exposure (i.e. in the 27th hr of development) the liquids in Petri dishes were replaced, and the mobility of embryos in spawn was observed under a microscope. Mortality tests were completed after 48 hours' exposure (for spawn and larvae — 51 and 123 hrs of development, respectively); however, spawn was kept longer, in order to record, during the subsequent 39 hrs, the hatching of larvae, without any further replacement of the liquids in Petri dishes. For each experimental variant the results of duplicate tests were treated jointly.

2. Long-term tests I — Development and morphopathology. Observations were made during 816 hrs (34 days) after fertilization, i.e. they lasted throughout "Pielik" application and after its cessation. The development of carp was in vivo observed under a microscope, in a drop of water. Samples of larvae for morphopathologic examinations were taken daily and fixed in a 2% formalin solution.

3. Long-term tests II — Metabolism. Observations were made only during "Pielik" application, i.e. throughout 216 hrs of development (9 days).

A total of 20 series of oxygen consumption measurements were carried out, two series being performed each day. Every series comprised three measurements involving control animals and three — involving those treated with "Pielik" solution (50 mg A.I./l). The total number of oxygen consumption measurements was 120. Oxygen consumption was measured in constant pressure microrespirometers (for description of apparatus and procedure cf. Klekowski 1968). The total air volume in the microrespirometric system amounted to approximately 20 ml. Each respiration chamber held 0.5 ml of either water or of the "Pielik" solution as well as the animals tested. About 120 non-fertilized eggs were used in the first measurement; in the subsequent ones their number was gradually reduced, in parallel with the increase in oxygen consumption, to drop to five larvae in the last measurement. Oxygen consumption was measured at constant temperature $23°C \pm 0.1°$. These values were converted to oxygen consumption at the temperature of cultures (Fig. 2), according to the procedure of Winberg (1956), based on Krogh's "normal curve" (cf. discussion in: Kamler 1972 a and b).

RQ measurements were performed every day; in total, nine series of measurements were carried out. Each series comprised three control measurements and three experimental ones. The apparatus and procedure were analogous to those used for oxygen consumption measurements (Klekowski 1968). In accordance with the conclusions of the discussion presented by Kamler (1972 b), for the oxy-calorific coefficient a constant value of $4.74 \cdot 10^{-3}$ cal/μl O_2 was accepted, instead of its being derived from the measured *RQ* values. In this case, similarly as in the previous study (Kamler 1972 b), use was made of Kleiber's (1961) data regarding heat production in fasting animals katabolizing only fats and protein. Subsequent studies (Kamler, unpublished data) seem to indicate that, in fact, at the stage of endogenous feeding carps can be regarded as animals katabolizing only fast and protein.

Samples for the determination of the production and excretion of lactic acid were taken daily. In total, nine series of analyses were performed. Samples (500 mg) of the live material of eggs or of larvae were incubated for 3 hrs in 5 ml of water (controls) or in 5 ml of "Pielik" solution containing 50 mg A.I/l (animals taken from the experimental Weiss glass of from the experimental aquarium). Each experimental variant was run in duplicate. After incubation, the animal material was separated from the liquid by filtration, and both were separately stored in plastic vials. Liquids in which no animals had been incubated, viz. "Pielik" free water and "Pielik" solution containing 50 mg A.I./l, served as additional controls. All materials were stored and transported in solid carbon dioxide. Previous tests on young Anguilla (Kamler, unpublished data) have shown that storage in solid carbon dioxide does not affect lactic acid content; the differences between the results do not exceed 5%, i.e. they are not greater than those between duplicate analyses of the same material. Lactic acid content was determined by the method of Barker and Summerson (1941) (cf. also Natelson 1961). For deproteinization of tissue homogenate samples, equal volumes of 10% trichloroacetic acid were added. Colorimetric readings were taken on a Pulfrich photometer, using filter S56 (560 nm). No lactic acid excretion was demonstrated, as lactic acid contents in water and "Pielik" solution, in which the animals had been incubated, failed to exceed those in control liquids. Thus, the results comprise only lactic acid retained in the bodies of embryos and larvae. The technical difficulties presented by the determination of lactic acid excretion to aqueous medium have been stressed by Augenfeld (1967).

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[1] The weight of one egg was somewhat less than 4 mg and that of one larva — nearly 2 mg (Kamler 1972 b); thus, the ratio liquid volume/body volume amounted for eggs to over 50:1 and for larvae to over 100:1.

3. RESULTS

A. ACUTE TESTS—COMPARISON OF THE EFFECT OF "PIELIK" AND OF PURE SODIUM SALT OF 2,4-D

In control (Fig. 3, curves 1 and 3) and at all the concentrations of both preparations studied (e.g. Fig. 3, curves 2 and 4, "Pielik" 1600 mg A.I./l) high mortality was observed at the beginning of the exposure period. This prevented plotting of the time-mortality curves (Bliss 1937). Moreover, since the effect of toxicants on the mortality became manifest only at the highest concentrations used, plotting of the concentration-mortality curves (Bliss 1935) was also dispensed with. The treatment of the results was confined to an only very generalized presentation, involving an evaluation of the toxic (threshold) concentration, as formulated by Luk'yanenko (1973), i.e. of the concentration causing pathologic changes in any arbitrarily chosen, individually considered functional system in the organism.

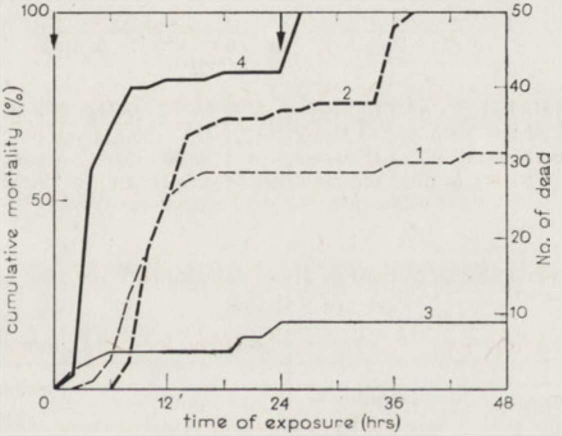

Fig. 3. Mortality of carp eggs and larvae in "Pielik", 1600 mg A.I./l. 1 — eggs, control, 2 — eggs, "Pielik", 3 — larvae, control, 4 — larvae, "Pielik". Vertical arrows — new solution added after 0 and 24 hrs. (Each point is a mean of two duplicate measurements)

Up to the concentration of 800 mg A.I./l, neither pure 2,4-D-Na nor "Pielik" brought about, during 48 hrs, an increase in the mortality of embryos (survival presented in Fig. 4A, curves 1 and 2) and larvae. The concentration of 1600 mg pure 2,4-D-Na/l also failed to affect the mortality of embryos (Fig. 4 A curve 1) and larvae, whereas the same concentration of "Pielik" already increased the mortality of embryos (Fig. 3, curves 1 and 2, Fig. 4A curve 2) and, even more so, that of larvae (Fig. 3, curves 3 and 4). The concentration of 3200 mg A.I./l of either preparation caused rapid death of both embryos (100% dead within 4 hrs of exposure to pure 2,4-D-Na and within 16 hrs — to "Pielik") and larvae (100% dead within 2 hrs in both toxicants).

Microscopic observations of embryos (Table I) revealed that both pure 2,4-D-Na and "Pielik" reduce their mobility, and that at higher concentrations they inhibit normal development. Concentrations evoking the same response were lower for "Pielik" than for pure 2,4-D-Na.

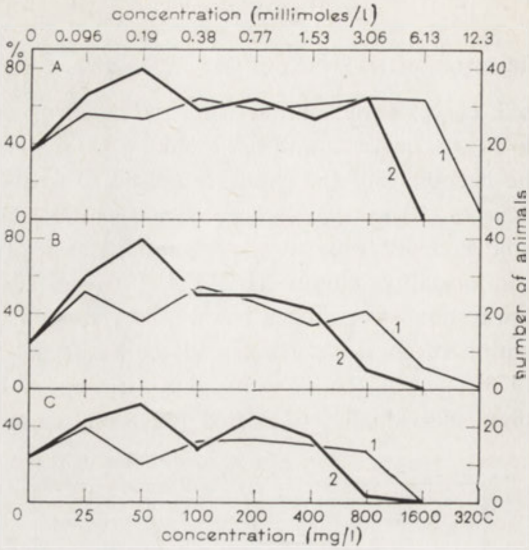

Fig. 4. Influence of sodium salt of 2,4-D on hatching. A — survival of eggs after 48-hr exposure to pure sodium salt of 2,4-D (1) and to "Pielik" (2); B — total larvae hatched within 87 hrs of exposure to pure sodium salt of 2,4-D (1) and to "Pielik" (2); C — normally developed, live larvae hatched within 87 hrs in pure sodium salt of 2,4-D (1) and in "Pielik" (2). (Each point is a mean of duplicate measurements)

Table I. Microscopic observations of 27-hrs-old carp embryos after 24 hrs exposure to toxicants

Embryos		2,4-D-Na, form and concentration (mg/l)	
development	mobility	pure	formulated product "Pielik"
normal	good	0–200	0–100
normal	poor	400–1600	200–800
no normal embryos	none	3200	1600 and 3200

In the 87th hr of exposure to pure 2,4-D-Na (90th hr of development), at concentrations 25–800 mg/l the total numbers of larvae hatched were not lower than in controls (Fig. 4 B, curve 1), whereas at concentration 1600 mg/l (which did not yet affect the survival in the 48th hour of exposure — cf. Fig. 4 A, curve 1) the total number of larvae hatched was already markedly lower (Fig. 4B, curve 1). For the animals exposed to "Pielik" the over-all picture was analogous, except that the effective concentrations were two times lower: concentrations 25–400 mg A.I./l failed to affect the number of larvae hatched, and concentration 800 mg A.I./l caused a reduction of this number (Fig. 4B, curve 2), though the survival in the 48th hr of exposure (Fig. 4A, curve 2) did not differ from control values.

From the standpoint of practice, the number of larvae hatched alive and showing normal development is of interest. It was found that not all the larvae hatched

Table II. Influence of pure sodium salt of 2,4-D and "Pielik" on carp

concentration (mg sodium salt of 2,4-D/l)	25	50	100	200	400	800	1600	3200
mortality eggs and larvae (48 hrs test)								
pure	—	—	—	—	—	—	—	++
"Pielik"	—	—	—	—	—	—	+	++
larvae hatched (total and normal)								
pure	—	—	—	—	—	—	+	++
"Pielik"	—	—	—	—	—	+	++	++
embryos (microscopic observations at 24 hrs of exposure)								
pure	—	—	—	—	+	+	+	++
"Pielik"	—	—	—	+	+	+	++	++

Table III. Embryonal development of carp. Fertilization — June 6th, 3 P.M., 1972

Duration of development hrs min	24-hr periods after fertilization	Mean temp. of water during 24-hr periods	Degree-days	Developmental stage
0 40				Swelling of egg, formation of perivitelline space
1 05				Blastodisc
1 10				2 blastomers
2 20				16 blastomers. Eggs placed in Weiss glasses ("Pielik", 0 and 50 mg A.I./l)
5 30				Multicell morula
7 30				Early gastrula
9 00				Late gastrula
12 00	1	23.3°C		Neurula
16 30				Formation of embryo, fine outline of eyes and somites
23 30				First faint movements of embryo at ca 12 sec intervals
26 30				Bud of fin fold, somites clearly outlined
27 30				Embryo moves vigorously in various directions
30 00				Embryo is in active motion, being able to move the egg by body contractions. Losses of eggs between fertilization and 30th hr of development are: control — 26.3%; "Pielik", 50 mg A.I./l — 35.7%
35 00	2	22.7°C	46.0	Pigmentation of eyes. Blood circulation
57 00				First larvae in control
61 00				First larvae in "Pielik", 50 mg A.I./l
63 30				Very high numbers of larvae in control; as heretofore, few larvae in "Pielik", 50 mg A.I./l
73 30			69.0	Complete hatching; catching of larvae in control Weiss
74 00			69.0	Complete hatching; catching of larvae in Weiss with "Pielik", 50 mg A.I./l

(Fig. 4B) were normally developed (Fig. 4C). The effect of both preparations on their number was similar as in the case of the total number of larvae hatched (Figs. 4C and 4B), and became manifest for pure 2,4-D-Na at concentration 1600 mg and for "Pielik" at concentration 800 mg A.I./l.

The results are presented as a schema in Table II. "Pielik" proved to be more toxic than pure 2,4-D-Na. In all the tests applied, the toxic (threshold) concentrations of "Pielik" calculated per active ingredient were two times lower than those of pure 2,4-D-Na. The 48-hr mortality test was found to be the least sensitive one of all tests used.

B. LONG-TERM TESTS I — DEVELOPMENT AND MORPHOPATHOLOGY

The life history of embryos is presented in Table III. The effect of the permanent presence of herbicide at concentration 50 mg A.I./l manifested itself by somewhat increased mortality of the treated embryos during the initial phase of embryonal development and by slightly delayed hatching of larvae.

The life history of larvae is presented in Table IV. The toxicity of herbicide is more strongly marked in the larval than in the embryonal development. The larvae developing in herbicide solution showed behavioral changes: restlessness, equilibrium disturbances and depression (Table IV). Their food intake was lower (Table IV) and some failed to feed at all (Table V); the number of nonfeeding larvae increased even after discontinuation of any further addition of herbicide (Table V, 240th hr). In the control aquarium the larvae developed normally, whereas in that containing the herbicide they exhibited morphopathologic changes (Table IV, Fig. 5B): hydrocoele embryonalis, ventral deformation of the spinal cord, rounding of mouth profile, displacement of the eyeballs towards the front of the head, protrusion of eyeballs. It is noteworthy that during the development in the presence of herbicide the percentage of larvae with hydrocoele embryonalis gradually increased (Table V); this process continued also after cessation of herbicide addition: in the 240th hr of development hydrocoele embryonalis was found in 91% (Table V), and in the 336th hr — in as many as 98% of larvae (Table IV). The larvae exposed to herbicide exhibited hemorrhages in various parts of the body and anemia. The herbicide delayed filling of the swimming bladder with air (Table V). The growth in length of control and treated larvae is recorded in Fig. 6. The body length was not very variable. In eight different samples of control larvae ($n=35$) the coefficient of variation amounted to 4.09–7.08% of the mean, and in samples of treated larvae it was 5.27–10.25% of the mean; thus, the variability of body length was somewhat higher in the presence of herbicide. The larvae exposed to herbicide grew slower; their body lengths measured on the 1st, 3rd, 5th, 6th and 7th day after hatching were lower, as compared with controls (Fig. 6), the differences being highly significant ($p<0.001$, t-test). The larvae cultured in herbicide solution began to die in the 156th hr of development, dying in great numbers between the 216th and 240th hr.

The fates of the surviving larvae were different. A part of them (about 19% in the 504th hr of development) showed signs of recovery: disappearance of the

Table IV. Larval development of carp. Hatching — June 9th, 4^{30}–5^{10} PM, 1972 (Data below the horizontal line — cessation of herbicide addition)

Duration of development	24-hr periods after		Mean temp. of water during 24-hr periods °C	Course of development
	fertilization	hatching		
93	4	1	22.8	After having been placed in aquariums, the larvae settle at the bottom, resting there for nearly 48 hrs. By the end of this time some of them try, from time to time, to rise. Somewhat later, many larvae become vertically attached to the walls of aquariums. Their mouths are closed, swimming bladders — airless and yolk sacs — spherical. The behavior of larvae in the control aquarium is normal, while those in the "Pielik" - containing aquarium (50 mg A.I./l) are alarmed.
117	5	2	22.9	In the major part of control and treated larvae the swimming bladders are filled with air. Their mobility increases, and they move freely all over the aquarium. They briskly react, by flight, to dipping of some object in the aquarium. The yolk sac is pear-shaped and contains a fairly great store of yolk. The behavior of control larvae is normal. In the "Pielik"-containing aquarium, the first larvae with symptoms of hydrocoele embryonalis of the yolk sac are observed.
144	6	3	22.3	In all control larvae and in about 90% of the treated ones, one chamber of the swimming bladder is filled with air. In the "Pielik"-containing aquarium the number of larvae with signs of hydrocoele embryonalis of the yolk sac continually increases.
156	7	4	23.1	The yolk sac is almost completely resorbed. No feeding is applied. In the "Pielik"-containing aquarium first deaths of larvae are noted. Some larvae with hydrocoele embryonalis show hemorrhages in various parts of the body.
192	8	5		In the "Pielik"-containing aquarium the number of larvae with symptoms of hydrocoele embryonalis of the yolk sac steadily increases. Control larvae show normal behavior. The yolk sac is resorbed.
216	9	6	20.2	For the first time plankton is added to the aquariums, and the control larvae feed intensely. There is a high number of treated larvae with equilibrium disturbances, paralyzed or even dead; they accumulate near or at the bottom of the aquarium. Only few larvae are swimming in the water layer. The para-

Duration of development	24-hr periods after		Mean temp. of water during 24-hr periods °C	Course of development
	fertilization	hatching		
				lyzed larvae show, in addition to hemorrhages, deformations of the spinal chord, rounding of the profile of the mouth or dislocation of the eyeballs towards the anterior part of the head (Fig. 5B). Larvae with hydrocoele embryonalis are resistlessly carried away by the stream of water produced by the aerator.
240	10	7	20.9	Test solutions of "Pielik" are added no more. Further mass deaths of larvae with signs of hydrocoele embryonalis are observed. The behavior of control larvae is normal.
312	13	10	20.6	The control aquarium still contains large numbers of very lively and feeding larvae. Only 60 of the treated larvae still survive.
336	14	11	21.5	In both aquariums larvae show great differentiation in body length. Apart from larvae with well filled alimentary canals, there are some with less filled or empty ones. Plankton predominates in the intestinal contents; there are also relatively many Chironomus and Chaoborus larvae. Also some treated larvae with hydrocoele embryonalis of the yolk sac are feeding; however, the degree of filling of the intestine is lower, as compared with controls. Swimming bladders are one-chamber. The control aquarium still contains a high number of larvae which are very lively and feed intensely. In the aquarium with "Pielik" solution 52 larvae still survive; 98% of them exhibit signs of hydrocoele embryonalis of the yolk sac, of different severity. Concomitant symptoms include: ventral deformation of the spinal cord, a larger or smaller hydrocoele (very large in 6% of larvae), depression, equilibrium disturbances, lateral position of the body, hemorrhages in the region of the heart, under the eyes, in the caudal and ventral parts; hemorrhages occur in 59,5% of larvae.
504	21	18	21.5	The behavior of control larvae is normal. In some of them the swimming bladders are two-chamber. There is a high differentiation in their body length. In the "Pielik"-containing aquarium disappearance of the hydrocoele is observed in 18.75% of larvae, which also show well filled alimentary canals. The remaining treated larvae exhibit, in addition to the above-mentioned pathologic symptoms, pronounced protrusion of the eyeballs and anemia.

Duratinn of development	24-hr periods after		Mean temp. of water during 24-hr periods °C	Course of development
	fertilization	hatching		
552	23	20	21.9	In the control aquarium all the larvae have died, because of a defect in ventilation. Among the treated larvae there are deaths with severe pathologic symptoms. Larvae, in which disappearance of the hydrocoele had been observed, show normal behavior and keep feeding. In larvae with hydrocoele embryonalis further progress of disease and deaths are observed.
648	27	24	23.0	In the "Pielik"-containing aquarium there survive 11 larvae with widely differentiated body length, including 1 larva without symptoms of hydrocoele embryonalis.
816	33	31	23.9	End of observations. The surviving larvae were fixed in formalin.

Table V. Influence of sodium salt of 2,4-D on carp larvae. 0 — control, 50 — "Pielik" 50 mg A.I./l. Data below the horizontal line — herbicide addition stopped

Duration of development	No. of larvae in sample		Percentages of larvae showing:					
			empty alimentary canals		hydrocoele embryonalis		swimming bladders not filled with air	
	0	50	0	50	0	50	0	05
72	35	35	—	—	0	0	—	—
96	35	35	—	—	0	0	—	—
120	35	73	—	—	0	9.6	22.9	26.0
144	35	65	—	—	0	53.8	0	10.8
156	35	76	—	—	0	63.2	0	0
192	35	74	—	—	0	68.9	0	0
216	35	35	6.0	28.5	0	71.4	0	0
240	35	17	5.0	41.0	0	91.1	0	0

hydrocoele bladder and good filling of the alimentary canal. In the remaining larvae the disease was found to progress. Observations on the morphopathology and development were completed in the 816th hr of development (31 days after hatching). Some of the larvae remained alive: those in which the regress of disease was observed, those in which hydrocoele was not observed at all as well as few larvae with advanced disease.

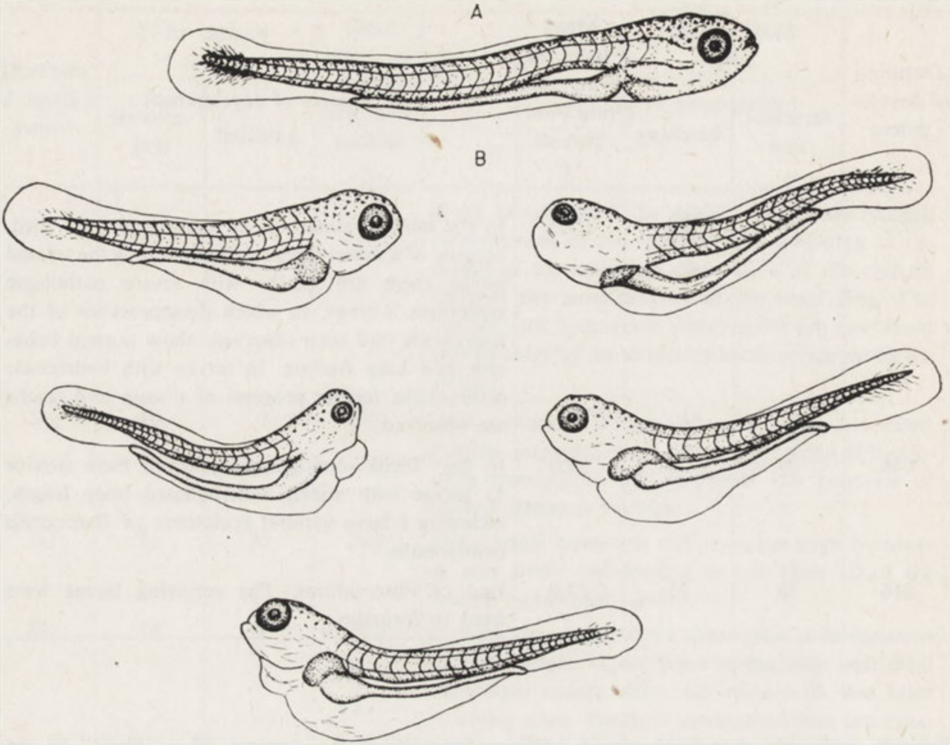

Fig. 5. Carp larvae. A — normal larva, from control aquarium, B — examples of different morphological changes in larvae cultured in "Pielik", 50 mg active ingredient/l

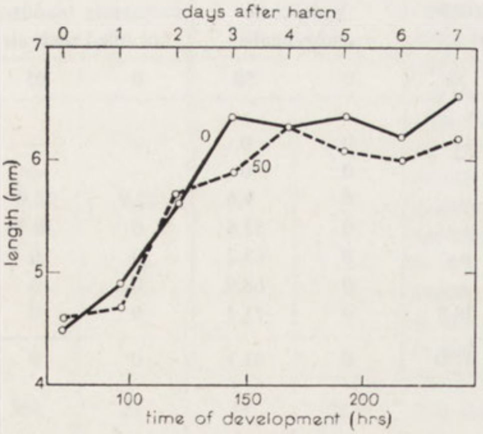

Fig. 6. Body length of carp larvae cultured in "Pielik", 0 and 50 mg A.I./l

C. LONG-TERM TESTS II — METABOLISM

In unfertilized eggs oxygen consumption was 0.051 µl O_2/ind.·hour (Fig. 7). Instantaneous oxygen consumption rose exponentially till the 97th hr of development; after the 130th hr it began to decrease, owing to depletion of nutrient storse in the yolk sac. Mean oxygen consumption of both control embryos and control

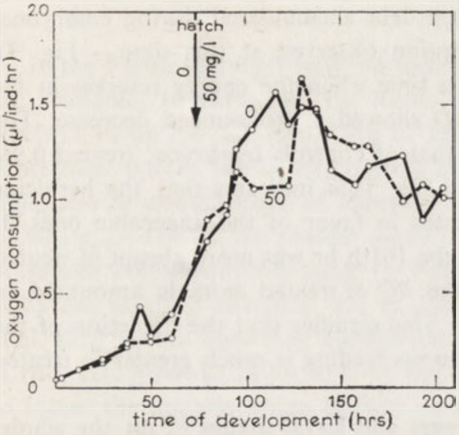

Fig. 7. Instantenous oxygen consumption at 23°C ± 0.1° in carp embryos and larvae cultured in "Pielik", 0 and 50 mg A.I./l. (Each point is a mean of three measurements)

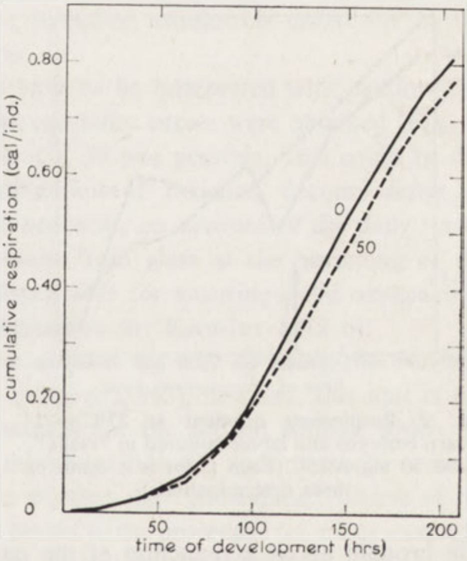

Fig. 8. Respiration cumulated for carp embryos and larvae cultured in "Pielik", 0 and 50 mg A.I./l; temperature of cultures

larvae was somewhat higher, as compared with those treated with herbicide (embryos: control 0.183 μl O_2/ind.·hour, treated 0.151; larvae: control 1.187, treated 1.173).

Fig. 8 shows the respiration cumulated from time 0 to time *n*. It was calculated from the instantaneous oxygen consumption measured at 23°C (Fig. 7), subsequently converted to oxygen consumption at cultures temperature (Fig. 2), multiplied by the oxy-calorific coefficient and cumulated to the successive hours of life. The energy expenditure by respiration was reduced in treated animals, compared with controls: in the 50th, 100th and 150th hr of development by 12, 5 and 9%, respectively.

Respiratory quotient (*RQ*) of embryos, determined a few hours after fertilization, was high and amounted to about 1 (Fig. 9), this presumably pointing to considerable participation of glycolysis in the metabolic processes. Later on, *RQ* showed a gradual drop. In the larvae it persisted at a low level of 0.5–0.7; this could testify to going on of the repayment of the oxygen debt accumulated during embryonal development (cf. the high oxygen consumption observed at this time — Fig. 7). After the 161st hr of development, i.e. at a time when the energy reserves in the yolk sac are about to become depleted, *RQ* showed a pronounced decrease. The mean *RQ* of the treated animals exceeded that of controls (embryos: treated 0.96, control 0.89; larvae: treated 0.61, control 0.53). This indicates that the herbicide reduces the participation or aerobic processes in favor of the anaerobic ones. It is noteworthy that the decrease in *RQ* after the 161th hr was more abrupt in treated animals than in controls; in the 190th hr the *RQ* of treated animals amounted to 0.26, this being an exceptionally low value. This signifies that the depletion of the energy reserves during the period of endogenous feeding is much greater in treated than in untreated animals.

Lactic acid content in herbicide-treated eggs and larval tissues is, on the whole, markedly higher than in controls (Fig. 10). This indicates that the exposure to herbicide brought about a reduction of the participation of aerobic processes in favor of the anaerobic ones. There is a noteworthy drop in lactic acid content after the 150th hr of development, i.e. in the period of depletion of the energy reserves. This drop was more pronounced in treated larvae, simultaneously showing also particularly low lactic acid values, in the 171th and 189th hr averaging 0.220 and 0.291 μg/ind., respectively. This testifies to the depletion of the energy reserves in treated larvae.

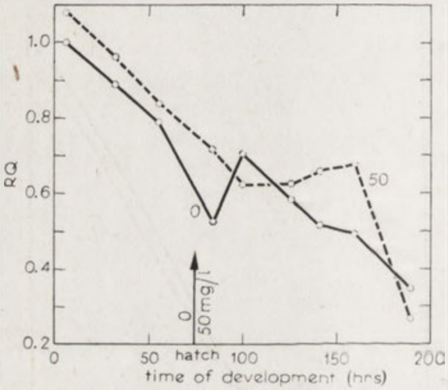

Fig. 9. Respiratory quotient at 23°C ± 0.1° in carp embryos and larvae cultured in "Pielik", 0 and 50 mg A.I./l. (Each point is a mean of three determinations)

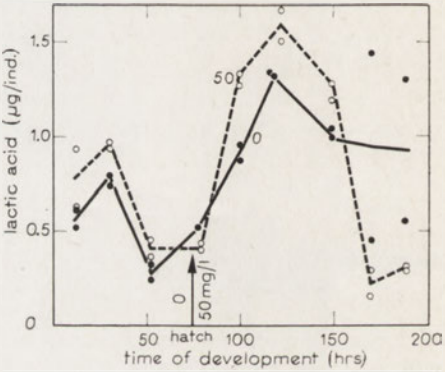

Fig. 10. Lactic acid content in carp eggs and larval tissues; animals cultured in "Pielik", 0 and 50 mg A.I./l. Temperature of cultures

It is stressed that the direction of the herbicide-induced metabolic changes, as indicated by all three determinations, is consistent: the herbicide impairs the aerobic processes in favor of the anaerobic ones, this being reflected by a drop in oxygen consumption, with a simultaneous increase in *RQ* and lactic acid content. Consequently, the herbicide brings about more rapid depletion of the energy reserves in treated animals, this being expressed by a more abrupt drop in *RQ* and lactic acid content in treated animals by the end of the endogenous feeding period.

4. DISCUSSION

In the Polish literature only few data on the occurrence of 2,4-D in surface waters are available. In studies of the lower Vistula Taylor et al. (1972 a) have found levels of 10 µg 2,4-D/l. According to Taylor et al. (1972 b), as a result of the plant protection actions the contamination of the neighboring water bodies is up to 40 µg/l, and in consequence of chemical maintenance of drainage ditches — it is up to 60 µg 2,4-D/l. In the present study the authors resolved to investigate the effects of higher concentrations, since in Poland the derivatives of arylalcanecarboxylic acids are in most common use as herbicides (Lipa, Głogowski 1970, Taylor et al. 1972 b), and since their application is expected to become even more widespread (Lipa, Głogowski 1970), and–on the other hand — because herbicides are known to injure the aquatic fauna by way of slow chronic poisoning caused by toxicant cumulation. However, it is stressed that the main aim of the present studies was to elucidate the mechanisms underlying the action of the herbicide studied under arbitrarily chosen laboratory conditions; therefore, caution is called for in the interpretation of the toxic concentrations.

The results of the present acute tests have to be interpreted with caution also because of the fact that no typical sigmoid mortality curves were obtained (Fig. 3), and thus no accurate calculation of 48-hr CL 50 was possible. This could be due to some experimental conditions: volatilization of toxicant, decomposition of active ingredient (this factor being hardly probable, on account of the daily replacement of liquids), leaching of some poisons from glass at the beginning of the experiment, small volume of liquid (indispensable for ensuring good oxygenation conditions for developing spawn — cf. discussion in: Kamler 1972 b).

Most of these shortcomings could be avoided by way of using the excellent flow apparatus designed by Alabaster, Abram (1965), however, this unit is too elaborate to warrant its construction especially for the present studies, which are only complementary to the long-term morphopathologic and metabolic investigations. Moreover, it is not at all certain whether the "non-typical" course of the mortality curves results only from faults inherent in the procedure (cf. many examples of a "non-typical" course of the curve in Luk'yanenko (1967).

In the literature the following CL 50 values for pure sodium salt of 2,4-D can be found:

As regards pure sodium salt of 2,4-D, its mortality threshold concentration (24-h) has been estimated by Alabaster (1969) for *Rasbora heteromorpha* at concentration 1020 mg/l. From the data of Okołowicz et al. (1970) this concentration can be inferred to remain, for *Asellus aquaticus* (Crustacea), within the range of 1000–2000 mg/l (48-h). According to Korde, Zvirgzds (1971), for embryos of *Misgurnus fossilis* (Pisces) placed in toxicant immediately after fertilization this concentration varies between 1300 and 2600 mg/l (18-h), whereas for embryos placed in toxicant 4 hrs after fertilization (stage of 32–64 blastomers) it exceeds 3900 mg/l. The present results (Table II) permit the conclusion that for carp embryos placed in toxicant 3 hrs after fertilization (stage of 16 blastomers) and carp larvae this concentration remains within the range of 1600–3200 mg/l (48-h). Obviously,

Table VI. C L 50 values for pure sodium salt of 2,4-D

Time	mg/l	Test organism	Authors
24-h	1160	*Rasbora heteromorpha* (Pisces)	Alabaster (1969)
24-h	ca 2000*	*Simocephalus vetulus* (Cladoceta)	Klekowski, Zwigzds (1971)
48-h	ca 650*	*Simocephalus vetulus* (Cladocera)	Klekowski, Zwirgzds (1971)
48-h**)	780	*Cloeon dipterum* (Ephemeroptera)	Solski (1968 b)

*) estimated on the basis of the survival curves presented in the original paper.
**) C 50.

at longer exposures the mortality threshold concentrations are much lower, for *Asellus aquaticus* varying between 180 and 320 mg/l (15 days, Okołowicz et al., 1970), and for *Lepomis macrochirus* (Pisces) exceeding 100 mg/l (12 days, Hiltibran 1967, 1969).

Formulated product "Pielik" is noxious for the aquatic fauna at much lower concentrations. According to Solski (1968 b), 48-h C 50 for *Cloeon dipterum* is 400 mg/l. Okołotowicz et al. (1970) have found in 48-h tests that "Pielik" used in concentration 112 mg A.I./l exerts no effect on the mortality of Asellus, and in concentration 200 mg A.I./l causes its increase. The lot of "Pielik" used in the present study was not very toxic, the mortality threshold concentration remaining within the range of 800–1600 mg A.I./l (Table II). Long-term tests indicate that also low concentrations of "Pielik" are toxic. Trzebiatowski (1969) has studied the formulated product "Antyperz" (38% of sodium salt of TCA), as well as "Antyperz" in mixture with "Pielik". "Antyperz", at a concentration of 190 mg A.I./l, exerted no effect on carps, whereas the same concentration of "Antyperz" with an addition of "Pielik", 42.5 mg A.I./l resulted in mortality of 33.4% in 1-year-old carps after 104 hrs of treatment. According to the data of Okołotowicz et al. (1970), the mortality threshold concentration for "Pielik", at 15 days' exposure, varies between 20 and 36 mg A.I./l. The present results testify to mass mortality of carp larvae cultured for 216–240 hours in "Pielik", 50 mg A.I./l (Table IV).

The above data clearly indicate that "Pielik" is more toxic than the pure compound. The mortality test, as well as the other tests applied (Table II) prove that the toxicity of the examined lot of "Pielik" was two times higher than that of the pure compound. Identical results have been obtained by Solski (1968 b), who compared various C 50 (from 3 to 96 hrs) of "Pielik" and of pure sodium salt of 2,4-D for *Cloeon dipterum*. The toxicity of "Pielik" can exceed that of the pure compound even more than twice, as pointed to by the above-mentioned findings of Okołotowicz et al. (1970). Braginskij (1972) quotes experiments on monuron, showing that the toxic substance contained in a formulated product did not consist of monuron itself, but of contaminants including parachloraniline, a highly toxic transient product of the synthesis. From a very extensive survey of the literature, Mullison (1970) also draws the conclusion that additional components of a formulated product may be more toxic than the active ingredient itself.

If it is kept in mind that:

a) "Pielik" concentration of 800 mg A.I/l did not increase the mortality and that of 1600 mg A.I./l did, whereas pure sodium salt of 2,4-D in concentrations up to 1600 mg/l failed to do so (Table II),

b) A concentration of 20 mg chlorophenols/l is lethal for fish (Luk'yanenko 1967),

c) The composition of "Pielik" is as follows: not less than 85% of sodium salt of 2,4-D and up to 3% chlorophenols (Polish Standard),

then the following reasoning can be put forward. At "Pielik" concentration of 800 mg A.I./l, the concentration of formulated product is 940 mg/l. If it were assumed that the chlorophenol contaminants account for, alternatively, 3%, 2% and 1%, then chlorophenol concentrations would amount to 28, 19 and 9.4 mg/l, respectively. Thus, it can be concluded that in the lot of "Pielik" used the chlorophenol contamination was $<2\%$. This theoretical conclusion was further confirmed by the analysis made by the Institute of Organic Industry, Warsaw. About 1% of chlorophenols, mainly 2,4-dichlorophenol, was found in the investigated lot of "Pielik". At "Pielik" concentration of 1600 mg A.I./l, the chlorophenol concentration would be 38 mg/l (if 2%) or 19 mg/l (if 1%), i.e. it would already be noxious, while pure sodium salt of 2,4-D at a concentration of 1600 mg/l is not. Thus, in this case the contaminants are doubtless also more toxic than the active ingredient itself. This indicates that testing of the active ingredients themselves can be of theoretical interest, while being of no practical importance, and that it is advisable to test formulated products, as applied in the field.

Long-term studies of the morphopathology and development demonstrated higher resistance, to "Pielik" concentration of 50 mg A.I./l, of eggs than of larvae. This result is in good agreement with our previous studies (Matlak 1972, Kamler 1972 b) as well as with the findings of other authors (Davis 1961, Hiltibran 1967, Solski 1968a). This proves that sometimes the egg case and the perivitelline fluid considerably protect the embryo from noxious substances. However, "Pielik" was found to prolong embryonal development, this being consistent with our earlier results (Matlak 1972, Kamler 1972 b) and with those of Korde, Zvirgzds (1971) who have observed delayed hatching of *Misgurnus fossilis* exposed to pure sodium salt of 2,4-D.

"Pielik"-induced diseases of larvae were the same as those previously observed by Matlak (1972). However, the pathologic changes are not specific for this poison. Similar symptoms resulting from oxygen deficiency have been observed in fish larvae by Gottwald (1960) — hydrocoele embryonalis, and by Gulidov (1971) — hemorrhages. Also butyl ester of 2,4-D (0.0125–5 mg/l) has been found by Shcherbakov, Chemova (1972) and Shcherbakov et al. (1973) to cause hemorrhages in young carps. Solski (1968 a) has demonstrated the occurrence of hydrocoele embryonalis in carp larvae developing in solutions of atrazine, and ventral deformation of the spinal cord and difficulties in filling of the swimming bladder with air in carp and pike larvae. Cooke (1972) has reported a DDT-induced delay of the development and weight gain of *Rana temperaria* tadpoles.

Mass deaths of larvae cultured in "Pielik" were observed between the 216th and 240th hr of development. Obviously, the noxious effect of toxicant became manifest with special sharpness in the depression period particularly critical for all fish larvae, being caused by resorption of the yolk sac, with its nutrients and blood vessels (Olifan 1949). At this time, also the control animals exhibited no weight gains (Fig. 6) and depressed respiration (Fig. 7). In previous studies (Matlak 1972, Kamler 1972 b) "Pielik" has been observed to also cause mass deaths of larvae at the critical stage of development, though somewhat earlier (in the 200th hr of development). Likewise, Solski (1968 a) has reported that 5-day-old larvae represent the most sensitive stage in the development of carp and pike.

The few larvae having survived the experiments showed symptoms of recovery. Solski (1968 a) has reported signs of recovery in carp larvae transferred from an atrazine solution to pure water. According to Wolf (1957), the recovery is irregular and, on the whole, involves low percentage of larvae.

Investigations on the dynamics of oxygen consumption fully confirmed the regularities observed in 1971 (Kamler 1972 b), viz. an exponential increase in oxygen consumption during embryonal and early larval development, followed by a high plateau of oxygen consumption, with a subsequent drop resulting from the depletion of the yolk sac prior to the switch-over to endogenous feeding (Fig. 7). The inhibitory effect of herbicide on oxygen consumption (Fig. 8), though slightly less pronounced than in the previous year, was confirmed. Owing to the action of herbicide, the cumulative energy expenditures for respiration were reduced relative to control by:

hour of development	100	150	180
1971 (Kamler 1972 b)	13%	11%	13%
1972 (present study)	5%	9%	6%

Perhaps, the fact of a smaller decrease in respiration, as observed in the present study, is due to the above-mentioned lower chlorophenol contamination of the lot of herbicide used.

Privolnev (1964) has reported that at the early stages of the embryonal development of fish *RQ* is high, attaining or exceeding 1. According to this author, in the period of fish embryonal development *RQ* fails to be a satisfactory index of the composition of the katabolyzed substances. These findings are consistent with the present results.

In the period of fish hatching, lactic acid content shows an increase (Fig. 10, as well as Trifonova (1949) and Milman (1965)). In Table VII compilation is made of lactic acid contents in control fishes and Chironomidae larvae (cultured under conditions similar to the natural ones), as found in this study and reported by other authors. These data indicate that eggs show low lactic acid levels, probably because of the small mobility of embryos. In fishes and Chironomidae larvae lactic acid contents were similar; surprisingly, in the present study lactic acid content in endogenously fed carp larvae was found to approach that in older fishes. The herbicide induced an increase in lactic acid content (Fig. 10).

Table VII. Lactic acid content in control groups of some fish species (1–5 and 8–10) and midge larvae (6 and 7) (computed data)

Species and tissue	Approx. mean live wt. (g/ind.)	Lactic acid content (mg/100 g live wt.)	Reference
1. *Misgurnus fossilis*, whole eggs	0.0030*)	3.3–10.0	Milman (1965)
2. *Cyprinus carpio*, whole eggs	0.0037–0.0038	6.7–21.2	present paper
3. *Carassius carassius*, muscles	8.0	26.7	Blažka (1958)
4. *Salmo trutta m. fario*, muscles	—	30.4	Blažka (1958)
5. *Anguilla anguilla*, whole body	0.25	11.0–44.0	Jędryczkowski (unpubl.)
6. *Chironomus plumosus*, whole body	—	8.7–49.0	Augenfeld (1967)
7. *Glyptotendipes polytomus*, whole body	0.01	28.3–45.0	Kamler, Srokosz (1973)
8. *Cyprinus carpio* larvae, whole body	0.0015–0.0018	34.3–85.8	present paper
9. *Gadus morhua*, muscles	552.0	66.8	Beamish (1968)
10. *Salmo gairdneri*, muscles	66.2–140.8	85.3–181.8	Burton et al. (1972)

*) Computed from diameter data (Rass 1971).

The present findings on the effect of "Pielik" on the metabolism of embryos and larvae unequivocally testify to the impairment, by this herbicide, of aerobic, processes in favor of the anaerobic ones (drop in oxygen consumption, rise of *RQ* and lactic acid content). These results are in good agreement with the previous ones (Kamler 1972 b) showing that "Pielik" caused a more intense increase in the total energy expenditure (i.e. a greater drop in the calorific equivalent of one organism, resulting from both a greater reduction of dry weight of one animal and a greater decrease in the calorific value of 1 mg dry weight). It is well known that glycolysis is energetically less effective than complete aerobiosis.

The mechanism underlying the effect of the herbicides studied on the metabolism has been elucidated some time ago. Namely, it has been demonstrated that 2,4-D (acid and its sodium salt) uncouples phosphorylation from substrate oxidation in the liver mitochondria system (Brody (1952) — rat; Whitehouse (1964) — rat; Hiltibran (1966) — fish; Zvizgzds et al. (1971) — fish).

Acknowledgements

We were greatly aided in our work by Mrs Emilia Duszak-Karczewska, by Miss Hanna Sztrantowicz, by Miss Maria Janik, by Dr. Stanisław Lewkowicz and by Dr. hab. Jan M. Włodek. Dr. Stanisław Łakota gave us valuable advice. We extend our thanks to all of them.

5. SUMMARY

1. The present studies are a continuation, within a wider scope, of the investigations reported in papers of Matlak (1972) and Kamler (1972 b).

2. Comparison was made of the toxicity of pure sodium salt of 2,4-D and of formulated product "Pielik" containing this salt as active ingredient (85%). Carp embryos and larvae were the object of study. Three acute tests applied in parallel showed that "Pielik" is about two times more toxic than pure sodium salt of 2,4-D (Table II). This is due to the high toxicity of contaminants present in the formulated product.

3. The sensitivity of three acute tests was compared. The 48-hr mortality test proved to be least sensitive (e.g., differences between "Pielik" and control became evident only at concentration 1600 mg A.I./l), whereas the test involving the mobility of embryos at the age of 27 hrs, cultured in test solutions during 24 hrs, was most sensitive (e.g., differences between "Pielik" and control were perceptible at concentration 200 mg A.I./l) (Table II).

4. Long-term tests evaluating the development, morphopathology and metabolism demonstrated the toxicity of "Pielik" at concentration 50 mg A.I./l, i.e. greatly exceeded the acute tests in sensitivity.

5. In long-term tests "Pielik" used at concentation 50 mg A.I./l proved not to be very harmful for carp, at the stage of embryonal development (Table III). On the other hand, in larvae it induced behavioral changes, disturbances in feeding, a number of morphopathologic changes, hemorrhages, delay of linear growth and, finally, deaths in the period of changing to exogenous feeing (Tables IV and V, Figs. 5 and 6). After discontinuation of the exposure to herbicide, the very few surviving larvae exhibited symptoms of recovery.

6. In long-term tests "Pielik" applied at concentration 50 mg A.I/l caused a reduction of oxygen consumption (Figs. 7 and 8), a rise of *RQ* (Fig. 9) and an increase in lactic acid content in the tissues (Fig. 10). This indicates that the herbicide imparis the metabolic aerobic processes in favor of the anaerobic ones. The resulting more rapid depletion of energy reserved in treated larvae proves to be lethal or at least imparis their further development.

6. STRESZCZENIE

1. Obecne badania stanowią kontynuację i rozszerzenie badań prezentowanych w pracach Matlak (1972) i Kamler (1972 b).

2. Porównano toksyczność czystej soli sodowej 2,4-D i preparatu handlowego, Pielika, którego powyższa sól stanowi substancję aktywną (85%). Obiektem doświadczalnym były embriony i larwy karpia. Paralelnie stosowane trzy ostre testy wykazały, że Pielik jest ok. dwukrotnie toksyczniejszy od czystej soli sodowej 2,4-D (Tabl. II). Przyczyną tego jest wysoka toksyczność zanieczyszczeń występujących w preparacie handlowym.

3. Porównano czułość trzech testów ostrych. Najmniej czułym okazał się 48-godzinny test śmiertelności (np. w Pieliku różnice z kontrolą pojawiły się dopiero w stężeniu 1600 mg/l), zaś najbardziej czułym testem — ruchliwość embrionów w wieku 27 godz., przetrzymywanych w roztworach testowych przez 24 godz. (np. w Pieliku różnice z kontrolą pojawily się w stężeniu 200 mg/l) — Tab. II.

4. Testy długotrwałe: rozwój, morfopatologia i metabolizm wykazały szkodliwość Pielika w stężeniu 50 mg/l, tzn. okazały się znacznie bardziej czułe od ostrych.

5. W testach długotrwałych Pielik w stężeniu 50 mg/l okazał się niezbyt szkodliwym dla karpia w okresie rozwoju embrionalnego (Tab. III). Natomiast u larw powodował zmiany behavioru zakłócenia w pobieraniu pokarmu, liczne zmiany morfopatologiczne, krwotoki, opóźnienie wzrostu liniowego i wreszcie śnięcia w okresie przechodzenia na pokarm egzogenny (Tab. IV i V, Fig. 5 i 6). Po zaprzestaniu stosowania herbicydu u bardzo nielicznych larw, które przeżyły obserwowano objawy zdrowienia.

6. W testach długotrwałych Pielik w stężeniu 50 mg/l spowodował obniżenie zużycia tlenu (Fig. 7 i 8), podniesienie *RQ* (Fig. 9) i podniesienie zawartości kwasu mlekowego w tkankach (Fig. 10). Świadczy to o upośledzeniu procesów aerobowych na korzyść anaerobowych w metabolizmie tych zwierząt pod wpływem badanego herbicydu. Doprowadza to do szybszego wyczerpania rezerw energetycznych u larw rozwijających się w obecności herbicydu i jeśli nawet nie doprowadza do śmierci, to upośledza dalszy rozwój tych zwierząt.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.) | 21 | 3/4 | 503–518 | 1974

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EXPERIMENTAL FISH CULTURE STATION AT GOŁYSZ-ZABORZE, CIESZYN DISTRICT; LABORATORY OF WATER BIOLOGY OF POLISH ACADEMY OF SCIENCES IN CRACOV

BIBLIOGRAPHY (1953–1973)

CONTENTS

1. INTRODUCTION

The Experimental Fish Culture Station at Gołysz-Zaborze is the Centre of pond ecosystem investigations of the Laboratory of Water Biology of the Polish Academy of Sciences in Cracov. It was founded in 1953.

It consists of three experimental farms. Their total area is 1252 ha with 796.86 ha of ponds. Two of them, namely Gołysz-Zaborze (443.65 ha of ponds) and Ochaby (94.09 ha of ponds) are situated in Cieszyn District and the third — Landek (259.12 ha of ponds) in Bielsko-Biała District. Both Districts belong to Silesia. All the laboratories and administration are at Gołysz-Zaborze. There are also here a library consisting of 7600 volumes.

The main fish bred is carp, but there are also other species: tench, crucian carp, the hybrid of crucian carp and carp, pike, pikeperch, grass carp, silver carp, welch and large mouth bass.

The research programme is concerned with the problems of intensification of pond production on wide hydrobiological basis. Those problems are subjected to elaborate work in the following sections: phytoplankton, zooplankton, hydrochemistry, hydrometeorology, microbiology as well as fish breeding, technology of fish feeding and making fish foods.

The bibliography contains publications dealing with subjects worked out at the Experimental Station of Gołysz-Zaborze, written by its workers or the workers of the Laboratory of Water Biology in Cracov. It contains as well as publications issued by other person making experiments at Gołysz-Zaborze or person using materials worked out at all the above mentioned experimental farms.

This bibliography does not contain short notices (informations) published in various papers. The publications are listed alphabetically after their authors names'.

It has been made up on the occasion of the coming 25th anniversary of the foundation of the Laboratory of Water Biology of the Polish Academy of Sciences in Cracov and is dedicated to all, who during the past years by their works have contributed to the development of the Experimental Fish Culture Station.

2. ABBREVIATIONS OF PERIODICALS

Acta hydrobiol. — *Acta Hydrobiologica*, Kraków.
Acta geophys. pol. — *Acta Geophysica Polonica*, Warszawa.
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4. SUBJECT INDEX

5. AUTHORS' INDEX

Acknowledgements

I am very grateful to Dr Ewa Kamler and M. A. Renata Głowacka for many valuable suggestions and bibliographic comments as well as for reviewing the manuscript.

(Pol. Arch. Hydrobiol.) POLSKIE ARCHIWUM HYDROBIOLOGII	21	3/4	519	1974

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In 1944 he was awerded the degree of Candidate of Agricultural Sciences and in 1951 — of Doctor of Biological Sciences, whereoupon he was appointed to the post of professor.

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Prof. B. S. Kuzin died on April 26, 1973.

He was the author of about 30 scientific paper of great theoretical and partical importance. He personally edited 22 volumes of publication "Trudy Borka", 27 issues of the information bulletin of the Biological Institute for Inland Waters Research and nine various monographs.

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