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Paper arrangement. A paper should be arranged as follows: 1. ABSTRACT — a brief report of the topic, results and conclusions, not exceeding 100 words, 2. INTRODUCTION — containing a formulation of the subject, a statement on its actual stage of elaboration, and a clear definition of the aim of the paper, 3. MATERIAL and METHODS — the description must be sufficiently detailed to enable a repetition of the procedure by other researchers, 4. RESULTS — only the results attained in the work should be dealt with under this heading, 5. DISCUSSION — a comparison of the results brought by the paper with results of other works, an elucidation of theoretical and logical aspects, deductions and conclusions,

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	18	4	325–345	1971

J. B. GOLACHOWSKA[1]

THE PATHWAYS OF PHOSPHORUS IN LAKE WATER

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ABSTRACT

This paper reports the results of some investigations carried out in Poland in 1965 and 1966, in different types of lakes. The quality, vertical distribution and conversion of total phosphorus and its various forms was estimated in the lake waters. Special attention was given to some sources of the dominant forms of phosphorus as well as to the influence of calcium, iron, and humus compounds on the processes of phosphorus circulation. It was found that sorption of phosphorus by lake bottom sediments depends to a large degree on the chemical properties of the muds.

CONTENTS

1. INTRODUCTION

Phosphorus concentration in the waters of Polish lakes is very little known. A little data on this subject can be found in the papers of Stangenberg (1936), Patalas (1960), Solski (1964), and Golachowska (1968). Little is also known the phosphorus content in the lakes· of the other region of the world. A little data, with no comment with regard to total phosphorus or organic phosphorus, is found in Aberg and Rodhe (1942), and Thomas (1949). There is a lack of literature about the quantity and the forms of phosphorus occurring in the water of midland basins. Hutchinson (1957) summarized some of the data, and drew the conclusion that total phosphorus content in the lake water of the world was very low (0.000–0.140 mg/l). The work of Ohle (1934) showed that the concentration of total phosphorus in the waters of some regions of the world must be significantly higher.

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2. TERRAIN DESCRIPTION AND METHODS

Thirtheen Polish lakes were chosen for investigation. They were classified according to Stangenberg (1936). This classification is based partly on the thesis of Naumann (1932).

Water and sediments were collected in the summers of 1965 and 1966. Water was taken with a Ruttner sampler at the deepest part of each from the following depths: surface, 3, 5, 8, 10, 15, 20, 25 m and at 10 m intervals to the bottom.

A vertical water column was investigated each month during the summer and autumn in lakes Charzykowo, Konarzyny and Ostrowite. The surface waters of Charzykowo Lake (10 km long) were also investigated at 15 points. Samples of the bottom sediment were taken with an Ekman dredge at the deepest part of each lake. At the same time, samples of water from each lake were taken for chemical analysis and determination of chlorophyll content. A total of 2660 water samples were analysed. Only the results of the analyses for phosphorus are presented here. The other data will be presented in a later paper. The various fractions of total phosphorus were regarded according to the ideas stressed by Ohle (1938), Strickland and Parsons (1960), Armstrong (1965) and Olsen (1966).

Total phosphorus was estimated by the method of wet digestion after Strickland and Parsons (1960). Phosphates were estimated after the method of Vogler (1965), which is based on the widely known method of Murphy and Riley (1962).

Lake water was filtered on glass filter paper (Whatman GF/C) just after sampling. Total phosphorus and phosphates were estimated directly both in unfiltered and in filtered water. Some of the forms of phosphorus were estimated directly (540 analyses) while others were estimated indirectly as is stated below.

The term "total phosphorus" includes all forms of phosphorus occurring in water both in organic and mineral form as well as in the dissolved and the particulate states.

In total phosphorus are included four basic forms which have significant importance for interpretation of biological processes which take place in a water basin. These were defined as follows: 1. dissolved orthophosphate — directly determined phosphate in filtered water; 2. dissolved organic phosphorus — estimated as the difference between the total phosphorus in filtered water and the phosphate directly determined in filtered water; 3. mineral particulate phosphorus — estimated as the difference between the directly determined phosphate in unfiltered and filtered water; 4. organic particulate phosphorus — estimated as the difference between the total particulate phosphorus and the mineral particulate phosphorus.

The results of the field and laboratory studies showed that different chemical environments influence phosphorus uptake by algae. In an attempt to solve this problem, the algae *Scenedesmus quadricauda* was cultivated in various media following Rodhe (1948) and Overbeck (1962 a, b). After observations of algae growth on different media the following composition was chosen: $Ca(NO_3)_2$ — 0.207 g, KNO_3 — 0.025 g, KH_2PO_4 — 0.010 g, $MgSO_4 \cdot 7H_2O$ — 0.050 g, K_2CO_3 — 0.034 g, $Fe_2(SO_4)_3$ — 0.00125 g. When the above quantities of components were dissolved in 1 l of distilled water, the pH of the medium was 6.8.

Experiment 1. To the above medium was added various quantities of iron as $FeCl_2$, calcium as CaO, and humus substances in the form of peat extract (Szymański 1962). Seven different experiments were set up and each of the experiment consisted of seven different concentrations of each component. All experiments were duplicated and the results given as the mean. The control consisted of only the above basic medium and the algae. Algae chlorophyll and total phosphorus content were used as indices of growth. A photostat with the light intensity of 4500 lux at the level of cultures was used for experiment. Light was supplied by "glittering" lamps. The temperature during all experiments was 20°C.

Experiment 2. Experiments on the uptake and release phosphorus by algae, were carried out in light and in darkness. Algae cultured on the medium with the above mentioned composition were centrifuged and washed with distilled water. They were then transferred to freshly prepared medium with the content of 0.54 mg PO_4 in 100 ml of medium. On this medium algae were kept for 12 hr in the photostat (4500 lux). After this time they were centrifuged and washed again with distilled water. In this way the phosphorus in the medium was separated from the phosphorus filled cells. The algae were then transferred quantitatively to Erlenmayer flasks containing 200 ml of water each. The experiments were carried out in two series — in the light and in the darkness. Each experiment was repeated and the result expressed as the mean of the two values. Each series consisted of 13 samples. Exposure time was 5760 min (96 hr). In the samples taken, estimations were made of: total phosphorus content in the algae biomass and content of total phosphorus, dissolved orthophosphate and dissolved organic phosphorus in the surrounding water.

In order to measure the sorption ability of the bottom sediments of the lakes studied a very simple experiment was carried out. This experiment consisted of several parts. Each part of the experiment was carried out on the sediments of ten lakes. In the first part, ten grams of dry and pulverized sediments of known chemical composition were mixed with 200 ml of KH_2PO_4 solution (1.5 mg PO_4/l) and distilled water, then kept in a dark bottle at a temperature of 20°C. In the second part of the experiment, the sorption system was acidified with HCl to pH 6. In the third part of the experiment the sorption system was made more basic with NaOH to pH 8.0–9.6. In the fourth part of this experiment the sediments were enriched with iron as $FeCl_3$ or with calcium as CaO or with both of them together. The pH of water with sediments, if not changed purposely, was 7.1 to 7.5 for all experiments. Flasks with sediments were shaken by hand from time to time. After two and after ten hours, the samples were filtered on Whatman GF/C filters and the phosphates in the solution were estimated. All measurements were duplicated and the results are presented as the mean.

3. RESULTS

Total phosphorus

Total phosphorus content in the lakes examined varied from 0.022 mg/l PO_4 in the mountain oligotrophic Lake Morskie Oko, to 4.64 mg/l PO_4 in the eutrophic Lake Konarzyny (Table I).

The total amount of phosphorus in the vertical water column of Charzykowo Lake increased from spring to the summer stagnation period, then decreased (Fig. 1). During the spring–summer period, the first rapid increase of phosphorus in the entire vertical water column occurred in June. This increase coincided with the first high values of chlorophyll in this lake (Fig. 2). The vertical distribution of total phosphorus is shown in Fig. 3. From the presented data, it can be seen that, although there is a tendency of total phosphorus content to increase toward the bottom, at middle depths there are very significant maxima and minima of total phosphorus. They are probably connected with the high density of plankton, with the epilimnion-metalimnion and metalimnion-hypolimnion borders and also with underwater currents.

Dissolved orthophosphate

The content of dissolved orthophosphate in lake water varied from 0.006 mg/l PO_4 in the oligotrophic Lake Morskie Oko to 3.52 mg/l PO_4 in the eutrophic Lake Konarzyny (Table I). Dissolved orthophosphate content increased toward the bottom (Fig. 4). This form of phosphorus does not have sharp maxima or minima at particular depths of the lake.

Table I. Extreme values of phosphorus and chlorophyll in a vertical water column of some characteristic lakes in the summer of 1965

Lake	Limnological type	Surface (ha)	Maximum depths (m)	Phosphorus (mg/l PO_4)			Chlorophyll (μg/l)
				Total	mineral	organic	
Morskie Oko	oligotrophic	33.4	50.8	0.022—0.112	0.006—0.010	0.012—0.102	1.3—4.6
Hańcza	oligotrophic	305.8	108.5	0.088—0.152	0.035—0.063	0.053—0.089	1.2—8.7
Wigry	α-mesotrophic	2166.8	73.0	0.084—0.114	0.041—0.052	0.043—0.245	1.3—12.7
Babięty Wielkie	α-mesotrophic	251.5	56.0	0.300—1.200	0.057—0.097	0.228—1.123	2.8—8.5
Piłakno	α-mesotrophic	30.1	38.0	0.200—0.860	0.057—0.087	0.143—0.797	2.1—9.0
Ostrowite	α-mesotrophic	280.7	43.0	0.144—0.336	0.088—0.129	0.056—0.207	2.5—14.0
Charzykowo	β-mesotrophic	1348.1	30.5	0.520—3.336	0.410—2.764	0.110—0.572	5.5—5.6
Mikołajskie	eutrophic	470.5	27.8	0.450—2.250	0.063—0.940	0.250—1.310	2.1—33,0
Białe Sejneńskie	eutrophic	23.2	12.5	0.096—0.202	0.031—0.095	0.056—0.107	8.1—16.5
Konarzyny	eutrophic	6.8	12.1	1.400—4.640	0.520—3.520	0.880—1.120	42—360
Lisunie	—	25.0	7.5	0.528—0.688	0.040—0.057	0.488—0.646	7.0—14.5
Flosek	acidotrophic	0.5	6.0	0.312—1.680	0.052—0.088	0.227—1.592	8.2—12.1
Smolaczek	sidero-acido-dystrophic	1.0	4.5	0.208—0.408	0.880—0.880	0.120—0.320	19.7—52.0

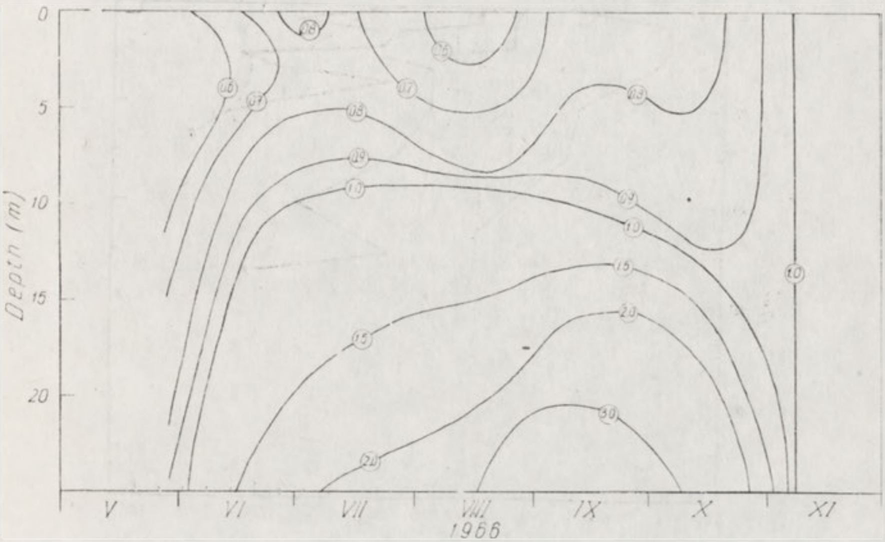

Fig. 1. Seasonal variation in concentration of total phosphorus in a vertical water column in Charzykowo Lake in 1966

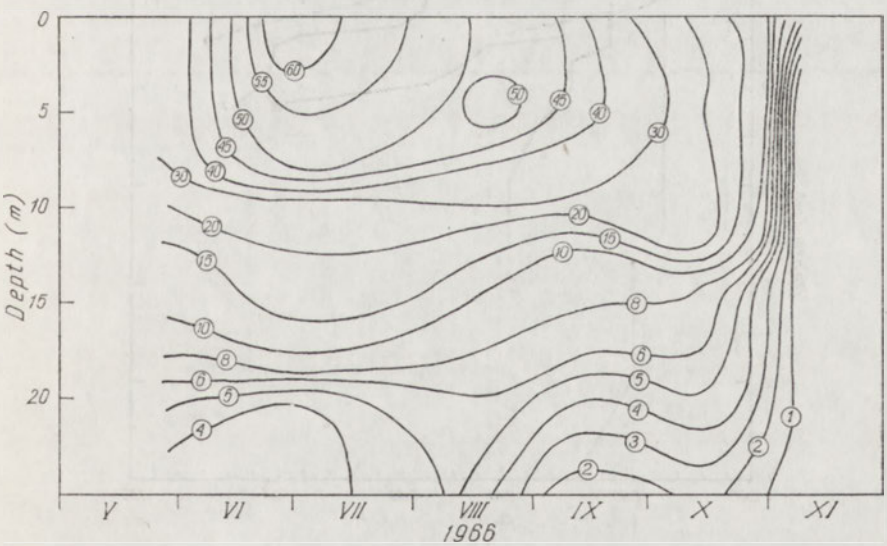

Fig. 2. Chlorophyll concentration in a vertical water column in Charzykowo Lake in 1966

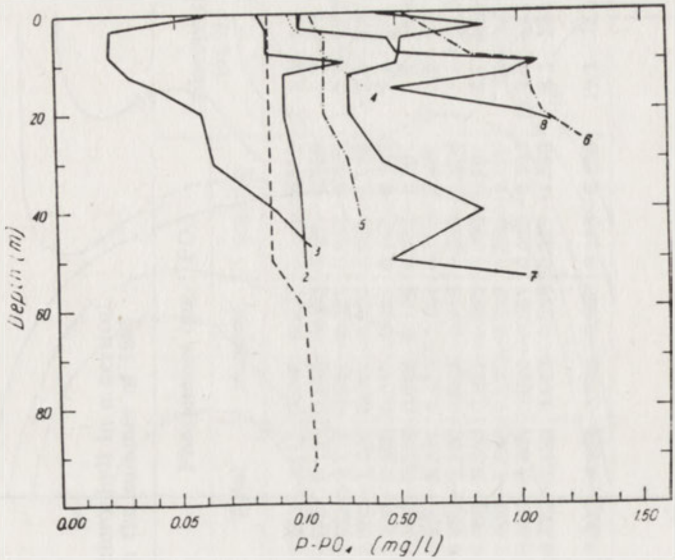

Fig. 3. Vertical distribution of total phosphorus in the summer of 1965 in lakes: 1 — Hańcza, 2 — Wigry, 3 — Morskie Oko, 4 — Białe Sejneńskie, 5 — Ostrowite, 6 — Charzykowo, 7 — Babięty Wielkie, 8 — Mikołajskie

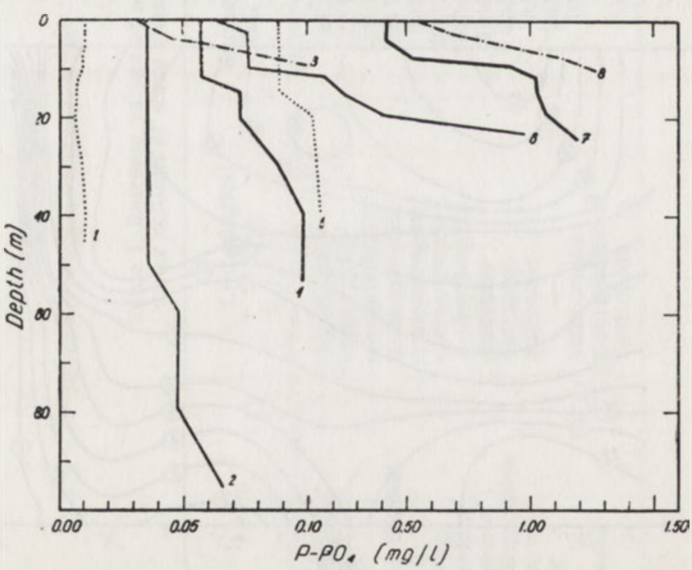

Fig. 4. Typical vertical distribution of dissolved orthophosphate in 1965 in Polish lakes: 1 — Morskie Oko, 2 — Hańcza, 3 — Białe Sejneńskie, 4 — Babięty Wielkie, 5 — Ostrowite, 6 — Mikołajskie, 7 — Charzykowo, 8 — Konarzyny

From comparison of the vertical distribution of orthophosphate and dissolved oxygen it can be seen that increase in orthophosphate content is accompanied by decrease in oxygen content (Fig. 5). In eutrophic Mikołajskie Lake it was observed that increase in the concentration of orthophosphate was accompanied by an increase of iron (Fig. 6). The variation in the horizontal distribution of orthophosphate in Charzykowo Lake was within the range 0.36 to 0.47 mg/l PO_4. The mean quantities were 0.40 mg/l PO_4 for 15 sampling stations, the location of which is shown in Fig. 7. The percentage of the means of the different forms of phosphorus in total phosphorus is shown in Fig. 8. Orthophosphate comprises the largest part of total phosphorus, while dissolved organic phosphorus occupies the second position. The conversion of phosphate to the other forms of phosphorus differed in the various types of lakes (Fig. 9).

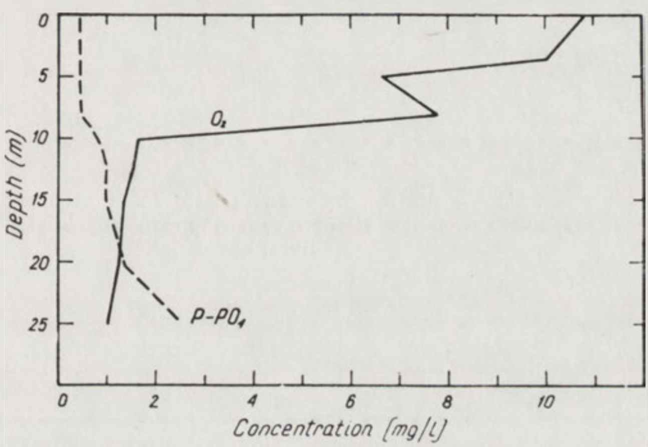

Fig. 5. Vertical distribution of dissolved orthophosphate and oxygen in 1966 in Charzykowo Lake

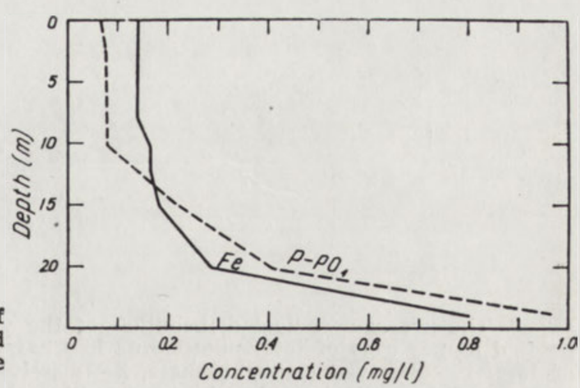

Fig. 6. Vertical distribution of dissolved orthophosphate and iron in 1965 in Mikołajskie Lake

In α-mesotrophic lakes in summer phosphate represented 35–50% of the total phosphorus, in β-mesotrophic lakes 60–80% and in the eutrophic lakes 20–80%.

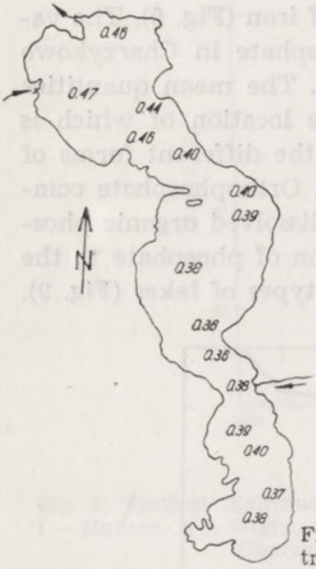

Fig. 7. Station locations (1–15) for horizontal distribution of dissolved orthophosphate in 1966 in Charzykowo Lake

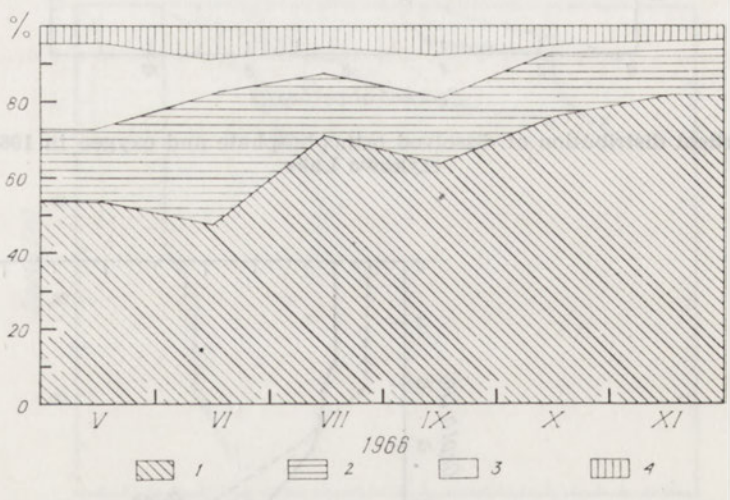

Fig. 8. The average vertical distribution of the various forms of phosphorus, expressed as per cent of total phosphorus in Charzykowo Lake, during the summer of 1966. 1 — dissolved orthophosphate, 2 — dissolved organic phosphorus, 3 — organic particulate phosphorus, 4 — mineral particulate phosphorus

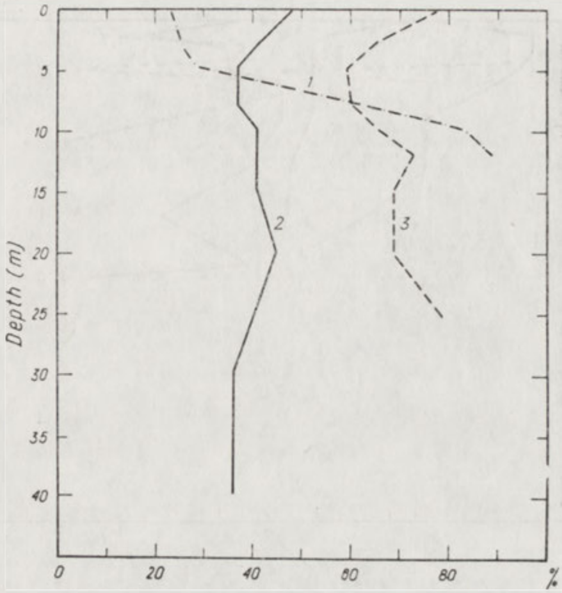

Fig. 9. Dissolved orthophosphate as per cent of total phosphorus in different types of the lakes, during the summer of 1966. 1 — Lake Konarzyny (eutrophic), 2 — Lake Ostrowite (α-mesotrophic), 3 — Lake Charzykowo (β-mesotrophic)

Total organic phosphorus

The content of total organic phosphorus (dissolved and particulate) ranged from 0.012 mg/l PO_4 in Lake Morskie Oko to 1.592 mg/l PO_4 in Lake Flosek (Table I). Organic phosphorus content had its maximum at various depths in the lakes investigated (Fig. 10). The main part of the total organic phosphorus consisted of dissolved organic phosphorus. The percentage of dissolved organic phosphorus in the vertical profile of total phosphorus of the lakes investigated was independent of the type of lake (Fig. 11). In the eutrophic Lake Konarzyny at a depth of 5 m, dissolved organic phosphorus composed 56% of the total phosphorus. This percentage decreased with increase in depth. In the α-mesotrophic Lake Ostrowite dissolved organic phosphorus increased with increasing depth. In β-mesotrophic Charzykowo Lake, the percentage of this form of phosphorus was low, with a maximum of 25% of the total phosphorus at the depth of 5 m. Table II gives an example of the seasonal changes of dissolved organic phosphorus in Charzykowo Lake. The highest content of this form of phosphorus was noted in the spring–summer period and the lowest was noted during the winter under the ice. The part which

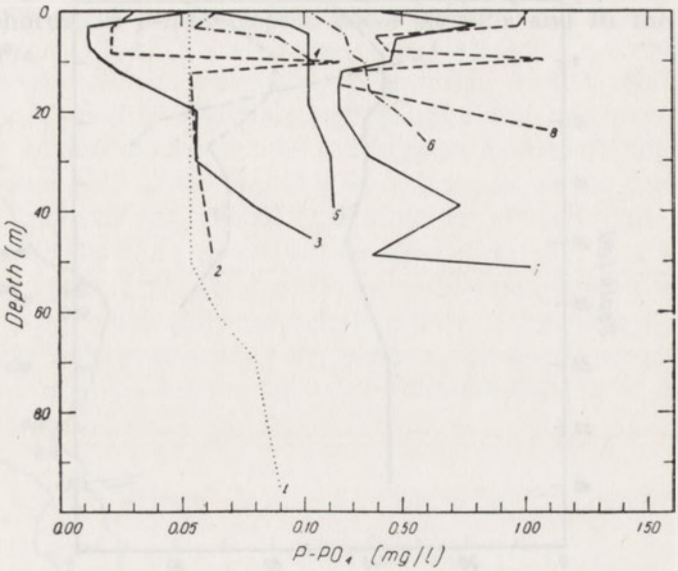

Fig. 10. Vertical distribution of total organic phosphorus (dissolved and particulate) in 1965 in lakes: 1 — Hańcza, 2 — Wigry, 3 — Morskie Oko, 4 — Białe Sejneńskie, 5 — Ostrowite, 6 — Charzykowo, 7 — Babięty Wielkie, 8 — Mikołajskie

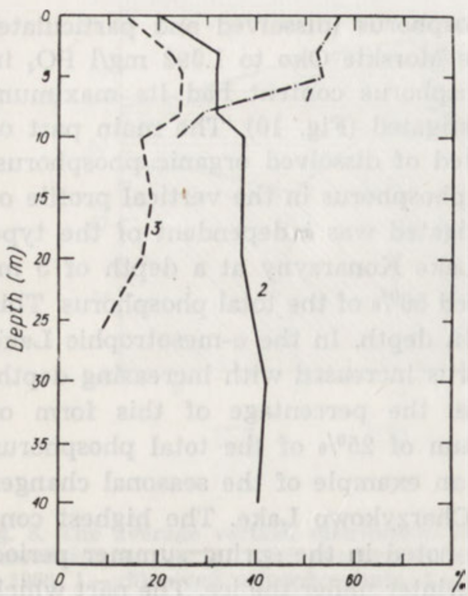

Fig. 11. Vertical distribution of dissolved organic phosphorus expressed as per cent of total phosphorus in typical lakes in 1966. 1 — Lake Konarzyny (eutrophic), 2 — Lake Ostrowite (α-mesotrophic), 3 — Lake Charzykowo (β-mesotrophic)

Table II. The average content of the chlorophyll and the various forms of phosphorus in Lake Charzykowo, 1966–1967

Date	Chlorophyll (μ/l)	Dissolved orthophosphate (%)	Dissolved organic phosphorus (%)	Organic particulate phosphorus (%)	Mineral particulate phosphorus (%)
28.V.1966	19.9	53.56	18.80	24.96	2.84
28.VI	33.8	46.67	34.59	10.35	8.42
16.VIII	29.1	70.37	16.58	7.72	5.10
8.IX	25.1	62.96	18.22	10.79	8.29
10.X	19.4	76.15	17.48	1.55	5.63
8.XI	1.0	81.82	10.90	3.60	3.60
27.II.1967	0.0	91.81	0.91	0.27	7.01

total organic phosphorus (dissolved and particulate) played in the total phosphorus in all the investigated lakes is shown in Table III. The average values of total organic phosphorus in most of the investigated lakes were higher than the content of the total mineral phosphorus. Total organic phosphorus was sometime as high as 90% of total phosphorus. An exceptionally high relationship of mineral to organic phosphorus (1 : 0.3) was observed in Lake Charzykowo which is polluted by domestic sewage from the town. This relationship in Lake Konarzyny which is enriched by extensive use of superphosphate fertilizers in the drainage basin was 1 : 0.6.

Table III. The average concentration of total mineral and total organic phosphorus in a vertical water column in lakes in the summer of 1965

Lake	Phosphorus ($mg/l PO_4$)			Phosphorus (%)		Ratio of P mineral to P organic
	total	mineral	organic	mineral	organic	
Morskie Oko	0.050	0.008	0.042	16.0	84.0	1 : 5.2
Hańcza	0.099	0.039	0.060	39.4	60.6	1 : 1.5
Babięty Wielkie	0.555	0.072	0.483	13.0	87.0	1 : 6.7
Piłakno	0.512	0.063	0.449	12.3	87.7	1 : 7.1
Wigry	0.112	0.042	0.070	37.2	62.8	1 : 1.7
Ostrowite	0.222	0.097	0.125	43.9	56.1	1 : 1.3
Charzykowo	1.330	1.006	0.324	75.5	24.5	1 : 0.3
Białe Sejneńskie	0.135	0.056	0.079	41.5	58.5	1 : 1.4
Mikołajskie	1.012	0.232	0.780	22.7	77.3	1 : 3.4
Konarzyny	2.588	1.571	1.017	60.7	39.3	1 : 0.6
Lisunie	2.420	0.181	2.239	7.5	92.5	1 : 12.4
Flosek	2.348	0.225	2.123	9.6	90.4	1 : 9.4
Smolczek	0.275	0.088	0.187	32.0	68.0	1 : 2.1

Phosphorus uptake and release by algae

From data mentioned above, it can be seen that phosphate content in the trophogenic zone of different types of investigated lakes was low. Also, the phytoplankton production varies and was not always directly proportional to the orthophosphate concentration (Table I). Therefore, the number of cycles of orthophosphate exchange between phytoplankton and lake water cannot be the same.

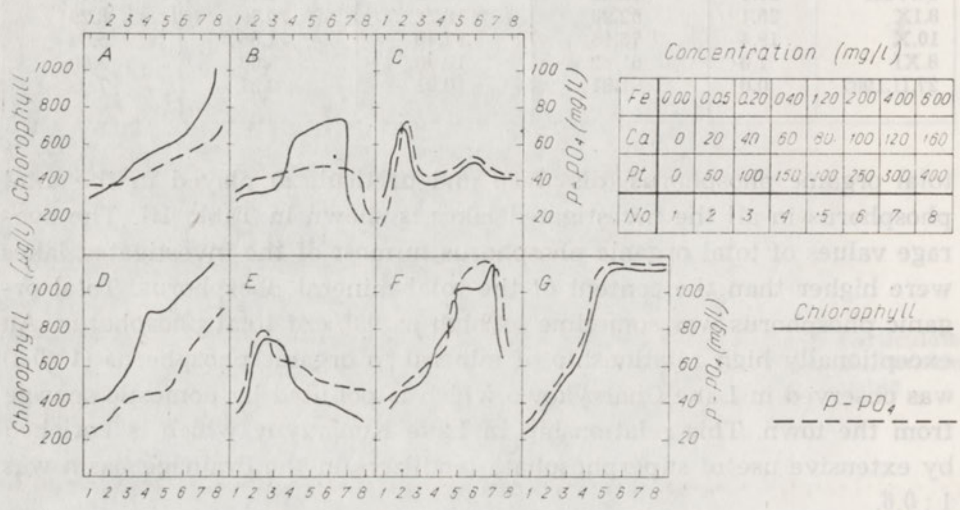

Fig. 12. Chlorophyll and phosphorus concentration in the biomass of algae in the standard medium in relation to that with added quantities of: A — iron, B — calcium, C — humus, D — iron and humus, F — iron and calcium, G — iron, calcium and humus

The results of the experiment investigating the influence of calcium, iron and humus compounds on the orthophosphate uptake and release by algae are shown in Fig. 12. From this it can be seen that:

A. The addition of $FeCl_3$ in the range of 0.05–6.0 mg/l Fe, was favourable to the growth of *Scenedesmus quadricauda*.

B. The addition of CaO was optimal within the limits of 60–120 mg/l Ca.

C. The addition of humus extract in small quantities (50 mg/l Pt), supported growth of algae, but higher quantities were not favourable to growth of the culture.

D. The addition of iron with humus extract always gave better results than iron alone in the same quantity.

E. The addition of calcium together with humus extract in small quantities was favourable to the growth of algae, but amounts above

80 mg/l Ca or more than 200 mg/l Pt restrained development of the culture.

F. The addition of calcium and iron together was very favourable up to quantities of 120 mg/l Ca and 4 mg/l Fe. Higher amounts rapidly stopped development.

G. The addition of humus extract, calcium and iron always gave good results and the cultures reached maximal development.

A second series of experiments was undertaken. In these experiments algae were kept in distilled water in the light and in the dark. Total phosphorus content in the biomass of the algae in each sample before transfer to the flasks containing distilled water was 800 µg PO_4. After transfer of the algae to the flasks, they were exposed to the light and the quantities of various forms of phosphorus were measured (Table IV).

Table IV. Release of phosphorus (mg/l PO_4) from *Scenedesmus quadricauda* cultures in light

Time (min)	Phosphorus in algae biomass	Phosphorus in distilled water		
		total	dissolved orthophosphate	dissolved organic phosphorus
0	800	430	416	14
5	—	460	416	44
15	—	460	416	44
30	—	460	432	28
60	—	460	440	20
90	—	460	440	20
120	750	460	440	20
180	—	460	440	20
360	—	490	416	74
720	—	480	408	72
1440	725	470	400	70
2880	700	410	392	18
5760	825	350	344	6

After the shortest exposure time the total amount of phosphorus found in the solution were 430 µg/l PO_4. Of this amount 416 µg/l were orthophosphates and 14 µg/l were in the form of dissolved organic phosphorus. The above mentioned quantities of phosphorus are therefore released by the algae immediately upon transfer. During this experiment there was noted a gain of dissolved organic phosphorus by the distilled water and a loss of total phosphorus by the algae biomass (Table IV, V). This process was particularly significant in the series of samples kept in the darkness (Table V).

Table V. Release of phosphorus (mg/l PO_4) from *Scenedesmus quadricauda* cultures in darkness

Time (min)	Phosphorus in algae biomass	Phosphorus in distilled water		
		total	dissolved orthophosphate	dissolved organic phosphorus
0	800	490	432	58
5	—	500	432	68
15	—	510	432	78
30	—	510	432	78
60	—	530	448	82
90	—	560	448	112
120	675	570	456	114
180	—	590	456	134
360	620	620	464	156
720	—	630	472	158
1440	600	670	504	166
2880	425	680	512	168
5760	400	710	512	198

Sorption by lake sediment

The experiment with sediments was done to investigate the sorption of phosphorus by the mud. Air dried sediments were used. Before starting the experiments, the basic chemical composition of the sediments was determined (Table VI). Deposits which had above 50% of organic

Table VI. Chemical composition (%) of air-dried bottom sediments of the examined lakes in the summer of 1965

Lake	Mineral matter					Organic matter
	SiO_2	$Fe(OH)_3$	$CaCO_3$	$P-PO_4$	total	
Smolaczek	4.03	5.34	1.58	0.22	11.17	88.27
Flosek	5.72	4.27	6.50	0.35	16.86	83.52
Lisunie	14.48	14.96	6.20	0.46	36.10	62.39
Piłakno	33.42	10.68	24.50	0.25	68.85	31.13
Białe Sejneńskie	46.51	11.37	16.12	0.78	74.78	25.39
Mikołajskie	53.30	10.68	13.50	1.10	78.58	21.84
Babięty Wielkie	23.56	13.89	40.40	0.78	78.63	21.33
Hańcza	66.94	9.20	3.50	1.32	80.94	20.44
Wigry	13.13	7.48	61.42	0.35	82.38	15.60
Morskie Oko	72.12	10.82	6.20	0.30	89.44	11.90

matter such as those from lakes Smolaczek, Flosek and Lisunie were defined as "organic deposits". Those which had less than 50% organic matter (the rest of the lakes) were defined as "mineral deposits". The results of the experiment show that only mineral sediments have any

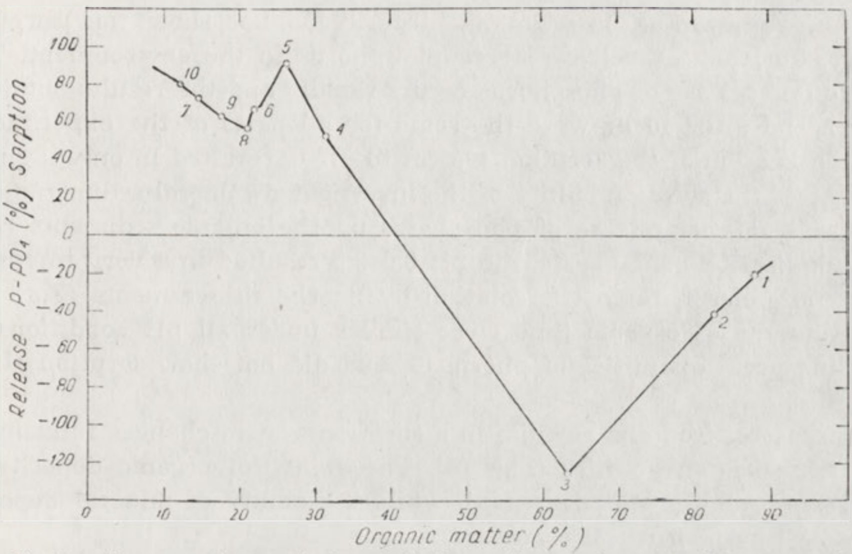

Fig. 13. Sorption or release of phosphate by sediment from the lakes: 1 — Smolaczek, 2 — Flosek, 3 — Lisunie, 4 — Piłakno, 5 — Białe Sejneńskie, 6 — Mikołajskie, 7 — Babięty Wielkie, 8 — Hańcza, 9 — Wigry, 10 — Morskie Oko

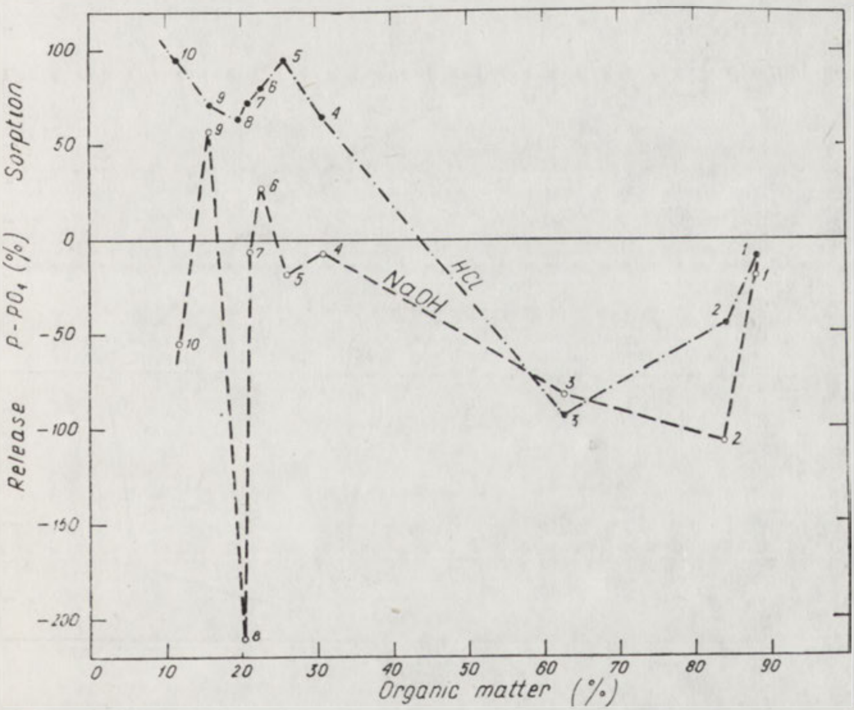

Fig. 14. The influence of acidification or alkalization on sorption of phosphate by sediments of the lakes: 1 — Smolaczek, 2 — Flosek, 3 — Lisunie, 4 — Piłakno, 5 — Białe Sejneńskie, 6 — Mikołajskie, 7 — Babięty Wielkie, 8 — Hańcza, 9 — Wigry, 10 — Morskie Oko

sorption ability (Fig. 13). Organic deposits do not show this sorption ability, but they do release stored phosphorus to the environment. The sorption or release of phosphorus occurs rapidly and the results obtained after 2 and after 10 hr were the same for all parts of the experiment.

Acidification of the sorption system to pH 6, resulted in only a slight increase of the sorption ability of the investigated mineral sediments and a decrease of the release of phosphates by the organic sediments (Fig. 14). Alkalization of the system to pH 8.0–9.6 resulted in a very intensive release of phosphate to the solution by nearly all sediments (Fig. 14).

Sediments of organic type were similar under all pH conditions in releasing great quantities of phosphate and did not show sorption abilities.

Increase of iron and calcium in a sorption system changes fundamentally the sorption abilities (Fig. 15). The ability of organic deposits to release phosphorus was decreased, while the ability of mineral deposits to absorb phosphorus was increased.

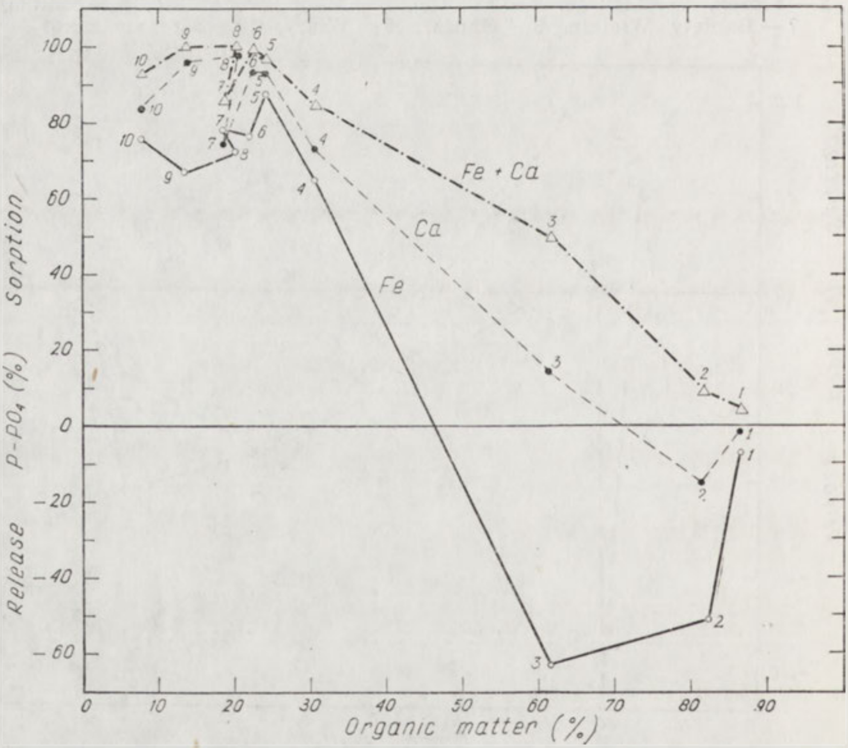

Fig. 15. The influence of enrichment of bottom sediments with iron and calcium on the sorption of phosphate. 1 — Smolaczek, 2 — Flosek, 3 — Lisunie, 4 — Piłakno, 5 — Białe Sejneńskie, 6 — Mikołajskie, 7 — Babięty Wielkie, 8 — Hańcza, 9 — Wigry, 10 — Morskie Oko

4. DISCUSSION

Total phosphorus content in the Polish lakes investigated in 1965 and 1966 varied in the range from the lowest values found in the oligotrophic mountain Lake Morskie Oko (0.022–0.112 mg/l PO_4) to the highest values found in the eutrophic Lake Konarzyny (1.400–4.640 mg/l PO_4). These limits apply also to the vertical distribution of total phosphorus in all Polish lakes which have been studied (Table I). These values are significantly higher in total phosphorus than those given by Hutchinson (1957) for the lakes of the world. Aberg and Rodhe (1942) mentioned that total phosphorus content in a vertical column of water increased with depth. Present data for vertical distribution of total phosphorus shows that the maximum of total phosphorus may occur at any depth in a lake.

Dissolved orthophosphates do not have maxima or minima at different depths (Fig. 1). The increase in content of this form of phosphorus from the surface to the bottom is gentle in oligotrophic lakes but sharp in eutrophic lakes. Transformation of phosphate into other forms of phosphorus is different in the waters of different types of lakes (Fig. 9). In euthrophic lakes and those lakes approaching eutrophy, the per cent of phosphate in total phosphorus is especially high. In oligotrophic lakes this percentage is lower which is due to slower rate or different type of mineralization of organic matter in these lakes.

Vertical distribution of total organic phosphorus (dissolved and particulate) in the lake examined (Fig. 10) is similar to the vertical distribution of total phosphorus (Fig. 3). The percentages of the particular forms of phosphorus in total phosphorus in the lakes of the different types were found to be: 40—60% for dissolved orthophosphate, 16—43% for dissolved organic phosphorus, 3–20% for organic particulate phosphorus and 4.2–11.1% for mineral particulate phosphorus. During the summer in the water of the investigated lakes the major form of total phosphorus was dissolved orthophosphate, while the second most important form was dissolved organic phosphorus. Hutchinson's (1957) data, showed that dissolved organic phosphorus made up about 61% of total phosphorus in northern Wisconsin lakes and about 29% in Lisney Pond. The above mentioned forms of phosphorus have a basic significance for the interpretation of the biological processes which take place in the water. The above mentioned forms of phosphorus are not exactly the same as these forms as classified by Strickland and Parsons (1960) for sea water forms of phosphorus. It is possible, however, to use these forms to sketch the transformation and circulation pathways of phosphorus in lakes.

The field studies mentioned above showed that the uptake of orthophosphate by algae must be influenced to a large degree by the chemical differentiation of the water environment, especially the presence of humus substances, iron and calcium. A partial explanation of this matter was found in the laboratory (Fig. 12). It was found that large amounts of humus compounds (greater than 200 mg/l Pt) inhibited development of algae. This supports the hypothesis of Gessner (1934) about the biological inactivity of phosphate in the presence of large amounts of humus substances. A full test of this hypothesis requires more data.

On the basis of the results obtained here, it is possible to suppose that the presence of iron and calcium remove, in some manner, the biological inactivity of orthophosphate caused by an excess quantity of humus compounds. In all cases iron had a positive influence on orthophosphate uptake by algae.

The data obtained show that orthophosphate content in the trophogenic layer of the lakes of different types is, as a rule, low (Table I). Therefore, if production of phytoplankton in the lakes is different (high in eutrophic and low in oligotrophic), this means that turnover of phosphorus, although always rapid, is different in the different lakes. The results of the isotope experiments of Harris (1957), Golterman (1960) and Watt and Hayes (1963) support this statement.

The fact that algae release dissolved organic phosphorus to the water in the light and in the dark (Table IV, V) explains to some degree the large per cent (16–43%) of this form of phosphorus in the total phosphorus of the water of the lakes investigated. This form of phosphorus occurs mostly in the summer period and has a quantitative relationship with the chlorophyll content (Table III). Release of phosphorus to the water by algae probably proceeds at two levels. The first level is the release of reserve phosphorus which is stored loosely in the algae cell in the form of orthophosphate and dissolved organic phosphorus. The second level is the release of phosphorus which is strongly bounded to the organic substances of the cell. Loosely stored phosphate can oscillate between the algae cells and the environment depending upon conditions. Algae are therefore one of the main sources of dissolved organic phosphorus in lake waters. A low content of chlorophyll and high content of organic phosphorus in Piłakno and Babięty Wielkie Lakes (Table I), permit the supposition that the second important source of organic phosphorus in lake waters could be bacteria. There is a lack of such types of data in the limnological literature.

One of the important factors which remove phosphorus from circulation in lake is sorption of bottom deposits. This is evident during the autumn period when the phosphorus content in the water decreases

(Fig. 1). The little work Olsen (1958, 1964), Hayes et al. (1958) on this subject has shown the complexity of the laws of phosphorus balance between water and the lake bottom. Mortimer (1941) gave a large significance to ferric hydroxide in the phosphorus absorption by the deposits, and MacPherson and Sinclair (1958) called attention to the influence of pH in release of phosphorus from the deposits. Experiments presented here showed that phosphate was absorbed only sediments of the "mineral" type (Fig. 13). Sediments of "organic" type, do not show this phenomenon, but in some conditions (after alkalization) they are able to release stored phosphates from deposits. After alkalization, the sediment of Lake Hańcza, the most oligotrophic lake of Poland released more than 220% of phosphate to the solution. The sediments of the oligotrophic mountain Lake Morskie Oko give similar results. The above show that bottom deposits of oligotrophic lakes store significant amounts of phosphorus which may be released in specific conditions.

Increase of iron and calcium in a sorption system changed fundamentally the sorption abilities of the lake sediments. Sediments of the organic type lost the ability to release phosphorus but began to show specific characteristics of mineral deposits such as the ability for sorption of phosphorus from the environment (Fig. 15). This research again stresses the complexity of the laws concerning the balance of phosphorus between the water and bottom sediments.

5. SUMMARY

The picture of the circulation of phosphorus in lakes presented here shows that the production ability of a water body is determined not only by the absolute level of phosphorus content but also the form in which it occurs.

In lakes of different types total phosphorus in the summer period consists of 40–60% dissolved orthophosphates, 16–33% dissolved organic phosphorus, 3–20% particulate organic phosphorus and 4.2–11.7% particulate mineral phosphorus. These four forms have a basic significance for the interpretation of biological processes. Attention is given to the fact in some of the lakes in the summer period a significant position in total phosphorus balance is played by dissolved organic phosphorus. It is supposed that the sources of this form are algae and bacteria.

It is shown that humus compounds, iron and calcium strongly influence the phytoplankton production. These compounds may act either as inhibitors or stimulators.

Significant loss of phosphorus from lake water in the autumn period was caused by sorption by bottom deposits of the "mineral" type. Deposits of "organic" type show very weak sorption and, therefore, in some conditions they release phosphates to the water.

6. STRESZCZENIE

Przedstawiony obraz krążenia fosforu w jeziorach wykazał, że w ocenie zdolności produkcyjnej zbiornika wodnego decyduje nie tylko absolutny poziom zawartości fosforu, ale również postać w jakiej on występuje.

W jeziorach różnych typów w okresie letnim 40–60% fosforu całkowitego sta-

nowią rozpuszczone w wodzie ortofosforany, 16–33% rozpuszczony fosfór organiczny, 3–20 fosfór organiczny sestonu i 4,2–11,7% fosfór mineralny sestonu. Wyróżnione 4 postaci fosforu mają zasadnicze znaczenie dla interpretacji procesów biologicznych. Zwraca uwagę fakt, że w niektórych jeziorach w okresie letnim znaczną pozycję w bilansie fosforu całkowitego zajmuje rozpuszczony fosfór organiczny. Przypuszcza się, że źródłem tej postaci fosforu są glony i bakterie.

Wykazano, że związki humusu, żelaza i wapnia wywierają silny wpływ na produkcję fitoplanktonu. Związki te mogą działać hamująco lub pobudzająco.

Znaczny ubytek fosforu z wody jezior w okresie jesieni jest spowodowany sorpcją przez osady przydenne typu mineralnego. Osady typu organicznego wykazują bardzo słabą sorpcję, a nawet w pewnych warunkach uwalniają fosforany do wody.

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TRANSPIRATION OF REED (*PHRAGMITES COMMUNIS* TRIN.)

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ABSTRACT

The changes of transpiration intensity of *Phragmites communis* Trin. as depending on the time of day, month of the vegetation, presence or lack of the panicle and the age of leaves were investigated. The influence of infection by *Ustilago grandis* Fr. was also investigated. It was found that all above factors influence the intensity of reed transpiration. Transpiration of reed can influence the lake water balance.

CONTENTS

1. INTRODUCTION

Transpiration, i.e. the evaporation of water from the plant tissues, is known, according to Strebeyko (1966) for more than 200 years. Gravimetric methods are used for the estimation of the transpiration intensity in natural and experimental conditions. The description of the methods of transpiration measurements is given by: Burgerstein (1920), Huber (1927), Ivanoff (1928), Stocker (1929), Hygen (1951) and others.

The amount of water transpired by plants is significant for the water balance of plant and its environment. In the case of helophytes, i.e. also the reed, in order to estimate the water balance of the water body it is necessary to know the ratio of the amount of water lost by transpiration to the amount evaporated from the free water surface, especially for the artificial reservoirs with controlled water level (Novikova 1963).

The paper aimed at the estimation of the following: amount of water transpired by reed (*Phragmites communis* Trin.) during a day and a season, the influence of the age of leaves and of panicle formation on transpiration, the share of leaves and panicle in transpiration, influence of the infection by *Ustilago grandis* Fr. on reed transpiration. *Ustilago grandis* Fr. occurring mainly in the northern Poland (Durska 1969), attacks young reed during spring, but the infection is visible at the end of July. Infected reed is bent and weak, and never flowers (Durska 1970).

2. MATERIAL AND METHODS

The measurements of transpiration intensity were carried out during the vegetation season of 1969 in a reed-belt of Mikołajskie Lake (Masurian Lakeland). The upper parts of reed shoots, 110 cm long, with and without a panicle, cut from green healthy plants were used for the measurements. The leaves (without the leaf sheaths) and panicles were collected separately. The samples were collected by from two states, a terrestrial and aquatic one, located on the south-west shore of Mikołajskie Lake. The terrestrial state was 5 m from the lake shore, the aquatic one was in a reed-belt at the depth of 50 cm. One sample consisted of 6 similar fragments of plants. A total of 260 measurements was made.

The transpiration was measured with the help of a weight method of Huber (1927). A cut shoot, leaf or panicle was immediately weighed. Then these parts of plants were placed on the sampling site in a position similar to the natural one, and weighed again after 5 minutes. The difference between these two weights was the weight of water transpired during 5 minutes. As the transpiration of cut fragments was not compared with that of growing plants, cutting in the paraffine cover was not applied, as the increase of transpiration of a cut fragment during the first minute was surely compensated by its slowing down in last two minutes of exposition, according to the results of Ivanov et al. (1950).

The samples were weighed on a rapid microbalance after Arland ("Mikro--Schnellwaage zur Anwelkmethode n. Prof. Dr. Arland für Feldversuche" — firm Paul Polikeit, Halle), the accuracy of which was 10 mg. The cut parts of reed were immediately placed in a beaker with a 5 mm water layer to prevent the air getting into the damaged tissues during the measurements. The balance was placed in a closed compartment 10 m from the lake shore.

The temperature and relative humidity of air (with the help of Assman psychrometer), solar radiation (with the help of universal solarimeter after Gorczyński) and wind velocity (with the help of anemometer after Robinson) were estimated paralelly with the transpiration measurements.

The transpiration intensity was calculated per unit of plant area (leaves and shoots with leaves but without a panicle) and per unit of fresh weight (for all samples) per one hour.

The leaf area was calculated acc. to the formula (Ondok 1968):

$$P = 0.57 \cdot d \cdot r$$

where: P — leaf area, d — leaf length (with the accuracy of measurement of 1 mm), r — maximal leaf width (with the accuracy of measurement of 1 mm), 0.57 — coefficient determining the difference between the area of a rectangle and a real leaf area calculated with the help of this method.

Coefficient 0.57 used in this paper was calculated by Szczepański (unpublished), and according to Ondok it is 0.54.

Assuming that a reed shoot has a shape of a sharp cone, its area was calculated according to the formula:

$$P = \pi \cdot 0.5\, \varnothing \cdot l$$

where: P — shoot area, $\varnothing$ — lower diameter of shoot (with the accuracy of measurement of 0.1 mm), l — shoot length (with the accuracy of measurement of 10 mm).

The changes of transpiration intensity of reed during a day were estimated by measurements carried out on a sunny day in hour intervals from 4 a.m. to 8 p.m.

During the investigation period, every ten days the transpiration and the length of reed were measured at $12.^{34}$ a.m., i.e. at the moment of the highest position of sun in this area.

3. RESULTS

The climatic conditions during the vegetation period of 1969 slightly differed from the average ones (Table I).

Table I. Climatic conditions during the summer and autumn of 1969 (data of the Synoptical Station of State Hydrological and Meteorological Station in Mikołajki)

Month	Decade	Air temperature (°C)			Relative humidity %	Sum of rainfall (mm)
		max.	min.	mean		
June	I	17.1	8.2	12.6	77	19.5
	II	22.5	13.3	17.7	78	6.4
	III	22.6	12.2	17.5	67	0.2
July	I	22.2	12.4	16.8	73	22.1
	II	22.3	12.3	16.9	71	4.1
	III	25.6	15.4	20.4	71	0.0
August	I	24.7	13.1	18.8	59	0.0
	II	21.4	12.9	16.9	78	12.9
	III	18.7	11.3	16.7	82	35.8
September	I	19.2	10.4	14.6	78	5.2
	II	19.8	9.7	14.2	74	8.4
	III	15.0	7.5	10.9	81	15.3
October	I	13.2	6.3	9.4	81	5.4
	II	13.1	6.4	9.3	89	0.0
	III	9.9	3.7	6.4	80	33.9

Daily changes of transpiration intensity of reed

The highest transpiration intensity was found between 11 a.m. and 1 p.m. on both terrestrial and aquatic sites (Fig. 1 and 2). During the day reed from terrestrial site transpired on the average 155.8 mg/g per hour and 4.1 mg/cm² per hour, with the average values of air temperature 23.2°C, relative air humidity 65%, and solar radiation 0.06 cal/cm² per min.

For reed from the aquatic site these values were relatively: 298.9 mg/g per hour, 6.9 mg/cm² per hour, 20.3°C, 76% and 0.35 cal/cm² per min.

The measurements were carried out on a windless day.

A positive dependence of transpiration intensity on the air temperature and solar radiation, and a negative one on relative air humidity was found for plants from the aquatic site. On the terrestrial site these dependences were not so visible, and especially that of transpiration on solar radiation, because the reed on this site was shadowed by trees in the afternoon.

Apart from the upper parts of reed with leaves and panicles, the leaves and panicles were studied independently from the aquatic site.

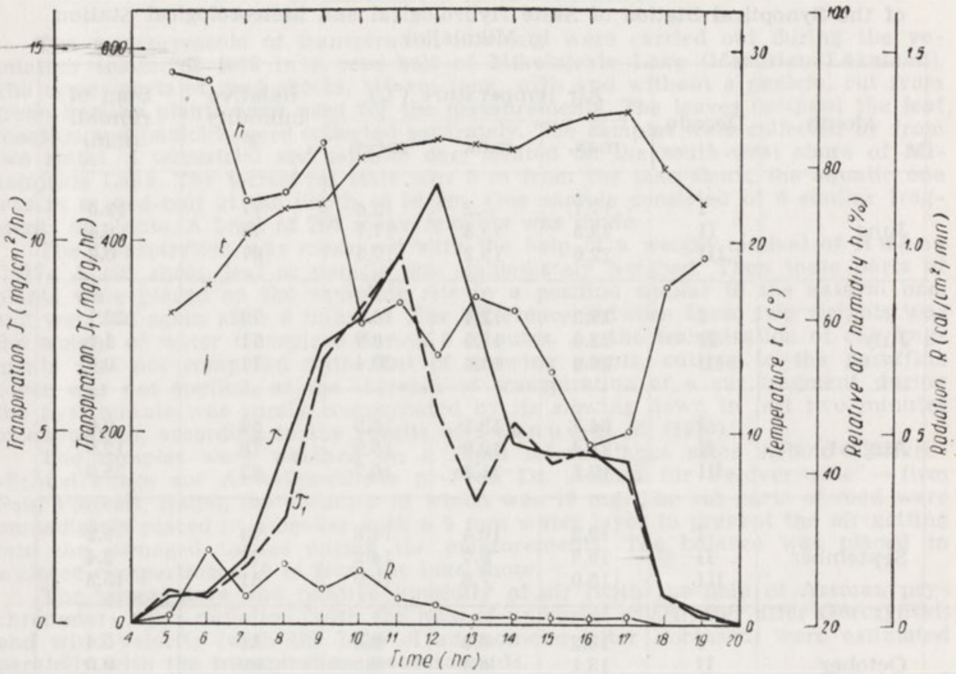

Fig. 1. Changes of reed transpiration on the terrestrial site during a day, and changes of the environmental conditions

It was found, that the leaves had the highest transpiration intensity (Fig. 3). The panicles transpired 90% less than leaves, shoots about 50% less than leaves (daily average).

Seasonal changes of transpiration intensity of reed

The most intense transpiration on a terrestrial site was observed in the third decade of July, while on the aquatic site — in the first decade of August (Fig. 4 A). In these periods the relative humidity on both sites was low (Table II): terrestrial site, 30 July — 51%, aquatic site, 9 August — 60%; there was no rain falls (Table I).

During the investigated period the reed growing on land transpired 108.9 mg/g hr at the average temperature 17.2°C, average relative air humidity 72%, and average wind velocity 0.8 m/sec. The average values for the reed growing in water were relatively: 305.9 mg/g/hr, 17.5°C, 74% and 0.9 m/sec. Thus the transpiration of reed from land was not more than 36% of the transpiration of reed from the lake.

It was also found that the reeds growing on the shore were about 28% shorter than the lake ones (Fig. 4 B).

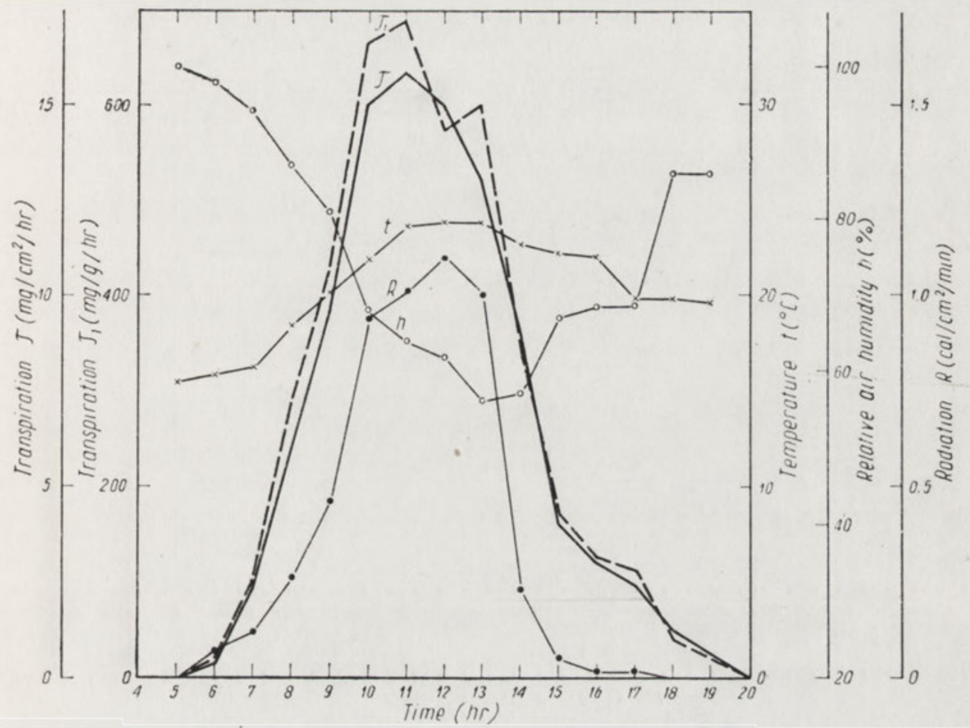

Fig. 2. Changes of reed transpiration on the aquatic site during a day, and changes of the environmental factors

Table II. Climatic data at the time of reed transpiration measurements (1969)

Date	Air temperature (°C)		Relative humidity (%)		Wind velocity (m/sec)	
	1	2	1	2	1	2
20.VI	22.0	22.4	87	76	0	0
30.VI	17.2	18.6	74	57	0	1
10.VII	17.4	18.2	83	65	0	1
20.VII	17.8	18.0	78	83	1	1
30.VII	23.0	21.8	51	73	2	2
9.VIII	18.0	18.4	70	60	1	0
19.VIII	20.2	21.0	60	73	2	2
29.VIII	16.4	17.4	77	76	1	1
8.IX	17.6	17.4	74	89	0	0
19.IX	12.8	12.2	62	72	1	1
30.IX	10.8	10.8	84	66	2	1
13.X	13.0	12.2	67	93	0	1

1 — on the terrestrial site.
2 — on the aquatic site.

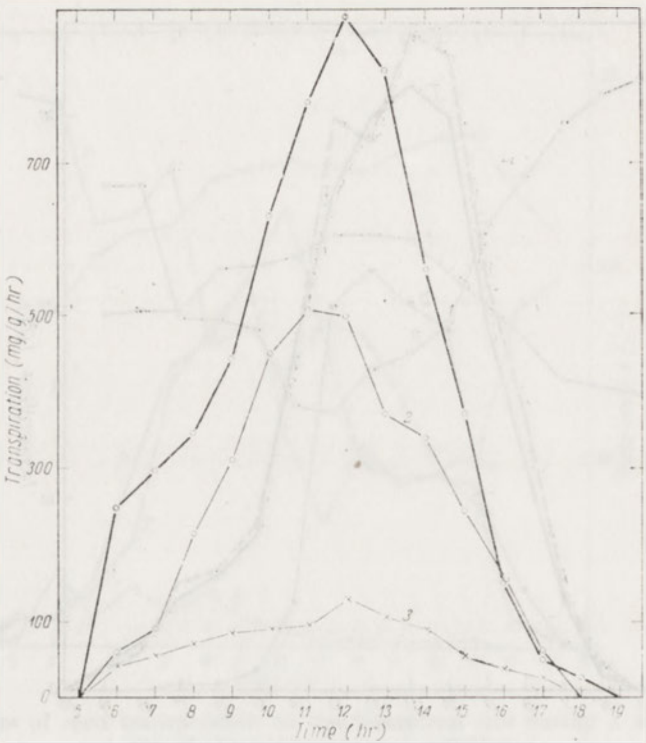

Fig. 3. Changes of transpiration of upper parts of reed shoots with leaves and panicles compared with transpiration of leaves and panicles. 1 — leaves, 2 — shoots, 3 — panicles

Transpiration intensity of successive leaves of reed

The number of leaves was counted on plants with and without a panicle. A reed shoot without a panicle had on the average 11 leaves while a flowering shoot — 14. For the reed growing in water the transpiration intensity of successive leaves was estimated. Shoots with and without a panicle were studied separately. The first green leaf at the lower end of the shoot was assumed to be the oldest one. The assimilation area and fresh weight of each leaf were estimated. It was found that the leaves of the flowering plants transpire less than the leaves of shoots without a panicle (Fig. 5 A), apart from their greater assimilation area (Fig. 5 B) and fresh weight (Fig. 5 C). It was also found that the upper leaves (younger) transpire more than the lower (older) ones (Fig. 5 A: 1 and 2).

The leaves of reed shoots without panicles transpired on the average 981.1 mg/g/hr and 15.44 mg/cm²/hr, while the leaves of flowering shoots — 669.4/mg/hr and 11.5 mg/cm²/hr. The above results show that the leaves of reed without panicle transpire about 50% more than the leaves of

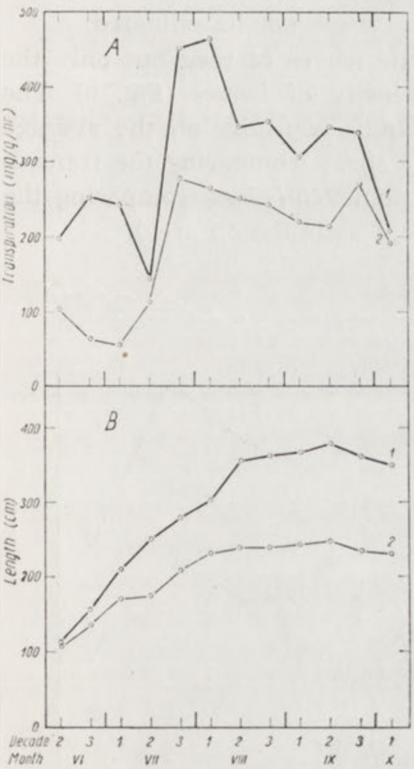

Fig. 4. Seasonal changes of transpiration (A), and length (B) of reed. 1 — on the terrestrial site, 2 — on the aquatic site

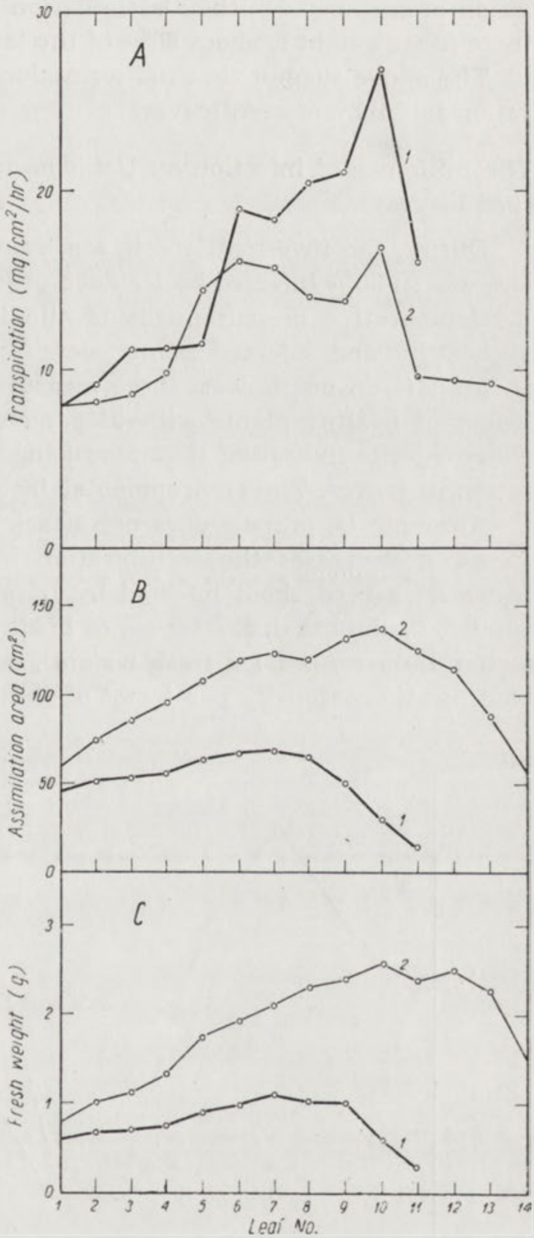

Fig. 5. Transpiration intensity (A), assimilation area (B) and fresh weight (C) of successive leaves on a reed shoot (from the oldest to the youngest leaf). 1 — shoots without panicle, 2 — flowering shoots

flowering reed (calculated per fresh weight) or over 30% more (calculation area), however their assimilation area is about 40% smaller, and their fresh weight is about 60% of the latter.

The above suggest that the formation of a panicle lowers the transpiration intensity of reed leaves.

The influence of infection by *Ustilago grandis* Fr. on transpiration of reed leaves

During the investigations it was found that the reed on the aquatic site was in 30% infected by *Ustilago grandis* Fr.

Comparative measurements of the transpiration intensity of leaves of healthy and infected plants were made. As the shoots infected by *U. grandis* do not flower, these measurements were carried out on the leaves of healthy plants without panicles, taking into consideration the above results indicating that producing a panicle decreases the transpiration of leaves. The environmental factors were not investigated.

Although *U. grandis* does not attack the leaves of reed but only the stems, it decreases the transpiration intensity of leaves (Fig. 6). The leaves of a reed shoot infected by *U. grandis* transpire on the average about 50% less than the leaves of healthy shoot (comparing the transpiration intensity per 1 g fresh weight), or about 40% less (comparing the transpiration intensity per 1 cm^2 of the leaf assimilation area).

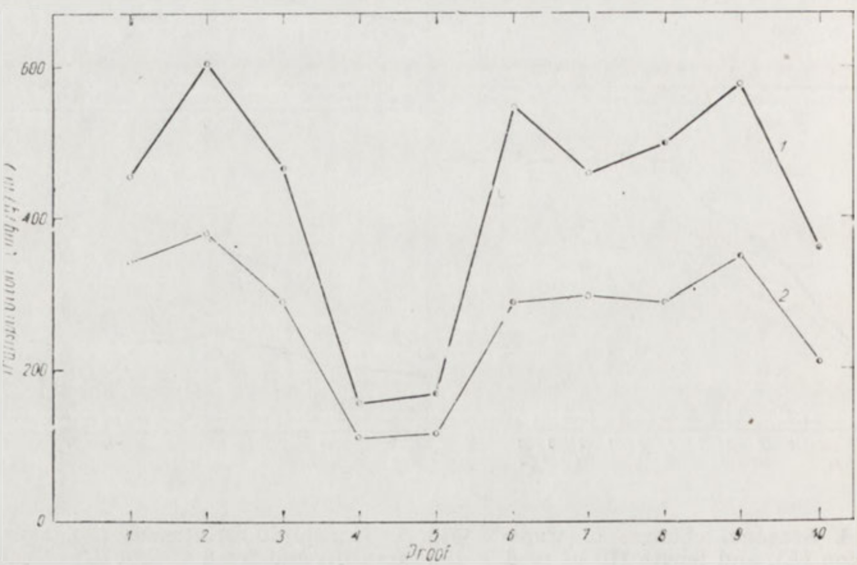

Fig. 6. The influence of the infection by smut (*Ustilago grandis* Fr.) on the transpiration of reed leaves. 1 — healthy shoots, 2 — infected

Such a great influence of the infection of reed by *U. grandis* on the reed transpiration intensity should be taken into consideration when calculating the water balance of reed-belt infected by this species.

4. DISCUSSION

The obtained results on reed transpiration intensity depend on the humidity of the plant soil, on the aquatic or terrestrial growth. This agrees with the data of Beydeman (1960), who found that transpiration intensity of reed growing on land and in brackish waters is lower than that of reed from fresh waters. However, it should be pointed out that the reed on an aquatic site formed a pure community of Scirpo-Phragmitetum association, typical for the littoral zone of eutrophic lakes (Tomaszewicz 1969), while the reed on a terrestrial site was in an association with 23 other plant species, thus competed for light and water. Also water forms a natural environment for reed.

According to the investigations of Demidovskaja and Kiričenko (1964) the number of stomata on the lower side of leaf is greater if the humidity of the soil is lower, therefore the transpiration intensity is greater. However, transpiration of the reed growing in water was found to exceed that on terrestrial site. This can be explained by a lack of "defence" against the excessive transpiration by reed growing in water. The stomata are not closed even on hot sunny days, and the reed transpires all day with half open stomata (Novikova 1963). However, it does not effect the transpiration, as Von den Honert (1948) found that at the 50% of the stomatal aperture, the stomata are effective regulators of the transpiration process.

The investigations of the transpiration of successive leaves showed that the upper (younger) leaves transpire more than lower (older) ones. This can result from the presence of greater amount of stomata on younger reed leaves (Demidovskaja and Kiričenko 1964). Also it can be due to the ageing process, smaller possibilities of protein synthesis, stronger hydrolysis, changes of the amount of chlorophyll and loss of the ability of cells division (Peach 1950).

Transpiration of reed during the night was not found. However, it is possible that it was below the accuracy of measurements, due to high relative humidity of air and dew settling on the leaves.

According to Uryvaev (1953) transpiration of helophytes can be even three times higher than the evaporation of water from the free surface of a reservoir. The importance of reed transpiration for the water balance of the investigated lake was analysed. In Mikołajskie Lake of a surface area 460 ha, 39.2 ha are overgrown by emergent macrophytes, reed occupies 32.3 ha (Kowalczewski and Wasilewski (1966). The shoot density of a studied reed-belt was 54 shoots per 1 m^2 (Szczepański unpublished). Taking into consideration the average value of the transpiration intensity of reed during the vegetation season and the shoot density in the investigated reed-belt it was found that the reed from 1 m^2 can transpire 2.23 kg of water per day. Such an intensity of transpiration, as well as the evaporation from the free water surface resulted in a lower water level in the lake, which during the investigations dropped some 25 cm.

Rudescu et al. (1965) calculated that a single reed shoot in Danube delta transpires in a vegetation season 33 to 50 kg of water, when the reed-belt density is 30 shoots per 1 m^2 — the reed transpires about 5 kg of water per 1 m^2 per day.

Thus the daily value of transpiration per 1 m^2 found in this paper (2.23 kg) does not differ much from the one found by Rudescu et al. (about 5 kg). The difference can be the result of various environmental conditions, as e.g. climate, solar radiation in a reed-belt depending on the reed density, etc. The average length of reed found in this paper was about 270 cm, thus it was lower than the reed from the Danube delta investigated by Rudescu et al. (450 cm).

Acknowledgements

The author acknowledges with thanks valuable help of Ass. Prof. Dr A. Szczepański during the preparation of this paper.

5. SUMMARY

Transpiration of reed *Phragmites communis* Trin. in a reed-belt of Mikołajskie Lake was measured on two sites: terrestrial and aquatic one during the vegetation season of 1969.

The following were found: the intensity of reed transpiration increases with the increase of its fresh weight; it also increases with the rise of air temperature and of solar radiation values, but decreases with the rise of relative air humidity; transpiration rises rapidly in the morning and gradually lowers in the afternoon — maximal values were found between 11 a.m. and 1 p.m.

The transpiration intensity increases from spring till mid-summer, and then decreases; the maximum was found at the end of July and beginning of August.

The reed growing on land transpires less than the one in the lake, and is also shorter. The leaves of the upper part of shoot (younger ones) transpire more than the lower (older) ones. The formation of panicle slows down the transpiration of reed leaves. Also the infection by *Ustilago grandis* Fr. lowers the intensity of reed transpiration.

Transpiration of reed is of an intensity which can influence the water balance of the lake.

6. STRESZCZENIE

Przeprowadzono pomiary transpiracji trzciny *Phragmites communis* Trin. w trzcinowisku nad Jeziorem Mikołajskim na stanowiskach lądowym i wodnym w sezonie wegetacyjnym 1969 r.

Stwierdzono, że intensywność transpiracji trzciny zwiększa się ze wzrostem świeżej masy, ze wzrostem temperatury powietrza, ze wzrostem radiacji i ze spadkiem wilgotności względnej powietrza. Transpiracja trzciny gwałtownie wzrasta przed południem i stopniowo obniża się w godzinach popołudniowych — maksymalne wartości stwierdzono w godzinach 11–13.

W ciągu okresu wegetacyjnego transpiracja wzrasta do środka lata i następnie maleje; maksimum transpiracji stwierdzono na przełomie lipca i sierpnia.

Trzcina rosnąca na lądzie transpiruje mniej niż rosnąca w wodzie, oraz osiąga mniejszą wysokość. Liście położone w górnej części źdźbła (młodsze) transpirują więcej niż dolne (starsze). Wykształcenie kwiatostanu wpływa hamująco na transpirację liści trzciny. Porażenie trzciny przez *Ustilago grandis* Fr. obniża intensywność transpiracji.

Transpiracja trzciny osiąga tak duże wartości, że może wpływać na bilans wodny jeziora.

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INFLUENCE OF REGULATION OF THE ŁĄCZANKA RIVER ON THE OCCURRENCE OF HIRUDINEA

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ABSTRACT

The influence of regulation of the river on the number of species and the number of individuals of Hirudinea was investigated. Before regulation of the river the numbers of species and individuals were great and similar at all stations. In the part of the river-bed that was regulated, the numbers of species and individuals were significantly decreased, although in the unregulated part of the river-bed, the numbers of species and individuals were basically similar. The next time, it was observed that colonization of the regulated part of the river-bed by Hirudinea had occurred.

CONTENTS

1. INTRODUCTION

Information about transformations of river environments and changes of biocenotic relationships as a result of river regulation are contained in the papers of Mikulski and Tarwid (1951) and Sakowicz (1951). Kufel (1967) gave a brief note about the occurrence of Hirudinea in regulated rivers. This problem still has received little study. The aim of this study was to determine how river regulation influences the occurrence of Hirudinea.

2. MATERIALS AND METHODS

Research was carried out on the River Łączanka from the outflow from Lake Gaładuś to the inflow to Lake Hołny (Fig. 1). Research was carried out in 1964, before regulation of the River Łączanka and then in the years 1966–1968 following regulation. In the year 1965, while regulation took place, no research on Hirudinea was conducted. Observations were made at 10 sampling stations. Stations 1 and 2 were in the unregulated part of the river, and stations 3 to 10 were in the regulated part. In the period of research, 40 samples were taken including 2579 specimens of Hirudinea.

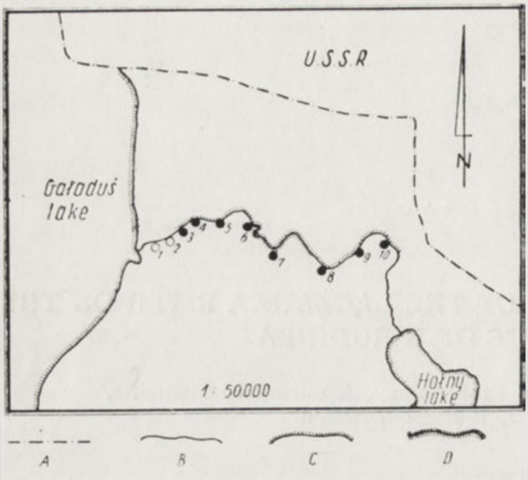

Fig. 1. Map of the Łączanka River. A — Polish border, B — unregulated parts, C — regulated parts, D — straightened parts. 1, 2 — sampling stations on the unregulated part, 3—10 — sampling stations on the regulated part

Animals were collected by the "time" method. From all stations Hirudinea were collected for a fixed period of time, e.g. 45 min, following, among others, Pawłowski (1936), Sandner (1951) and Wojtas (1959) and used in the earlier studies (Wilkialis 1962, 1970 b). In many environments where Hirudinea occur in quantities, for example, solid substrata such as stones and wood debris, standard methods for quantitative study of benthos fauna cannot be used. For this reason, we used the above mentioned methods. Bottom dredging was not used to catch Hirudinea on sand and gravel substrata because in these areas Hirudinea rarely occur.

3. RESULTS

Research revealed that regulation of the River Łączanka caused changes in the Hirudinea fauna. The numbers of individuals of the species investigated (Fig. 2) in the regulated part of the river was smaller after regulation (in the years 1966–1968) than before regulation (in 1964). In Table I, are given the numbers of species of Hirudinea and the numbers of individuals of all species together for the regulated and unregulated zones. It can be seen that in the unregulated zone (sampling stations 1 and 2) the numbers of species were rather high and stable during all the research period (6–9). The numbers of individuals were equally high and stable (68–219). On the regulated part of the river (sampling stations 3–10) in the year 1964, and thus in the year before regulation the numbers of species and individuals were, generally speaking, near to that found in the unregulated part during all four of the years sampled. After regulation of the part containing sampling stations 3–10 the samples taken in 1966–1968 showed a significantly smaller number of species (2–6) and individuals (15–80). This decrease in the number of individuals following after regulation and the quantitative relationships in the following years are presented in Fig. 3. They are presented as the

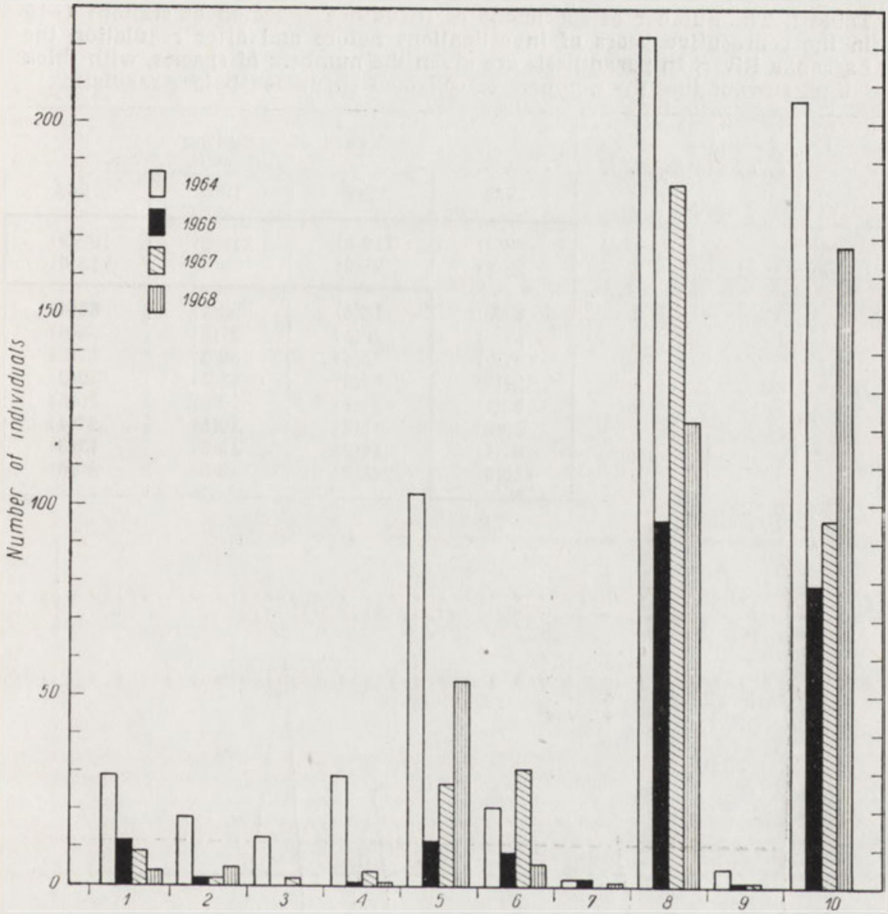

Fig. 2. The total number of individuals of particular species of Hirudinea caught on the regulated part of the river (stations No. 3–10). 1964 — before regulation, 1966–1968 — after regulation.
1 — *Hemiclepsis marginata*, 2 — *Theromyzon tessulatum*, 3 — *Theromyzon maculosum*, 4 — *Haementeria costata*, 5 — *Glossiphonia complanata*, 6 — *Helobdella stagnalis*, 7 — *Haemopis sanguisuga*, 8 — *Erpobdella octoculata*, 9 — *Erpobdella testacea*, 10 — *Erpobdella nigricollis*

number of individuals at a given sampling station in comparison to the number of individuals from the period before regulation, expressed as a per cent. Only in 3 cases of the twenty four studied, was the number of individuals higher after regulation than before. In all these 3 cases the number of individuals before regulation was exceptionally low (sampling station No. 8 — 30 specimens, sampling station No. 7 — 50 specimens and sampling station No. 4 — 55 specimens, Table I).

The data in Fig. 3 generally confirm that the smallest number of individuals in the regulated part occurred in 1966, even though this was

Table I. The number of specimens of Hirudinea collected at stations 1–10 in the consecutive years of investigations before and after regulation the Łączanka River. In paranthesis are given the numbers of species, with thick lines surrounding the numbers of collected Hirudinea before regulation

No. of station		Years of sampling			
		1964	1966	1967	1968
Unregulated	1	68(9)	119(8)	219(8)	192(9)
	2	88(8)	95(9)	105(8)	113(6)
Regulated	3	87(9)	18(6)	60(4)	65(4)
	4	55(7)	20(5)	27(5)	56(6)
	5	127(9)	23(4)	30(3)	57(5)
	6	94(8)	39(5)	38(5)	29(5)
	7	50(7)	21(3)	59(6)	30(5)
	8	30(6)	15(2)	49(5)	27(4)
	9	87(7)	35(6)	45(6)	17(3)
	10	119(8)	42(5)	49(5)	80(6)

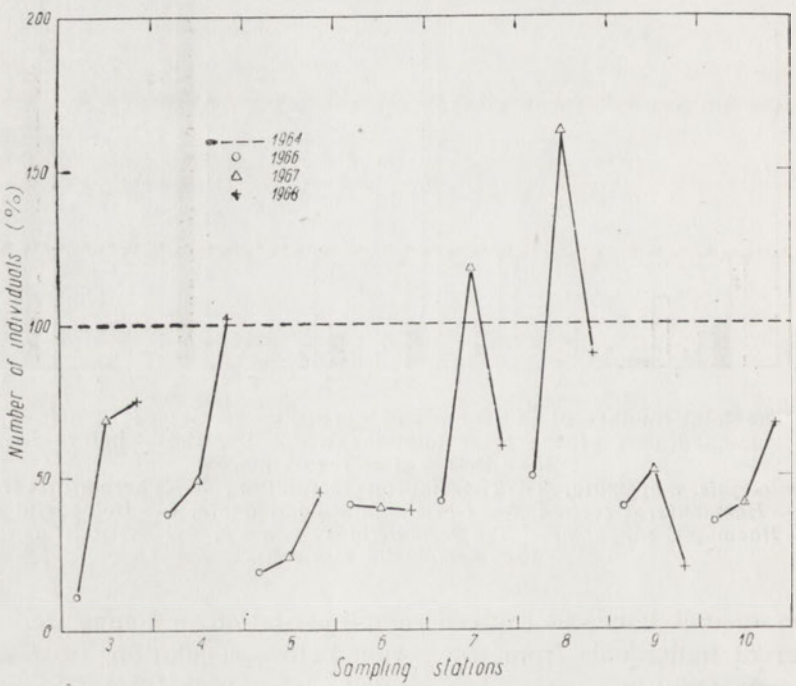

Fig. 3. The per cent of Hirudinea individuals collected on the regulated part of the river (stations No. 3–10) before regulation (1964 — 100%) and after regulation (1966–1968)

the first year after regulation. The data for all species collected at all sampling stations before and after regulation are given in Table II. Generally, in the first year after regulation of the river (1966) there were about 33% of the individuals that were present before regulation. In the following years, the number of individuals in the regulated part increased but did not reach the numbers found in the period before regulation.

Table II. Total numbers of Hirudinea in sampling stations 3–10. The period of four years (1964 — before, and 1966, 1967, 1968 — after regulation of the Łączanka River)

	1964	1966	1967	1968
Number of collected Hirudinea	649	213	357	361
%	100	32.8	55.0	55.6

It can be seen that after regulation, in the regulated parts in the proximity of the unregulated parts (stations 3 and 10), the number of individuals was slightly higher than at the stations far from the unregulated parts of the river.

The common species and the good swimmers: *Glossiphonia complanata* (L.), *Erpobdella octoculata* (L.) and *Erpobdella nigricollis* (Brandes) enter the ameliorated terrain more frequently and in greater numbers than the rare species: *Theromyzon maculosum* (Rathke), *Theromyzon tessulatum* (O. F. Müll.) and *Haementeria costata* (Fr. Müll.).

4. DISCUSSION

Changes in bed and bank configuration, decrease in water level, lack of places for attachment, lack of reproductive sites, disappearance of stagnant environments (backwaters), and disappearance of differentiated environments very significantly affected the occurrence of Hirudinea in the river. Lack of mud and detritus probably caused a decrease in the number of Chironomidae, Oligochaeta and Mollusca which are food for Hirudinea predators. Regulation also caused a decrease in the number of places where water birds feed. The birds are a source of food for bloodsucking Hirudinea. The number of invertebrates, fish and amphibians, which form food for some species of Hirudinea, also decreased.

The number of Hirudinea in the regulated part was limited by the removal from time to time of submerged plants and accumulated mud deposits. This removal was a source of changes in quantitative occurrence of Hirudinea at some stations in the regulated section (Fig. 3, st. 6, 7, 8, 9). Not the least role may be played by the fall in the water level of the river such as that following a long dry period, which caused the exposure of parts of the river shore regulated parts. This all results in a decrease in the number of hiding and attachment places for Hirudinea.

A factor favourable to the reintroduction of Hirudinea into the regulated part of the river (Fig. 3, st. 3, 4, 5, 10) was the accumulation of solid objects on the river-bed, such as logs, tree branches, pieces of regulation material, different wrecks, stones and others. Around objects stuck to the river-bed, particularly on the side away from the current, mud and detritus particles accumulated. In such places algae and underwater macrophytes settle and provide favourable conditions for sedentary and moving benthos fauna. Of great importance here are woven-branch-breakwaters used in regulation, which produce stagnant pools near the bank. Mud sediments are collected there which offer favourable conditions for the development of benthos fauna requiring firm places attachment.

There was a greater number of Hirudinea in the regulated station near the unregulated part than in the stations far from the unregulated parts. This shows that migration of Hirudinea is connected with occupation of "free" ecological niches in the river.

Probably one of the causes of the significant decrease in the number of bloodsucking species (*T. tessulatum* and *H. costata*) after river regulation was due to a decrease in the number of their prey. That the occurrence of the above mentioned species depends mostly on the presence of their prey was shown by the results of Wilkialis (1970 a).

The reason that the number of *H. marginata* (O. F. Müll.) was particularly small in the last year of the investigations was, among others, the fall in the water level which made it difficult for fish to visit the regulated parts of the river. The fall in the water level also had a detrimental effect on the occurrence of *H. stagnalis* (L.) and *E. octoculata* by uncovering the richest marginal parts of the river which contain the attachment, feeding, hiding and reproduction sites. The number of individuals of *G. complanata* and *E. nigricollis* regularly increased in the years following river regulation (Fig. 2). The occurrence of *G. complanata* was connected with the presence of Gastropoda and objects laying on the river-bed. *E. nigricollis* is often found in dense growths of *Elodea canadensis* (Rich.) which was one of the first plants to colonize the regulated parts. *Glossiphonia heteroclita f. papillosa* (Braun) did not occur in the regulated parts, but was noted in the unregulated station near Lake Gaładuś. The author's earlier investigations (Wilkialis 1964) showed that this species is characteristic of lake environments from which it can also pass to rivers. This accounts for the presence of this leech at the above mentioned station.

5. SUMMARY

As a result of changes of the environment following the regulation of the River Łączanka a decrease in the number of species and of individuals of Hirudinea was observed in the regulated parts (Table I, II).

At the regulated stations, the eurytopic species occurred in great numbers, but the rare species which need almost stagnant environments and the presence of prey from the surrounding environments (water birds, mammals) were observed sporadically or not at all (Fig. 2).

Changes in the number at particular stations of the regulated part (Fig. 2, 3) were influenced by both periodical cleaning of the regulated parts and the fall in water level during dry periods.

The author is of the opinion that the factors favourable for more rapid recolonization of the regulated stations in the river by Hirudinea are: accumulation of mud sediments and detritus, the presence of good places for attachment, the reappearance of macrophytes and availability of suitable food.

6. STRESZCZENIE

W wyniku zmian środowiska, które wystąpiły po zmeliorowaniu rzeki Łączanki, obserwowano zmniejszanie się liczby gatunków i liczebności Hirudinea na zmeliorowanych odcinkach (Tab. I, II).

Na stanowiskach zmeliorowanych dominowały gatunki eurytopowe, spotykane w dużej ilości osobników, natomiast gatunki rzadsze, wymagające środowisk lenitycznych i obecności żywicieli z zewnątrz (ptaki wodne, ssaki), spotykano sporadycznie lub ich nie spotykano w ogóle (Fig. 2).

Zmiany liczebności na poszczególnych stanowiskach odcinka uregulowanego (Fig. 2, 3) uwarunkowane były okresowym oczyszczaniem zmeliorowanych cieków, jak również opadaniem lustra wody spowodowanym suszą.

Autor uważa, że czynnikami sprzyjającymi szybszemu ponownemu opanowywaniu zmeliorowanych stanowisk w rzece przez Hirudinea są: gromadzenie się osadów mułowych, detrytusu, obecność dogodnych miejsc przyczepu, ponowne pojawianie się roślin podwodnych oraz dostępność odpowiedniego pokarmu.

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INDICATOR VALUE OF BIOSESTON AND PERIPHYTON FOR EVALUATION OF POLLUTION DEGREE OF FLOWING WATERS

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ABSTRACT

Bioseston and periphyton of 3 rivers were compared, and the results were interpreted saprobiologically. Curves of bioseston saprobity of the River Odra were different at different seasons. The range of periphyton saprobity differences in individual sampling stations was much smaller. Bioseston saprobity of the rivers Ślęza and Oława in sampling stations situated below sugar-mills gradually increased during the sugar campaign and it returned to its pre-campaign state almost immediately after its end. Periphyton sets attained their maximal saprobity very quickly during the campaign and their increased saprobity was maintained for a long period after the end of the campaign. The results prove that in evaluating a present state of pollution of flowing water, indications of bioseston should be used, while indications of periphyton saprobity refer to a past period and to the conditions in near-to-bottom or littoral fluvial zones.

CONTENTS

1. INTRODUCTION

In some countries, biological evaluation has already been acknowledged as one of the criteria of quality of water, according with a postulate of the 1969 symposium of the Council of Mutual Economic Aid. Up to now, the saprobiological evaluation of flowing surface water has been most often based on investigations of only one set belonging to the biocenosis, i.e. of periphyton or benthos; much less frequently it has been extended upon both of these sets, and only in rare cases it included bioseston.

Presently, an increasing precision of results is expected from saprobiology, and above all their comparability with the results of physical and chemical assessments of water pollution state is required. In this connection it becomes vital to determine the indicator value of the basic sets constituting the biocenosis of investigated waters for evaluation of their pollution degree. It seems particularly necessary to carry out comparative studies of the sets whose development occurs in different conditions. These are: bioseston, dispersed and floating in water and moving with it along the stream, and periphyton and benthos as the settled sets, washed around and through by water which exerts its influence upon them.

The present paper is a report of comparative saprobiological investigations of bioseston and periphyton.

2. FIELD DESCRIPTION AND METHODS

Three rivers were selected as the field for comparative researches: Odra, Ślęza and Oława.

Odra was investigated along 210 km, from the state border with Czechoslovakia (Chałupki) to the 230th km of the river's stream (above Wrocław). Along this section Odra is strongly polluted by sewage and trade wastes from Czechoslovakia and Poland (Mańczak 1966). Bioseston samples were drawn on 25 stations. Four tests were carried out: in February (17 samples), August (19 samples) and November (18 samples) in 1963 and in March 1964 (20 samples). The range of periphyton investigations had to be limited for technical reasons. Samples of this set were drawn in February, August and March only, each time on the same 12 stations.

Ślęza was investigated along 59 km, from a sampling station about 7 km above the town Łagiewniki to its outlet into Odra. In the autumn and winter seasons, during sugar campaigns, the Ślęza River is very heavily polluted with effluents from the sugar factories at Łagiewniki and Klecina, while between sugar campaigns its water is polluted with only a small amount of sewage and it can be considered as naturally pure (Pawlaczyk and Solski 1965). Samples of bioseston and periphyton were drawn on 7 stations at one month intervals from May 1962 until May 1963. In total, 87 samples of bioseston and 51 samples of periphyton were taken from the Ślęza River.

Oława was investigated along 94 km of its length, from a station situated above the town Ziębice to its estuary into Odra. During sugar campaigns Oława is very strongly polluted along 30 km from Ziębice to Strzelin. Between sugar campaigns Pawlaczyk and Solski (1967) found a heavier pollution, attaining 44 mg O_2/l of BOD_5, only along 19 km, between 60.5 and 79.5 km of water stream. Bioseston and periphyton samples were drawn on 11 stations in total, 81 samples of bioseston and 58 samples of periphyton were taken from the Oława River.

Bioseston samples were drawn by filtering mainstream water through a plankton net made of milling gauze No. 25, in the amount of 20 l (in summer and autumn) or 40 l (in spring and winter). The obtained material was supplemented to the volume of 10–25 ml depending on its density, and then 0.1 ml of carefully mixed material was divided to 5 object glasses and covered with 24×24 mm cover-glasses. Each preparate was scanned stripe by stripe, and the encountered organisms were labelled and counted in vivo. The rest of the material was preserved by etaform to be used for the labelling and counting of organisms that had not required elaboration in vivo (mainly diatoms). The results were recomputed to the volume of 1 l of natural river water.

Samples of periphyton were scraped from a similar surface of a few stones on each sampling station and they were supplemented with water to identical volumes. After a very careful mixing of the samples by shaking up, they were scanned under a microscope to determine the percentages of the several species. 3 microscope preparates were made from each sample, and in each of them the encountered were counted as long as species were found which had appeared for the first time in the given preparate. If in 6 consecutive stripes no new species were found, the counting was given up, and the scanning of the preparate was brought to completion only to supplement the list of the species. The data thus obtained were recorded in percentages, and the total amount of individuals counted on the 3 preparates of the given sample was considered as 100%.

The qualitative and quantitative analysis of the samples of bioseston and periphyton allowed for their saprobiotic assessment based on the system of saprobic organisms revised by Liebmann (1962) and performed by means of the vector method of saprobity determination (Gołowin 1968 a). In the method the numbers of indicator organisms, or percentages of particular index groups, are plotted on specific axes, the subsequent lengths of which are then added together to give a vector $\vec{S}$. The inclination angle of this determines the saprobiotic value of the living community of the water being studied.

The results of researches are presented in form of longitudinal profiles, representing the changes in saprobity of seston and periphyton occurring along the rivers' streams in the several research seasons.

3. RESULTS

The River Odra (Fig. 1). Investigations of bioseston carried out in various seasons proved that saprobity of this set oscillated between

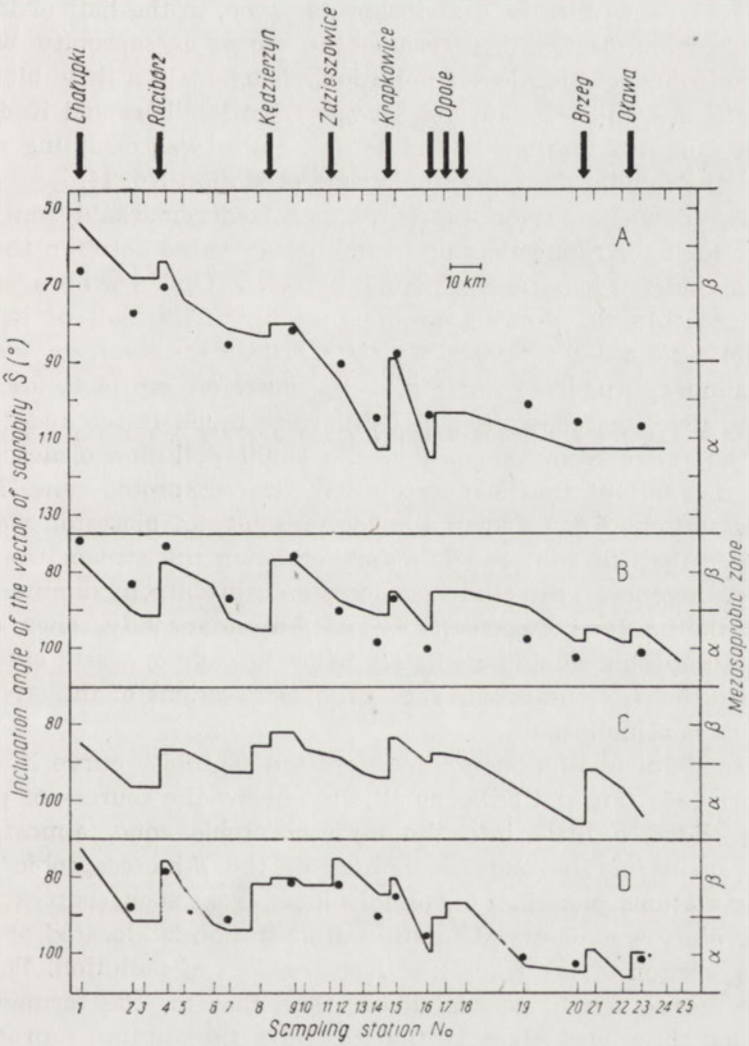

Fig. 1. The River Odra. A saprobiological longitudinal profile of bioseston and periphyton. Line — bioseston, points — periphyton. Black arrows — sewage and/or trade wastes inflows. A — February 1963, B — August 1963, C — November 1963, D — March 1964

α-meso and β-mesosaprobic zones. The curves of saprobity show its increase just below the sewage or waste outlets, and its gradual decrease with the distance down the stream from the sources of pollution.

The largest range of bioseston saprobity along the studied section of Odra was observed in winter, i.e. in February 1963 (Fig. 1 A), when saprobity was changing along the river from the half of the heavy-pollution region of α-mesosaprobic zone to the middle β-mesosaprobity. During that period the saprobity of bioseston at sampling station No. 1 (Chałupki) was kept within the α-mesosaprobic zone, in the half of its heavy pollution region. Down the streams the curve of saprobity was gradually declining towards lesser pollution, rising again a little bit at sampling stations 4 and 9 because of sewage from Racibórz and Kędzierzyn. Between sampling stations 9 and 14 the curve was declining so as to pass into the middle β-mesosaprobic zone at station No. 14.

Further down the river, saprobity increased remarkably only twice: at No. 15 (below Krapkowice) up to the border value between the α- and β-mesosaprobity zones, and at No. 18 (below Opole) where saprobity increased within the β-mesosaprobic zone up to the half of its heavy--pollution region.

In summer, August 1963 (Fig. 1 B) bioseston saprobity oscillations along the investigated section of Odra were much lesser and they fell within the range from the half of the slight pollution α-mesosaprobic zone to the half of the heavy-pollution β-mesosaprobic zone. Between sampling stations No. 1 and 9, a lower saprobity of bioseston was found in summer than in winter, while further down the stream the reverse was true. In general, the curve of bioseston saprobity in summer oscillated near the border between the α- and β-mesosaprobity zones, entering the α-mesosaprobic zone immediately below sewage or waste outlets, and shifting to the β-mesosaprobic zone in longer sections of the stream free from sources of pollution.

The range of oscillations of the bioseston saprobity curve in autumn, November 1963 (Fig. 1 C) was small. Just below the sources of pollution the line slided a little into the α-mesosaprobic zone, almost always shifting again just beyond the border of the β-mesosaprobic zone at sampling stations placed at larger distances from such sources. The lowest saprobity was observed in autumn at station 20, located at the end of a long section of the river free from sources of pollution. The whole saprobity curve ran in general lower than that for the former period. Only along three very short sections of Odra the autumn saprobity was found to be higher than in Summer.

In spring, March 1964 (Fig. 1 D), sewage and waste flowing into the river influenced bioseston saprobity more strongly than in earlier seasons. This was particularly remarkable at stations 1, 4, 12, in which bioseston saprobity increased much more than in autumn under the influence of the pollution sources located there.

Periphyton sets of the Odra River (Fig. 1) were very poorly developed, which often made their saprobiotic evaluation difficult. An interpretation of the results of microscopic analysis proved that periphyton, similarly as bioseston, assumed the saprobiological state appropriate for a zone of stronger pollution just below the sources of pollution rather than above them or in greater distances from them. However, in opposition to bioseston, changing its saprobity very much on the same sampling stations during the cycle of the four tests, saprobity of periphyton on each station at different seasons oscillated within a much narrower range.

The differences between minimal and maximal saprobity of bioseston observed on the particular sampling stations during the four tests (Fig. 2)

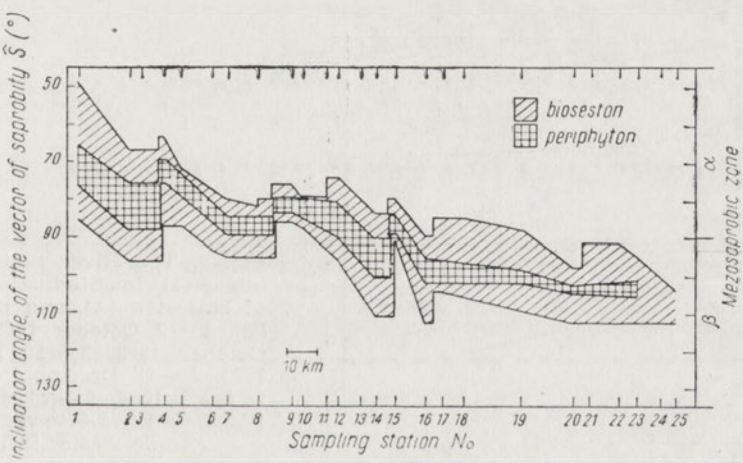

Fig. 2. The River Odra. Differences between maximal and minimal saprobity of bioseston and periphyton found during a year in individual sampling stations

were in some cases very large, approaching 37° on some stations, which constituted about 82% of a zone's range (equivalent to about 0.82 on the index by Pantle and Buck 1955). So large oscillations of bioseston saprobity were observed on sampling station No. 1: from extreme α-mesosaprobity bordering with the polysaprobic zone, to β-mesosaprobity bordering with the β-mesosaprobic zone. The range of differences observed on the other stations, e.g. No. 2, 15 etc., were not much lesser. An corresponding range of differences for periphyton was apparently less extensive, as it did not exceed 12°, or only 26% of zone's range (0.26 on Pantle and Buck index). The range of differences periphyton saprobity along the whole investigated section of the river during the year was kept within a narrow limit close to the mean values of the maximum and minimum indications for bioseston.

The River Ślęza (Fig. 3). The results of a saprobiological evaluation of bioseston collected on October 2, 1962, i.e. before the beginning of the sugar campaign, revealed minor oscillations within the β-mesosaprobic zone. In the sugar-mills pouring their effluents to the Ślęza River, the campaign started in the second half of October. The first test performed at the beginning of the campaign, on October 29, 1962, proved a remarkable increase of bioseston saprobity, shifting towards and into the middle α-mesosaprobity under the impact of effluents

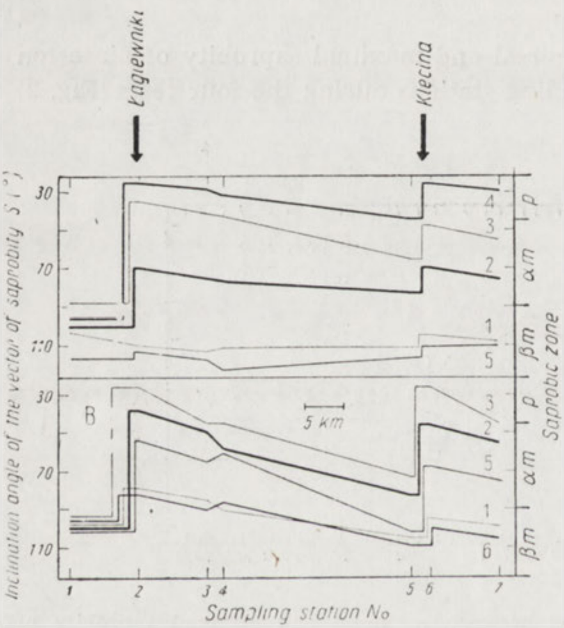

Fig. 3. The River Ślęza. Saprobiological longitudinal profiles of bioseston (A), and periphyton (B). 1 — 2 October 1962, 2 — 29 October 1962, 3 — 26 November 1962, 4 — 28 December 1962, 5 — 2 April 1963, 6 — 23 June 1963. Black arrows — sewage and/or trade wastes inflows

from sugar factories at Łagiewniki (station 2) and Klecina (station 6). The next test on November 26, 1962, evidenced an increasing influence of sugar-mills effluents during the campaign. By this the factory at Łagiewniki suddenly increased by its waste the saprobity of bioseston to the polysaprobic zone at sampling station No. 2. Down the stream, along a section of the river of about 35 km between stations 2 and 5, the degree of pollution fell down, which was expressed in a shift of saprobity into the middle region of α-mesosaprobic zone, and then the next source of pollution, the sugar factory at Klecina, increased bioseston saprobity again to a border value between α-meso and polysaprobic zones at station No. 6. The test carried out on December 28, 1962, evidenced another increase of the influence of the campaign waste, as bioseston saprobity mounted to extreme polysaprobity at stations 2 and 6. The decrease of pollution between stations 2 and 5 was expressed by a minor fall in saprobity, shifting by that time at station No. 5 to the border value between poly and α-mesosaprobic zones. Almost immediately after the sugar campaign, bioseston saprobity returned to its state from before the campaign. A curve representing the after-campaign bioseston saprobity is presented by way of example (the test performed on April, 2, 1963).

An interpretation of the analysis of periphyton, however, shows that the reaction of this set of living organisms to a change of environmental conditions brought about by sugar campaigns is different from the reaction of bioseston.

The first test performed at the beginning of the sugar campaign (October 29, 1962) proved a much stronger impact of sugar-mill effluents upon periphyton than upon bioseston. By that time Ślęza bioseston below the sugar factory at Łagiewniki, from station No. 2 to the outlet of the river, showed relatively minor oscillations within α-mesosaprobic zone, while saprobity of periphyton suddenly increased from β-mesosaprobic zone at station No. 1 to polysaprobic zone at station 2 (below the sugar factory at Łagiewniki). Along a 35 km section down the stream, periphyton saprobity gradually increased to the half of light-pollution region of the α-mesosaprobic zone. Under the influence of waste from the sugar factory at Klecina, periphyton saprobity below that source of pollution increased to the border value between the α-meso and polysaprobic zones. Bioseston at stations 2 and 6 attained so high level of saprobity only at the end of November. In that month, at stations 2 and 6, an extremely polysaprobic character of periphyton was found, which was attained by bioseston only in December. After the sugar campaign, periphyton could not return to its state and pattern from the moment immediately before the campaign for a long time. As late as on April 2, periphyton saprobity at stations 2 and 6, situated below sugar factories which had remained inactive for a few months, was very much higher than immediately before the campaign, i.e. on November 2, 1962. It was only on the June 23, 1963 test, that the pre-campaign state of periphyton saprobity was restored.

The River Oława (Fig. 4). During the season between sugar campaigns, bioseston saprobity in this river oscillated from middle β-mesosaprobity to the half of the heavier pollution region of this zone. During the campaign, a reaction of bioseston analogous to that observed in Ślęza was recorded. Along about 35 km from station 2 to station 7, bioseston saprobity of Oława increased as the campaign was going on,

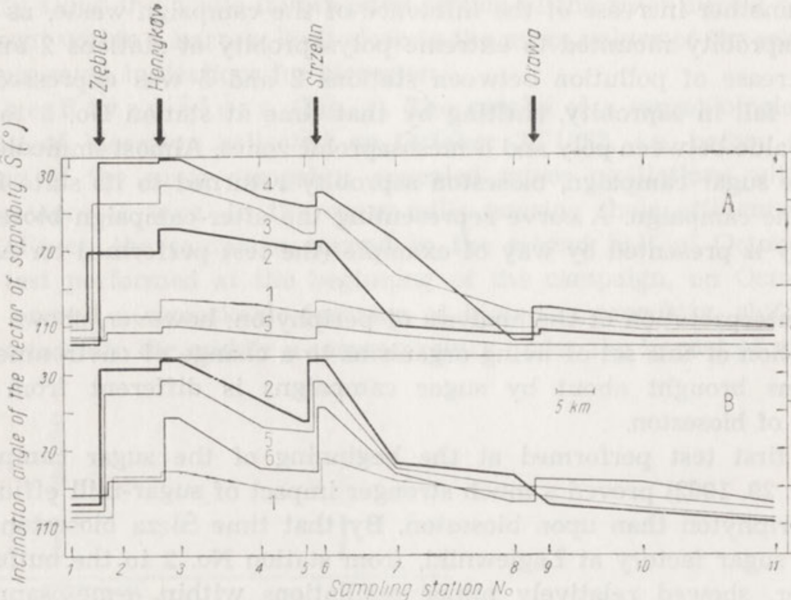

Fig. 4. The Oława River. Saprobiological longitudinal profiles of bioseston (A), and periphyton (B). 1 — 2 October 1962, 2 — 29 October 1962, 3 — 26 November 1962, 4 — 28 December 1962, 5 — 2 April 1963, 6 — 29 April 1963. Black arrows — sewage and/or trade wastes inflows

and at the end of December it attained maximal values at stations 2, 3 and 6, i.e. immediately below the waste outlets from the sugar factories at Ziębice and Strzelin. Almost immediately after the end of the campaign, bioseston saprobity in the Oława River returned to its state from before the campaign, similarly as in the Ślęza River.

The curve of bioseston saprobity after the campaign represents the test carried out on April 2, 1962, but the curve for the half of March (not presented in Fig. 4) was almost identical.

It was proved that Oława periphyton reacted to increased loads of waste poured into the river during the sugar campaign in the same way as in the Ślęza River. Already at the beginning of the campaign a maximal increase of saprobity, maintained at the same level during the whole campaign, was observed at sampling stations No. 2, 3 and 6, situated below the near-by sugar factories. In the Oława River, too, the level of saprobity has been very slow in returning to its state from before the campaign after it was ended. As late as April 2 an apparently polysaprobic character of periphyton sets was observed at stations, 2, 3 and 6, below the sugar factories which had not been working for a few months. At the end of April, periphyton saprobity at stations 3 and 6 was remarkably higher than before the campaign. It was only in June 1963 (data not presented in Fig. 4) that the saprobiotic character of these sets was found to approach the pre-campaign level recorded on October 2, 1962.

4. DISCUSSION

Among authors of saprobiological works prevails the view that bioseston, being a set of living organisms floating with the current of water, does not allow for judgements on the degree of pollution of flowing waters, or on its changes occurring along the stream. Such a view was expressed by Reinhard (1931), Caspers and Schultz (1960, 1962), Cabejszek et al. (1961), Klapper (1961), Fjerdingstad (1964), Klose (1968) and others. Among the arguments in favour of this position, the basic and most common are the following:

a. Living organisms from "untypical" environments can be brought into, and mixed with, bioseston of the investigated stream, biasing the image and leading to fallacious conclusions.

b. A composition of bioseston and its saprobiotic state observed at a sample station is considered as a reflection of environmental changes occurring above the station. Such "retardation" is said to hinder the ascribing of results to actual places, and thereby to make impossible, e.g., an elaboration of saprobiological longitudinal profiles validly reflecting the actual conditions in a river.

These arguments in favour of unrepresentativeness of bioseston for evaluating the degree of pollution of flowing waters do not seem to be convincing for the following reasons.

a. Organisms from the so-called "untypical" environments can indeed be mixed into bioseston of an investigated stream, but together with water from such "untypical" environments, and usually in proportion to its amount. If, then, a pure tributary is considered as "untypical" with reference to a strongly polluted river, a given amount of water from the tributary will introduce to the river's bioseston a proportional amount of the tributary's bioseston. Its pure water will dissolute the polluted water of the river, and the introduced bioseston, typical in its contents for pure waters, will decrease the saprobity of the river's seston set. On the other hand, if a tributary, being "untypical", is heavily polluted, and the river is pure, then the supply of bioseston from the tributary will also be, in general, proportional to the supply of water, and thus the resulting changes in the saprobiotic state of the river's bioseston will usually be proportional to the respective changes in the physical and chemical properties of water.

b. Bioseston, as a set of living organisms floating in water and flowing along the stream, is not subject to accidental changes in the physical and chemical properties of water, but its transformations, as it is floated away from a source of pollution, are continuous. These transformations are defined as a process of self-purification. Mutual relationships between bioseston and its water environment must be kept in mind; on one hand a composition of bioseston depends on the prevailing conditions in water around it, but on the other, it is the living sestonic organisms which are an instrumental, and in a great extent the decisive factor bringing about the changes that occur in water. Changes in biocenosis, and the changing properties of river waters, are strictly interrelated phenomena, influencing each other. Thus the threat of the 're-tardation" effect said to impair the indicator value of bioseston is in general negligible in lowland rivers.

In saprobiological works there are also statements in favour of bioseston tests as useful for evaluating the quality of water. Liebmann (1960) describing the changes in biocenosis of the Danube and Mein under the impact of sewage and waste, explicitly remarks that these changes are evidenced by the composition of plankton as well as periphyton. The same author studying *Sphaerotilus natans* Kütz (Liebmann 1953) observed that this bacteria developed in periphyton and plankton of river waters. He observed the development of threads of *Sphaerotilus natans* in flowing river water almost immediately below a sewage outlets, and at about 1 km down the stream bundles of its threads were inhabited by a set typical for *S. natans* flocs. At 1 km distance below a source of pollution, the set of living organisms settled on flocs of *S. natans,* developing as a periphyton constituent, was identical with the set inhabiting the flocs floating downstream, and it was constituted by polysaprobic species: *Beggiatoa alba* (Ehr.) Schew., *Bodo putrinus* (Stokes) Lemm., *Colpidium colpoda* Stein., *Glaucoma pyriformis* (Ehr.) Schew., *G. scintillans* Ehr., *Paramaecium putrinum* Clap. et Lachm., *Vorticella microstoma* Ehr. Such a composition of the set inhabiting both types of flocs was maintained in the Danube along about 10 km sections below sources of pollution, and then on the floating flocs changes occurred such as an appearance of α-mesosaprobic species: *Aspidisca lynceus* Ehr., *Lionotus fasciola* Ehr., and morphological changes leading to a disintegration of a floc. These findings of Liebmann bear witness to a quick adaptive reaction of bioseston to environmental changes brought about by pollution and self-purification of water, and thereby they promote the utility of bioseston analysis for the evaluation of water quality. Walter and Scharf (1961) also consider bioseston as useful in saprobiological researches. These authors, who studied the lower Odra and Nysa Łużycka, found that periphyton sets in the Odra

River were very poorly developed and thus they postulated the investigation of plankton as the only means of attaining round biological evaluation of water in the lower part of the river. The consistency, found by these authors, between physico-chemical results and biological ones obtained by means of bioseston investigations, is an argument in favour of their position.

An interpretation of the present results leads to a conclusion that in saprobiological investigations of the three rivers bioseston must have been taken into account. Results of saprobiological evaluation of bioseston, presented as longitudinal profiles, reveal a pattern which may be explained by the prevailing conditions in these rivers, and by the changes in the quality of water along their streams. The remarks by Walter and Scharf as to the poor development of periphyton in the lower stream of Odra were confirmed by our findings in the upper section of the river.

Saprobity as indicated by periphyton at the several sampling stations along the Odra River in general approached the mean values between the extremes of saprobity as indicated by bioseston at the respective stations. However, the indications of periphyton saprobity in the rivers Ślęza and Oława, periodically very strongly influenced by sugar-mills effluents, revealed a different pattern. The fact that periphyton can indicate average values of pollution during past periods, considered by some authors as its advantageous property, in the cases of Ślęza and Oława has made it impossible to evaluate the present quality of water by means of testing this set of organisms. Tests of periphyton from rivers, carried out many months after the end of the sugar campaign, were still revealing significantly increased saprobity at sampling stations situated below the inactive sugar-mills. Thus it seemed absurd to postulate any relationship between periphyton saprobity and a present, or even average for a few recent months, state of water pollution.

A similar phenomenon was described by Cabejszek and Stanisławska (1962) who found the following organisms in periphyton of the River Wkra a few months after a sugar campaign: *Spaerotilus natans* Kütz., *Leptomitus lacteus* Ag., *Paramaecium caudatum* Ehr., *Vorticella microstoma* Ehr. The presence of these species, even if in lesser amounts than during the campaign, must have influenced the saprobiological state of periphyton sets.

Indications of periphyton are extremely important for a general and full saprobiological description of rivers; they can be highly symptomatic for the conditions in littoral or near-to-bottom fluvial zones (Gołowin 1968 b), prevailing in a past but indefinite period of time, but they cannot be used for evaluating an actual state of water purity, and thus they are useless for establishing relationships between biological and chemical indicators of water pollution.

6. SUMMARY

Investigations of bioseston and periphyton of 3 rivers: Odra, Ślęza and Oława, were interpreted saprobiologically by means of vector analysis. The results are presented as longitudinal saprobiological profiles, illustrating the changes in saprobity along the river's streams at different testing seasons. Extensive differences in the indications of each of the analysed sets of organisms were found.

The curves of bioseston saprobity of the River Odra were different in the several seasons, and thus extensive oscillations of saprobity in individual sampling stations were observed during a year cycle. The ranges of periphyton saprobity in individual sampling stations were much lesser. During a year cycle, periphyton saprobity in individual stations oscillated within a relatively narrow range of values approaching the averages from extreme bioseston indications.

At the stations situated below the outlets of sugar-mills effluents, bioseston saprobity in Ślęza and Oława gradually increased during the sugar campaign, and it attained its maximum in December. Almost immediately after the end of the campaign, bioseston saprobity at these stations fell down to its pre-campaign level. Periphyton sets, on the other hand, reached its maximum saprobity below pollution sources very quickly during the campaign, and they kept their increased saprobity for several months after its end.

The results of comparative analyses prove that in evaluating a present state of purity of water, indications of bioseston can and should be used, while indications of periphyton saprobity refer to an indefinite past period and to the conditions in near-to-bottom or littoral fluvial zones.

6. STRESZCZENIE

Przeprowadzone badania biosestonu i zespołów poroślowych 3 rzek: Odry, Ślęzy i Oławy poddano saprobiologicznej interpretacji stosując metodę wektorową Wyniki przedstawiono jako podłużne profile saprobiologiczne ilustrujące zmiany saprobowości zachodzące wzdłuż biegu rzek w różnych okresach badań. Stwierdzono znaczne różnice we wskazaniach obu analizowanych zespołów.

Krzywe saprobowości biosestonu Odry miały przebieg różny w różnych okresach, stąd w poszczególnych przekrojach stwierdzono znaczne wahania saprobowości w cyklu rocznym. Zakres różnic saprobowości zespołów poroślowych w poszczególnych przekrojach był znacznie mniejszy. Saprobowość zespołów poroślowych w cyklu rocznym w poszczególnych przekrojach wahała się na ogół w stosunkowo wąskim przedziale wartości zbliżonych do wartości średnich z ekstremalnych wskazań biosestonu.

W przekrojach zlokalizowanych poniżej ujścia ścieków cukrowniczych, saprobowość biosestonu Ślęzy i Oławy wzrastała stopniowo w okresie kampanii cukrowniczej, osiągając maksimum w grudniu. Niemal bezpośrednio po zakończeniu kampanii saprobowość biosestonu w tych przekrojach obniżała się do stanu z okresu przedkampanijnego. Zespoły poroślowe natomiast w czasie kampanii bardzo szybko osiągały maksymalną saprobowość poniżej źródeł zanieczyszczenia, a silnie podwyższona ich saprobowość utrzymywała się przez okres kilku miesięcy po zakończeniu kampanii.

Wyniki przeprowadzonych badań porównawczych wykazują, że przy aktualnej ocenie stanu czystości wód można i należy opierać się na wskazaniach biosestonu, natomiast wskazania saprobowości zespołów poroślowych odnoszą się do bliżej nieokreślonego okresu minionego i warunków w przydennych albo przybrzeżnych strefach rzeki.

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COMPARISON OF POLLUTION DEGREE OF FLUVIAL WATERS EVALUATED BY MEANS OF THE BOD_5 TEST AND A SAPROBIOLOGICAL METHOD

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ABSTRACT

Correlations were established between bioseston saprobity and BOD_5 values in samples drawn parallelly from three rivers. The correlations were different for each river, and seasonal changes of pollution degree made them different for the same river during sugar campaign and the other months. They were identical, however, for rivers exposed to similarly purified sugar-mill effluents. Thus, saprobity is not an absolute and direct measure of the state of pollution of a river, but rather it is a measure of reaction of biocenosis of a river to the degree of pollution or its change. A reaction of the set of saprobes to an increase of pollution depends on the degree of adaptation of such set to increased concentrations of organic substances vulnerable to decomposition. Better adjustment is expressed by stronger increases of percentages of α-meso and polysaprobes related with smaller increases of BOD_5 as compared with a river with non-adapted biocenosis.

CONTENTS

1. INTRODUCTION

A modern evaluation of a degree of pollution and of self-purification processes of surface waters should be based on physical and chemical criteria, as well as on biological ones. The parallel application of both types of methods requires the application of such saprobiological methods, and above all such interpretation of their results, as to make possible a correlation and comparison of the physico-chemical and biological indices.

The aim of the present work was a comparison of saprobiological evaluations of pollution degrees of river waters and BOD_5 tests results. In most researches, BOD_5 is the basic index of physico-chemical classification of water, and for that reason it has been selected for a comparison with biological results.

2. METHODS

The present elaboration is based on data for bioseston, collected during a study of three rivers of Lower Silesia: Odra, Ślęza and Oława. Odra, on the investigated section from the state borderline with Czechoslovakia at Chałupki to the 230 km of the river's stream, is heavily polluted by sewage and trade wastes from urban centres in Czechoslovakia and Poland (Mańczak 1966). Ślęza is very heavily polluted during sugar campaigns in autumn and winter seasons. In the other seasons its water is natural in character and it receives only moderate amounts of sewage (Pawlaczyk and Solski 1965). Oława is very heavily polluted by sugar-mills efluents along 30 km of its stream during sugar campaigns. During the remaining part of a year its water was found to be polluted up to the BOD_5 value of 44 mg O_2/l only along 19 km of its stream (Pawlaczyk and Solski 1967).

Bioseston samples were drawn from Odra at 25 stations. During 4 tests, in February, August and November 1963 and in March 1964, 74 samples were collected in total. The investigations of Ślęza and Oława have been carried out during 1 year, and samples have been drawn at 1 month intervals, from 7 stations at Ślęza (87 samples in total) and from 11 stations at Oława (81 samples in total).

Bioseston samples were drawn by filtering mainstream water through a plankton net made of milling gauze No. 25, in the amount of 20 l (in summer and autumn) or 40 l (in spring and winter). The obtained material was supplemented to the volume of 10—25 ml depending on its density, and then 0.1 ml of carefully mixed material was divided to 5 object glasses and covered with 24×24 mm cover-glasses. Each preparate was scanned stripe by stripe, and the encountered organisms were labelled and counted in vivo. The rest of the material was preserved by etaform to be used for the labelling and counting of organisms that had not required elaboration in vivo (mainly diatoms). The results were recomputed to the volume of 1 l of natural river water. The results were evaluated by means of the system of saprobic organisms revised by Liebmann (1962), with an application of the vector method of saprobity determination (Gołowin 1968). In this method the numbers of indicator organisms, or the percentage of particular index groups, are plotted on specific axes, the subsequent lengths of which are then added together to give a vector Ŝ. The inclination angle of this vector determines the saprobiotic value of living community of the water being studied (Gołowin 1968).

BOD_5 values were taken from physico-chemical investigations by Mańczak (1966) for Odra, and by Pawlaczyk and Solski (1965, 1967) for Ślęza and Oława. Biological and physico-chemical field tests were performed parallelly, i.e. biological samples of bioseston and physico-chemical samples were drawn simultaneously at the same stations.

Pairs of results: BOD_5 (mg O_2/l) and saprobity (Ŝ) were considered. Separate calculations were made for each of the investigated rivers. The degree of association between the two variables was calculated by means of the correlation method. Correlation coefficients (r_{xy}), probabilities (α) and critical values of correlation coefficients (r_{cr}) were computed. A computer ODRA-1003 was employed.

3. RESULTS

The correlation between BOD_5 and saprobity expressed by the vector of saprobity $\hat{S}^\circ$ established for the Odra River is shown by curve 1 on Fig. 1. It is characterized by a small dispersion of points arranged in a continuous sequence. The interrelation of the two variables, expressed by the equation:

$$\hat{S}^\circ = 188.9 - 67.75 \cdot \log BOD_5$$

has a high correlation coefficient ($r_{xy} = -0.9436$) at the critical value of correlation coefficient $r_{cr}=0.3938$ and probability $\alpha<0.001$, which proves a tight relation and a significant interdependence of the two considered indices of pollution, i.e. biological and physico-chemical. The border value of BOD_5 between α-mesosaprobic and β-mesosaprobic zones was for Odra 28 mg O_2/l. At higher BOD_5 values, the general saprobiotic character of the set of saprobes assumed the features of α-mesosaprobity, while at lower BOD_5 values it passed into the β-mesosaprobic zone.

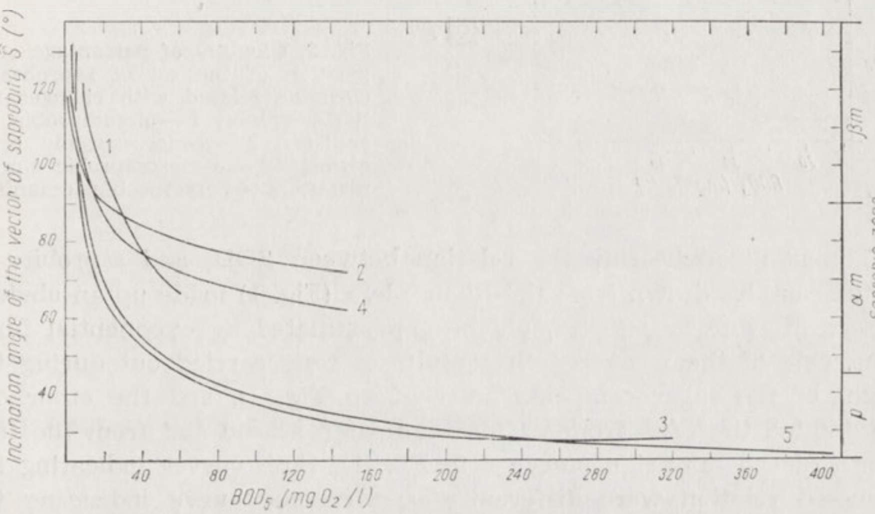

Fig. 1. A comparison of correlations between bioseston saprobity and BOD_5. 1 — Odra, 2 — Ślęza, non-campaign season and the beginning of the campaign, 3 — Ślęza, during the campaign, 4 — Oława, non-campaign season and the beginning of the campaign, 5 — Oława, during the campaign

In Figure 2 there are presented changes in percentage composition of bioseston set of saprobes of the River Odra, related with changes in BOD_5 values. At BOD_5 values from 8 to 26 mg O_2/l it was found that about 4% of the total amount of saprobes were oligosaprobes, while at higher BOD_5 values mean proportions of oligosaprobes fell down to fractions of a per cent (about 0.2%). The most numerously represented were the groups of β-meso and α-mesosaprobes, which accounted for the largest percentage of the whole set of saprobes. At BOD_5 about 8 mg O_2/l the amount of β-mesosaprobes was about 78% of the total amount of saprobes, and as BOD_5 increased, this group was falling down to 20% at BOD_5 equal to 57 mg O_2/l. At the same time the share of α-mesosaprobes increased from about 13% to about 60% of the total amount of saprobes. The group of polysaprobes, which constituted about 6% of the total amount of saprobes at BOD_5 about 8 mg O_2/l, went up to 18% at BOD_5 about 57 mg O_2/l.

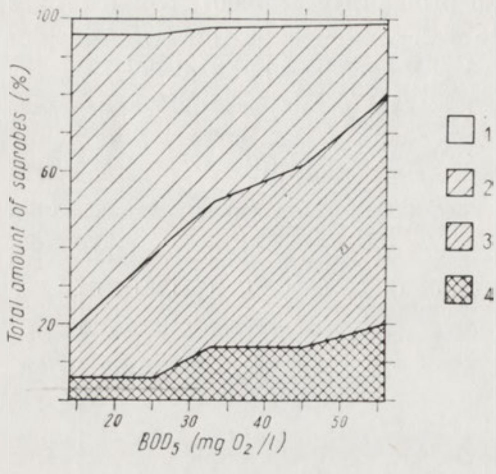

Fig. 2. Changes of percentage composition of the set of saprobes of Odra as related with changes of BOD_5 values. 1 — oligosaprobic organisms, 2 — β-mesosaprobic organisms, 3 — α-mesosaprobic organisms, 4 — polysaprobic organisms

The points indicating the relation between BOD_5 and saprobity of all the samples drawn from the River Ślęza (Fig. 1) made up an obvious pattern of two sets which could be approximated by exponential functions. One of them covered the results of tests carried out during the height of the sugar campaign (curve 3 in Fig. 1), and the other one represented the tests from the non-campaign season and from the first month of the campaign (curve 2 in Fig. 1). Both curves indicating the discussed relation were differently shaped. The curve indicating the relation between BOD_5 and saprobity for the Ślęza River at the height of the sugar campaign was defined by the equation:

$$\hat{S}^{\circ} = 145.3 \cdot BOD_5^{-0.23}$$

at $r_{xy} = -0.9860$, $r_{cr} = 0.7084$, $\alpha < 0.001$.

During the non-campaign season, the relation was defined by the equation:

$$\hat{S}^{\circ} = 122.9 \cdot BOD_5^{-0.1}$$

at $r_{xy} = -0.9964$, $r_{cr} = 0.3938$, $\alpha < 0.001$.

During the campaign, the value of BOD_5 corresponding to the border saprobity between α-meso and β-mesosaprobic zones was about 5.5 mg O_2/l, while during the remaining part of the year, saprobity of bioseston shifted to α-mesosaprobic zone only at BOD_5 values exceeding 20 mg O_2/l. During the campaign, BOD_5 of about 60 mg O_2/l caused the shift of saprobity to polysaprobic zone, while during the non-campaign season even BOD_5 as high as 150 mg O_2/l did not bring about sufficient changes in the set of saprobes to make their composition typical for polysaprobic zone.

Changes of quantitative relations between the several groups of saprobic organisms, occurring under the influence of changes of pollution degree during both periods are presented in Fig. 3. During the non-cam-

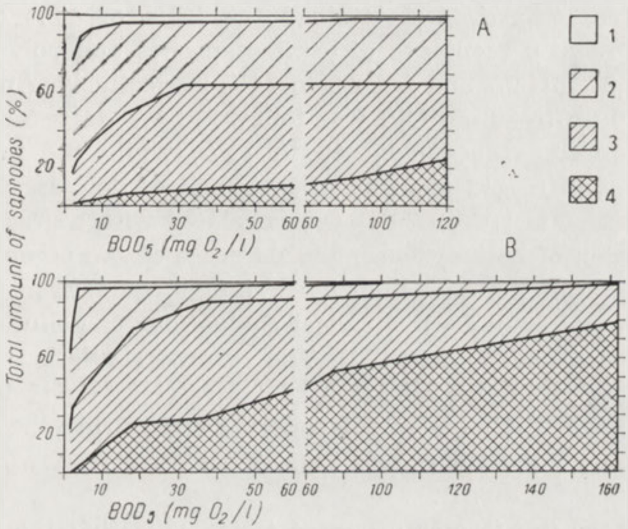

Fig. 3. Changes of percentage composition of the set of saprobes of Ślęza as related with changes of BOD_5 values. 1 — oligosaprobic organisms, 2 — β-mesosaprobic organisms, 3 — α-mesosaprobic organisms, 4 — polysaprobic organisms. A — non-campaign season and the first month of the campaign, B — during the campaign

paign period and the first month of the campaign (Fig. 3 A), when the biocenosis of the river has not yet been adapted to large amounts of organic compounds subject to biochemical decomposition, even high concentrations of polluting matter (expressed in BOD_5) could bring about only incompatibly small changes in the composition of the set of saprobic organisms. An increase of BOD_5 from 2 up to 10 mg O_2/l correspond to a decrease of the percentage of oligosaprobes from about 24% to about 6%, and of β-mesosaprobes from about 60% to about 55% of the total amount of the saprobes. At the same time, the percentage of α-mesosaprobes increased from about 15% to about 36%, and of polysaprobes from about 0.6% to about 3%. A further increase of BOD_5 from 10 up to 30 mg O_2/l was followed by a small decrease of the percentage of oligosaprobes (from 6 to 5%), of β-mesosaprobes from 54 to 33%, and an increase of the percentage of α-mesosaprobes from 36 to 55%, and of polysaprobes from 3 to 7%. An increase of BOD_5 beyond 30 mg O_2/l did no longer influence the percentages of oligosaprobes and β-mesosaprobes, and it was reflected only by a gradual and slow increase of the percentage of polysaprobes at the cost of α-mesosaprobes.

During the height of the sugar campaign (Fig. 3 B) changes in the composition of the set of saprobic organisms as influenced by increasing pollution were more radical. An increase of BOD_5 from 1.7 to 10 mg O_2/l was related with a decrease of the percentage of oligosaprobes from 36 to 3%. β-mesosaprobes maintained the level of 40% of the total amount, while the percentages of α-mesosaprobes and polysaprobes increased from 23 to 42% and from 0 to 14% respectively. An increase of BOD_5 from 10 up to 30 mg O_2/l was followed by a decrease of the proportion of β-mesosaprobes from 40 to 13%, while the proportion of α-mesosaprobes increased from 42 to 56%, and of polysaprobes from 14 to 28%. A further increase of BOD_5 was related with a gradual increase of the proportion of polysaprobes, and decrease of the other groups.

For bioseston of the River Oława a similar comparison of saprobiological results and of physico-chemical evaluations of pollution was performed (curves 4 and 5 in Fig. 1). The results, similarly as for Ślęza, could be arranged in two separate sets, covering the results of tests carried out during the non-campaign period and during the first month of the sugar campaign (curve 4, Fig. 1), and the results of campaign season tests (curve 5, Fig. 1).

The curve for the former group of tests, indicating the relation between BOD_5 and $\dot{S}^\circ$, was defined by the following equation:

$$\dot{S}^\circ = 162.0 \cdot BOD_5{}^{-0.31}$$

at $r_{xy} = -0.9865$, $r_{cr} = 0.4078$, $\alpha < 0.001$. During that period the BOD_5 value corresponding to the border value between α-meso and β-mesosaprobic zones was about 15 mg O_2/l.

At the height of the sugar campaign the relationship was defined by the equation:

$$\dot{S}^\circ = 141.3 \cdot BOD_5{}^{-0.16}$$

at $r_{xy} = -0.962$, $r_{cr} = 0.693$, $\alpha < 0.001$. During that period, the set of bioseston organisms revealed a composition characteristic for α-mesosaprobic zone at a level of pollution expressed by BOD_5 as 7 mg O_2/l. During the campaign, BOD_5 of about 60 mg O_2/l caused a polysaprobic pattern of the set of saprobes, while during the non-campaign season even at BOD_5 as high as 150 mg O_2/l a remarkably α-mesosaprobic character of bioseston was maintained.

The seston saprobes of the River Oława, similarly as of Ślęza, reacted differently to increasing pollutions during the non-campaign and early campaign season, in opposition to the full campaign period (Fig. 4). Dur-

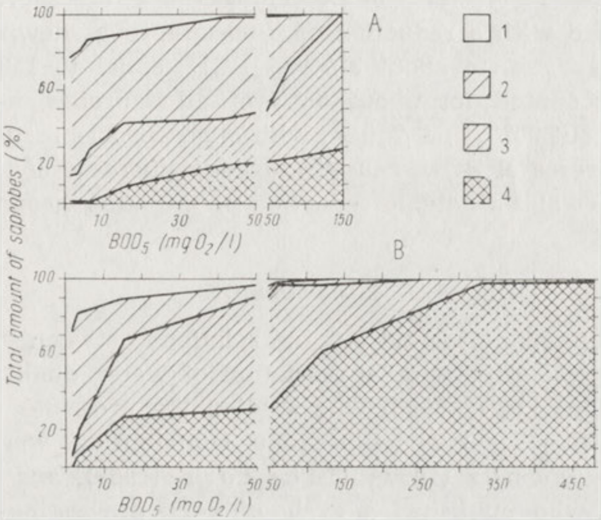

Fig. 4. Changes of percentage composition of the set of saprobes of Oława as related with changes of BOD_5 values. 1 — oligosaprobic organisms, 2 — β-mesosaprobic organisms, 3 — α-mesosaprobic organisms, 4 — polysaprobic organisms. A — non--campaign season and the first month of the campaign, B — during the campaign

ing the non-campaign season, an increase of BOD_5 from 2 to 15 mg O_2/l was followed by an increase of the proportion of polysaprobes from a fraction of a per cent to about 8%, α-mesosaprobes increased from 14 to 34%, and β-mesosaprobes fell down from about 63 to 47%, while oligosaprobes from about 22 to 10%. An increase of BOD_5 from 15 to 50 mg O_2/l was related with a gradual increase of the percentage of polysaprobes up to 22%, at the cost of a decrease of the percentage of β-mesosaprobes down to 28%. At the same time there has occurred a small increase of the proportion of α-mesosaprobes up to 50% at the cost of oligosaprobes which decreased to a tiny fraction of a per cent. An increase of BOD_5 from 50 to 150 mg O_2/l was followed by a minor increase of the proportion of polysaprobes (by 9%), while the percentage of α-mesosaprobes increased notably (from 28 up to 72%), while oligosaprobes and β-mesosaprobes were reduced to fraction of a per cent.

At the height of the sugar campaign (Fig. 4 B) the set of saprobes reacted to the changes of pollution degree (expressed in BOD_5) by much more remarkable changes of its composition. During the campaign, an increase of BOD_5 from 2 to 15 mg O_2/l caused an increase of the proportion of polysaprobes from 0 to 26%, α-mesosaprobes from 4 to 42%, and a decrease of β-mesosaprobes from about 69 to 21%, and oligosaprobes from about 27 to 10%. An increase of BOD_5 to 50 mg O_2/l was followed by a small increase of the percentage of polysaprobes (by about 5%) and a very large increase of the percentages of α-mesosaprobes (to about 59%) associated with a reduction of β-mesosaprobes down to about 7% and of oligosaprobes to about 3%. At BOD_5 equal to 150 mg O_2/l polysaprobes accounted for about 68% of all saprobes, α-mesosaprobes constituted about 30%, and β-meso with oligosaprobes made up only 2%. A further increase of BOD_5 caused a gradual increase of the percentage of polysaprobes and a notable reduction of the other groups.

4. DISCUSSION

Attempts are made to find out the relations between physico-chemical and biological criteria of appreciation of water quality. They were initiated by Leclerc (1960) who studied the processes of self-purification of the River Geer, and subsequent contributions were made in recent years by Ventz (1967), Nehrkorn (1967), and above all by Tümpling who published a cycle of very interesting works (1965, 1966, 1967, 1968) and by Sládeček (1965, 1966). Particularly interesting are the attempts to establish the relationships between saprobity and BOD_5. Investigations by Klotter and Hantge (1966), Liebmann (1965) and Sládeček (1965) seem to suggest that there are some correlations between these two indices. Tümpling (1967, 1968) using the data of the just mentioned authors and his own results, ascribed the ranges of BOD_5 to the several classes of quality of water and zones of saprobity.

Wuhrmann (1951) suggests that a reaction of a set of living organisms to pollution depends on the kind of waste to which the set is exposed and on the type of their purification (the degree of disintegration of organic substances). In natural conditions, every river is a receiver of more or less purified waste, and it purifies itself in different manners, related to its morphological, hydrological and hydrochemical properties. Thus, taking Wuhrmann's suggestion for granted, it can be expected that a reaction of biocenosis to similar BOD_5 values will be different in each river. Reactions may be similar or identical only in two similar rivers exposed to analogous sources of pollution.

Results of saprobiological investigations of the rivers Odra, Ślęza and Oława prove that there is a remarkable and exact correlation between their pollution as indicated by chemical BOD_5 tests, and their saprobity determined by the vector method. A comparison of correlations observed for the rivers Ślęza and Oława during the sugar campaigns and during the remaining seasons (including the first month of a campaign), and for the River Odra (Fig. 1) suggests that the existing relations between saprobity and BOD_5 are different for each of these rivers, and besides that they are different for Ślęza and Oława during and after campaigns. Reactions of sets of living organisms to various concentrations of pollution, expressed by the correlations between BOD_5 and saprobity, though different for each of the rivers, became almost identical for Ślęza and Oława during the sugar campaign, when both rivers exposed to heavy concentrations of identical effluents.

These observations lead to a conclusion that saprobity is not an absolute index of a state of pollution, but rather a measure of a reaction of biocenosis to the degree of pollution, and thus it can be an index of relative pollution.

A reaction of biocenosis to increased loads of organic waste is different at the beginning and during the period of increased pollution. At the beginning, when biocenosis is not yet adapted to the increased loads, its reaction towards higher concentrations of waste matter reflected by BOD_5 values, is very slow, and even BOD_5 values approaching 150 mg O_2/l have not been sufficient to shift the saprobity of Ślęza and Oława beyond the middle α-mesosaprobic zone. After a month from the beginning of the sugar campaign, however, biocenosis of these rivers has been adapted to increased concentrations of organic substances, so that its reaction has become quicker, and BOD_5 values of an order of 60 mg O_2/l have been able to shift saprobity into polysaprobic zone.

In a system of coordinates representing the relationship between BOD_5 and saprobity, the degree of adaptation of biocenosis of a river to increased concentrations of polluting organic substances capable of being decomposed is expressed by the shape of the line defining the said relationship (Fig. 1). As the process of adaptation of biocenosis proceeds, the relationship changes in such a manner that a smaller increase of BOD_5 value causes a stronger shift of saprobity towards the zones of heavier pollution; i.e., the curve indicating the relationship becomes more steep.

Remarkable differences can be seen between reactions of the sets of saprobes to an increase of organic pollution as expressed by the BOD_5 index in a river with adapted and non-adapted biocenoses (Figs. 3 and 4). In a river with adapted biocenosis, a much smaller increase of the BOD_5 value causes a stronger increase of percentages of α-meso and polysaprobes, i.e. organisms adapted to life in conditions of heavier pollution and actively participating, directly or indirectly, in decomposition of organic waste, in comparison with a river with non-adapted biocenosis. This fact confirms the phenomenon established by Müller (1951) that burdened rivers are more capable of self-purification than pure ones.

5. SUMMARY

Investigations of three rivers in Lower Silesia constituted a basis for establishing correlations between saprobity of bioseston and BOD_5 values, determined at the same time.

The observed correlations turned out to be different for each of the rivers, and for changing degrees of pollution they were different for the same river at different seasons (sugar campaign period and the other months). However, the correlations were almost identical for different rivers subject to identical or similar influences (two rivers exposed to similarly purified sugar-mill effluents). Thus, saprobity cannot be said to be an absolute and direct measure of the state of pollution of a river, but it is rather a measure of an individual reaction of biocenosis of a given river to pollution or its change. Saprobity does not allow for comparison of the states of pollution of two rivers, or even sometimes of the same river in different test seasons.

A reaction of a set of saprobes to an increase of pollution depends on the degree of adaptation of the set to heavier concentrations of organic substances vulnerable to decomposition. The degrees of adaptation can be expressed by a curve defining the relationship between saprobity and BOD_5. Its more steep shape is an evidence of a better adaptation of biocenosis. A better adaptation is in its turn expressed by a more strong increases of the percentages of α-meso and polysaprobes related with smaller increases of BOD_5 in comparison with a river with non-adapted biocenosis.

6. STRESZCZENIE

Wyniki przeprowadzonych badań trzech rzek Dolnego Śląska stanowiły podstawę do ustalenia związków korelacyjnych pomiędzy saprobowością biosestonu a jednocześnie oznaczaną wartością BZT_5.

Uzyskane związki korelacyjne okazały się różne dla każdej z rzek, a przy zmiennym okresowo stanie zanieczyszczenia były różne dla jednej i tej samej rzeki w różnych okresach (okres kampanii cukrowniczej i okres pozakampanijny). Związki te natomiast okazały się niemal identyczne dla różnych rzek poddanych identycznym lub bardzo zbliżonym warunkom (dwie różne rzeki poddane wpływowi jednakowo oczyszczonych ścieków cukrowniczych). Saprobowość nie jest więc bezwzględną i bezpośrednią miarą stanu zanieczyszczenia rzeki, lecz stanowi miarę indywidualnej reakcji biocenozy danej rzeki na stan zanieczyszczenia czy jego zmianę. Na podstawie saprobowości nie można porównywać stanu zanieczyszczenia dwu różnych rzek, a niekiedy tej samej rzeki w różnych okresach badań.

Reakcja zespołu saprobów na wzrost zanieczyszczenia uzależniona jest od stopnia dostosowania się tego zespołu do zwiększonych stężeń zdolnych do rozkładu substancji organicznych. Stopień dostosowania się wyraża krzywa wyznaczająca zależność pomiędzy saprobowością a BZT_5. Bardziej stromy jej przebieg świadczy o lepszym dostosowaniu biocenozy. Lepsze dostosowanie wyraża się silniejszym wzrostem procentowej liczebności α-mezo i polisaprobów przy mniejszym wzroście wartości BZT_5, niż w rzece, której biocenoza nie jest jeszcze dostosowana.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	18	4	393–400	1971

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THE INFLUENCE OF HERBICIDE 2,4-D-Na ON RESPIRATION AND SURVIVAL OF *SIMOCEPHALUS VETULUS* (O. F. MÜLLER) (CLADOCERA)

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ABSTRACT

The effect of herbicide, 2,4-D-Na, on oxygen consumption by matured females of *S. vetulus* was studied using Cartesian diver technique. The metabolism rate was found to decrease but there was no linear dependence between oxygen consumption and herbicide concentration. These results were compared with those of survival, obtained in different herbicide concentrations and exposure time. Dramatic changes of metabolism were observed in herbicide concentrations higher than 5.0 mM and of survival — in concentrations higher than 7.5 mM.

CONTENTS

1. INTRODUCTION

An acute experiment of physiological or biochemical character plays recently an important role in investigation of aquatic toxicology since it permits to disclose first responses of an organism to the action of a toxic substance. The amount of oxygen consumed which reflects bioenergetical processes is a good integrating indicator of the physiological state of an organism. Therefore studies were undertaken to examine the effect of broadly applied herbicide 2,4-D-Na (sodium salt of 2,4 dichlorophenoxyacetic acid) on oxygen consumption by a cladoceran, *Simocephalus vetulus*.

2. METHODS

Matured, non-ovigerous females, which had been kept in an aquarium on a mixed food consisting of dry yeast, milk powder, egg yolk, and algae, were used for experiments in culture containers. Aquaria were kept in room temperature (at about 18°C). The animals were acclimated to temperature of 20°C for 24 hr before the experiments began. The technical herbicide used in farming contains some admixtures, among which up to 3% of 2,4 dichlorophenol, which is a strong uncoupler of oxidative phosphorylation. In order to examine the effect of pure herbicide on metabolism in aquatic animals 2,4-D-Na was extracted from technical substance according to an earlier described method (Zvirgzds and Bāliņa 1969).

Different concentration of 2,4-D-Na were prepared with tap water[1] which had been conditioned by aeration in the laboratory for at least 3–4 days. The following concentrations in mM were used: 1.25, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0.

Oxygen consumption of single individuals was measured in stoppered cartesian divers after Zeuthen (Zeuthen 1950, for details see Klekowski 1971), with air bubble volume of 13–21 μl, at a temperature of 20°C. The results concern the third hour of stay of the animals in a solution of 2,4-D-Na of a given concentration. Oxygen consumption by control animals was measured in divers filled with conditioned tap water. In general, oxygen consumption was measured in 216 animals.

After 3 hr-experiments the length of animals was measured and their dry weight was estimated from the length-weight regression line for *S. vetulus* (Klekowski and Ivanova in print). Dry weight of majority of examined animals ranged from 50 to 70 μg. The results of the experiments were expressed as μl O_2 per μg dry weight per hr $\cdot 10^{-5}$. Since there were rather small differences in weight of animals used in the experiments, their weight was used for determining intensity of metabolism without taking into account the regression between the respiratory rate and the body weight of animals.

Experiments on survival of *S. vetulus* in different concentrations of 2,4-D-Na were made as follows. Twenty animals were placed in vials containing 20 ml of 2,4-D-Na solution of an appropriate concentration. The vials were kept in incubator at 20°C for 6, 12, 24, and 48 hr after which the animals were transferred to pure water of the same temperature and number of surviving animals was determined after next 24 hr. The animals were not fed during this experiment. The obtained results were analysed statistically. The degree of variation within experimental groups was expressed as standard deviation δ. For evaluation of significance of difference between any two groups of experiments the criterion of non-parametric statistics for independent data was accepted Wilkoxon-Mann-Whitney U test, Gubler and Genkin 1969, Walch 1965).

3. RESULTS AND DISCUSSION

The results of experiments (Table I, Fig. 1) indicate a considerable effect of 2,4-D-Na on oxygen consumption by *S. vetulus* under conditions of acute experiments. However, as it is evident from the curve of changes in the intensity of respiration (Fig. 1), there is no direct dependence between increase in herbicide concentration in the medium and the decrease in oxygen consumption by examined animals. The lowest concentration applied (1.25 mM) caused a statistically significant depletion of the curve, whereas at next concentration (2.5 mM) the metabolism in-

[1] Water of Warsaw supply system had the following indices in October 1969: total hardness 5.3 meq/l, Cl — 120 mg/l, total Fe — 0.05 mg/l, oxygen content — 6.0 mg O_2/l, carbonate hardness 3.3 meq/l, SO_4 — 70 mg/l.

Table I. Respiration rates of *S. vetulus* in different 2,4-D-Na concentrations

Herbicide concentration (mM)	No. of measurements	Respiration ($\mu l\ O_2 \cdot 10^{-5}$ μg dry wt $\cdot$ hr) mean$\pm$S.D.	Significance of difference between means p
0.0	89	133 ± 29	
1.25	17	106 ± 35	<0.01
2.5	20	145 ± 25	<0.001
5.0	20	108 ± 30	<0.001
7.5	17	69 ± 12	<0.001
10.0	16	93 ± 20	<0.001
12.5	16	91 ± 21	>0.1
15.0	20	85 ± 20	>0.1

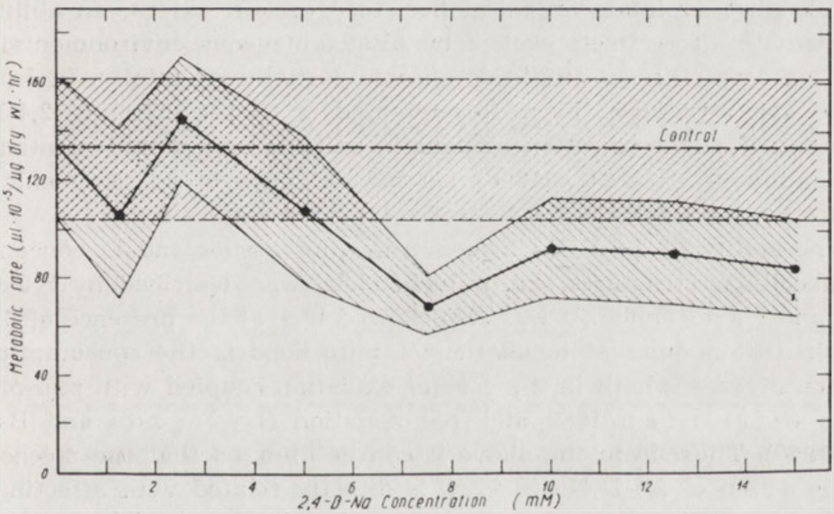

Fig. 1. Influence of 3 hr stay in solutions of herbicide 2,4-D-Na on *S. vetulus* respiration rate. Dashed area indicate standard deviations

tensity returned nearly to the control level. Further increase in concentration of the toxic substance in the medium (5.0 and 7.5 mM) brought about a sharp and uniform decrease in O_2 consumption. Still higher concentration of the toxine in the medium (10.0 mM) led to a clearly expressed increase of the curve up to the level not changed at still higher concentrations (12.5 and 15.0 mM). All examined animals during the experiment showed locomotory and filtration activities.

The initial decrease in respiration rate at relatively low concentration of the toxic substance, in the authors' opinion, should be related with typical shock reaction of the animal to the action of external irritating agent. Such concentration apparently does not create significant threat for basic metabolic function of the organism and during short-lasting experiment defensive-adaptative mechanisms did not operate. The doubling of experimental concentration (up to 2.5 mM) leads to overcoming the first shock even during the experiments, and oxygen consumption increases to the values characteristic for respiration of the control animals. It can be assumed that the described initial variation in the respiration rate reflects rather general response of the organism to irritating agent than a specific reaction to the influence of 2,4-D-Na. This can be interestingly compared with studies on the effect of different concentrations of 2,4-D-Na on oxygen consumption by mitochondria of carp liver (Zvirgzds and Bāliņa 1969). The curve depicting respiration by mitochondria at increasing herbicide concentration did not show similar fluctuations, neither in its initial nor in any other part. It is obvious that an intact organism has, to a certain extent, an ability to compensate after-effects evoked by disadvantageous environmental factors whereas such an ability is absent, partially or totally, in isolated subcellular structures. From infrequent data on the effect of 2,4-D-Na on survival and respiration of aquatic animals one should mention investigations by Solski (1968), carried out on a broad taxonomic material.

The ability of 2,4-D-Na to penetrate into tissues and to concentrate in subcellular structures, e.g. mitochondria, was described by Suchitra and Fang (1966). It was also showed that at the presence of 2,4-D-Na in the medium of incubation of mitochondria the consumption of oxygen decreases both in the case of oxidation coupled with phosphorylation (Hiltibran 1966) and free oxidation (Zvirgzds and Bāliņa 1969). Thus, from the above it can be inferred that the mechanism of the action of 2,4-D-Na in vitro is directly related with affecting the respiratory chain. Similar type of the effect is also possible in a whole organism. Therefore, 2,4-D-Na at higher concentrations penetrating into cells reacts as an inhibitor of oxidative processes at subcellular level. The authors are inclined to consider a clear drop of the curve of respiratory rate in *S. vetulus* at the range of concentrations 2.5–7.5 mM as a complex mechanism.

The lowest values of O_2 consumption at the applied concentrations of herbicide was found at 7.5 mM and it formed 50% of the control level. Poisoning of the organism reaches apparently a degree of direct danger for its life, but with further increases in concentration of toxic substance the respiration rate increases to about 70% of the initial level and practically remains constant up to the highest concentration (15.0 mM). The observed phenomenon is not quite clear, but such defensive mechanism

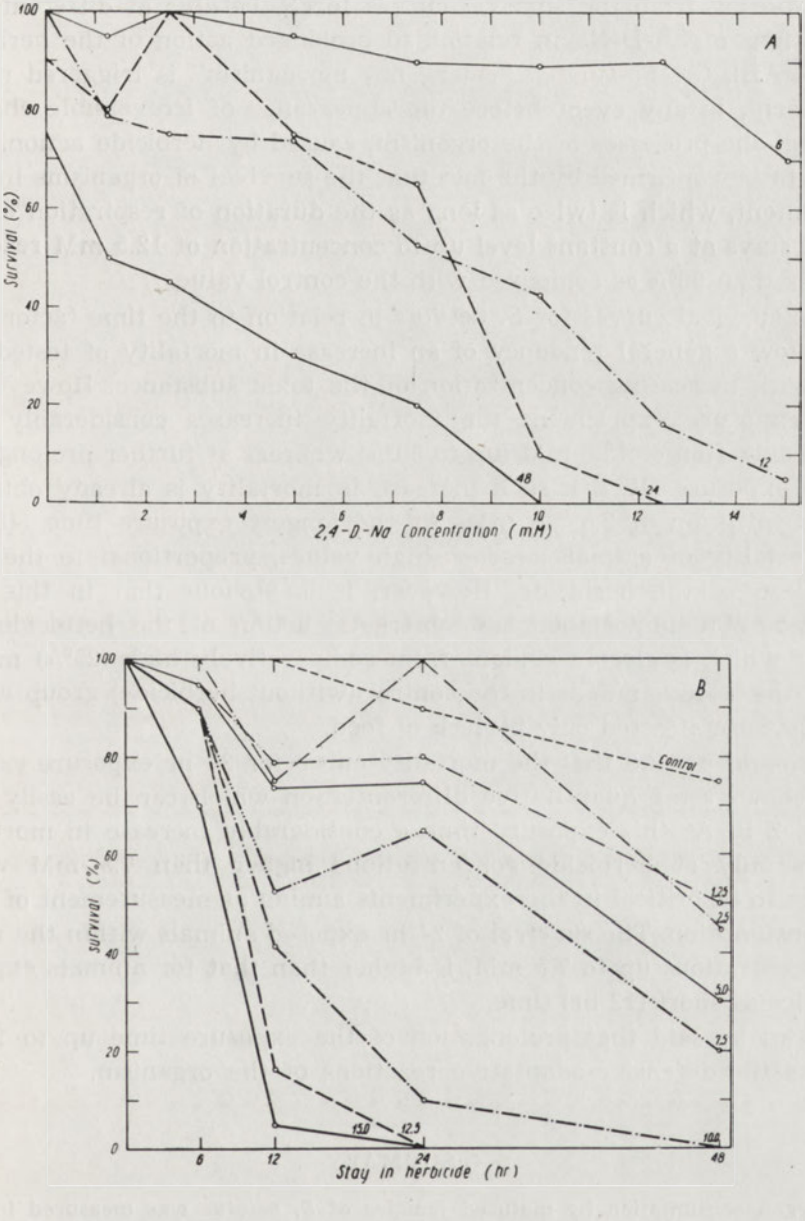

Fig. 2. Survival of *S. vetulus* after stay in different concentrations of 2,4-D-Na. A — presented as a function of concentration of herbicide (figures on curves — hours of stay in 2,4-D-Na solutions). B — presented as a function of exposure time (figures on curves — concentrations of herbicide — mM)

seems to help to sustain basal metabolic processes under disadvantageous conditions.

Gathering from the survival curves for *S. vetulus* at different concentrations of 2,4-D-Na in relation to prolonged action of the herbicide (Fig. 2 AB), the postulated "emergency mechanism" is triggered relatively early, in any event before the appearance of irreversible changes in metabolic processes of the organism, caused by herbicide action. This seems to be confirmed by the fact that the survival of organisms in 6 hr experiment, which is twice as long as the duration of respiration experiment, stays at a constant level up to concentration of 12.5 mM reaching not less than 90% as compared with the control value.

The survival curves for *S. vetulus* in relation to the time factor (Fig. 2 B) show a general tendency of an increase in mortality of tested animals with increasing concentration of the toxic substance. However, at 6 hr exposure experiment the mortality increases considerably only at concentration of 15.0 mM (up to 30%) whereas at further prolongation of the exposure (12 hr), such increase in mortality is already obtained at concentration of 2.5–5.0 mM. At the longest exposure time (48 hr), the mortality of animals reaches high values, proportional to the concentration of the herbicide. However, it is obvious that in this case a considerable importance has synergetic action of the herbicide and hunger which is clearly evident from comparatively high (25%) mortality of the tested animals in the control (without herbicide) group where animals were affected only by lack of food.

It should be said that the mortality curve for 24 hr exposure experiment shows some quantitative differentiation which can be easily seen in Fig. 2 B. At this exposure time a considerable increase in mortality follows only at herbicide concentrations higher than 7.5 mM which appears to be critical in the experiments aiming at measurement of oxygen consumption. The survival of 24 hr exposed animals within the range of concentrations up to 7.5 mM, is higher than that for animals exposed for twice as short (12 hr) time.

It can be said that prolongation of the exposure time up to 24 hr triggers the defensive-adaptative reactions of the organism.

4. SUMMARY

Oxygen consumption by matured females of *S. vetulus* was measured in cartesian divers. Herbicide 2,4-D-Na brought about a general inhibition of the intensity of metabolism in the studied cladocerans, however in short-lasting experiments, the dependence of oxygen consumption from the herbicide concentration has no linear character (Fig. 1). At 2,4-D-Na concentrations from about 7.5 mM, a compensative mechanism operates in the cladoceran organism, which ensures, at least for several hours of exposure to the herbicide, a constant level of metabolism. The

results on survival of cladocerans in relation to concentration of the herbicide and the time of exposure to this herbicide (Fig. 2) speak in favour of the fact that such compensative mechanism exists. At low concentration of the herbicide, cladocerans survive over 24 hr exposure better than 12 hr exposure which may reflect a short--lasting, adaptative and compensative reaction.

5. STRESZCZENIE

Zużycie tlenu przez dorosłe samice *S. vetulus* mierzono w nurkach kartezjańskich. Herbicyd 2,4-D-Na powodował na ogół zahamowanie intensywności metabolizmu badanych skorupiaków, jednakże w krótkotrwałych eksperymentach zależność intensywności zużycia tlenu od stężenia herbicydu nie miała w zasadzie charakteru liniowego (Fig. 1). W stężeniach 2,4-D-Na od ok. 7,5 mM w organizmach wioślarek działa pewien „mechanizm kompensacyjny", który co najmniej w okresie kilkugodzinnym oddziaływania toksyny zapewnia stały poziom metabolizmu. Za istnieniem takiego mechanizmu kompensacyjnego przemawiają wyniki doświadczeń nad przeżywalnością wioślarek w zależności od stężenia herbicydu i czasu pobytu w jego roztworach (Fig. 2). W niskich stężeniach herbicydu wioślarki przeżywają 24 godz. ekspozycję lepiej niż 12 godz., co może być przejawem krótkotrwałej reakcji adaptacyjno-kompensacyjnej.

6. REZUMĒJUMS

Mūsu eksperimentos tika pārbaudīta 2,4-D-Na ietekme uz *S. vetulus* skābekļa patērina intensitāti, lietojot Dekarta pludina mikrorespirometrisko metodi. Pētitais toksikants bremzē summārā metabolisma intensitāti, taču nav novērojama lineāra sakarība starp patērētā O_2 daudzumu un toksikanta koncentrāciju (Fig. 1). Pie koncentrācijas 7,5 mM organismā iesledzas „kompensācijas mehanisms", kas īslaicīga eksperimenta apstāklos nodrašina galveno metabolisma procesu tālāku norisi. „Kompensācijas mehanisma" esamību pierāda arī rezultāti, kas iegūti pētot *S. vetulus* izdzīvotību dažādās 2,4-D-Na koncentrācijās, atkarībā no laika faktora (Fig. 2). Pie relatīvi nelielām toksikanta koncentrācjiām 24 stundu eksperimentā organismi uzrāda lielāku dzīvotspēju kā 12 stundu eksperimentā, kas norāda uz adaptīvo mechanismu īslaicīgu mobilizēšanos.

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TEMPERATURE PREFERENCE OF FATHEAD MINNOW *PIMEPHALES PROMELAS* (RAFINESQUE) AND ITS CHANGES INDUCED BY COPPER SALT $CuSO_4$

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ABSTRACT

The selected temperatures of fathead minnow (*Pimephales promelas*) acclimated to 10, 15, 20, 25 and 30°C were 24.0, 26.3, 27.3, 28.0 and 28.5°C, respectively. Final temperature preferendum was found to be 28.5°C. Exposure of fish acclimated to 10 and 20°C to sublethal doses of copper (0.20–0.25 mg/l) brought about significant decrease in the selected temperature.

CONTENTS

1. INTRODUCTION

In spite of the fact that fathead minnow (*Pimephales promelas*) has no importance from commercial fisheries point of view, this species is commonly used for laboratory experiments and its significance as bioassay animal is still increasing. As far as the present author is aware, no data are available in literature concerning temperature selection of this species. The final temperature preferendum, i.e. the temperature at which the selected temperature is identical with the acclimation level, and the relation between acclimation and preferred temperature is characteristic for the species (Fry 1947, Ferguson 1958) and the knowledge of these data can be helpful in planning experiments where the temperature factor is involved.

The preferred temperature is not a constant value. It can depend upon the acclimation temperature (Doudoroff 1938, Pitt et al. 1956, Garside and Tait 1958), season (Sullivan and Fisher 1953), condition of fish (Javaid and Anderson 1967) and also pre-exposure to some chemicals. Ogilvie and Anderson (1965) found that exposure of Atlantic salmon underyearlings to sublethal doses of DDT for 24 hr produced a shift in the selected temperature. In the present work, an influence of copper as $CuSO_4$ on preferred temperatures has been in-

[1] Experiments were carried out at Freshwater Institute, Fisheries Research Board of Canada, Winnipeg, Man., Canada.

vestigated. According to Mount (1968) the common use of copper salts for vegetation control in water supplies, the frequent presence of copper in certain industrial wastes and its high toxicity to fishes make copper a toxicant that should be cautiously considered in any habitat that is to be used for fish production.

2. MATERIAL AND METHODS

Individuals of fathead minnow 0.6–2.1 g, mean 1.3 g, were used for the experiments. Fish were brought from a pond situated at Glenea Experimental Farm, University of Manitoba, in October 1968. They have been kept under constant acclimation temperatures 10, 15 and 20°C beginning from 6th April 1969, i.e. for more than six weeks before the experiments started. For 25°C acclimation temperature, fish were taken from 20° and have been kept at 25° for at least one week before the experiment. The same routine was applied for 30°C acclimation level; fish were taken from 25° acclimation temperature and have been kept at 30°C one week before the experiments were carried out. During acclimation the temperature was maintained within ±0.5°C. Fish were kept in continuously running, dechlorined city water. Table I contains the results of water analyses. At the beginning of the acclimation period, fish were fed on dry fish food. They were reluctant to accept it, and it was replaced by finely ground beef liver.

Table I. Chemical characteristics of tap water *

Calcium	(mg/l)	12.9
Magnesium	(mg/l)	5.70
Sodium	(mg/l)	1.53
Potassium	(mg/l)	1.33
Iron	(mg/l)	0.02
Copper	(mg/l)	< 0.01
Zinc	(mg/l)	0.031
Hardness	(mg/l $CaCO_3$)	55.1
Alkalinity	(mg/l $CaCO_3$)	84.3

* Analyses made by Dr. F. A. Armstrong, Freshwater Institute, Winnipeg.

As the source of copper, cupric sulfate $CuSO_4$ was used (J. T. Baker Chemical Co., Lot No. 37597). On the base of previous experiments, sublethal copper concentrations 0.20 mg/l for fish acclimated to 10°C and 0.25 mg/l for fish acclimated to 20°C were applied. Fish were exposed to copper in glass jars, 22 cm in diameter, to which cupric sulfate dissolved in 8.8 l of water had been added. Water has been always taken from an acclimation tank where the experimental fish were kept. Seven fish were put to each jar. The jars were immersed in acclimation tanks in order to maintain constant water temperature during 24 hr of exposition to copper. In each jar, water was aerated and kept close to 100% oxygen saturation. pH ranged from 8.0 to 8.7. During the acclimation time as well as the copper pre-exposure, light regime was 12 hr light and 12 hr dark.

A vertical temperature gradient apparatus was used. It consisted of a glass aquarium 91×46×41 cm marked with horizontal lines to form 10 equal cells. A tele-thermometer probe was horizontally situated in the middle of each cell. To set up and maintain the temperature gradient, warm water was conducted through a copper coil from the top to the bottom of the aquarium. Through a similar copper coil cold water was pumped in the opposite direction. For a better cooling effect at the lower end of the apparatus, a third flat coil conducting cold water was placed on the bottom. Such a coil has already been used for experiments with cold-water species (Opuszyński in prep.) and it made possible to maintain the temperature of 3–4°C on the cooler end of the gradient. The coils were coated with a green-plastic paint.

The above arrangement provided an approximately linear temperature gradient. By adjusting the rate of flow as well as the temperature of cold and hot water, any desired temperature gradient within the range of about 20°C could be obtained. Before the temperature gradient was set up, water had been well aerated and during the experiments oxymeter tests proved that there were practically no differences in water oxygen content (mg/l) within the gradient. The experiments were carried out in a dark room. A preferred temperature tank was lighted by two fluorescent bulbs placed vertically at both sides of the tank. Bulbs were screened off by means of green translucent fiber glass sheets. The intensity of light was below 10 Lux. No light gradient was observed within the tank.

Five fish were tested each time. They were placed in temperature similar to the previous acclimation temperature. Observations were made when the temperature gradient was completely established but not earlier than at least 4 hr after stocking. Fish distribution was noted every minute for 20 min. Three sets of 20 min observations were made with 15 min intervals between them. Temperatures were recorded just before and after each set of observations and then average temperatures of individual zones were calculated. Selected temperatures were calculated as modal values of the frequency distribution, according with the most common practice. Experiments were carried out between May 21 and July 29, 1969.

3. RESULTS

Selected temperatures of fathead minnow acclimated to different temperatures are shown in Table II. Fish acclimated to 10, 15 and 20°C were tested three times, and those acclimated to 25 and 30°C were tested twice. Selected temperatures were expressed as the means of individual modal points. The final preferendum was found to be 28.5°C (Fig. 1).

Table II. Selected temperatures of *Pimephales promelas* acclimated to different temperatures

Acclimation temperature (°C)	Date of experiment (1969)	Total number of observations	Selected mean temperature±S.D. (°C)	Selected modal temperature (°C)	Mean of individual modals (°C)
10	2.VI	198	21.6 ± 1.83	23	24.0
	10.VI	393	23.0 ± 3.19	25	
	11.VI	238	24.3 ± 1.12	24	
15	22.V	401	27.0 ± 1.38	28	26.3
	23.VI	296	23.0 ± 3.58	25	
	24.VI	393	25.7 ± 1.93	26	
20	21.V	447	28.2 ± 2.56	29	27.3
	3.VI	191	25.4 ± 3.38	26	
	26.VI	398	27.0 ± 1.64	27	
25	30.VI	385	27.7 ± 1.54	28	28.0
	4.VII	394	27.6 ± 1.87	28	
30	8.VII	372	28.7 ± 2.17	29	28.5
	9.VII	384	29.3 ± 2.26	28	

Fish acclimated to 10 and 20°C and exposed to copper were subject to five experiments. Fish acclimated to 20°C were exposed to 0.25 mg/l

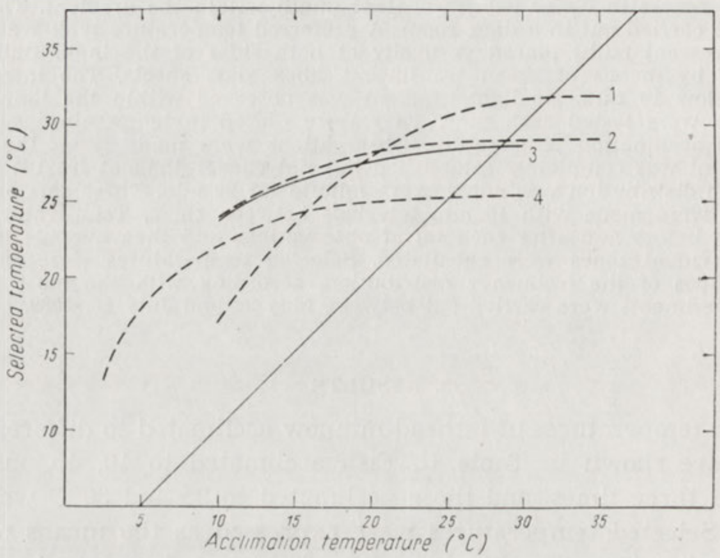

Fig. 1. Selected temperatures of *Pimephales promelas* compared with other fish. 1 — *Cyprinus carpio* (Pitt et al. 1956), 2 — *Carassius auratus* (Fry 1947), 3 — *Pimephales promelas* (present paper), 4 — *Notropis atherinoides* (Cambell, unpubl). Diagonal line — isothermal line

copper solution. No mortality or changes of fish behaviour had been observed until the fourth day after experiments. Selected temperatures for individual experiments ranged from 18 to 26°C, the mean was 21.3°C. Fish acclimated to 10°C were also exposed at the beginning to 0.25 mg/l copper solution. Unexpectedly, the toxic effect of copper at 10°C was found to be greater than at 20°C. During exposure, momentary disturbances of fish balance were observed and even in one case a permanent loss of balance was found. For this reason copper concentration at 10°C was lowered to 0.20 mg/l. Selected temperatures for individual experiments ranged from 20 to 22°C, the mean was 20.9°C.

Figure 2 shows pooled results of all the experiments for control and copper exposed fish. In both groups of experiments, pre-exposure to copper resulted in a shift of preference towards the cooler temperatures. Its significance has been tested statistically by the chi-squared test. For both acclimation temperatures, the differences between pre-exposed and untreated fish were found to be statistically significant. The probability that the calculated values of χ^2 (234 and 522) would occur by chance alone was <0.001.

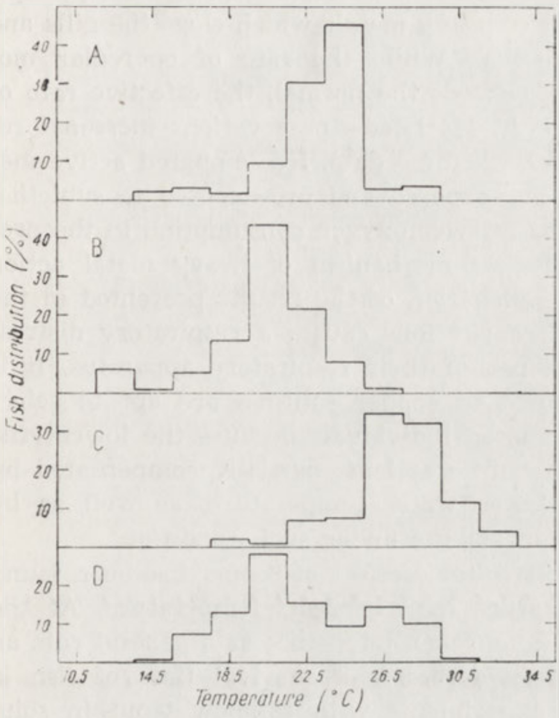

Fig. 2. Frequency of observations of untreated and Cu-exposed *Pimephales promelas* at the gradient temperatures. A — acclimation to 10°C — control, B — acclimation to 10°C, pre-exposed to 0.20 mg/l Cu, C — acclimation to 20°C — control, D — acclimation to 20°C, pre-exposed to 0.25 mg/l Cu

4. DISCUSSION

Figure 1 shows the selected temperatures of fathead minnow as compared with other warm-water species. Two types of relationship between acclimation and selected temperature can be observed. The first one is represented by *Cyprinus carpio*. Selected temperatures gradually increase with increasing acclimation temperature. This is represented by the slope of the selection curve. The second relationship is typical for *Carassius auratus*. Fishes acclimated to low temperatures tend to select a relatively high temperature. A further increase of an acclimation temperature does not cause a great increase of the selected temperature, and the selected temperature curve becomes flat in shape. The selected curve of fathead minnow fits very well into the second type. It is very similar to goldfish curve not only in its shape but also in its absolute values.

Pre-exposure of fathead minnow to copper solution decreased the selected temperatures. Carpenter (1927) has found that the effective action of dissolved heavy metal salts on fish is not "toxic" in the ordinary acceptance of the term, as implying changes in internal body fluids, but rather it is purely mechanical in character, consisting in a precipitation of an organic compound of heavy metals which clogs the gills and inhibits their respiratory function. While the rate of opercular movements in the exposed fishes exceeds the normal, the effective rate of gaseous exchange is far below it. MacLeod's observations (personal report) are in a good agreement with these data. He compared active metabolism of fathead minnow non-exposed and pre-exposed to sublethal copper concentration and found a lower oxygen consumption in the pre-exposed group. This physiological mechanism of heavy metal action seems to justify a following explanation of the results presented in the present paper. The action of copper ions causes a respiratory distress of fish by lowering the efficiency of their respiratory apparatus. It is probable that fishes pre-exposed to copper solution are apt to select lower temperatures than unexposed individuals because the lower efficiency of their gill apparatus can be at least partially compensated by higher oxygen solubility at lower water temperature, as well as by a decrease of fish oxygen requirements under such conditions.

In the present work, a stronger toxic effect of copper has been found at 10°C pre-exposure temperature than at 20°C temperature. At the first glance, this fact seems incomprehensible, since as a general rule an increase in temperature usually decreases a survival time of fish in heavy metal solutions. E.g., experiments with rainbow trout in solutions of zinc (L l o y d 1962) showed that the survival times increased 2.35 times when the temperature was reduced from 22 to 12°C.

An explanation can be found by a more detailed scrutiny of the mechanism of toxic influence of copper solution upon fish, and by considering that only as little as 8.8 l of this solution has been used in our experiment. As it has been mentioned above, the lethal action of heavy metals on fishes consists in the formation of an insoluble compound of metalic ions with some constituent of the mucus which coats on the skin and gill and finally causes death by suffocation. Mucus appears not only on the surface of a fish body but it is also excreted to the surroundings. It is very probable that mucus can tie up the toxic ions of copper in water and make them harmless for fish. When the volume of the solution used for fish pretreatment is small and the total amount of soluble copper salt is not large enough, as it probably was in our case, the initial copper ions concentration may decrease significantly due to the fish activity during pre-exposure. This phenomenon may intensify as water temperature increases because of more intensive mucus excretion at higher temperature.

To prove the above hypothesis, a simple additional experiment was carried out. Under the same conditions as described above, fish had been exposed to 2.00 mg/l copper solution at 10, 15, 20, 25 and 30°C. This concentration was lethal for all fish at 10°C but only one fish (14%) died at 30°C. Great differences in water turbidity were observed. Whereas the water transparency at 10°C remained almost unchanged, at 30°C mucus excretion caused so great turbidity that it was difficult to see the fish inside 10 l glass jar.

It seems interesting to compare the copper concentration used in this work, 0.25 mg/l at 20°C and 0.20 mg/l at 10°C, with the maximum acceptable copper concentration for fathead minnows. Such a study was done by Mount (1968) on the basis of continuous 11 months copper exposure, using the "laboratory fish production index" (LFPI) as the measure of copper effect. The author found that the threshold concentration for the effect of copper on reproduction in fathead minnows lied between 0.030 and 0.015 mg/l. Mean weekly temperatures during the experiment varied from 18 to 23°C.

Copper concentrations used in this work were about ten times higher in comparison with Mount's data. It should be remembered that this comparison is a very rough one. In our tests the real copper concentrations during fish pre-exposure were probably much lower than the initial level. Toxicity of copper salts depends on chemical character of water. Heavy metals have been shown to be more toxic in soft water than in hard water (Lloyd 1962). Water used by Mount had a hardness of 198 mg/l $CaCO_3$ and it contained 54 mg/l of calcium. Ours was more soft (Table I).

In the present work, copper concentrations below 0.25 to 0.20 mg/l have not been used. It is too early to decide, how useful the selected temperatures test can be for an estimation of copper concentration acceptable by fish. Further tests with fish pre-exposed to lower copper concentrations should be carried out to estimate the treshold copper concentration, still causing shifts of the selected temperatures. In order to prevent changes in copper concentration during the fish pre-exposure, a large enough volume of the solution should be employed, or exposure should be performed under continuous-flow constant concentration conditions.

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The author is thankful for a Postdoctorate Fellowship from the National Research Council of Canada. I wish to thank Drs. L. Johnson and J. C. MacLeod, Fisheries Research Board of Canada, Freshwater Institute, Winnipeg, Man., for providing research space for this study, helpful discussion and critical reading of the manuscript. For technical assistance in the laboratory I am indebted to J. Whitaker and E. Pessah.

5. SUMMARY

1. Final temperature preferendum of fathead minnow (*Pimephales promelas*) was found to be 28.5°C. The selected temperature curve was flat in shape and very similar to one observed for goldfish.

2. 24 hr pre-exposure of fathead minnow to copper concentration resulted in a downward shift of the selected temperatures. The selected temperatures were found to be 3°C lower for fish acclimated to 10°C and pre-exposed to 0.20 mg/l initial copper concentration, and 6°C lower for fish acclimated to 20°C and pre--exposed to 0.25 mg/l Cu.

3. As a probable cause of the observed phenomenon, an influence copper ions on the gill apparatus of fish, inhibiting its respiratory action, was considered.

6. STRESZCZENIE

1. Ostateczna temperatura wybieralna *Pimephales promelas* wynosiła 28.5°C. Krzywa temperatur wybieralnych była kształtu płaskiego i bardzo przypominała krzywą stwierdzoną dla karasia.

2. Przetrzymywanie *Pimephales promelas* przez 24 godziny w roztworze wodnym siarczanu miedzi spowodowało obniżenie temperatur wybieralnych. Dla ryb aklimatyzowanych w 10°C i przetrzymywanych w roztworze o początkowej zawartości miedzi 0.20 mg/l temperatura wybieralna uległa obniżeniu o 3°C, a dla ryb aklimatyzowanych w 20°C i przetrzymywanych w roztworze 0.25 mg Cu/l temperatura wybieralna uległa obniżeniu o 6°C.

3. Jako prawdopodobną przyczynę obserwowanego zjawiska uważa się oddziaływanie jonów miedzi na aparat skrzelowy ryb. Jony miedzi obniżają wydajność respiracyjną aparatu skrzelowego.

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A. ŁYSAK

THE INFLUENCE OF ENVIRONMENT TEMPERATURE AND OF THYROTROPHIN ON IODINE METABOLISM IN CARP (*CYPRINUS CARPIO* L.). PART I. RESULTS OF INVESTIGATIONS IN VIVO

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ABSTRACT

Present paper intends to reveal the translocations of parenterally administered radioiodine ($Na^{131}I$) in one and two-year carp under the influence of thyrotrophin in different environment temperatures (10–12 and 20–22°C). In the experiment lasting sixteen days, in vivo determinations were made of the disposition of administered radioiodine s. c. iodograms. The results showed in the thyrotrophin-treated carps a new radioiodine accumulation center located in head kidney. The main role, however, in iodine metabolism in carp plays the center in thoracic kidney. This role depends also on the factor of temperature. Moreover, thyrotrophin, administered before and after radioiodine injection gives an opposite effect.

CONTENTS

1. INTRODUCTION

The phenomenon of the renal position of thyroid centers in some fish of the Cyprinidae family, rather untypical for vertebrates, has been recently pointed out by many authors (Chavin 1956 a, b, c — on crucian carp, Sugiyama and Sato 1960, Łysak 1962, 1964, 1966, Chang Shih-Jung 1963 — on carp). Further investigations aimed to gain a better understanding of this phenomenon, and to reveal its physiological mechanism. In the poikilotherms the temperature of an environment is a factor complicating the picture, as it influences in a decisive way the life processes of this group of animals.

The present paper aims to bring out, in what manner outer factors (environment temperature) and inner ones (thyrotrophic activity of hypophysis) influence the intensity of iodine metabolism in carp.

The experimental part of the work was performed in Great Britain, Torry Research Station and Marine Laboratory, Aberdeen, during the author's British

Council scholarship. The results were further elaborated and prepared for print in the Department of Fishery, Cracow Agricultural College.

2. MATERIAL AND METHODS

Subject to the experiment were one and two-year old carps (K_1 and K_2), 55 and 270 g of average weight respectively, kept before the experiment in plastic tanks 5 m in diameter with conditioned (aerated and sedimented) river water. During the period before the experiment the fish had been fed with granulated fodder made in Sweden (Trout Pellets Co.), and during the experiment no food at all was administered. The tanks were stored in a closed room, at a steady temperature $16 \pm 1°C$, and light was provided during 10 hr per day. As the investigations were made in two temperature ranges, the respective groups of fish had been acclimated to the temperatures of 10 to 12°C or 20 to 22°C during 10 to 14 days before the experiment proper. An exact scheme of the experiment is presented in Table I. After acclimation, during the six days preparatory period, the fish were

Table I. Diagram of the experiment (x — thyrotrophin injection, o — diluent injection, ↓ — radioiodine injection, + — bleeding)

Treatment of fish	Six days preparatory period (day)		Experimental period (day)									
	2	4	1	2	4	6	8	10	11	12	14	16
Experimental group	x	x	↓				x		x			+
Control	o	o	↓				x		x			+
Scanning of fish alive				I	II	III	IV	V		VI	VII	VIII

treated with two incjections, on the second and fourth day; thyrotrophin was administered to the experimental group, and diluent only to the controls. The dosis of thyrotrophin (TSH) was 0.1 mg for K_1, and 0.25 mg for K_2. On the first day of the experimental period all the fish were given parenterally radioiodine in form of $No^{131}I$ in physiological solution; the dosis was 40 $\mu C/K_1$ and 160 $\mu C/K_2$. An activity of the basic initial dosis (40 μC) was measured five times on a phantom, in identical conditions as those in which iodograms for the fish would be subsequently obtained. The mean value of these measurements was 256.5 ± 19.2 cm^2 (the diagram surface). This allowed to compute the amount of accumulated radioiodine in per cent of the dosis. During the 16 days experimental period, all the fish were scanned in vivo at 2 days intervals to assess radioiodine disposition in their bodies (the so-called iodograms were taken), and on the eighth and eleventh day all the fish were given a dosis of thyrotrophin equal to each of the two former doses. On the sixteenth day all the fish were killed by bleeding.

The technique of in vivo measurement of radioiodine disposition in the body was applied to fish for the first time by Hoffert and Fromm in 1959. It was developed and modified by the present author in earlier works. However, the measurements have always been done by passing a fish under a collimator by hand, which led to unprecise and highly unreliable results. In the present investigation, this inconveniency was eliminated by the use of a mechanical device for passing a fish under a sintillation counter, connected with an automatic recorder of the changes in radiation intensity. The whole set, arranged from elements produced by the Nuclear Chicago, is presented in Fig. 1. As the speed of the pulling of a fish under the counter's slit was relatively slow (0.5 to 1.5 inch per min), the time of scanning of a whole fish approached to 20 min. Thus, to warrant a sufficient oxygen supply, the animals were put into a plastic tubing with water flowing through it (Fig. 2). To make the fish completely immobile during the measurements, water contained an anaestethic (uretan or MS-222).

Automatically recorded iodograms, eight for each one-year old carp, and seven for each two-year old one (measurements were taken every two days during the

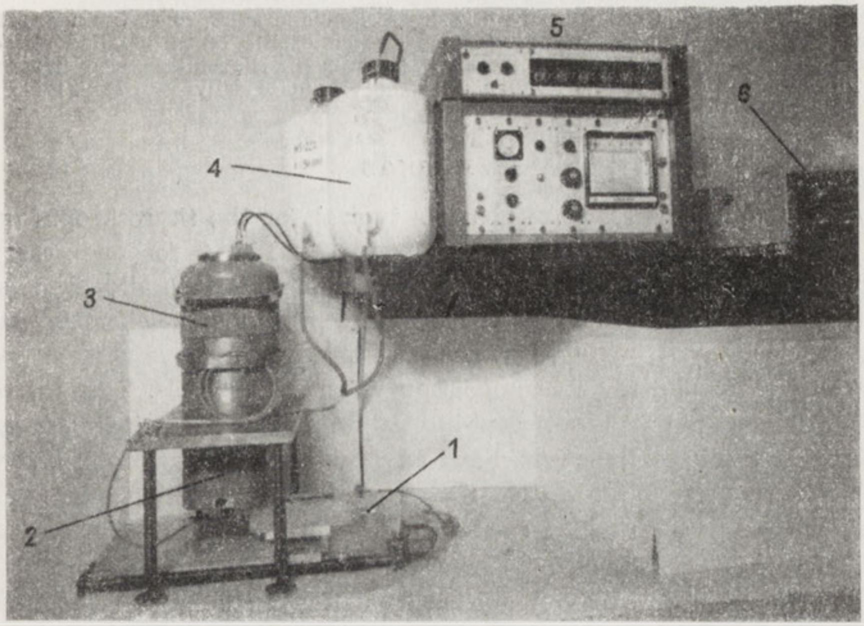

Fig. 1. Equipment set for in vivo measurement of radioiodine disposition in fish. 1 — carriage, 2 — collimator, 3 — sintillation counter, 4 — anaestethizing device, 5 — scaler, 6 — recorder

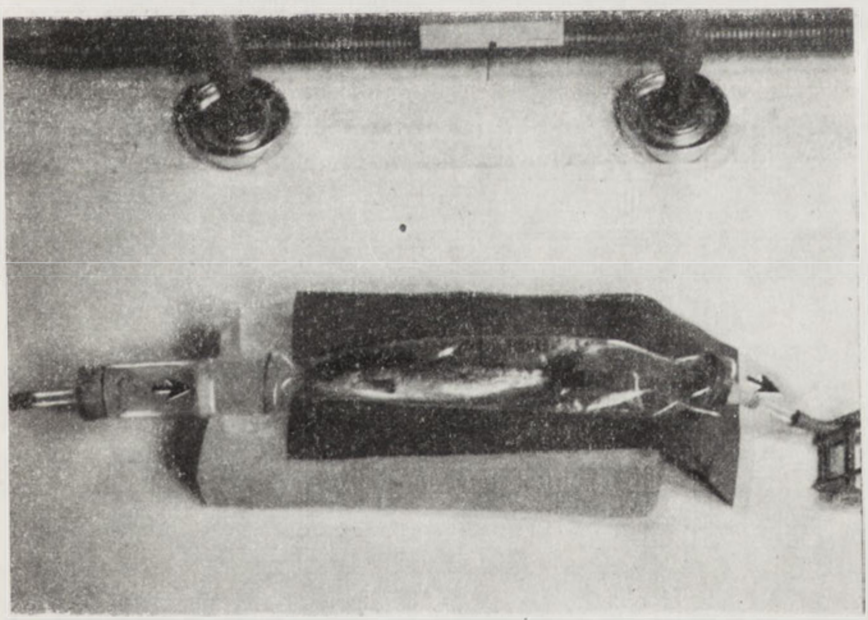

Fig. 2. Plastic tubing holding anaestethized fish during measurement. Arrows indicate water flow direction

experimental period) were subsequently properly elaborated. The obtained values were adjusted considering the physical half-period of life of the studied element, the range of recordings of the device, and the actual background of the measuring set. The adjusted diagrams were then arranged chronologically for each individual.

3. RESULTS

An example of a series of changes typical for the thyrothropin treated groups as compared with the controls, in Fig. 3 for one-year old carps is presented. An analysis of the confronted results allows to establish that the first two injections of thyrothropin during the preparatory period in experimental fish (in both age groups) have very strongly increased iodine uptake in comparison with the controls. This increase was most marked in the thoracic kidney region, and less in pharyngeal region. Besides these two centers of accumulation (thoracic and pharyngeal), the thyrotrophin-treated group revealed a small, but remarkable in all diagrams, additional region of iodine accumulation which was situated in the head kidney, not performing excretory functions in teleost fishes. This new center was observed in all the TSH fishes belonging to both of the age groups. All the discussed differences are much more remarkable at the higher temperature range (20–22°C) than at the lower one (10–12°C).

The adjustment of the records reduced them all to a comparable form, allowing for immediate quantitative comparisons. Such a comparison was made by a planimetric determination of the total surfaces of the diagrams and their components: a. the surface corresponding to the thoracic kidney activity, and b. the surface corresponding to the activity of the rest of the body, including the pharyngeal region and the kidney.

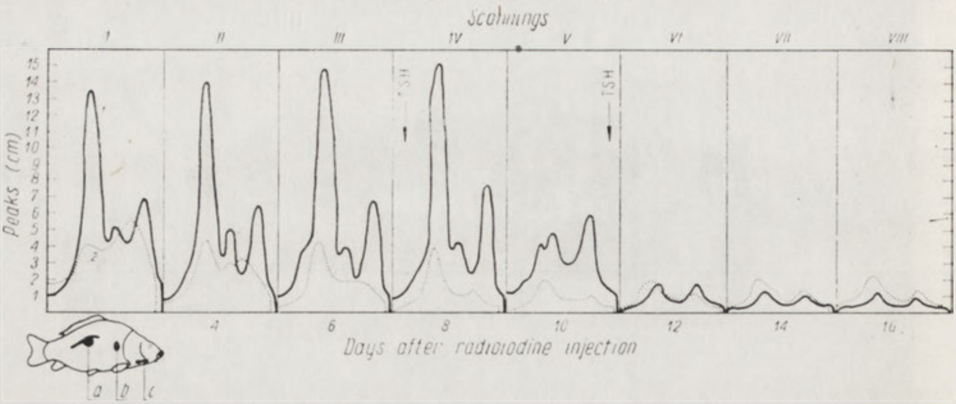

Fig. 3. An example of typical changes in the shape of the iodograms of one year old carp (20–22°C) during the experimental period. a — thoracic kidney, b — head kidney, c — pharyngeal region, corresponding with three subsequent peaks on each iodogram. 1 — TSH-treated, 2 — control

The mean results of these measurements and their respective standard deviations, obtained during the 16 days since the radioiodine injections for both age groups and for both temperature ranges (10–12 and 20–22°C), are presented in Tables II and III. In these tables, the values for all the two-year old carps are four times smaller with respect to the planimetric data because of the differentiation of the initial dose of radioiodine, mentioned above (K_1 — 40 μC, K_2 — 160 μC per specimen).

A horizontal comparison of the mean values of iodine uptake (Tables II and III) allows to make the following observations: a. significantly higher values of iodine uptake ($p<0.03$) in thyrotrophin-treated carps in comparison with the controls; b. significantly higher iodine uptake values ($p<0.03$) in carps investigated at 20–22°C in comparison with those kept at 10 to 12°C.

However, a comparison of the discovered vertical relationships (Fig. 4), i.e. of the patterns of changes in individual fishes in time (scannings I, II, III etc.) reveals the presence of a quite different pattern of changes in the renal thyroid center in the TSH-treated carps than in the controls in all cases. An increase of temperature from 10 to 20°C tends to make this difference even more marked. On the other hand, the chan-

Table II. Uptake of the renal thyroid region on the iodogram (cm^2)

Treatment of fish				Scannings							
				I	II	III	IV	V	VI	VII	VIII
One-year old carp (K_1)	20–22°C	TSH-treated $n=9$	$\bar{x}$	44.6	41.6	42.9	43.0	32.4	20.4	17.2	15.1
			± S. D.	8.46	10.7	14.4	10.77	11.3	8.92	6.78	7.6
		Control $n=9$	$\bar{x}$	29.3	17.8	16.2	16.3	12.2	11.0	7.7	11.6
			± S. D.	10.3	4.17	5.93	5.11	3.91	4.34	3.46	2.8
	10–12°C	TSH-treated $n=2$	$\bar{x}$	20.4	17.5	18.0	16.8	14.6	10.2	6.1	4.0
			± S. D.	4.2	2.58	5.0	2.36	0.5	2.0	1.0	1.1
		Control $n=4$	$\bar{x}$	13.4	10.6	7.9	8.6	5.7	5.0	5.4	6.0
			± S. D.	1.9	0.91	1.7	2.75	0.96	0.75	1.1	1.4
Two-year old carp (K_2)	20–22°C	TSH-treated $n=5$	$\bar{x}$	46.6	42.1	39.4	36.2	21.9	19.9	20.5	—
			± S. D.	14.1	10.8	9.72	7.63	5.27	2.65	4.07	—
		Control $n=5$	$\bar{x}$	29.4	27.2	29.8	28.1	24.7	23.7	19.5	—
			± S. D.	8.1	8.4	8.88	8.23	7.99	7.85	5.28	—
	10–22°C	TSH-treated $n=2$	$\bar{x}$	26.2	20.2	20.4	20.5	18.8	14.8	10.1	—
			± S. D.	5.4	3.2	3.75	1.25	2.65	0.38	1.67	—
		Control $n=4$	$\bar{x}$	14.6	11.6	11.0	9.8	8.8	8.2	8.3	—
			± S. D.	5.3	2.3	2.13	3.01	1.34	1.22	1.75	—

ges in uptake in the rest of the body, including the pharyngeal region and the head kidney, reveal a pattern which is quite similar to that in the controls, which seems to point to a much lesser contribution of these organs to iodine metabolism of the investigated carps.

Table III. The rest of the body region on the iodogram (cm^2) (including pharyngeal and head kidney regions)

Treatment of fish				Scannings							
				I	II	III	IV	V	VI	VII	VIII
One-year old carp (K_1)	20–22°C	TSH-treated $n=9$	$\bar{x}$	59.2	31.9	29.7	27.4	27.0	—	—	—
			± S. D.	12.7	5.8	10.7	6.95	7.19	—	—	—
		Control $n=9$	$\bar{x}$	57.7	22.5	14.8	12.0	9.4	8.3	7.8	8.0
			± S. D.	26.46	6.53	4.43	3.98	3.03	2.13	1.9	2.4
	10–12°C	TSH-treated $n=2$	$\bar{x}$	40.1	27.2	22.1	17.2	14.0	12.8	10.8	11.0
			± S. D.	9.1	6.6	1.0	5.85	1.0	3.58	2.0	1.5
		Control $n=4$	$\bar{x}$	34.6	17.1	8.9	6.8	4.8	4.7	5.7	4.8
			± S. D.	12.5	8.0	1.96	1.61	0.86	0.76	1.1	0.43
Two-year old carp (K_2)	20–22°C	TSH-treated $n=6$	$\bar{x}$	26.0	18.5	21.4	19.8	18.8	13.4	12.2	—
			± S. D.	4.22	6.3	5.57	5.93	3.92	2.32	3.24	—
		Control $n=6$	$\bar{x}$	25.2	17.4	14.0	11.2	10.6	11.1	9.4	—
			± S. D.	6.76	4.02	2.07	2.95	4.85	4.45	4.68	—
	10–12°C	TSH-treated $n=2$	$\bar{x}$	38.5	20.3	22.0	18.1	18.5	21.7	16.5	—
			± S. D.	8.7	1.7	8.9	0.6	0.12	3.47	3.83	—
		Control $n=4$	$\bar{x}$	32.6	16.6	12.5	10.5	8.1	3.7	3.6	—
			± S. D.	9.39	5.07	1.38	2.22	2.15	0.85	0.23	—

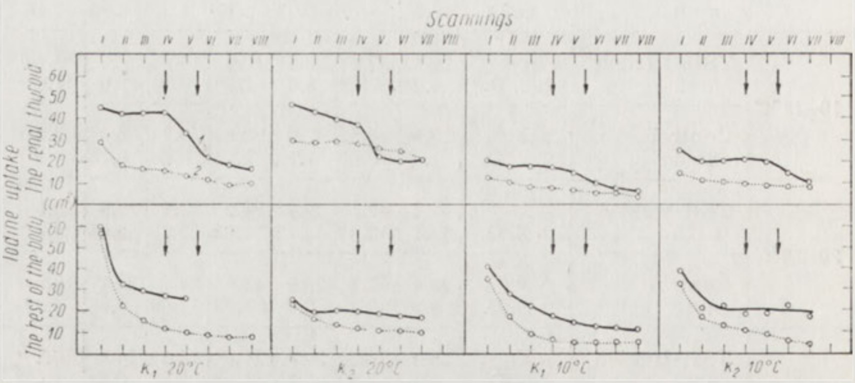

Fig. 4. Average changes in iodine uptake (cm^2) in renal thyroid region and in the rest of the body in TSH-treated (1) and control (2) carps, in two temperatures, during the experimental period. Arrows indicate TSH injection

4. DISCUSSION

Considering the relationship, mentioned in the introduction, between all the life processes in poikilotherm animals and the temperature of their environment, the ranges of temperatures have been selected so as to reflect different levels of metabolism intensity. The lower range (10 to 12°C) approaches the minimum thermal limit of normal existence for carps, while the upper range (20 to 22°C) represents their optimal conditions of feeding.

According to the recent views in endocrynology (Gorbman and Bern 1964, Pitt-Rivers and Tata 1959), the scheme of activity of the thyroid gland in all vertebrates proceeds, to simplify the matter, in the following stages: an accumulation of iodides from the bloodstream, a cycle of intramolecular transformation leading to a formation of thyroglobulins, a release of iodinated aminoacids (tyroxine and triiodothyronine with their derivatives) through the bloodstream to the organism where they work as thyroid hormones.

A question arises, at which point of this mechanism the thyrotrophic hormon comes into action. It has not been fully ascertained as yet. Berg et al. (1959), Chavin (1956 a, b, c), Fontaine and Fontaine (1962), and the unpublished investigations of the present author, point to a remarkable increase of thyroid uptake in fish (Cyprinus, Anguilla, Gadus) under the influence of hypophysis extracts and of purified TSH preparations. In the French works, the thyrotrophic activity of the hypophysis is defined by the regression coefficient between the thyroid uptake of radioiodine, measured after 48 hr since an injection, and the logarythm of concentration of an extract of the studied hypophysis. This coefficient always attains positive values, which indicates that an increase of the trophic hormon concentration leads to increases of the amount of iodine taken up by the thyroid cells.

However, somewhat earlier works by Rosenberg et al. (1960) point that TSH not only stimulates hormonal secretion of the thyroid tissue, but in some conditions it is apt to cause a sudden release of inorganic iodine from the thyroid gland.

The results of the present work seem to confirm both of these statements. They reveal such a situation in the studied carps that the same hormonal compound, administered in identical amounts to the same individuals, causes quite different reactions of their organisms. The first and second thyrotrophin administration significantly increased the uptake of the renal thyroid region in TSH-treated carps. An increase of the uptake, though lesser, was also observed in the other part of the body. However, the second and third administration of TSH on the eighth and eleventh day of the experimental period caused a very intensive secretion of iodine accumulated in the renal thyroid center in both age groups of fish. The intensity of this process was remarkably higher at the higher temperature 20–22°C than at the lower one (10–12°C).

Thus, the influence of thyrotrophin on carps in the present experiment can be presented in the following manner: the first two injections of this compound, preceeding the administration of radioiodine, had conditioned the thyroid tissue into an ability to accumulate this element at both of the investigated temperatures. The two further TSH injections performed on the eighth and eleventh day after the administration of radioiodine brought about its sudden secretion to the bloodstream.

The only explanation of the fact that one compound influences the same organisms in two extremely opposite ways can only be accounted for by various levels of the trophic hormon in the organisms at the beginning and during the experiment. The first and second injection is apt to condition the thyroid tissue into an accumulative readiness, while the third and fourth administration brings about favourable conditions for a sudden release of the accumulated element. Little can be said at the present stage about the form in which iodine has been secreted after the third and fourth TSH injections, as blood was sampled from fish for column chromatography at the end of the experiment only, i.e. on the 16th day. The results of chromatographic analysis are discussed in more detail in Łysak (1971).

According to the classical notions now prevailing in endocrynology, iodine accumulated in the thyroid gland can leave it only in form of thyroid hormons, i.e. thyroxine, triiodotyronine and their derivates. Recently, however, papers have appeared suggesting the possibility of secretion of iodine to the bloodstream by the thyroid tissue in the form of mono- and diiodotyronines, i.e. intermediate products of hormonal synthesis (Reilly et al. 1961, Block and Mandl 1961, Vigouroux and Jost 1965). The problem is worth to be carefully tested, since if it turned out that the accumulated iodine could leave the thyroid gland only in the form of a hormon, then by measuring the thyroid uptake at definite intervals we could judge about the intensity of the gland during a given period by the rate of disappearance of the accumulated iodine.

5. SUMMARY

In carps, besides the two familiar centers of iodine accumulation (the pharyngeal and the thoracic kidney regions) there is a third one situated in the head kidney.

The intensity of the uptake of the renal thyroid center in both age groups of carps is higher: a. in the TSH-treated group in comparison with the controls, b. at

the higher temperature (20–22°C) than at the lower one (10–12°C). In the other parts of the body (including the pharyngeal region and the head kidney) the observed differences in uptake were significantly less marked.

The renal thyroid center (the thoracic kidney) in carp reacts differently to the administration of the thyrotrophic hormon, depending on the timing. A dosis preceding an injection of radioiodine causes an increase of the uptake, while doses given on the 8th and 11th day after a radioiodine injection brings about an opposite effect, i.e. a very intensive release of iodine from the thyroid gland. The process is much more intensive at 20–22°C than at 10–12°C.

6. STRESZCZENIE

U karpi, oprócz dwóch dotychczas znanych ośrodków (gardzielowego i w nerce tułowiowej), istnieje jeszcze trzeci ośrodek akumulacji jodowej usytuowany w nerce głowowej.

Intensywność wychwytu nerkowego ośrodka tarczycowego u karpi jedno i dwuletnich jest wyższa: a) w grupie traktowanej uprzednio tyrotropiną, w porównaniu z kontrolą, b) w wyższej temperaturze (20–22°C) w porównaniu z niższą (10–12°C). Różnice w wychwycie pozostałych części ciała (wliczając w to ośrodek gardzielowy i nerki głowowej) obserwowano w znacznie mniejszym stopniu.

Nerkowe ośrodki tarczycowe (nerka tułowiowa) u karpia reagują różnorodnie w czasie na podanie hormonu tyrotropowego. Dawka poprzedzająca injekcję radiojodu powoduje zwiększenie wychwytu, a dawka podana w 8–11 dni po injekcji tego pierwiastka daje efekt odwrotny, mianowicie bardzo intensywne uwalnianie jodu z tarczycy. Proces ten zachodzi znacznie intensywniej w temperaturze 20–22° niż w 10–12°C.

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A. ŁYSAK

THE INFLUENCE OF ENVIRONMENT TEMPERATURE AND OF THYROTROPHIN ON IODINE METABOLISM IN CARP (*CYPRINUS CARPIO* L.). PART II. RESULTS OF CHROMATOGRAPHIC ANALYSIS

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ABSTRACT

Column chromatography (Sephadex G-25) of blood plasma of carps treated with thyrotrophin in two temperatures (10–12 and 20–22°C) showed that sixteen days after radioiodine ($Na^{131}I$) injection the major part of this element circulates in the bloodstream in a form of iodide. The level of hormonal fraction depends on thyrotrophin treatment and on temperature range.

CONTENTS

1. INTRODUCTION

Detection, separation and quantitative determination of thyroid hormons is primarily a concern of human medicine. The present communicate is an attempt at an application of methods recently widely used in the field to determine the level of radioiodine labelled thyroid hormons and their derivates in the bloodstream of carps subject to the influence of various temperatures of an environment and to the action of thyrotrophin. The changes taking place in their bodies, observed in vivo, were discussed in Łysak (1971). A double approach to the problem of iodine compounds metabolism in carp ought to bring a broader and more dynamic picture.

2. MATERIAL AND METHODS

The most useful technique for quantitative determination of the presence of the sought iodine compounds is offered by thin-layer chromatography, making use of silica gel, powdered cellulose, or diethyl-amino-methyl-cellulose (DEAE). After reviewing numerous systems offered in literature for the separation of iodine compounds, the method of Faircloth et al. (1965) was used, consisting in two-dimensional chromatography of the investigated mixtures on cellulose plates. As solvent I (Fig. 1) formic acid and bi-distilled water were used (1 : 5) after Björkstén et al. (1961), and as solvent II tertiary butanol, 2N ammonia and chloroform were applied in a mixture of the following proportions: 376 : 70 : 60 after Hollingsworth et al. (1963).

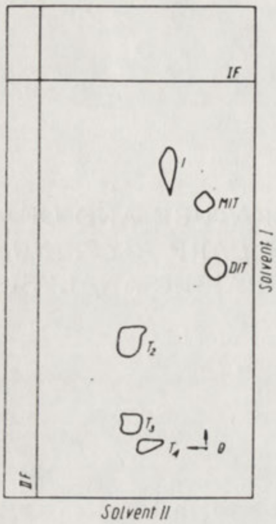

Fig. 1. An example of two-dimensional thin-layer chromatography of a mixture of iodine compounds. 0 — starting point, I F, II F — solvents' fronts, I — iodine, T_2 — diiodothyronine, T_3 — triiodothyronine, MIT — monoiodotyrosine, DIT-diiodotyrosine, T_4 — thyroxine. Solvent I, II — explanations in the text

After drying, the chromatograms were developed by the method of reduction of ceric sulphate by arsenic acid, with the use of iodine compounds as catalyzers, after Gmelin and Virtanen (1959). Intensively blue hatches of the separated substances against a light-green background were obtained. An example of such a division is presented in Fig. 1. Thin-layer divisions allowed only to find, what compounds were present in the investigated solutions. For quantitative determinations, the technique of column chromatography was used.

The procedure ran as follows. The whole experiment consisted of three basic periods: acclimation, when the fish were kept in the constant laboratory conditions during 10 to 14 days; preparatory (6 days), when the experimental groups were treated with the two initial injections of thyrotrophin, and the controls were injected a diluent only, the experiment proper (16 days) when all the groups received parenterally a similar dosis of radioiodine ^{131}I, and on the 8th and 11th day, doses of thyrotrophin were administered to them; these were the third and fourth doses for the experimental group, and the first and second injections of this compound for the controls. The whole scheme just described was performed simultaneously on carps in two age groups: one-year old and two-year old (called here K_1 and K_2 respectively). A more detailed description of the design of the experiment is offered in Łysak (1971). On the 16th day of the experimental period, blood samples were taken from all the fish by a method described earlier (Łysak 1959). Plasma from the several blood samples was pooled together for each group of fish for further common chromatographic analysis; thus, 8 samples of plasma were obtained, representing all the experimental factors (2 temperatures × 2 age classes × 2 treatments).

Delipidation of plasma was performed by the method described by Vigouroux and Jost (1965), and enzymatic digestion with pancreatine (Viokase) was carried out in the manner adviced for similar purposes by Tong and Chaikoff (1958). Column chromatography was carried out by a method suggested in Jacobson and Widström (1962).

The column (15×2.5 cm) was loaded with Sephadex G 25 (5 g per 50 ml), already swelled up in distilled water. A 3.0–5.0 ml sample of radioactive plasma, formerly delipidated and enzyme-hydrolyzed, was put at the top of the column by means of a Pasteur pipette. For washing, two solvents were used: 1. acetate ammonium buffer with pH 7.0 during about 2 hr (it washed out the first three fractions of iodine compounds), and 2. tertiary amyl alcohol, saturated with ammonia, during about 30 min (it washed out the last hormonal fraction). All the four fractions supplied by the column, 30 to 70 ml of capacity, were collected into glass bottles with ground necks for immediate distillation in vacuum. The dry residue was subsequently washed out from the bottles with the following washes:

wash I — 0.5 ml N-NH_4OH
wash II — 0.5 ml 4N-NH_4OH
wash III — 0.5 ml 4N-NH_4OH
wash IV — 0.5 ml etanol : 4N-NH_4OH (50 : 50)

Total 2.0 ml

Radioactivity of the fraction thus obtained, with constant capacity of 2.0 ml was measured with a Tracerlab sintillation well counter.

3. RESULTS AND DISCUSSION

The recovery of radioiodine supplied to the column in plasma samples was very high, as it reached 95.6–98.9%. The results for each of the eight pooled samples of plasma representing the respective experimental classes are presented in Table I; it shows the per cent distribution of radioactivity in the several fractions of each sample.

Table I. Disposition of carp plasma radioactivity placed on G-25 Sephadex column

Age class	Temp.	Treatment	Fractions from column (%)			
			I	II	III	IV
K_1	10–12°C	Thyrotrophin	8.2	88.8	1.3	1.7
		Control	4.6	93.6	1.7	0.1
	20–22°C	Thyrotrophin	3.1	90.4	1.0	5.5
		Control	2.6	95.4	0.7	1.3
K_2	10–12°C	Thyrotrophin	3.7	93.2	1.0	2.1
		Control	3.1	95.3	0.8	0.8
	20–22°C	Thyrotrophin	4.5	88.4	1.9	4.7
		Control	4.2	92.9	1.2	1.7

The first fraction, labelled tentatively as PBI (protein bound iodine), accounting for 2.6 to 8.2% of the initial radioactivity, represents radioiodine bound with the plasma protein. Its high values are evidence of incompletion of the process of proteolysis. According to Tong et al. (1963), the degree of enzymatic proteolysis of iodo-protein compounds depends in a great measure on the quality of the applied enzymatic compound on the proper proportion of the added catalyzers, and on the conditions in which the process of enzymatic hydrolysis occurs. The bulk of radioiodine was retained in the second fraction, which consisted of free iodides. It comprised 88.4 to 95.4% of the total amount of radioiodine. A high level of these compounds was also found by French investigators (Fontaine and Fontaine 1962) in plasma of salmon (*Salmo salar*), eel (*Anguilla anguilla*) and goldfish (*Carrasius auratus*). The third fraction, containing transfer products of enzymatic hydrolysis of iodo-protein compounds reached very low values in all the samples (0.7–1.9%), while the fourth fraction, washed out with amyl alcohol, which was the most important one as it contained thyroxine (T_4) and triiodothyronine (T_3) was the most diversified as to its quantity (0.1–5.5%). In general, it might be said that the most marked differences in fraction four appeared between the fishes of the thyrotrophin group and the control, in favour of the former. Similarly, much higher values of the hormonal fraction were observed in the samples from the fishes kept in 20–22°C as compared with those kept in 10–12°C. These differences are similar for both the one-year old and two-year old carps.

An insufficient number of the investigated individuals, particularly those of the thyrotrophin groups kept in 10–12°C (in K_1 and K_2 groups), and the single chromatographic division for each group, do not allow for a statistical analysis and require caution in drawing conclusions. However, the results permit for the following conclusions:

1. The bulk of radioiodine parenterally administered to one- and two--year old carps can be found in their plasma in the inorganic form (88.4–95.2%) 16 days after an injection.

2. The hormonal fraction accounts for 0.1–5.5% of radioactivity of plasma. Its values are remarkably higher in carps kept in 20–22°C than in those kept in lower temperature (10–12°C), and the action of thyrotrophin makes the difference even more apparent.

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4. SUMMARY

One and two-year old carps were treated with thyrotrophin against control fish in two temperatures (10 and 20°C). Blood samples were collected from all fish on the sixteenth day after radioiodine had been injected. Column chromatographic analysis of enzymatically digested plasma samples showed that in bloodstream of all fish parenterally administered radioiodine exists mostly in a form of free iodide. The last hormonal fraction washed out from the column with tertiary amyl alcohol amounts to only a few per cent of total radioactivity. The value of this fraction is however distinctly higher in carps kept in higher temperature (20°C), than in lower

one (10°C) and the action of thyrotrophin makes this difference even more marked.

5. STRESZCZENIE

Jedno i dwuletnie karpie poddano działaniu tyrotropiny w dwóch temperaturach (10 i 20°C), w których przetrzymywano również karpie kontrolne. Próbki krwi pobrano od wszystkich ryb szesnastego dnia po injekcji radiojodu. Chromatografia kolumnowa próbek enzymatycznie rozłożonej plazmy wykazała, że w krwioobiegu wszystkich ryb dootrzewnowo podany radiojod występuje przeważnie w formie jodku. Ostatnia frakcja hormonalna wymyta z kolumny za pomocą trzeciorzędowego alkoholu amylowego odpowiada zaledwie kilku procentom całkowitej radioaktywności. Radioaktywność tej frakcji jest jednak znacznie wyższa u karpi trzymanych w wyższej temperaturze (20°C) niż w niższej (10°C), a działanie tyrotropiny pogłębia jeszcze tę różnicę.

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etc., 6. SUMMARY — presenting the main results and conclusions in no more than 200 words. The summary will be printed in the author's native language and in the language of the main text.

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