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SEASONAL CHANGES IN NUMBERS OF SOME PHYSIOLOGICAL GROUPS OF MICROORGANISMS IN IŁAWA LAKES

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ABSTRACT

Bacterioplankton, heterotrophic bacteria and yeasts are more abundant in water depths of the polluted Lake Jeziorak Mały than in that of the eutrophic Lake Jeziorak. In the muddy and sapropelic bottom sediments of the investigated lakes the microorganisms are more abundant than in sandy soils. They are also abundant in the net plankton of Iława lakes. The maximum amounts of bacterioplankton, heterotrophic bacteria and yeasts in water depths and bottom sediments of Iława lakes were usually found in spring or at the beginning of summer and in autumn, the minimum amounts — in summer and winter. Extensive deviations from the general seasonal course were more frequently observed in Jeziorak Mały Lake. In the net plankton of the Iława lakes, seasonal variations in numbers of the investigated microorganisms were also observed.

1. INTRODUCTION

The number of microorganisms and their distribution in a lake depend on several factors: natural, climatic and meteorological conditions, type of bottom, season, water characteristics, quality and depth, and others. These subjects have been investigated several times. Quantitative dynamics of bacterioplankton and of heterotrophic bacteria in water were investigated most often; much less attention has been devoted to bottom sediments, water plants and plant and animal plankton.

Seasonal variations of numbers of bacterioplankton were observed in detail in the following lakes: White and Black lakes at Kosino (Kuznetsov 1934), Glubokoye Lake near Moscow (Kuznetsov 1937, Sokolova 1961, Shcherbakov 1967), a montane Lake Sevan (Gambaryan 1957), Naroch and Batorin lakes in Byelorussia (Belyatskaya-Potayenko 1964, Potayenko 1968), Constance Lake (Deufel 1967), Balaton Lake (Oláh 1969 a, b) and others. Yearly quantitative dynamics of heterotrophic bacteria were investigated in the following lakes: Alpine lakes (Minder 1918, Duggeli 1924, Deufel 1967, Goldman et al. 1968), eutrophic lakes at Kosino and mesotrophic Glukoboye Lake near Moscow (Kuznetsov 1934, 1958), Alexander Lake in Minnesota, U.S.A. (Henrici 1938), Balaton Lake (Oláh 1969 a, b) and others. However, there are few papers on lake yeasts, and specially on seasonal variations of their number (Hedrick, Soyugenc 1967, Meyers et al. 1970). In several of the above mentioned papers, an increase of the number of microorganisms in the spring-summer period was noticed, due to the raise of water temperature and to the mass development and the dying off of phytoplankton.

In the present paper, the numbers and seasonal variations of bacterioplankton, heterotrophic bacteria and yeasts in water, bottom sediments, and net plankton of Iława lakes of different pollution degrees, are presented.

2. TERRAIN DESCRIPTION AND METHODS

Materials were taken in 1967–1969 once a month, from 5 stations of Jeziorak Mały Lake and from 10 stations of Jeziorak Lake. The total characteristics of the investigated lakes and selected stations are given in Tables I and II. Due to

Table I. Some morphometric data on the investigated Iława lakes

Characteristics	Lake	
	Jeziorak Mały	Jeziorak
Latitude	53°36′7″N	53°35′30″N
Longitude	19°33′6″E	19°31′–19°35′E
Altitude (m a.s.l.)	100	99
Surface area (ha)	26	3230
Maximum length (m)	805	27,000
Maximum width (m)	365	2700
Length of shore line (m)	2330	148,500
Maximum depth (m)	6.4	11.1
Mean depth (m)	5.0	5.0
Water volume ($m^3 \cdot 1000$)	890.3	1415,945.2

Table II. Some hydrographic data on the investigated stations and bottom deposits type of the Iława lakes

Lake	Station	Distance to the shore (m)	Depth (m)	Bottom deposits type
Jeziorak Mały	1	15	4.5	muddy
	2	20	4.5	muddy
	3 *	in the middle of the lake	6.4	muddy
	4	20	2.0	muddy with a thick sawdust layer
	5	20	5.0	muddy
Jeziorak	6 *	150	5.0	muddy
	7	15	2.0	muddy
	8 *	300	2.5–3.0	sapropel
	9 *	15	2.0	sandy
	10	15	5.0	sandy
	11 *	650	11.1	muddy
	12	15	5.0	sandy
	13	15	6.0	sandy
	14	450	6.0	muddy
	15	15	5.0	sandy

* Microbiological data presented in this paper.

difficulties in presentation of all the results of microbiological investigations, only those of the five selected most typical stations (Fig. 1) are given in the

present paper: No. 3 in Jeziorak Mały Lake (muddy sediments, water heavy polluted by dairy and municipal sewages), and four others in Jeziorak Lake: No. 6 (muddy sediments, water slightly polluted by municipal sewages), No. 8 (slightly mineralized sediments of sapropelic type), No. 9 (1 mm thick sediments on sandy substratum), and No. 11 (muddy sediments, water unpolluted by any sewages). Organic matter contents was about 42% in muddy sediments and 1-2% in sandy soils.

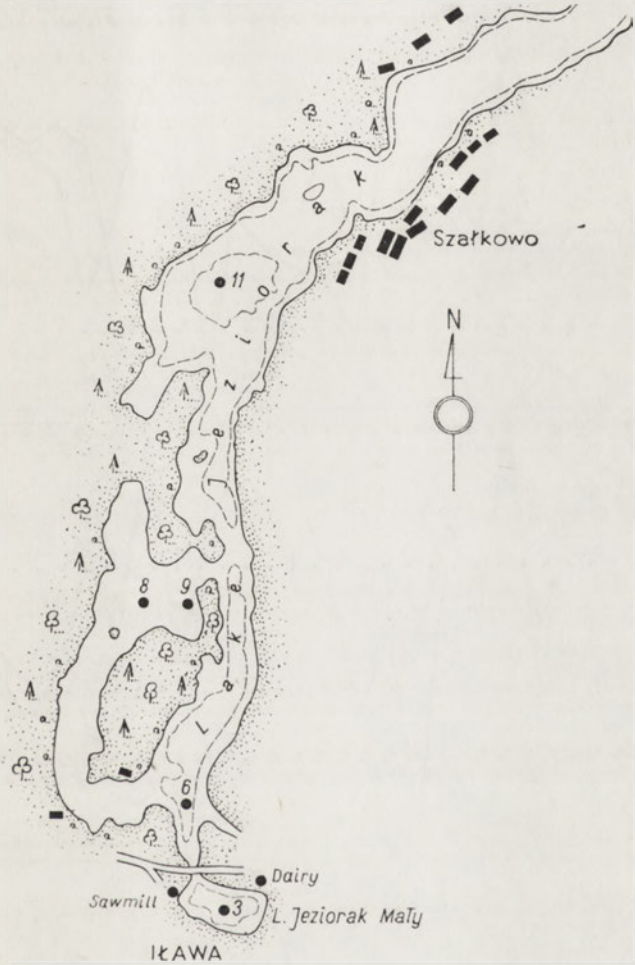


Fig. 1. Location sketch of the Iława lakes — Jeziorak Mały and Jeziorak. 3, 6, 8, 9, 11 — sampling stations

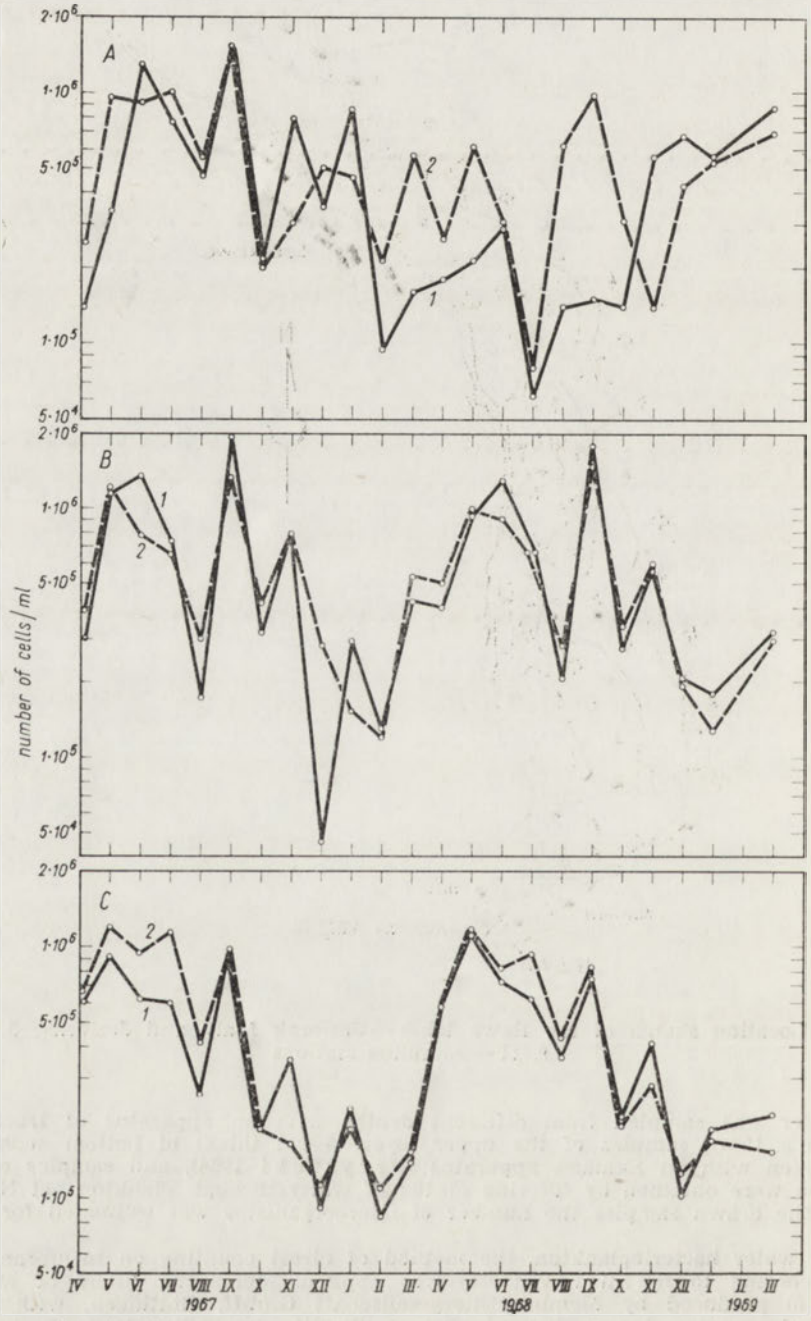
Water was sampled from different depths with an apparatus of Isachenko (Rodina 1965); samples of the upper layer (5 cm thick) of bottom sediments were taken with an Ekmann apparatus (Przyłęcki 1954), and samples of net plankton were obtained by filtering of 10 l of water through plankton net No. 25.

In the drawn samples the number of microorganisms was estimated for each group.

For water bacterioplankton, the method of direct counting on membrane filters according to Razumov (1932) was applied. Membrane filters of MF 15 (group 5) produced by Membranfiltergesellschaft GmbH, Gottingen, with pores of 0.275 μm in diameter, were used after sterilization by boiling in fresh distilled

water for 30 min. Each time 1 ml of the investigated water diluted with sterilized physiological NaCl solution in 1 : 10 proportion was filtered. Bacteria on filters were coloured on circles of filter paper placed on Petri dishes with 5% erythrozyne dissolved in 5% phenole solution, for 3 hr.

For bacterioplankton in bottom sediments the methods given by Rodina (1965) were applied. Each time 1 g of bottom sediment was placed in a sterili-



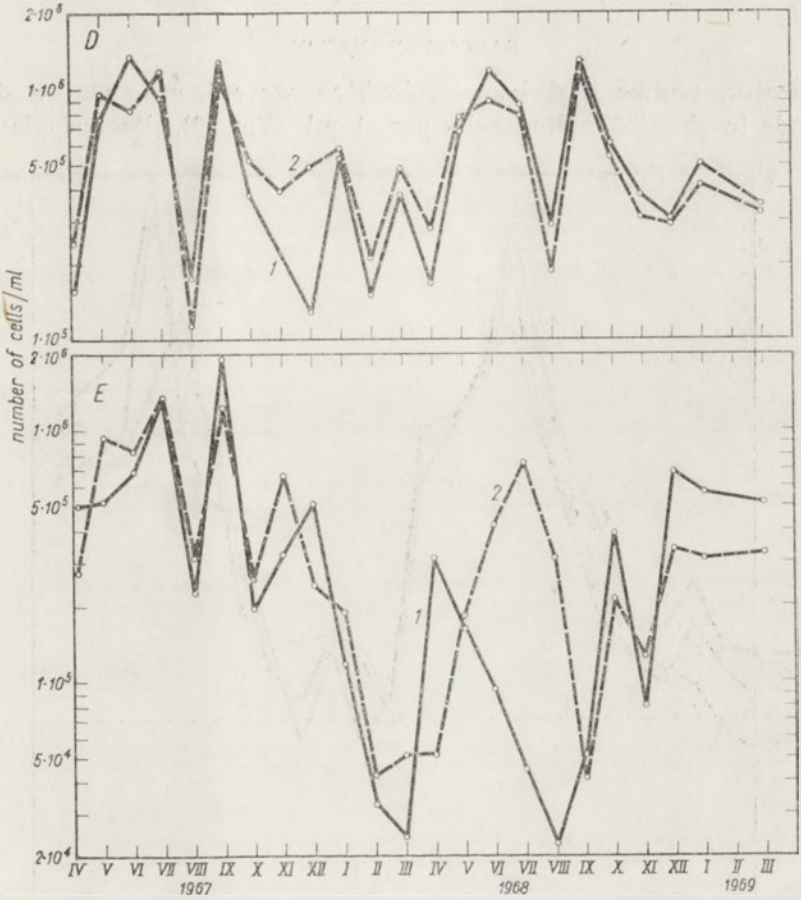


Fig. 2. Seasonal changes in number of bacterioplankton in the water of the Iława lakes. Each point is an average value of 3 parallel repetitions (semi-log scale). 1 — surface water, 2 — near bottom water. Jeziorak Mały Lake: A — station No. 3; Jeziorak Lake: B — station No. 6, C — station No. 8, D — station No. 9, E — station No. 11

zed bulb and submerged with sterilized 0.0004 n NaOH solution; then it was shaked for 20 min and put away for 1–2 min to allow the larger particles to settle down. 1 ml of suspension was filtered through a membrane filter and after colouring and drying the filters, microorganism cells were counted in a sample. The results were recalculated per 1 g dry weight of sediment.

Number of bacterioplankton in net plankton was estimated after smearing in a mortar and shaking of 1 g of fresh mass in a bulb with 100 ml of sterilized physiological NaOH solution.

Number of heterotrophic bacteria was estimated after cultivation on broth agar of pH 7.2, for 7 days at 20°C.

Number of yeasts was estimated after cultivation on malt agar for 5 days at 25°C.

Besides, bacteria fixing atmospheric nitrogen in aerobic conditions (Azotobacter) and anaerobic conditions (*Clostridium pasteurianum)* as well as oligonitrophilous bacteria were estimated; the results are given in N i e w o l a k (1972 a).

All the estimations were made on the basis of 3 parallel inoculations and the obtained results were recalculated per 1 ml of water, 1 g of dry weight of sediment or 1 g of fresh weight of phytoplankton.

3. RESULTS

BACTERIOPLANKTON

In water, numbers of bacterioplankton were from about a dozen thousands to about 2 milion cells per 1 ml (Fig. 2). Bacterioplankton

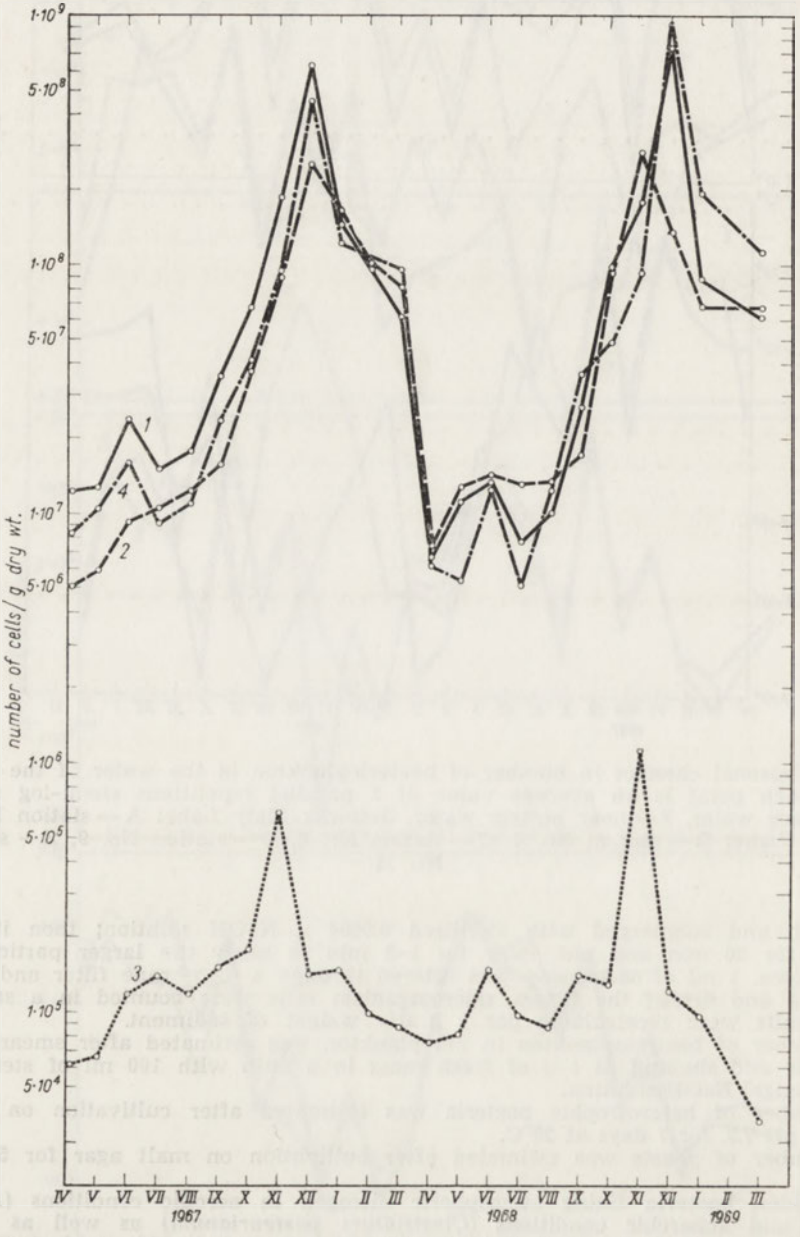


Fig. 3. Seasonal changes in number of bacterioplankton in the bottom deposits of the Iława lakes. Each point is an average value of 3 parallel repetitions (semi-log scale). 1 — station No. 3, 2 — station No. 8, 3 — station No. 9, 4 — station No. 11

was more abundant in Jeziorak Mały Lake (Fig. 2 A) and in shallow stations of Lake Jeziorak (Fig. 2 B-D) where, except in few cases, it amounted from 100,000 to 2000,000 cells per 1 ml of water. However, in the deepest station (No. 11) there was as few as several dozen thousands cells per 1 ml of water (Fig. 2 E).

In Jeziorak Mały Lake, bacterioplankton was abundant in different months, even in winter; in Jeziorak Lake it was regularly more abundant from May to July and in September, and only rarely in other months.

In both lakes, now and then there were pretty considerable differences in bacterioplankton distribution between surface and near to bottom water. In the vegetation period the microorganisms were mostly more abundant near the bottom, whereas in winter — in surface water.

In bottom sediments (the upper layer), bacterioplankton was many times more abundant in water (Fig. 3). Particularly rich with bacterioplankton were muddy soils of Jeziorak Mały Lake (station No. 3) and muddy and sapropelic sediments of Jeziorak Lake (stations No. 6, 8, 11), containing up to 916 million cells per 1 g of dry weight. The microorganisms were the least abundant (up to 1.1 million cells per 1 g of dry weight) in sandy soil (station No. 9). In all the soil types more bacterioplankton was observed in autumn and winter; moreover a slight increase of their number was recorded in June.

In net plankton, numbers of bacterioplankton were 4—75 million cells per 1 g of fresh mass (Fig. 4), i.e. from several dozen to several

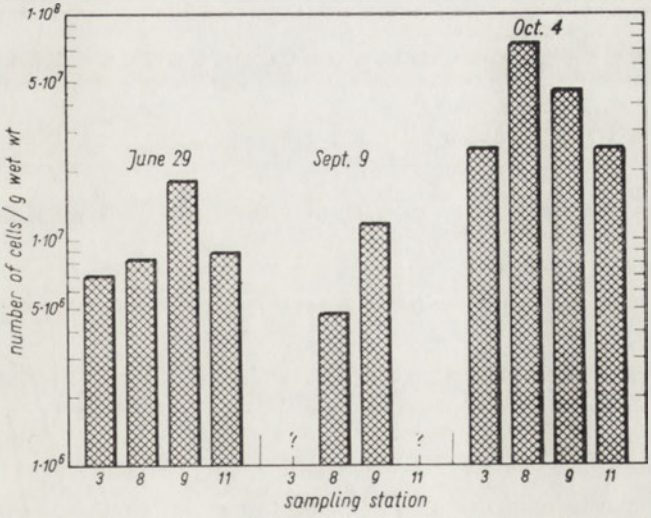
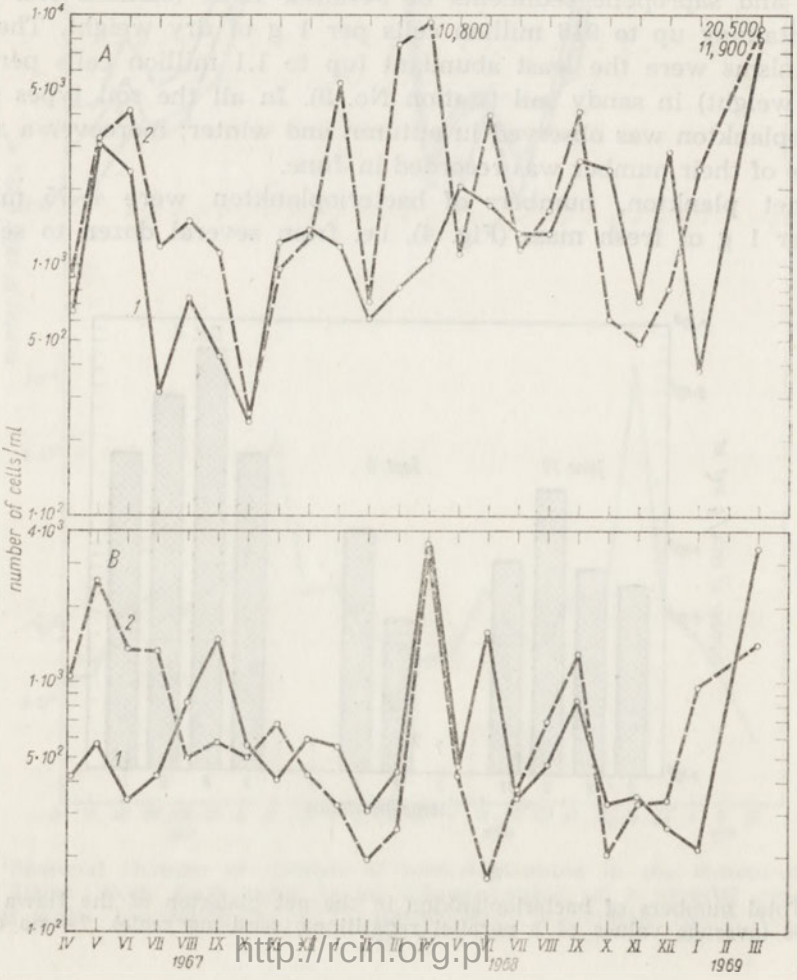


Fig. 4. Total numbers of bacterioplankton in the net plankton of the Iława lakes in 1968 (average values of 3 parallel repetitions, semi-log scale). ? — no data

hundred times more than in water. Maximum amounts were recorded in October 1968, minimum amounts in September 1968 (station No. 8).

HETEROTROPHIC BACTERIA

In water of Jeziorak Mały Lake no regularity was found, with some exceptions, in seasonal occurrence of heterotrophic bacteria counted in broth-agar. For example, in station No. 3 (Fig. 5 A) most heterotrophic bacteria were found in spring 1967, in both surface and near to bottom water, and in 1968 they were abundant in several months: in surface water in May, September, October and December (2000—2700 cells/ml), in near to bottom water mainly in January, March and April, and also in June and September (up to 10,800 cells/ml). In 1969 they were specially abundant in March (up to 20,500 cells/ml). Irregular occurrence of heterotrophic bacteria in this lake is connected with its pollutions with sewages from a nearby dairy and sawmill and with municipal sewages from the residential areas.



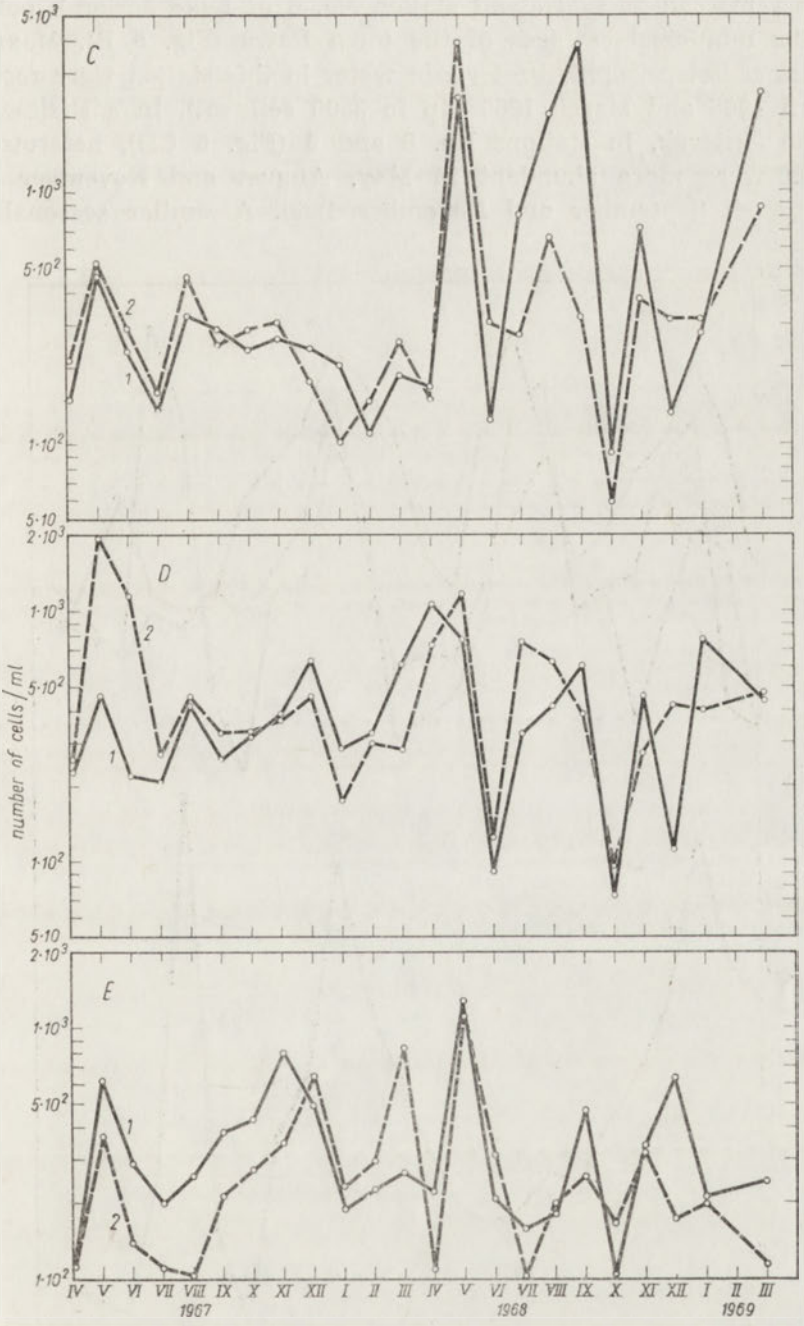


Fig. 5. Seasonal changes in number of heterotrophic bacteria in the water of the Iława lakes. Each point is an average value of 3 parallel repetitions (semi-log) scale). 1 — surface water, 2 — near bottom water. Jeziorak Mały Lake: A — station No. 3; Jeziorak Lake: B — station No. 6, C — station No. 8, D — station No. 9, E — station No. 11

The same can be said about station No. 6 of Lake Jeziorak polluted with the municipal sewages of the town Iława (Fig. 5 B). Maximum numbers of heterotrophic bacteria in water in this station were recorded in April 1968 and March 1969 (up to 3500 cells/ml). In a shallow bay of Lake Jeziorak, in stations No. 8 and 9 (Fig. 5 CD), heterotrophic bacteria were more abundant in May, August and November 1967, and August, September and November 1968. A similar seasonal pat-

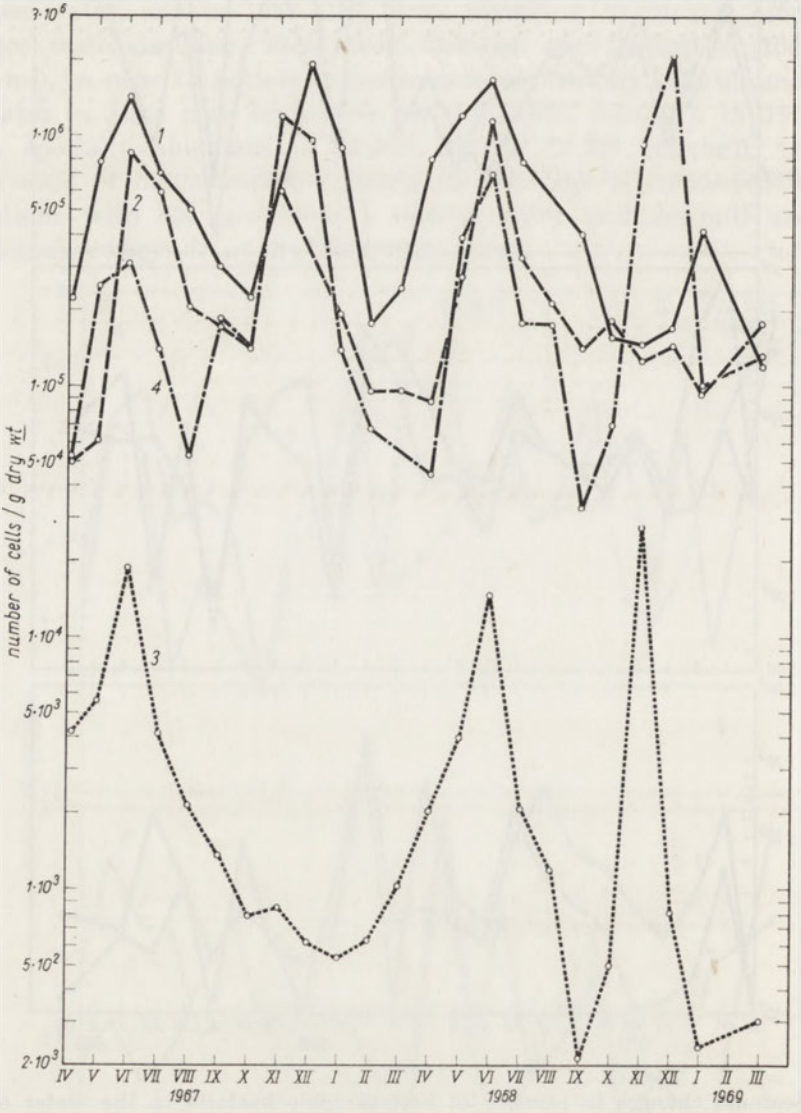


Fig. 6. Seasonal changes in numbers of the heterotrophic bacteria in the bottom deposits of the Iława lakes. Each point is an average value of 3 parallel repetitions (semi-log scale). 1 — station No. 3, 2 — station No. 8, 3 — station No. 9, 4 — station No. 11

tern of occurrence of heterotrophic bacteria was observed in the deepest station No. 11 (Fig. 5 E).

In bottom sediments of the lake, distribution of heterotrophic bacteria was as irregular as in water and it depended on bottom type (Fig. 6). In stations No. 3 and 11, in muddy soils of both lakes and

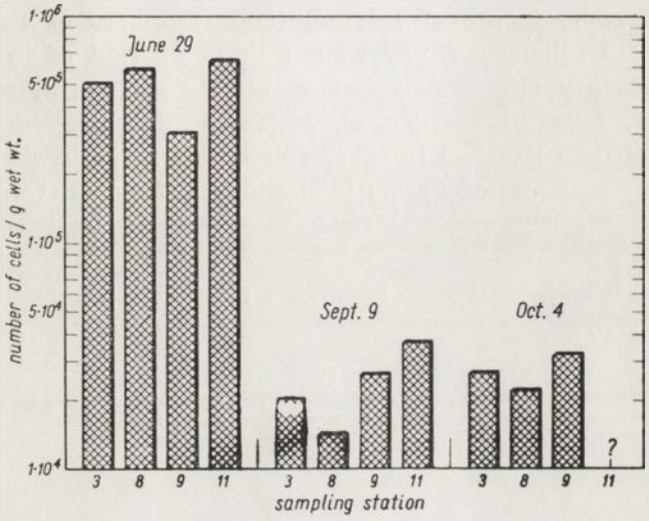


Fig. 7. Total numbers of heterotrophic bacteria in the net plankton of the Iława lakes in 1968 (average values of 3 parallel repetitions, semi-log scale). ? — no data

in station No. 8, in sapropelic sediments of Lake Jeziorak, more heterotrophic bacteria (up to 2 million cells per g dry weight) were found than in station No. 9 in sandy soils (up to 28,300 cells/g dry wt.). Maximum amounts of the heterotrophic bacteria in bottom sediments of the investigated lakes were found in June and November. In winter they were relatively less abundant and only in station No. 3 (Jeziorak Mały Lake) there was more than 4000,000 cells/g dry wt. in 1969.

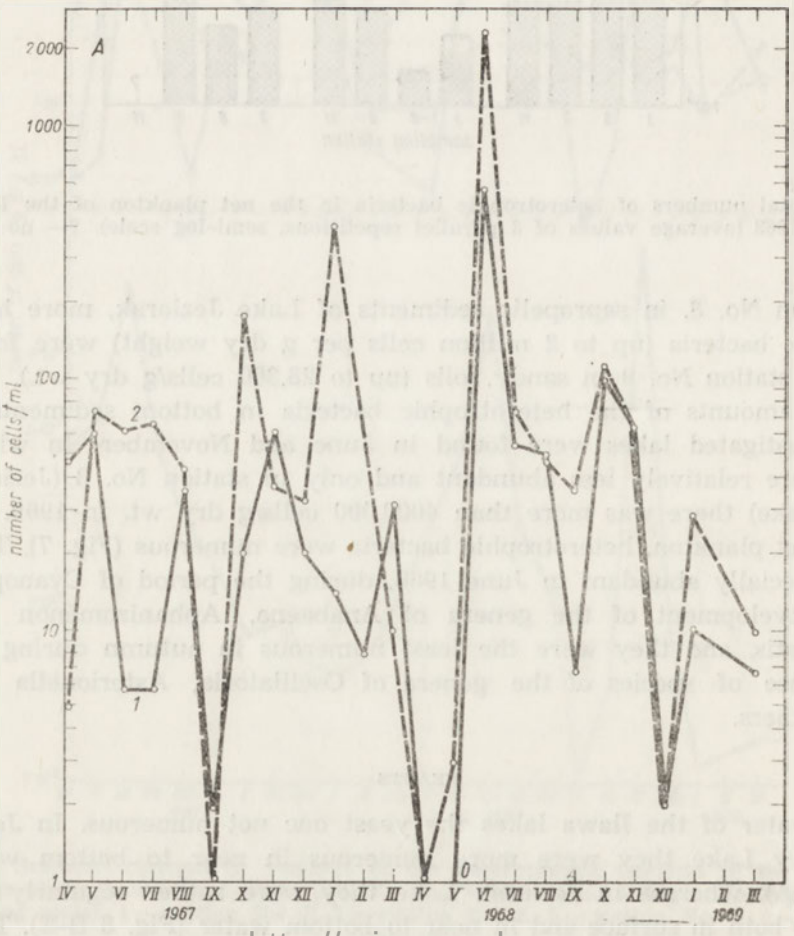
In net plankton, heterotrophic bacteria were numerous (Fig. 7). They were specially abundant in June 1968, during the period of Cyanophyceae development of the genera of Anabaena, Aphanizomenon and Microcystis, and they were the least numerous in autumn during the abundance of species of the genera of Oscillatoria, Asterionella and some others.

YEASTS

In water of the Iława lakes the yeast one not numerous. In Jeziorak Mały Lake they were more numerous in near to bottom water (Fig. 8 A), whereas in Jeziorak Lake they were rather regularly distributed both in surface and in near to bottom water (Fig. 8 B-E). They

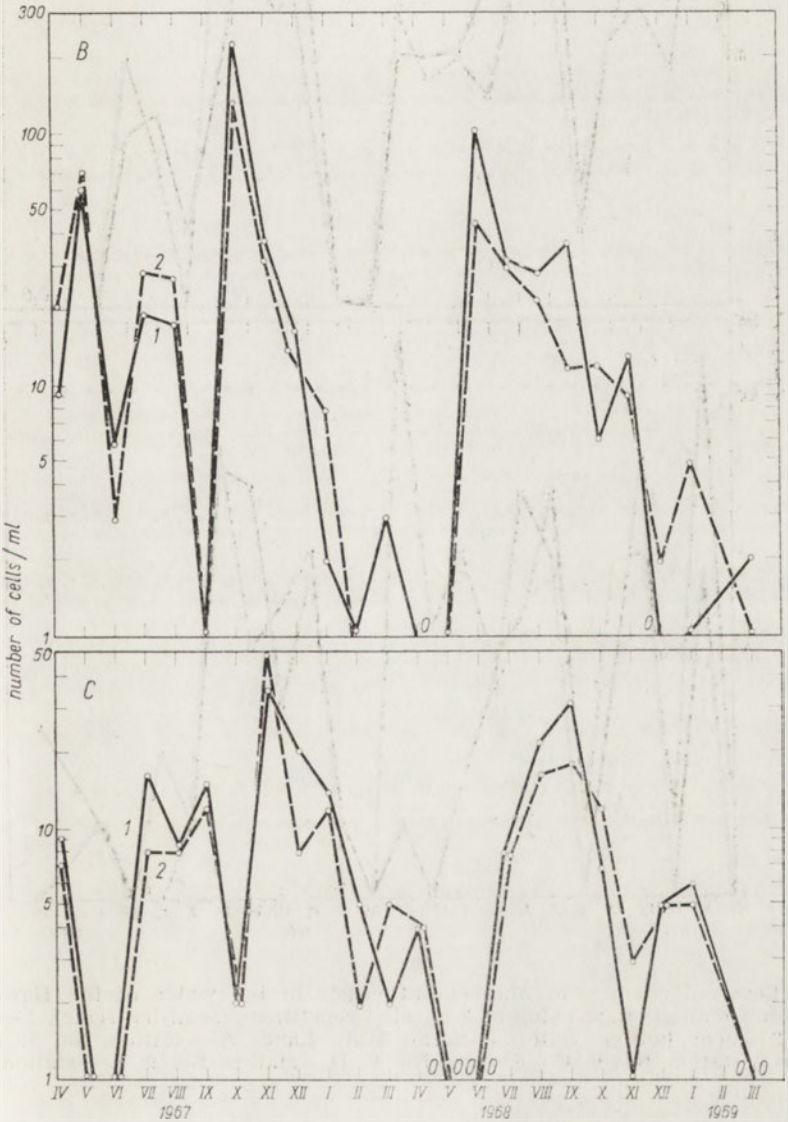
were very numerous in spring (May or June) and in autumn (October or November), and exceptionally in other months. In winter and early spring they were not numerous; several times they were not observed at all at 1 ml inoculations.

In bottom sediments the yeasts were relatively more numerous, particularly during the vegetation period (Fig. 9). Depending on a sediment type or a degree of its pollutions, numbers of yeasts varied from 0 to 6200 cells per g dry weight in the period of investigations. As a rule, more yeasts were found in muddy sediments, especially in Jeziorak Mały Lake (station No. 3), where the dairy sewages rich with carbohydrates flow in, than in other soil types. In Jeziorak Mały Lake, the maximum amounts of yeasts in bottom sediments were recorded in April and May, the minimum ones in winter. In Jeziorak Lake (sta-



tions No. 6, 8, 9, 11) the yeasts were more abundant in June and October, and the minimum amounts were found in winter.

Net plankton is also a convenient environment for yeasts (Fig. 10). They were found in amounts of 150—15,000 cells per g fresh weight. In Jeziorak Mały Lake they were more abundant in June, and in Jeziorak Lake — in September 1968.



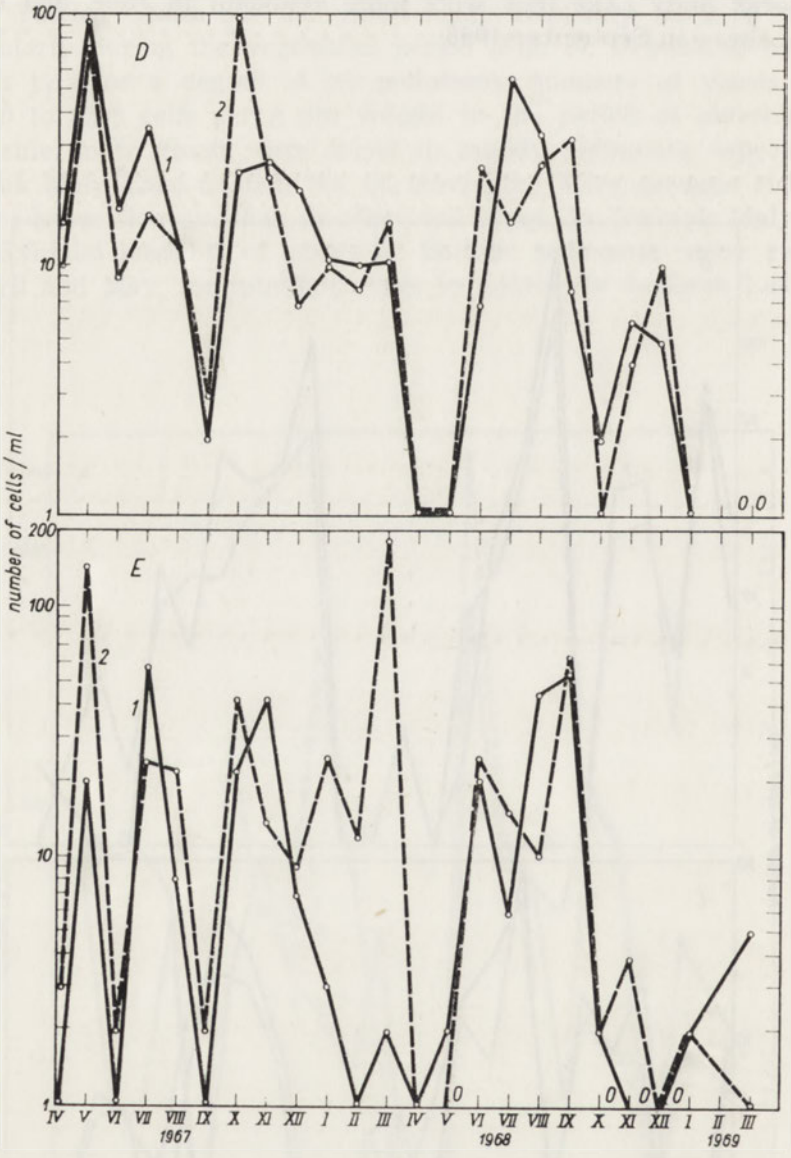


Fig. 8. Seasonal changes in numbers of yeasts in the water of the Iława lakes. Each point is an average value of 3 parallel repetitions (semi-log scale). 1 — surface water, 2 — near bottom water. Jeziorak Mały Lake: A — station No. 3; Jeziorak Lake: B — station No. 6, C — station No. 8, D — station No. 9, E — station No. 11

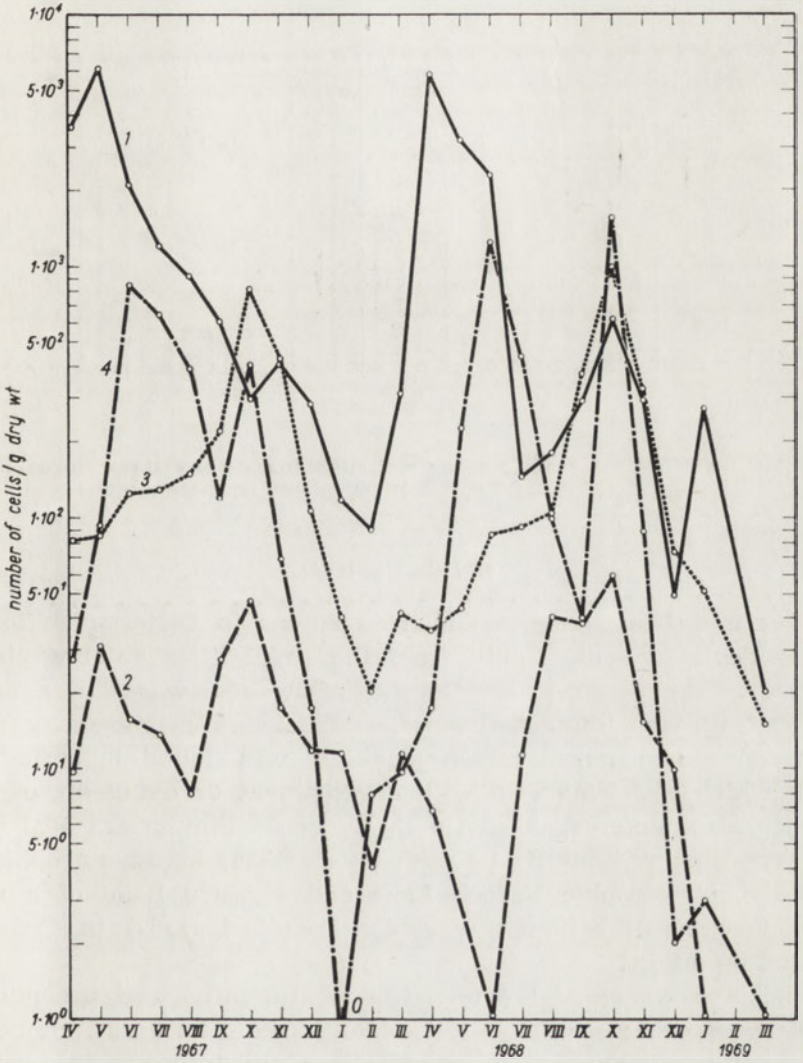


Fig. 9. Seasonal changes in numbers of the yeasts in the bottom deposits of the Iława lakes. Each point is an average value of 3 parallel repetitions (semi-log scale). 1 — station No. 3, 2 — station No. 8, 3 — station No. 9, 4 — station No. 11

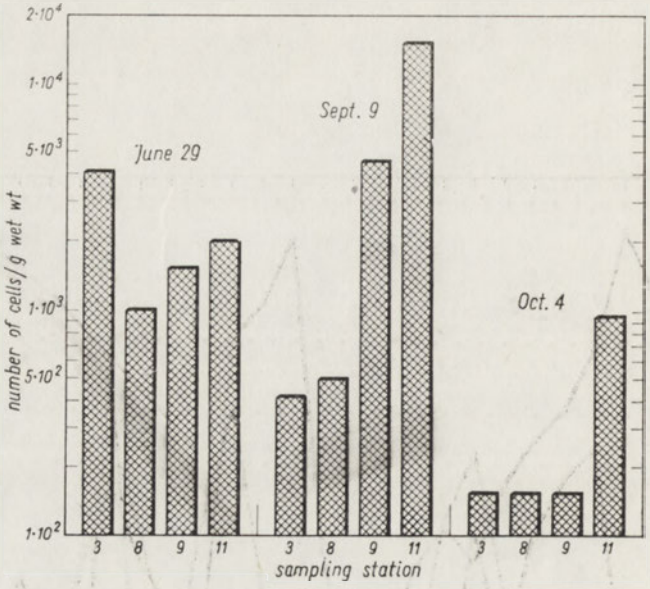


Fig. 10. The numbers of yeasts in the net plankton of the Ilawa lakes in 1968 average values of 3 parallel repetitions, semi-log scale)

4. DISCUSSION

In Jeziorak Mały Lake, seasonal variations in bacterioplankton occurrence deviated considerably from the regularities observed in the stations on Lake Jeziorak, due to the inflow of sewages of a nearby dairy, sawmill and municipal sewages from the neighbouring areas In Jeziorak Lake more bacterioplankton was found in spring and autumn which was connected with development or dying off of algae and other planktonic organisms in that periods. Similar seasonal variations were observed by Belyatskaya (1958) in an eutrophic Batorin Lake, mesotrophic Naroch Lake and Myastro Lake of a mesotrophic-eutrophic transitional type, by Deufel (1967) in Constance Lake, and by others.

Municipal sewages of the town Ilawa also influenced the numbers of heterotrophic bacteria in Jeziorak Mały Lake. The mean numbers of these bacteria in the period of investigations, amounting to 2200 cells/ml of surface water and 2930 cells/ml of near to bottom water, are typical for heavy polluted urban lakes of the Mazurian Lakeland (Niewolak 1972 b). However, these numbers are many times lower than those recorded in 1960—1963: on average, 45,000 cells/ml of surface water and 115,000 cells/ml of near to bottom water (Niewolak 1966). It was an initiation of a sewage treatment plants of dairy sewages (operating with a method of an activated sludge) in the middle of 1963 and bringing it to its full efficiency in the next years which influen-

ced the numbers of heterotrophic bacteria towards their decrease in the last period. Untill 1963 the unprocessed dairy sewages had been inflowing the lake directly.

Yeast numbers recorded in Jeziorak Mały Lake correspond to the values given for polluted waters (Ahearn et al. 1968, Simard, Blackwood 1971 a, b). Sewage nature of this lake has a bearing on irregularity of seasonal occurrence of all the three mentioned microorganism groups.

In water of Jeziorak Lake, particularly in stations No. 8, 9 and 11 situated far from Iława town, numbers of heterotrophic bacteria were lower, rarely exceeding 1000 cells/ml. Similar numbers of heterotrophic bacteria (up to 870 cells/ml) were recorded by Kuznetsov (1952) in a relatively pure Lake Swyatoye (Kosino), by Gerletti, Melchiorri-Santolini (1968) in lakes Monate and Mergozzo, and by others. Also yeast numbers in Lake Jeziorak (up to 225 cells/ml) corresponded to values given in Van Uden, Ahearn (1963), Meyers et al. (1970) for unpolluted lakes.

Seasonal variations dynamics of numbers of heterotrophic bacteria in water of Lake Jeziorak depends, first of all, on occurrence of available nutrients. Maximum amounts of these microorganisms in all stations of this lake, found in spring (May—June), were connected with increased intake of organic matter from neighbouring fields, as well as with increased production in the lake at higher temperature, while in autumn (September—November) their amount was related to the dying off of planktonic organisms being a substratum for these bacteria. Similar regularity of heterotrophic bacteria development in this lake was recorded also in 1960—1963 (Niewolak 1966). In other lakes similar regularities were found by Henrici (1938), Pokorny (1971) and others. Lasser densities of heterotrophic bacteria in lake waters during summer and winter stagnation periods are commonly known. For example, Pokorny (1971) observed such a phenomenon in Titisee Lake. A decrease of the number of heterotrophic bacteria in summer can be caused by more intensive development of zooplankton or other animal organisms feeding on bacteria, and/or by negative influence of toxic excretions of algae on bacteria. Such phenomena are often mentioned in the literature (Kuznetsov 1952, Paluch, Dobrzański 1956).

Bakterioplankton as well as heterotrophic bacteria and yeasts were more abundant in muddy and sapropelic bottom sediments than in sandy soils of the investigated lakes. This was apparently due to the higher contents of organic matter in muddy and sapropelic bottom sediments. As to the numbers of heterotrophic bacteria, both muddy and hardly mineralized sapropelic sediments of the Iława lakes are similar to those of some eutrophic lakes in the Soviet Union (Ekzertsev 1948, Zavarzina 1955). However, the total number of bacterioplankton was

never so high as the value given by Hartulari (1939) for bacterioplankton of the surface sapropelic layer of some lakes in the Soviet Union. The differences could be caused by different sampling methods.

Maximum numbers of heterotrophic bacteria in summer and autumn are connected with great amounts of organic matter stored at the bottom in these periods, resulting from mass dying off of phytoplankton after its spring blooming as well as from dying off of phyto- and zooplankton during autumn chills. The winter decrease of the numbers of these bacteria in bottom sediments of the Iława lakes is connected with the decrease of temperature of the upper layer of the bottom and/or with depletion of reserves of assimilable organic matter. A similar sequence of seasonal variations of development of this microorganism group was also described by Kuznetsov (1952) for bottom sediments of some lakes in the Soviet Union.

High amounts of bacterioplankton, heterotrophic bacteria and yeasts found in summer and autumn 1968 in net plankton of the Iława lakes were connected with diluted organic substances excreted by phytoplankton, composing a substratum for these microorganisms. On this account, several authors (Chan, McManus 1969, Coler, Gunner 1969, Strzelczyk, Mielczarek 1971) justly pointed to the analogy between root microflora of land plants and microorganisms living in water plants zone.

A relatively small number of the investigated samples of net plankton did not allow to determine the reasons of seasonal regularity of microorganisms in the studied biocenosis. Maybe they are related to the development of some algae groups and/or excretion of some nutrient or toxic products of algae metabolism.

Acknowledgements

The author wish to thank Professor H. Karnicka for her valuable suggestions concerning the present paper. Many thanks are due to Dr. L. Bittel for his help in sampling the material for investigations.

5. SUMMARY

The distribution of bacterioplankton, heterotrophic bacteria and yeasts in water of the Iława lakes depends mainly on the type of a lake, and that in bottom sediments — on the bottom type. All the investigated microorganism groups were more abundant in water of Jeziorak Mały Lake, polluted with sewages, than in the eutrophic Jeziorak Lake. In muddy and sapropelic bottom sediments of the investigated lakes all the groups were several times more abundant than in sandy soils. High amounts of all the microorganisms groups were also found in the net plankton of the Iława lakes. Usually the maximum amounts of the microorganisms were found in spring or in early summer and in autumn; the minimum amounts — in summer and winter stagnation periods. Remarkable deviations from the general pattern of seasonal occurrence of microorganisms were more often observed in Jeziorak Mały Lake. Seasonal variations of amounts of the investigated microorganisms were also observed in the net plankton of the Iława lakes.

6. STRESZCZENIE

W rozmieszczeniu bakterioplanktonu, bakterii heterotroficznych i drożdżaków w wodzie jezior iławskich zasadnicze znaczenie posiada typ zbiornika, a w osadach dennych charakter dna. Wszystkie badane grupy drobnoustrojów występują liczniej w toni wodnej ściekowego Jezioraka Małego, mniej licznie w eutroficznym jeziorze Jeziorak. W mulistych oraz sapropelowych osadach dennych badanych jezior, zarówno bakterioplankton jak też bakterie heterotroficzne i drożdżaki występują wielokrotnie liczniej, aniżeli w gruntach piaszczystych. Duże ilości bakterioplanktonu, bakterii heterotroficznych i drożdżaków występują również w planktonie sieciowym jezior iławskich. W sezonowym występowaniu badanych drobnoustrojów w wodzie i osadach dennych stwierdza się na ogół maksymalne ilości wiosną lub z początkiem lata i w jesieni, minimalne w okresie stagnacji letniej i zimowej. Większe odchylenia od ogólnie obserwowanej sezonowości rozwoju bakterioplanktonu, bakterii heterotroficznych i drożdżaków występują częściej w Jezioraku Małym. Stwierdza się również sezonowe wahania liczebności badanych drobnoustrojów w planktonie sieciowym jezior iławskich.

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PHYTOPLANKTON OF THE SOUTHERN BALTIC SEA

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ABSTRACT

In the period of investigations 97 planktonic plant species were identified in the southern Baltic. The analysis of the hydrographical background exhibited that during the period of investigations some changes took place in the southern Baltic sea. In 1969 a salt water influx took place from the North Sea into the Baltic. It caused an increase in salinity and brought with some species of phytoplankton, rarely noted hitherto. These changes concerned as well the increment of species number after each water influx from the North Sea, as the hydrobiological character of the southern Baltic phytoplankton.

1. INTRODUCTION

This contribution deals with the species composition of the southern Baltic phytoplankton in 1971 with respect to the horizontal and vertical distribution and the seasonality of appearance of particular plant species compared with analogous results obtained for some previous years.

The phytoplankton distribution in the southern Baltic in relation to hydrological conditions (temperature and salinity) has been investigated since 1946 (Rumek 1948, Mańkowski 1951, Ringer 1970, 1973). The present paper gives the results of investigations that are a continuation of the same kind of plankton studies. It primarily concerns the relations between the changes observed in the biocenosis of phytoplankton and the hydrological fluctuations that actually take in the Baltic sea. The obtained results would be the basis for an elucidation of a number of events that are relevant for the next link of the trophic chain in the Baltic.

2. TERRAIN DESCRIPTION, MATERIAL AND METHODS

The phytoplankton was sampled in 1971 in the months representative of particular seasons: March (winter), May (spring), August (summer), November (autumn) by vertical hauls performed with plankton net of Copenhagen type (mill gauze No. 25, 80 threads in 1 cm). Apart from the data for 1971 there are also those for 1967-1968 (Ringer 1970) and for 1970 (Ringer 1973). The hauls were made throughout the whole water column from the bottom to the surface but the samples were taken separately from the water layers: 0–15, 15–30, 30–50, 50–70, 70–90 m and from 90 m to the bottom. In 1971 on the whole 92 samples were taken all over the area of the southern Baltic at stations A_1 (Arkona Deep), B_1 (Bornholm Deep), B_2 (western part of the Słupsk Furrow), G_2 (Gdańsk Deep) and at K_2 (situated NE from Rozewie at about 70 m depth) (Fig. 1).

The material was preserved in 4% formalin. The analysis of salinity and temperature conditions was elaborated on the basis of measurements made at the same time as sampling plankton material.

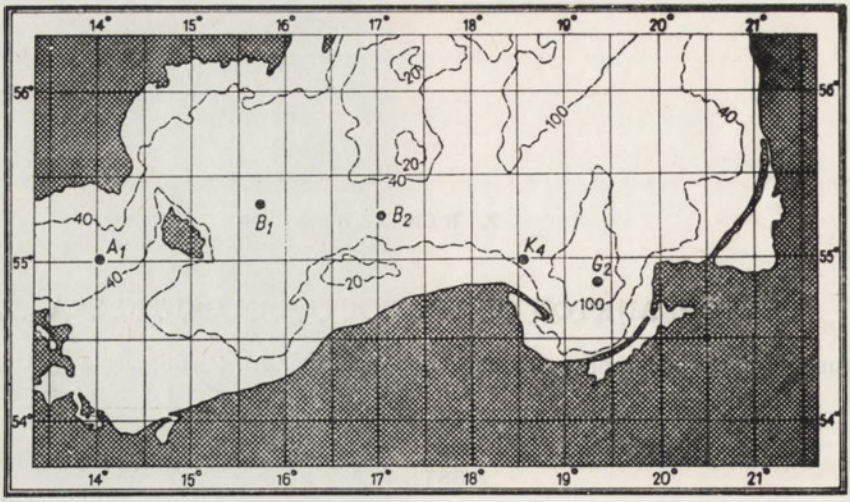

Fig. 1. Routine hydrographic and plankton sampling stations in the southern Baltic. Broken lines — isobaths. A_1, B_1, B_2, G_2, K_4 — sampling stations

The systematics has been elaborated after: Brandt, Apstein (1908), Pascher (1915), Lebour (1925, 1930), Wołoszyńska (1928), Lakowitz (1929), Huber-Pestalozzi (1938–1955), Hustedt (1962), Siemińska (1964).

3. RESULTS

SALINITY

The surface layer salinity during 1971 amounted, on the average, to 7.5‰ all over the southern Baltic, the exception was the Arkona Deep where in November the salinity was observed to be as high as 9.2‰. The bottom salinity in 1971 ranged from 7.9‰ in the area represented by station K_4 up to 16.3‰ found in the area of the Arkona Deep. In November a small influx of oceanic water from the North Sea into the Baltic took place. This influx caused increase of salinity in the Arkona Deep and it was also stated in the bottom water layer of the Bornholm Deep.

TEMPERATURE

In March 1971 the surface temperature in the southern Baltic ranged from about 1°C in the area of Arkona Deep to about 2°C in the Gdańsk Deep. In May the surface temperature ranged from about 6°C (in the Słupsk Furrow) to about 11.5°C (in the Gdańsk Deep). In August the surface temperatures were the highest of the year amounting to about 15°C in the Arkona Deep and increasing gradually eastward up to over 17°C noted in the Gdańsk Deep. In November the extreme surface temperatures were: 9.2°C (at A_1) and 8.4°C (at G_2).

PHYTOPLANKTON

The detailed phytoplankton compositions is given in Table I. On the whole, for these four years 83 forms were identified as to the species and 14 forms as to the genus only. Out of those 97 species, 1 belonged to the group of Chrysophyceae, 14 — to Dinoflagellatae, 3 — to Silicoflagellatae, 17 — to Cyanophyceae and 40 species to Bacillariophyceae.

In the surface water layer in 1971 the most numerous were the species: *Aphanizomenon flos-aquae, Nodularia spumigena, Coscinodiscus granii, Actinocyclus ehrenbergii, Chaetoceros borealis, Chaetoceros danicus, Peridinium pellucidum* and *Dinobryon balticum.*

From the point of view of their salinity demand the above cited species are euhalobes or mezohalobes (K o l b e 1927). In 1971 the phytoplankton was most numerously found in the top water layer of the sea. In the layer 40—60 m the number of species was markedly lower, apparently due to greatest fluctuations of temperature and salinity since at these depths there are both the halocline and the thermocline. Near the sea bottom the phytoplankton was quite poor. In the intermediate and bottom layers Bacillariophyceae and Cyanophyceae were relatively most abundant.

The species compositions changed during a year.

In winter the vegetation was rather poor, characterized by small quantities of plankton that belonged to following species: *Aphanizomenon flos-aquae, Nodularia spumigena, Chaetoceros borealis, Chaetoceros decipiens, Actinocyclus ehrenbergii, Coscinodiscus granii, Ceratium tripos* and *Sceletonema costatum.* In view of the salinity requirement these species are considered meso- or euhalobes. In March the phytoplankton of the surface layer was relatively most numerous in the western area of the Słupsk Furrow (at B_2). Following number of species were found there: 6 species of Bacillariophyceae, 3 — of Cyanophyceae, 2 — of Dinoflagellatae, 1 — of Chrysophyceae and 1 species of Chlorophyceae.

In May the most numerous species at that time were: *Dinobryon balticum, Aphanizomenon flos-aquae* and *Peridinium pellucidum.* All these species belong to mesohalobes. In this time the richest in phytoplankton was the Gdańsk Deep area with its 5 species from the group of Bacillariophyceae, 2 — from Cyanophyceae, 3 — from Dinoflagellatae, 1 — from Chrysophyceae and 3 species from Chlorophyceae.

In August the most numerous species were: *Aphanizomenon flos-aquae, Coscinodiscus granii* and *Actinocyclus ehrenbergii.* These species are either euhalobes or mesohalobes. The most dense phytoplankton was found at this time in the area situated NE from Rozewie (station K_4). It was represented by 4 species from the group of Cyanophyceae, 3 species from that of Bacillariophyceae and 4 species from Chlorophyceae.

In November following species did occur: *Coscinodiscus granii, Actinocyclus ehrenbergii, Ceratium tripos, Ceratium fusus, Chaetoceros bo-*

Table I. Species composition of phytoplankton in Southern Baltic

ol — oligohalobe, eu — euhalobe,
m — mezohalobe, pol — polyhalobe.

	Species	1967	1968	1970	1971
	Chrysophyceae				
m	*Dinobryon balticum* (Schütz) Lemm.	+	+	+	+
	Dinoflagellatae				
eu	*Dinophysis norvegica* Clap. Lemm.		+	+	+
m	*Dinophysis acuminata* Clap. Lachm.		+	+	+
m	*Dinophysis ovum var. baltica* Schütt.	+			
	Dinophysis sp.			+	
pol	*Peridinium stenii* Jörg.	+		+	+
m	*Peridinium divergens* Ehbg.				+
m	*Peridinium catenatum* Levander		+	+	+
m	*Peridinium pellucidum* (Bergh.) Schütz.	+	+	+	+
	Peridinium sp.	+			
m	*Peridinium granii* Ostenfeld	+	+		
m	*Ceratium fusus* (Ehrenb.) Clap. Lachm.	+			+
m	*Ceratium tripos* (O.F.M.) Nitsch.	+	+	+	+
pol	*Ceratium macroceros* Schrank			+	+
pol	*Ceratium arcticum* (Ehbg.) Cleve			+	+
	Silicoflagellatae				
eu	*Dictyocha fibula* Ehrenb.			+	
eu	*Distephanus speculum* (Ehrenb.) Haeckel			+	
m	*Ebria tripartita* (Schum) Lemm.	+	+	+	+
	Cyanophyceae				
ol	*Merismopedia punctata* Meyen		+		
m	*Merismopedia tenuissima* Lemm.				+
ol	*Merismopedia glauca* (Ehrenb.) Nägeli	+			
	Merismopedia sp.			+	
m	*Microcystis aeruginosa* Kützing	+	+	+	+
ol	*Microcystis grevillei* (Marsal) Elenkin	+	+		
m	*Microcystis flos-aquae* (Wittr.) Elenkin				+
	Chroococcus sp.	+			
ol	*Gleocapsa turgida* (Kützing) Holler	+			
	Gleocapsa sp.	+			
ol	*Oscillatoria tenuis* Agardt.				+
	Oscillatoria sp.		+		
m	*Aphanizomenon flos-aquae* L. Ralfs	+	+	+	+
m	*Nodularia spumigena* Mertens	+	+	+	+
m	*Anabaena spiroides* Klebahn		+	+	+
m	*Anabaena flos-aquae* (Lyngh.) Bréb.	+	+	+	+
	Anabaena sp.	+			
	Chlorophyceae				
ol	*Pediastrum clathratum* (Schr.) Lemm.			+	
ol	*Pediastrum boryanum* (Turpin) Manegh.	+	+	+	+
ol	*Oocystis solitaria* Wittr.		+	+	
m	*Oocystic submarina* Lagerh.		+	+	
ol	*Oocystis lacustris* Chodat		+		+
ol	*Oocystis borgei* Snow.			+	
	Oocystis sp.			+	
m	*Scenedesmus acuminatus* (Lagerh.) Chodat				+
m	*Scenedesmus quadricauda* (Turp.) Breb.			+	+
m	*Scenedesmus obliquus* (Turp.) Kg.			+	
ol	*Crucigenia tetrapedia* (Kirchn) W.n. G.S.West			+	
m	*Dictyosphaerium ehrenbergianum* Naeg.	+	+	+	+
m	*Ankistrodesmus falcatus* Corda Ralfs	+	+	+	+

	Species	1967	1968	1970	1971
m	*Ankistrodesmus falcatus var. mirabile* W.N. G.S.West			+	
m	*Ankistrodesmus nivalis* (Chod.) Brunnth.			+	
m	*Ankistrodesmus setigerus* (Schröd) G.S.West			+	
m	*Trochiscia brachiolata* (Möb) Lemm.	+	+	+	+
m	*Trochiscia multispinosa* (Moeb) Lemm.	+	+		
m	*Trochiscia clevei* Lamm.	+	+	+	+
m	*Hexasterias problematica* Cleve		+		
ol	*Kirchneriella subsolitaria* G.S.West			+	
ol	*Characium limneticum* Lemmerm.			+	
	Bacillariophyceae				
m	*Melosira nummuloides* (Dillw.) Agarth.		+		
m	*Melosira juergensii* Ag.	+	+		+
	Melosira sp.	+	+	+	
m	*Sceletonema costatum* (Grev.) Cl.	+	+	+	+
m	*Thallasiosira baltica* (Grun.) Ostf.	+	+	+	+
m	*Thallasiosira gravida* Cleve			+	
pol	*Thallasiosira nana* Lohmann			+	
m	*Cyclotella meneghiniana* (Kütz)	+	+	+	+
m	*Cyclotella stelligera* Cl. Grun			+	
m	*Cyclotella striata v. subsalina* Grunow			+	
m	*Coscinodiscus granii* Gough.	+	+	+	+
m	*Actinocyclus ehrenbergii* Ralfs	+	+	+	+
m	*Chaetoceros danicus* Cleve	+	+	+	+
eu	*Chaetoceros densus* Cleve				+
eu	*Chaetoceros borealis* Bail	+	+	+	+
pol	*Chaetoceros decipiens* Celve			+	
eu	*Chaetoceros affinis* Lauder			+	
m	*Chaetoceros laciniosus* Schütt.			+	+
m	*Chaetoceros brevis* Schütt.	+	+	+	+
m	*Chaetoceros holsaticus* Schütt.		+	+	+
m	*Chaetoceros wighami* Brightw	+	+	+	+
m	*Chaetoceros subtilis* Cleve				+
m	*Chaetoceros debilis* Cleve				+
m	*Chaetoceros socialis* Lauder				+
eu	*Chaetoceros gracilis* Schütt.				+
ol	*Diatoma elongatum* (Lyngh) Ag.		+		
pol	*Thallassiotrix nitzschioides* Grun.	+		+	+
ol	*Synedra ulna* (Nitzch.) Ehrenb.		+	+	
pol	*Asterionella gracillima* (Hantzch) Heib.			+	
m	*Asterionella zasumensis* (Cabejsz.) Lundh. Alm.			+	
m	*Fragilaria crotonensis* Kitt.			+	
m	*Ditylium brightwelli* (West)			+	
	Navicula sp.			+	
eu	*Achnanthes taeniata* Grun.		+	+	+
m	*Diploneis interrupta* (Kütz) Cl.			+	
	Gyrosigma sp.		+		
pol	*Guinardia flaccida* Castr.		+	+	
	Asterionella sp.			+	
	Lyngbya sp.		+		
	Chaetoceros sp.		+		

realis. All these species are euhalobes. In November the area of Arkona Deep had the richest plankton vegetation. The occurence of following species was observed there: 3 species from the group of Bacillariophyceae, 2 — from Dinoflagellatae, 1 — from Cyanophyceae and 1 species from Chlorophyceae.

After the influx of 1969 several species of phytoplankton have appeared in the southern Baltic, rarely noted hitherto. These are: *Dinophysis norvegica, Ceratium mocroceros, Ceratium arcticum, Dictyocha fibula, Distephanus speculum, Oocystis submarina, Thalassiorisa nana, Chaetoceros decipiens* and *Chaetoceros affinis*. After the next influx of 1971 which was markedly weaker in comparison with that of 1969, *Ceratium fusus* has been noted among the plankton forms. This species was already found in the Baltic after the influx of 1959 (Ringer 1973).

Whether these North Sea plankton species will remain in the Baltic for good — will be known after some other years to come.

4. DISCUSSION

The analysis of the hydrographical conditions (see above) as well as of the list of occurrence of phytoplankton species (Table I and II) leads to the conclusion that in the southern Baltic changes in phytoplankton biocenosis took place at that time. These changes in the time span of several years were going in two directions:

1. of a conspicuous increase in number of species in 1970 after salt water influx from the North Sea into the Baltic on 1969 (Table II),
2. of changes in hydrobiological character of the phytoplankton in the next years of research after influx.

In several last years a trend to salinity increase has been observed in the Baltic. This salinity increase has resulted in an augmentation of the number of euhalobe and polyhalobe (see Table II) species which appeared either for a limited period or — as it was observed recently — seemed to remain for good (at least within some regions of the southern Baltic) (Mańkowski 1963, Żmudziński 1968, Ringer 1973).

Table. II. Number of phytoplankton species belonging to particular groups for a number of years

Group	1967	1968	1970	1971
	Number			
Chrysophyceae	1	1	1	1
Dinoflagellatae	7	6	9	10
Silicoflagellatae	1	1	3	1
Cyanophyceae	10	8	6	8
Chlorophyceae	6	10	8	8
Bacillariophyceae	12	20	29	19
Total	37	46	66	49
	Percentage (%)			
Oligohalobe	10	12	12	5
Euhalobe + polyhalobe	10	10	15	10
Mesohalobe	70	68	63	75
Indefinited	10	10	10	10

5. SUMMARY

In 1971 studies on distribution and seasonability of the southern Baltic phytoplankton were conducted together with temperature and salinity measurements. These studies were completed by the data on species composition from 1967, 1968 and 1970. During those 4 years 97 phytoplankton species were identified: 1 species belonged to Chrysophyceae, 14 were those of Dinoflagellatae, 3 — of Silicoflagellatae, 17 — of Cyanophyceae, 22 — of Chlorophyceae, 40 — of Bacillariophyceae (Table I). The composition of phytoplankton varies together with region, depth and season. The changes in the species composition were stated as depending on water influxes from the North Sea which also resulted in general increment of species number, and their hydrobiological character (see Table I and II).

6. STRESZCZENIE

W 1971 r. przeprowadzono badania nad rozmieszczeniem oraz sezonowością występowania fitoplanktonu Bałtyku południowego na tle warunków hydrologicznych. Badania te uzupełniono danymi składu gatunkowego z lat 1967, 1968 i 1970. W ciągu tych czterech lat określono 97 gatunków roślin planktonowych, z tego 1 gatunek zaliczono do Chrysophyceae, 14 do Dinoflagellatae, 3 do Silicoflagellatae, 17 — Cyanophyceae, 22 — Chlorophyceae, 40 — Bacillariophyceae (Tab. I). Skład fitoplanktonu zmienia się w zależności od rejonów badań, od głębokości oraz od sezonu. Stwierdzono zmiany w składzie gatunkowym roślin planktonowych w zależności od wlewów wód słonych z Morza Północnego, które powodują wzrost ogólnej ilości gatunków oraz zmiany charakteru hydrobiologicznego występujących gatunków (Tab. I i II).

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CHANGES OF PHYTOPLANKTON BIOCENOSIS IN THE SOUTHERN BALTIC DURING THE LAST HALF-CENTURY

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ABSTRACT

On the basis of the review of the literature of 1927–1970 on phytoplankton species composition in the southern Baltic, it was found that there occurred some changes in the phytoplankton biocenosis under the influence of an increasing salinity.

The review of the literature of 1927—1970 leads to a conclusion that considerable changes of the environment and phytoplankton biocenosis in the Baltic have taken place. The aim of the present paper is to survey the changes of this biocenosis during the last fifty years, similarly as it was made for planktonic and bottom animals (Mańkowski 1951, 1963, Żmudziński 1968). Unfortunately, the Baltic phytoplankton had been studied only fragmentarily, in some years and in some zones of the southern Baltic; nevertheless the available investigations allow to recognize the general tendencies of changes occurring there. Changes in salinity presented in Fig. 1 show a general tendency to an increase of salinity in Baltic water. Both the phenomenon of periodical inflows and the mentioned general tendency towards the "ocean" are reflected in the phytoplankton composition (Table I). Periodical water inflows result in immediate appearance of some oceanic and North Sea phytoplankton species in the Baltic, which can be assumed to be the periodical biological indicators of inflows. Moreover, the general tendency to increasing salinity forms an environment for some of the species rarely or never found in the southern Baltic before to settle down there.

In 1927 the investigations were made in Bornholm Deep (Hessle, Vallin 1934, Hentschel 1951). Salinity of surface water was 7.11‰, and of near to bottom water — 16.1‰ (Fig. 1 A). Salinity values do not testify to any inflow having taken place in the period previous to the investigations. Anyway, those investigations of phytoplankton were fragmentary, and the number of recorded species was small. The determined brackish-water species were rather typical for the Baltic

and the only oceanic species found out were those which are usually recorded as originating from an inflow of cold and considerably salty waters. They were *Chaetoceros borealis* and *Chaetoceros decipiens*. Thus an inflow did take place however considerably earlier.

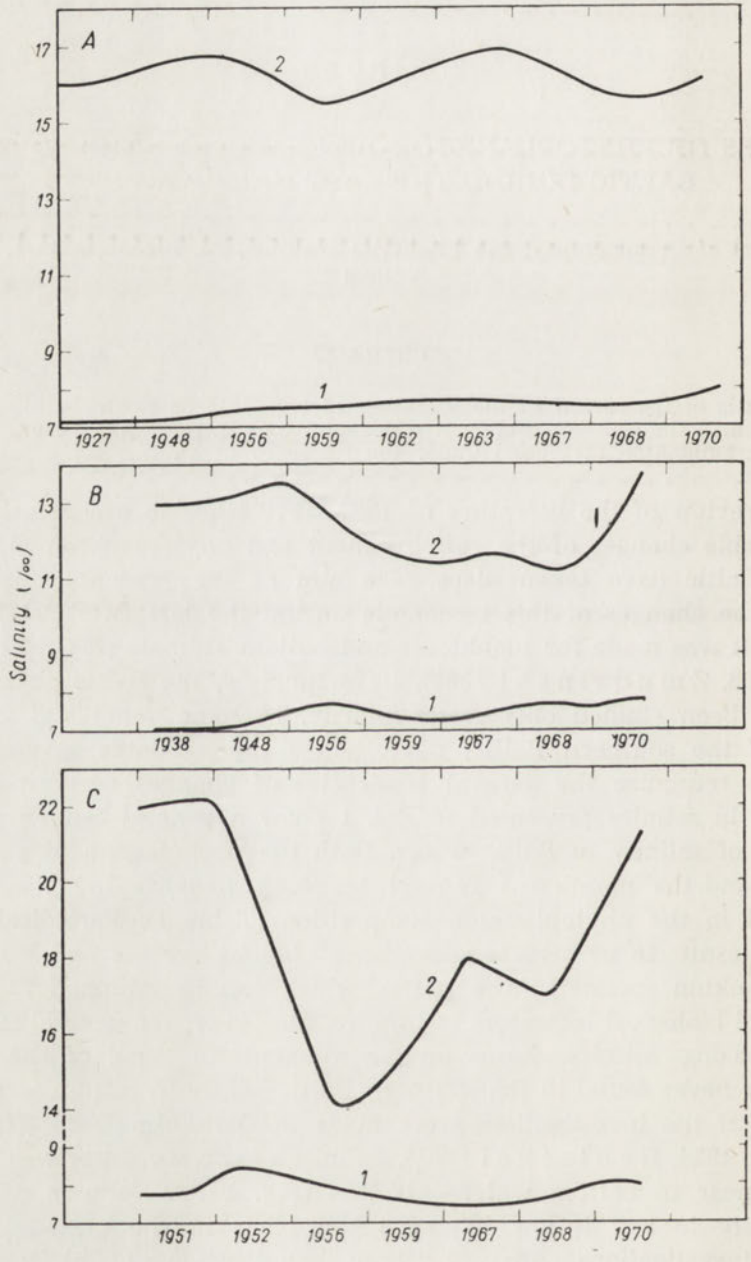

Fig. 1. Changes of salinity of the surface (1) and at depths (2) in: A — Bornholm Deep, (90 m), B — Gdańsk Deep (110 m), C — Arkona Deep (46 m)

In 1938 the investigations had been made in the Gdańsk Deep zone (Rothe 1942). Salinity of surface water was 7‰, however in the near to bottom water it was more than 13‰, so it testifies to an inflow having taken place in the period previous to the investigations (Fig. 1 B). In the region so distant from an inflow source — the North Sea — no oceanic species were found, however there was a considerable number of North Sea species. There were 25% of North Sea species and 74% of brackish-water species.

A similar pattern could be observed in 1948 (Rumek 1948, 1950) in the same region. Salinity of surface water was 7.3‰ or normal, and in the near to bottom water it was more than 13‰. There were 10% of North Sea species; some of them were those recorded in 1938 and the others were new. More of brackish-water species was then found, and still there were no oceanic species, which could be explained, as before, by a considerable distance from the inflow source.

In 1951—1952, the investigations were carried only in Arkona Deep (Lazar-Dmytrenko 1967), but they confirmed a great inflow from the North Sea. In March 1951 salinity of near to bottom water was 22‰, and in January 1952 — 22.3‰. There were two successive water inflows in this period. In the region, 10% of North Sea and oceanic species were found, among which *Chaetoceros densus* was recorded for the first time in this region. (*Chaetoceros gracilis*, also an oceanic species, was recorded as late as in 1956 and as far as in Gdańsk Deep (Ringer 1973) where the consequences of those inflows were felt for such a long time).

In 1956 the whole region of the southern Baltic was investigated (Ringer 1973), including Pomeranian Bay (Zembrzuska 1967). Apparently the hydrological conditions in the Bornholm Deep region of that period, thus also the salinity of near to bottom water being about 16‰ all the year, resulted from the heavy inflows of 1951—1952. In the other regions, the salinity was not observed to be increasing. The number of recorded species was rather low; there were 1% of oceanic and North Sea species, and 69% of brackish-water species.

In 1959 (Ringer 1973) the salinity of Baltic water decreased by about 0.5—3‰. In the region of Arkona Deep, an inflow of warm salty water took place in November, which brought, besides 3 oceanic species, already recorded in the Baltic, a new one, *Chaetoceros atlanticus*. There were found 6% of North Sea species and 92% of brackish-water species.

In 1962—1963 (Kijowska 1964) the investigations were made only in the Bornholm Deep region. The salinity was somewhat higher in this region by then, in comparison with the previous investigation period (in February 1961 there was a small inflow). 2% of North Sea species and 96% of brackish-water species were recorded.

Table I. Composition of phytoplankton of the southern Baltic in 1927–1970
ol — oligohalobe, m — mesohalobe, eu — euhalobe, pol — polyhalobe,
A — Arkona Deep, B — Bornholm Deep, C — Central Baltic, G — Gdańsk Deep,
P — Pomeranian Bay, S — Southern Baltic

	Species	Years of investigations										
		1927	1938	1948	1951–1952	1956	1958	1959	1962–1963	1967	1968	1970
		Hessle, Vallin 1934	Rothe 1950	Rumek 1948, 1950	Lazar-Dymitrenko 1967	Ringer 1973	Zembrzuska 1967	Ringer 1973	Kijowska 1964		Ringer 1970, 1973	
	Chrysophyceae											
m	*Dinobryon balticum* (Schütz) Lemm.	—	—	G	A	P	P	S	B	S	S	—
	Dinoflagellatae											
eu	*Dinophysis norvegica* Clap. Lachm.	—	G	G	A	B	P	C	—	—	S	S
m	*Dinophysis acuminata* Clap. Lachm.	B	G	G	A	S	—	S	B	S	S	S
m	*Dinophysis ovum var. baltica* Schütt.	—	—	—	A	G	—	—	B	S	S	S
m	*Dinophysis rotundata* Paulsen	—	G	—	—	B	—	B	—	S	S	S
m	*Peridinium catenatum* Levander	—	G	—	—	S	—	C	B	—	—	S
m	*Peridinium granii* Ostenfeld	—	—	G	—	—	—	A	—	—	—	—
m	*Peridinium pellucidum* (Bergh) Schütz.	—	G	G	A	S	P	S	B	S	S	S
m	*Peridinium finlandicum* Paulsen	—	—	—	—	S	—	—	—	—	—	—
m	*Ceratium tripos* (O.F.M.) Nitzch.	B	—	G	A	B	P	C	B	S	S	S
m	*Ceratium tripos f. hiemale* Paulsen	—	—	—	—	A	—	C	—	—	—	—
eu	*Ceratium furca* (Ehrenb.)	B	—	—	—	—	—	C	—	—	—	—
	Silicoflagellatae											
m	*Ebria tripartita* (Schum) Lemm.	—	—	G	A	—	—	C	—	—	—	—
	Cyanophyceae											
m	*Merismopedia tennuissima* Lemm.	—	—	—	—	S	—	S	B	S	S	B
ol	*Merismopedia glauca* (Ehrenb.) Nageli	—	—	G	—	—	P	G	B	S	—	—
m	*Microcystis aeruginosa* Kützing	—	—	G	A	S	P	S	B	S	S	S
m	*Microcystis flos-aquae* (Witrr.) Elenkin	—	—	—	—	B	—	—	—	—	—	—

ol	*Gleocapsa turgida* (Kützing) Holler	—	—	—	A	—	—	G	—	S	S	—	—	—
ol	*Lyngbya bipunctata* Lemmermann	—	—	G	—	—	—	S	—	—	—	—	—	—
ol	*Oscillatoria tenuis* Agardh.	—	—	—	—	B	—	—	—	—	—	—	—	—
ol	*Oscillatoria lacustris* (Klebahn) Geitler	—	—	—	—	G	—	—	—	—	—	—	—	—
ol	*Oscillatoria limosa* Agardh	—	—	—	—	—	—	G	—	—	—	—	—	S
m	*Aphanizomenon flos-aquae* L. Ralfs	B	G	G	A	S	P	S	B	S	S	S	S	S
m	*Nodularia spumigena* Mertens	B	G	G	A	S	P	S	B	S	S	S	S	S
m	*Anabaena spiroides* Klebahn	—	—	G	—	—	P	B	—	S	S	S	S	S
m	*Anabaena flos-aquae* (Lyngh) Bréb.	—	—	G	A	S	P	C	B	—	—	—	—	S
m	*Anabaena affinis* Lemmermann	—	—	—	—	B	P	—	—	—	—	—	—	—
	Chlorophyceae													
ol	*Pediastrum clathratum* (Sch) Lemm	—	—	G	—	—	—	G	—	—	—	—	—	S
ol	*Pediastrum boryanum* (Turpin) Manegh.	—	G	G	A	—	P	C	B	—	S	S	S	S
ol	*Oocystis solitaria* Wittr.	—	—	G	A	G	P	—	—	S	—	S	S	S
ol	*Oocystis submarina* Lagerh.	—	—	G	A	C	P	A	—	—	—	S	S	S
ol	*Oocystis lacustris* Chodat	—	—	—	—	A	—	A	B	—	—	—	—	S
m	*Scenedesmus acuminatus* (Lagerh.) Chodat	—	—	—	—	—	—	G	—	—	—	—	—	S
m	*Scenedesmus quadricauda* (Turp.) Bréb.	—	—	G	A	G	P	C	—	—	S	S	S	S
m	*Dictyosphaerium ehrenbergianum* Naeg.	—	—	—	A	S	P	S	B	—	S	S	S	S
m	*Ankistrodesmus falcatus* Corda Ralfs	—	—	G	A	S	—	S	B	—	S	S	S	S
m	*Ankistrodesmus nivalis* (Chod.) Brunnth.	—	—	—	—	G	—	C	—	—	—	—	—	S
m	*Trochiscia brachiolata* (Mob.) Lemm.	—	—	—	—	A	—	C	—	—	S	S	S	S
m	*Trochiscia multispinosa* (Moeb. Lemm.	—	—	G	A	G	—	S	B	—	S	S	S	S
m	*Trorochiscia clevei* Lemm	—	—	—	—	B	—	—	B	—	—	—	—	S
m	*Hexasterias problematica* Cleve	—	—	G	—	—	—	—	B	—	—	—	—	S
	Bacillariophyeae													
eu	*Melosira juergensii* Ag.	—	—	G	A	S	P	G	B	S	S	S	—	—
pol	*Melosira moniliformis* (Müll.) Ag.	—	—	—	A	—	—	C	—	—	—	—	—	—
eu	*Melosira borreri* Grev.	—	G	G	—	—	—	G	—	—	—	—	—	—
m	*Sceletonema costatum* (Grev.) Cl.	B	G	G	A	S	P	S	B	S	S	S	S	S
m	*Thallasiosira baltica* (Grun.) Ostf.	B	G	G	A	S	P	S	B	S	S	S	S	S
m	*Thallasiosira gravida* Cleve	—	—	—	—	—	—	A	—	—	S	S	S	S
m	*Cyclotella meneghiniana* (Kütz.)	—	—	G	—	—	P	C	B	—	S	S	S	S
ol	*Cyclotella planctonica* Brunnth.	—	—	—	—	B	—	—	—	—	—	—	—	S
ol	*Cyclotella socialis* Schütz.	—	—	G	—	B	—	—	—	—	—	—	—	S
m	*Cyclotella melosiroides* (Kirchn.) Lemm	—	—	G	A	G	P	—	B	—	S	S	S	S
m	*Coscinodiscus granii* Gouth.	—	—	—	A	A	P	—	—	—	S	S	S	S
m	*Coscinodiscus commutatus* Grunow.	—	—	—	—	A	—	G	—	—	—	—	—	—

Table I continued.

	Species	1927	1938	1948	1951-1952	1956	1958	1959	1962-1963	1967	1968	1970
pol	*Coscinodiscus jonesianus* (Grev.) Ostf.	—	—	—	—	—	—	C	—	—	—	—
m	*Actinocyclus ehrenbergii* Ralfs	—	—	G	A	S	P	S	B	S	S	S
m	*Actinocyclus ehrenbergii v. crassa* (W. Schmith) Hustedt	—	—	—	—	—	G	—	—	—	—	—
m	*Chaetoceros danicus* Cleve	B	—	G	A	S	P	—	—	S	S	S
eu	*Chaetoceros densus* Cleve	—	—	—	A	S	P	S	—	—	—	S
eu	*Chaetoceros borealis* Bail	B	—	—	A	S	—	S	—	S	S	S
pol	*Chaetoceros decipiens* Cl.	—	—	A	—	—	—	C	—	—	—	—
eu	*Chaetoceros affinis* Laud	—	—	—	A	—	—	B	—	S	—	—
m	*Chaetoceros laciniosus* Schütt.	—	—	—	A	S	—	G	—	—	—	S
m	*Chaetoceros brevis* Schütt.	—	—	—	A	G	—	C	—	S	S	S
m	*Chaetoceros holsaticus* Schütt.	—	G	G	A	C	P	S	B	—	S	S
m	*Chaetoceros wighami* Brightw.	—	—	G	A	S	P	S	—	S	S	S
m	*Chaetoceros subtilis* Cl.	—	—	—	—	—	P	S	—	—	S	S
m	*Chaetoceros debilis* Cl.	—	—	—	A	—	—	S	—	—	S	—
m	*Chaetoceros socialis* Laud.	—	—	—	—	G	—	—	—	—	—	S
eu	*Chaetoceros gracilis* Schütt.	—	—	—	A	S	—	—	—	—	—	—
m	*Chaetoceros eibenii* Meun.	—	—	—	—	—	—	—	—	—	—	—
m	*Chaetoceros diadema* (Ehr.)	—	—	—	—	—	—	—	G	—	S	—
pol	*Chaetoceros atlanticus* Cl.	—	—	—	—	—	—	B	—	—	—	—
m	*Chaetoceros pseudocrinitus* Ostf.	—	—	G	A	S	—	—	—	—	—	—
m	*Chaetoceros similis* Cl.	—	—	—	—	B	P	—	—	—	—	—
ol	*Diatoma vulgare* Bory	—	—	G	—	B	P	—	—	—	—	—
ol	*Diatoma elongatum* (Lyngb.) Ag.	—	—	G	A	—	P	C	—	—	S	—
pol	*Thallassiothrix nitzschioides* Grun.	—	—	G	—	C	—	C	—	—	S	—
ol	*Synedra ulna* (Nitzsch) Ehrenb.	—	—	G	A	B	—	A	—	—	S	S
ol	*Synedra pulchella* (Ralfs) Kütz.	—	—	—	—	A	—	—	—	—	—	—
m	*Synedra utermöhlii* Hust.	—	—	—	—	—	—	B	—	—	—	—
m	*Fragilaria crotonensis* Kitt.	—	—	G	—	B	P	—	—	—	—	—
ol	*Fragilaria nitzschioides* Grun.	—	—	G	—	B	—	—	—	—	—	—
m	*Achnanthes taeniata* Grun.	B	G	—	A	S	P	A	B	—	S	S
m	*Diploneis ovalis* (Hilse) Cl.	—	—	G	—	—	—	G	B	—	—	—

In 1967–1970 (Ringer 1970, 1973), the regular observations in all the southern Baltic regions were made in winter (March), spring (May), summer (July) and autumn (November). In the four-year period two inflows of considerable salinity were recorded. In the Arkona Deep region, salinity had increased up to 18‰ in May 1967, and after a heavy inflow in February 1970, it was up to 23‰. In 1967–1970 the number of oceanic and North Sea species was rather even, about 1–2%, and only after the 1970 inflow it increased (Table II).

Table II. Numbers of species found in the southern Baltic in 1927–1970 S — the whole southern Baltic, A — Arkona Deep, B — Bornholm Deep, G — Gdańsk Deep, P — Pomeranian Bay

Year	Region	Influx	Total number of species	Group of species		
				polihalobe and euhalobe (%)	mesohalobe (%)	oligohalobe (%)
1927	B	—	10	30	70	—
1938	G	+	14	25	74	1
1948	G	+	42	10	75	15
1951-1952	A	+	40	10	78	12
1956	S	—	57	1	69	30
1958	P	—	35	1	97	2
1959	S	+	60	6	92	2
1962-1963	B	—	27	2	96	2
1967	S	+	28	1	97	2
1968	S	—	43	2	96	2
1970	S	+	41	1	98	1

A tendency to increasing salinity in the Baltic was confirmed by a steady occurrence of the typically oceanic and North Sea species, never found in this region several years ago, as well as by a disappearance of several freshwater species, recorded in the Baltic before.

The newly settled, oceanic or North Sea species are as follows: *Chaetoceros borealis*, a species recorded in the northern Atlantic, North Sea, Arctic Ocean and Pacific Ocean. In 1927 it was found by Hessle, Vallin (1934) in the northern zone of Bornholm Basin; now it is abundant in the Gdańsk Deep and Gotland Deep. *Chaetoceros atlanticus* occurs in the northern Atlantic, North Sea, Mediterranean Sea. *Chaetoceros decipiens* is recorded in Arctic Ocean, northern Atlantic, English Channel and since 1951 in Arkona Deep. *Coscinodiscus jonesianus*, recorded in the seas of high salinity (even in Caspian Sea — Hustedt 1962), is found in all the Baltic regions and even in Vistula Bay. *Ceratium furca* is found in the northern Atlantic, North Sea, English Channel and in the recent period even in Słupsk Furrow region.

The above review confirms that a known Baltic feature — changea-

bility of hydrological conditions caused by inflows of salty water from North Sea — is reflected by changes in the Baltic biocenoses thus also in phytoplankton biocenosis. Similarly as in animal Baltic biocenoses (plankton, bottom fauna), also in phytoplankton there are some species being the inflow-indicators, which live in the Baltic for a short time (e. g. *Chaetoceros atlanticus*) as well as some settled species being the indicators of permanent changes in the Baltic resulting from the increasing salinity (e. g. *Chaetoceros borealis* and *Coscinodiscus jonesianus*). The increasing salinity caused retreat of several freshwater species, abundantly occurring in the Gdańsk Deeep till 1948, from the southern Baltic. They were: *Fragilaria capucina*, *Gomphosphaeria aponina*, *Anabaena contorta*, never found in the last period of investigations.

SUMMARY

A review of the literature of 1927–1970 leads to a conclusion on changeability of the southern Baltic environment and thus also on changeability of the phytoplankton biocenosis. Periodical inflows of North Sea water result in appearance of some oceanic and North Sea species in the Baltic. The species either disappear soon, thus being only periodical indicators, or adapt themselves to the environment conditions. During the considered period, a general tendency to an increasing salinity in the Baltic has been observed (Fig. 1), confirmed by the permanent occurrence of planktonic plant species originating from the inflows. Such species are: *Chaetoceros borealis*, *Chaetoceros decipiens*, *Chaetoceros atlanticus*, *Coscinodiscus jonesianus*, *Ceratium furca*. In the same period of increasing silinity, retreat of some freshwater species was recorded. which had been abundant in the Gdańsk Bay till 1948. They were *Fragilaria capucina*, *Gomphospaeria aponina* and *Anabaena contorta*.

STRESZCZENIE

Przegląd literatury z lat 1927–1970 prowadzi do wniosku o zmienności środowiska Bałtyku południowego oraz idącej za tym zmienności biocenozy fitoplanktonowej. Okresowe wlewy wód z Morz Północnego prowadzą do pojawienia się w Bałtyku gatunków oceanicznych lub północnomorskich. Gatunki te bądź to szybko giną i są wówczas tylko czasowymi wskaźnikami, bądź to przystosowują się do panujących warunków środowiska. Na przestrzeni rozpatrywanych lat zaznacza się tendencja do wzrostu zasolenia w Bałtyku (Fig. 1), co zostaje potwierdzone trwałym występowaniem gatunków roślin planktonowych, które dostały się do Bałtyku dzięki wlewom. Do takich gatunków należą: *Chaetoceros borealis*, *Chaetoceros decipiens*, *Chaetoceros atlanticus*, *Coscinodiscus jonesianus*, *Ceratium furca*. W tym samym okresie, kiedy zaznaczał się wzrost zasolenia, zanotowano zanik szeregu gatunków słodkowodnych, które jeszcze do roku 1948 spotykane były w Zatoce Gdańskiej dość licznie, były to gatunki: *Fragilaria capucina*, *Gomphoshpaeria aponina* i *Anabaena contorta*.

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ROLE OF ALGAE IN SEWAGE PURIFICATION. PART I. OXYGEN PRODUCTION

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ABSTRACT

The present paper deals with problems concerning determination of algae participation in oxygen production in biological ponds. On the basis of the complex biological and physico-chemical studies, including meteorological data, it has been stated that biologically purified sewage is favourable for development of algae. Algae are main producers of oxygen in the process of photosynthesis. This material estimated quantitatively can be utilized for designing and operating the ponds, working as the tertiary sewage treatment plants.

1. INTRODUCTION

In the process of sewage purification in the biological ponds, algae perform two essential functions, namely: furnishing oxygen indispensable for the normal course of the oxygen processes, and removing the nutrient substances from the sewage.

The object of the present study was to determine the participation of algae in the production of oxygen in the process of photosynthesis; whereas the role of algae in the removal of nitrogen and phosphorus from the sewage has been discussed in another paper (K a l i s z 1973).

In the most frequently used method of biological purification of sewage (activated sludge) oxygen, required for decomposition of organic waste matters in the sewage, is introduced in an artificial manner, whereas in the ponds the natural reaeration and photosynthesis are the main sources of oxygen in the water.

The photosynthesis of algae in the ponds is affected by three principal factors, intensity of light, depth of the pond, and density of algae. Sunlight is the direct source of energy for photosynthesis. Aquatic plants benefit from it to a higher degree than terrestrial plants, due to the fact that the former have supplementary colouring matters (phycocyanin and phycoerythrin) which absorb light energy and transmit it to chlorophyll.

Light, as one of the most important photosynthetic factors, must have a proper quality, intensity, and an adequate time of duration. The maximum intensity of the CO_2 assimilation in the range of the infrared light occurs in the 655–720 mμ wave length and in the ultraviolet range in the 400–500 mμ wave length.

O s w a l d, G o t a a s (1955) and O s w a l d et al. (1957) stated that algae seldom make use of 10–12% of the total sun radiation, in general those values are considerably lower. Moreover, those authors have observed that 3.68 cal of energy is required for production of 1 mg of oxygen in the process of photosynthesis.

The output of oxygen produced in the process of photosynthesis increases together with the increase in the intensity of light. It is assumed that at the

intensity of light of 257 lux the amount of oxygen released by algae (Chlorella) equals the quantity of oxygen used in metabolic processes. At a higher intensity of light the amount of the released oxygen increases proportionally until the intensity of light reaches the value of approximately 6500 lux.

The intensity of light ranging from about 11,000 to 43,000 lux has a restraining effect on the process of photosynthesis.

The light falling upon the surface of the water level penetrates into the deeper water layers only partially since a part of the radiation is absorbed by algae and other substances existing in water. The degree of absorption is determined by the local conditions, i. e. the character of the water body. Bartsch (1961) and Towne et al. (1957) declare that in the ponds of Dakota 99% of the light is absorbed already at the depth of 50–70 cm. Thus, the process of photosynthesis decreases with the increasing depth, in accordance with logarithmic curve, in result of the extinction of light.

When light passes through a suspension of algae its intensity decreases. That phenomenon is called mutual shadowing. The greater the algae are dense the greater is the mutual shadowing and the light penetrates less deep into the water depths. On the other hand, the oxygen production in the process of photosynthesis is strictly associated with the density of algae. Oswald et al. (1957) declare that with 1 g increase in the dry mass of algae a minimum of 1.6 g of oxygen is released in the process of photosynthesis.

The depth to which visible light penetrates can be expressed, after the Bear-Lambert law, by the following equation:

$$d = \frac{\ln J}{C_c \cdot a} \tag{1}$$

where: d — depth to which visible light penetrates (cm), J — incident light intensity (lux), C_c — concentration of algae (mg/l), a — absorption coefficient of the algae cells (usually about $1.5 \cdot 10^{-3}$).

In general the depth of the artificially formed ponds serving as sewage treatment plants is deeper than it would result from the above presented equation which is accounted for by the fact that ponds of greater depth are less sensitive to the effect of the atmospheric conditions.

According to Oswald et al. (1957) the amount of oxygen that may be produced depending on the photosynthetic efficiency of algae can be calculated on the basis of the following formula:

$$WO_2 = \frac{F \cdot S}{3.68} \text{ mg/l} \cdot \text{day}$$

or:

$$WO_2 = 100 \frac{F \cdot S}{3.68} = 27.1 \; F \cdot S \text{ kg/ha} \cdot \text{day} \tag{2}$$

where: WO_2 — weight of oxygen (mg), S — visible solar energy (cal/cm² · day), F — efficiency of light energy conversion into chemical energy (%), 3.68 cal — energy required to produce 1 mg of oxygen through photosynthesis.

The pond area required for production of a given amount of oxygen through photosynthesis depends on the visible solar energy and photosynthetic efficiency of algae. However, in the planning of artificial ponds one must take into account a limited area and sometimes also duration of the sewage detention time. In consideration of the physiology of algae the duration of sewage detention time required for the maintenance of adequate density of algae population should not be shorter than one day. Therefore, by including the detention time D (expressed in days) and the area A (expressed in cm²) into the equation (2) it will take the following form:

$$WO_2 = \frac{F \cdot S \cdot D \cdot A}{3.68} \tag{3}$$

where $S \cdot D \cdot A$ expresses the total visible solar energy reaching the pond in a defined period of time.

The volume of sewage brought into the pond in a defined period of time is expressed, as follows:

$$V = Q \cdot D \tag{4}$$

where Q — rate of flow per day.

When the volume of water in the pond is expressed in liters and its area in cm^2 then the area corresponding to 1 l of sewage can be expressed, as follows:

$$A = \frac{1000}{d} \tag{5}$$

where d — depth (cm).

By substituting the above expression into the equation (3) the following formula is obtained:

$$WO_2 = \frac{1000 \cdot F \cdot S \cdot D}{3.68 \cdot d} \tag{6}$$

or:

$$\frac{d}{D} = \frac{1000 \cdot F \cdot S}{WO_2 \cdot 3.68}$$

The last expression indicates that, when such factors as photosynthetic efficiency, density of algae population, etc. are constant then the total oxygen production in the pond is a function of the detention time and depth. The ratio d/D is expressed in cm/day.

The equation (6) allows to determine either the sewage detention time in the ponds with a known area and depth or the depth and area of the new designed artificial ponds, when characteristics of the sewage are known.

To determine the theoretical quantity of oxygen required for stabilization of pollution expressed as BOD_5 the following formula can be used:

$$WBOD_5 = 0.226 \cdot L_t \cdot \frac{d}{D} \cdot \frac{1.12}{2.54} = 0.100\, L_t \cdot \frac{d}{D} \tag{7}$$

$\frac{1.12}{2.54}$ — coefficient of conversion from lb/acre · day into kg/ha · day, where L_t is BOD_5 expressed in mg O_2/l.

By comparing the possible photosynthetic efficiency of algae (formula 2) with the required photosynthetic efficiency equalizing BOD_5 (formula 7) the critical photosynthetic efficiency is obtained, as follows:

$$27.1 \cdot F_c \cdot S = 0.100 \cdot L_t \cdot \frac{d}{D}$$

or:

$$F_c = 0.0037 \frac{L_t}{S} \cdot \frac{d}{D} \tag{8}$$

Assuming that photosynthesis produces the total amount of oxygen, F_c from the equation (8) expresses the critical efficiency. On the other hand, the actual photosynthesis efficiency F in the given conditions, as expressed by Oswald et al. (1957), is as follows:

$$F = \frac{H}{S'} \tag{9}$$

where: $S' = \frac{1000 \cdot S}{d} \cdot H = Y_c \cdot h$

$$Y_c = \frac{C_c}{D}$$

S' — available light energy (cal/l · day),
d — depth of the pond (cm),
h — heat of combustion (cal/mg) (normally 6.0),
Y_c — yield of cell material (mg/l · day),
C_c — concentration of algae (mg/l).

The ratio of values F/F_c termed oxygenation factor may serve as an index of the oxygenic conditions in a given pond. If $F/F_c=1.0$, it means the amount of oxygen that can be produced in the pond is such as required for stabilization of pollution, expressed as BOD_5.

$\frac{F}{F_c} < 1.0$ — the conditions in the pond are partially anaerobic.

$\frac{F}{F_c} > 1.0$ — there is a surplus of oxygen in the pond.

The above presented interrelations allow to control effectively the oxygen conditions in the ponds.

2. MATERIAL AND METHODS

Studies were carried out in the ponds located in the grounds of the Municipal Sewage Treatment Plant in Kielce, during the vegetative season of 1960, and in the ponds of the Experimental Sewage Treatment Plant in Częstochowa, during the yearly cycles of 1967–1968.

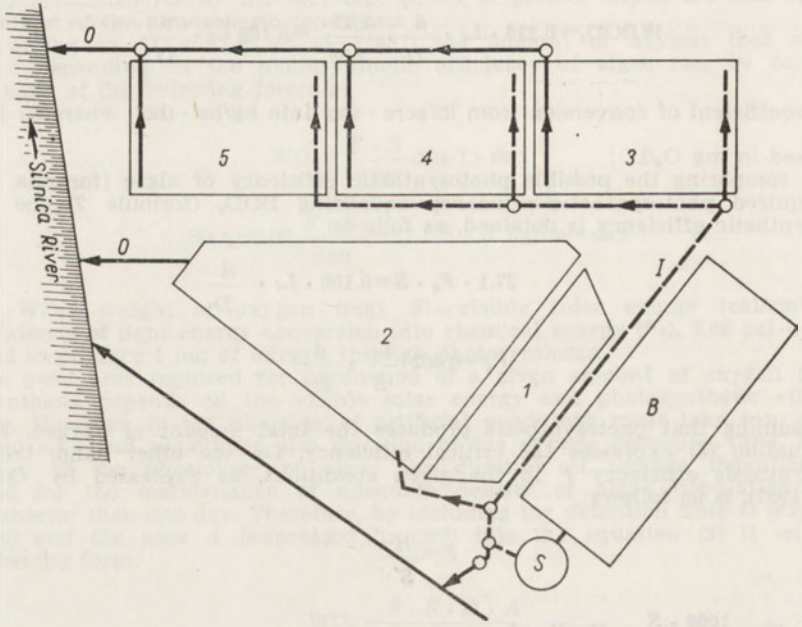

Fig. 1. The plan of the ponds in parallel system in the Sewage Treatment Plant in Kielce. B — aeration tank, I — inlet, O — outlet, S — secondary settling tank, 1–5 — pond numbers

Ponds in Kielce, presented schematically in Fig. 1, with total area of 5320 m² and average depth of 0.87 m were located near the Silnica River and worked in parallel system. Sewage after biological purification with activated sludge in Scheffield type aeration tanks was brought into the ponds.

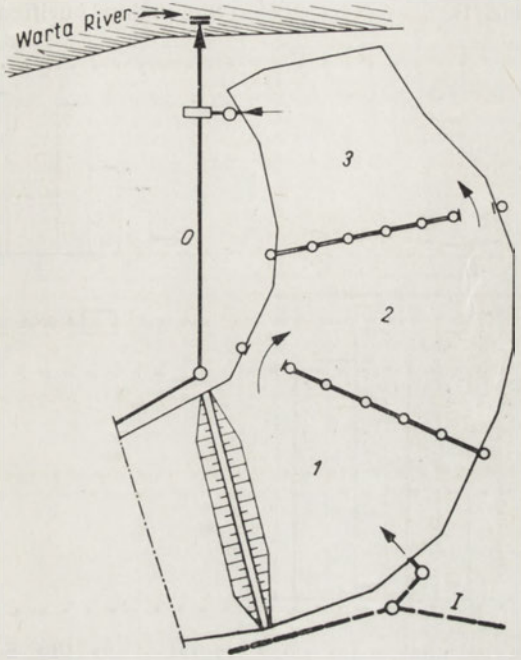

Fig. 2. The plan of the ponds in series system in the Experimental Sewage Treatment Plant in Częstochowa. I — inlet, O — outlet, 1–3 — pond numbers

Table I. Physico-chemical and bacteriological characteristics of sewages flowing into the ponds (mean values)

Characteristics	Unit	Sewage	
		Kielce	Częstochowa
Sewage temperature	°C	16.0	23.8
pH		7.9	7.8
Dissolved oxygen	mg O_2/l	3.4	—
BOD_5	mg O_2/l	5.2	27.5
Ammonia nitrogen	mg N-NH_4/l	1.1	20.2
Nitrite nitrogen	mg N-NO_2/l	0.08	0.13
Nitrate nitrogen	mg N-NO_3/l	9.1	0.11
Organic nitrogen	mg N_{org}/l	2.6	6.21
Total suspended solids	mg/l	103.0	34.2
Phosphorus	mg P_2O_5/l	0.85	4.9
Phenol	mg/l	—	0.20
Total number of bacteria per 1 ml after 72 ha at 20°C	cells/ml	3754	139,000
Total number of bacteria per 1 ml after 24 hr at 37°C	cellsm/l	2724	103,000
Colititre		10^{-3}	10^{-5}

— No data.

Ponds in Częstochowa, presented in Fig. 2, with total area of 4200 m² and average depth of 1.47 m, operated in series system. Municipal sewage of the town Częstochowa is carried off into the ponds after biological purification by the activated sludge method in aeration tanks of the Inka and Kessener system.

Sewage carried off into the ponds in Kielce differed considerably as regards its physico-chemical characteristics, from the sewage flowing into the ponds of Częstochowa (Table I). The former was thoroughly purified biologically, the

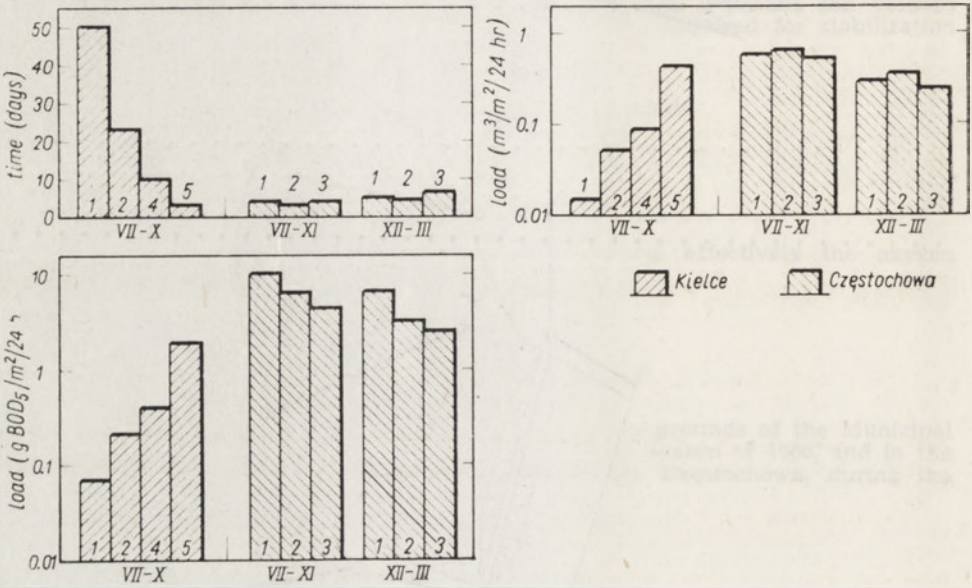

Fig. 3. Technological parameters of pond operation in the Sewage Treatment Plants in Kielce and Częstochowa. 1–5 — pond numbers. A — detention time of sewage in ponds, B — hydraulic loading, C — BOD_5 loading

Table II. Mean daily air temperatures and radiation characteristics for Kielce and Częstochowa (according to the State Hydro-Meteorological Institute in Warsaw)

Town	Year	Month	Mean daily temp. (°C)	Total radiation		Insolation period	
				cal/cm³ · month	cal/cm³ · day	hr/month	hr/day
Kielce	1960	July	16.0	10,421	336	144.5	4.65
		Aug.	16.1	10,340	334	174.7	5.63
		Sept.	11.7	7570	250	149.7	4.99
		Oct.	9.3	3781	122	81.2	2.52
Często-chowa	1967	July	19.4	14,337	460	279.9	9.0
		Aug.	17.3	11,568	373	212.1	6.85
		Sept.	14.2	7890	263	157.3	5.25
		Oct.	11.6	4635	149	125.2	4.05
		Nov.	3.9	2242	75	68.0	2.27
		Dec.	—1.7	1189	39	17.9	0.58
	1968	Jan.	—3.5	1578	51	35.6	1.15
		Febr.	0.2	2569	88	38.4	1.32
		March	3.2	7151	230	141.2	4.55

latter only partially, e.g. their BOD_5 amounted to 5.4 mg O_2/l and 27.5 mg O_2/l, respectively.

Data presented in Fig. 3 show that the ponds of Częstochowa operated at higher technological parameters, than the other ones.

Physico-chemical analyses for determination of sewage temperature, pH, CO_2, dissolved O_2, BOD_5, nitrogen (ammonium, nitrite, nitrate, and organic nitrogen) and phosphorus, gave the material indispensable for determining the basic technological parameters and the effects of the pond operation. Besides, they allowed to correlate closely the physico-chemical phenomena occurring in the ponds with the dynamics of algae development. The physico-chemical determinations were performed using methods given by Hermanowicz et al. (1967).

Biological studies consisted in determination of the species composition, population density, and biomass of the phytoplankton.

In order to get a more complete determination of the biocenotic character of the investigated ponds, besides the studies on phytoplankton, analyses of species composition of zooplankton and investigations on vascular plants and attached algae living at the bottom were carried out. A precise methodology of biological research was discussed by Kalisz (1972). Samples were collected at two-week intervals (sometimes at 3-day intervals). Meteorological data from the State Hydro-Meteorological Institute in Warsaw referred to the mean daily air temperature, monthly and daily total solar energy and insolation (Table II).

3. RESULTS

DYNAMICS OF PHYTOPLANKTON POPULATION IN THE PONDS OF KIELCE AND CZĘSTOCHOWA

Table III. The list of planktonic algae found in the ponds of the Sewage Treatment Plant in Kielce

Species	Pond				
	No. 1	No. 2	No. 3	No. 4	No. 5
Cyanophyta					
Oscillatoria pseudogeminata G. Smith	+	+		+	+
Bacillariophyceae					
Amphora ovalis Kütz.					+
Fragilaria crotonensis (Kitton) Kütz.				+	+
Gomphonema olivaceum Lyngb.				+	
Nitzschia dubia W. Sm.	+	+	+	+	+
Nitzschia palea (Kütz.) W. Sm.	+	+	+	+	
Synedra acus Kütz.		+	+	+	
Synedra ulna (Nitsch) Ehr.		+	+		
Chlorophyta					
Characium limneticum Lemmerman	+	–	+	+	+
Characium gracilipes F. D. Lampert	+	+	+	+	+
Coleastrum microporum Naegali	+	+	+		+
Dictiosphaerium pulchellum Wood		+		+	+
Ankistrodesmus falcatus (Corda) Ralfs		+		+	+
Actinastrum Hantzschii Lagerheim		+			
Pediastrum Boryanum (Turpin) Menigh	+	+	+	+	+
Pediastrum Tetras (Ehrenberg) Ralfs		+			
Scenedesmus quadricauda var. alternans G. M. Smith	+	+	+	+	+
Scenedesmus acuminatus (Lagerheim) Chodat		+		+	+
Scenedesmus obliquus (Turp.) Kützing.		+		+	+
Tetraëdron caudatum (Corda) Hans		+	+	+	
Tetraëdron quadratum (Reinsh) Hansgirg		+	+	+	
Oocystis sp.		+			+
Closterium moniliferum (Bory) Ehr.	+	+	+	+	
Pandorina morum (Müll.) Bory					+

In the material collected for the studies on phytoplankton from the ponds in Kielce, 23 species were determined and one component was classified in respect of its genus only. Plankton algae found in the investigated ponds were the representants of Cyanophyta, Chrysophyta (solely Bacillariophyceae), and Chlorophyta (Table III). Phytoplanktor from ponds No. 2, 4, and 5, differed radically from that collected in ponds No. 1 and 3. While in the former Chlorophyta were predominating, in the latter phytoplankton was represented by Cyanophyta, Bacillariophyceae and Chlorophyta, without a distinct predominance of any group, and showed a considerably smaller density of population. In general, it can be said that the phytoplankton density was decidedly higher in ponds No. 2 and 4 than in the other ones.

In the phytoplankton material collected from the ponds in Częstochowa, 25 species were determined (Table IV). In 3 components it was

Table IV. The list of planktonic algae found in the ponds of the Experimental Sewage Treatment Plant in Częstochowa

Species	Pond		
	No. 1	No. 2	No. 3
Cyanophyta			
Phormidium sp.	+	+	+
Oscillatoria sp.			
Euglenophyta			
Euglena viridis Ehrb.	+	+	—
Euglena proxima Dang.	+	—	—
Euglena acus Ehrb.	+	+	+
Phacus pleuronectus (O.F.M.) Duj	+	+	+
Phacus longicauda (E.) Duj.	+	+	—
Phacus caudatus Hübn.	+	—	—
Phacus pyrum (Ehrb.) Stein	+	—	—
Trachelomonas volvocina E.	+	+	+
Trachelomonas hispida (Perty) Stein	+	—	—
Lepocinclis Marsonii Lemm.	+	—	—
Lepocinclis ovum (E.) Lemm.	+	+	—
Bacillariophyceae			
Nitzschia palea Kütz. W. Sm.	+	+	—
Synedra ulna (Nitzch) Ehr.	+	+	—
Synedra acus Kütz.		—	—
Navicula cuspidata Kütz.	+	—	—
Navicula gracilis Ehr.	—	+	+
Chlorophyta			
Chlamydomonas sp.	+	+	+
Chlorogonium elongatum Dangeard	+	—	—
Pandorina morum (Müll.) Bory	+	+	+
Chlorella vulgaris Beijerinck	+	+	+
Scenedesmus quadricauda (Turp.) Breb.	+	+	+
Scenedesmus obliquus (Turp.) Kütz.	+	+	+
Scenedesmus acuminatus (Lagerh.) Chod.	—		+
Ankistrodesmus falcatus (Corda) Ralfs	—	+	+
Dictiosphaerium pulchellum Wood	+	+	+

possible to define genus only. The phytoplankton from those ponds was represented by the following systematic groups: Cyanophyta, Euglenophyta, Chrysophyta (Bacillariophyceae community) and Chlorophyta. Euglenophyta and Chlorophyta were predominating. Bacillariophyceae occurred relatively unfrequently and Cyanophyta only sporadically.

Differences observed in various ponds were mainly quantitative, the species composition of phytoplankton was in general approximate. The highest degree of phytoplankton development was observed in pond No. 1. In pond No. 2 algae were distinctly less frequent. Pond No. 3 was the poorest among the other ones, in respect of the frequency distribution as well as the population density. The maximum of the phytoplankton development in all investigated ponds was observed in summer and autumn.

GENERAL CHARACTERISTICS OF ZOOPLANKTON IN THE PONDS

To give a fuller picture of the biocenosis in the investigated ponds, attention was turned to zooplankton as one of its important components.

Three basic zooplankton groups: Rotatoria, Cladocera, and Copepoda, were observed in the ponds.

In the Kielce ponds (No. 2, 4 and 5) a greater variety of species and greater numbers of Rotatoria were found, as compared with other ponds. The most numerous representants of Rotatoria were *Brachionus calciflorus* Pall, *Philinia longiseta* Ehr., *Polyarthra sp.*, while slightly less numerous were *Lepadella patella* O.F.M., *Testudinella patina* Herr., *Rotaria rotatoria* Pallas, *Brachionus rubens* Ehr., *Brachionus angularis* Gosse, *Keratella quadrata* O.F.M., and *Keratella cochlearis* Gosse. The numbers of the representants of Copepoda were at a similar level in all the ponds, except one (pond No. 5). The greatest density of population of that group was reached directly after the filling up of the ponds with water and the greater part of the individuals occurring at that time were their larval forms. Cladocera were represented mainly by *Daphnia magna* (Lillj) and *Bosmina longirostris* O.F. Müll.

It is worth to emphasize that the numbers of zooplankton, especially of Cladocera, is inversely proportional to that of algae. In pond No. 2 the representants of Cladocera were not found at all, and in pond No. 4 they were observed but sporadically. The above mentioned ponds were characterized by intensive development of phytoplankton.

In the Częstochowa ponds, just the same as in the ponds of Kielce, Rotatoria was the most abundant component of zooplankton. The most frequently occurring species were *Brachionus calciflorus* Pallas, *Brachionus rubens* Ehrb., *Brachionus angularus* Gosse, *Lepadella patella* Müller, *Keratella cochlearis* Gosse, *Philodina roseola* Ehrb., and *Rotaria rotatoria* Pallas. Less frequently found were such species as *Polyarthra vulgaris* Carlin, *Asplanchna priodonta* Gosse, and *Testudinella patina* Hermann.

The maximal development was reached by Rotatoria in summer, then the number of individuals amounted to several thousands per 1 liter. The period of late autumn and winter appeared to be less favourable to their development. Copepoda and Cladocera were less abundant in comparison with Rotatoria, nevertheless, in general, they were observed as a regular component of zooplankton in the investigated ponds, especially in summer and autumn.

DESCRIPTION OF THE VASCULAR VEGETATION AND ATTACHED ALGAE LIVING IN THE PONDS

Rooted plants as well as algae living at the bottom of the pond, constitute a vital biotic factor in the characteristics of plankton.

In Kielce, in ponds No. 1 and 3 *Polygonum amphibium* L., *Rhumex hydrolapathum* Hudson, and *Equisetum sp.* were found. Pond No. 2 was devoid of rooted plants, while in ponds No. 4 and 5 only single specimens of *Typha latifolia* L., *Scirpus lacustris* L., *Rhumex hydrolapathum* Hudson and *Sparganium ramosum* Huds., were present. In pond No. 4 considerable number of bryophytes, water mosses, *Drepanocladus fluitans* (L) Warst. and *Fontinalis antipyretica* L., was also observed. In all the investigated ponds the bottom was covered with attached algae, *Cladophora fracta* (Vahl.) Kütz., which were growing most densely in pond No. 5.

Although the vascular plants covering the bottom of the ponds in Częstochowa were abundant, there was no marked differences in vegetation composition between the ponds. The plants found most frequently were *Ceratophyllum demersum* L., *Polygonum amphibium* L., and *Sparganium ramosum* Huds. The Częstochowa ponds showed a disposition to become overgrown with duckweed (*Lemna minor* L.), which by covering the surface of the water level hindered the penetration of light into the deeper water layers. In the summer-autumn season its growth was so thick that it was necessary to remove it mechanically from the surface of the ponds.

BIOMASS OF PHYTOPLANKTON

Figure 4 shows the mean monthly biomass of the various groups of algae (Cyanophyta, Bacillariophyceae and Chlorophyta) from the Kielce ponds. The maximum total biomass value in pond No. 1 amounted to as few as 0.0979 g/m^3, though apparently there were favourable conditions for the development of algae (small depth of the pond, good insolation, and a long detention time of sewage).

In pond No. 2, with hydraulic loading and the BOD_5 loading slightly higher as compared with pond No. 1, the maximum of the algae development occurred in October, then the biomass amounted to 257.527 g/m^3, as compared with its minimum value of 0.0037 g/m^3 in

July, i. e. at the initial stage of the experiment. Chlorophyta had the greatest share in the total biomass of algae.

In pond No. 3, where Cladocera occurred in abundance, the biomass values were low, just the same as in pond No. 1. The maximum biomass value of 0.288 g/m³ was noted in October. However, results obtained from this pond are not conclusive due to the considerable infiltration of water. The unfavourable subsoil (high infiltration coefficient) deranged thoroughly the working of the pond, in consequence, after some time it was excluded from the service.

Pond No. 4, working at the technological parameters twice as high as those in the other ponds, showed a high increase in biomass ranging

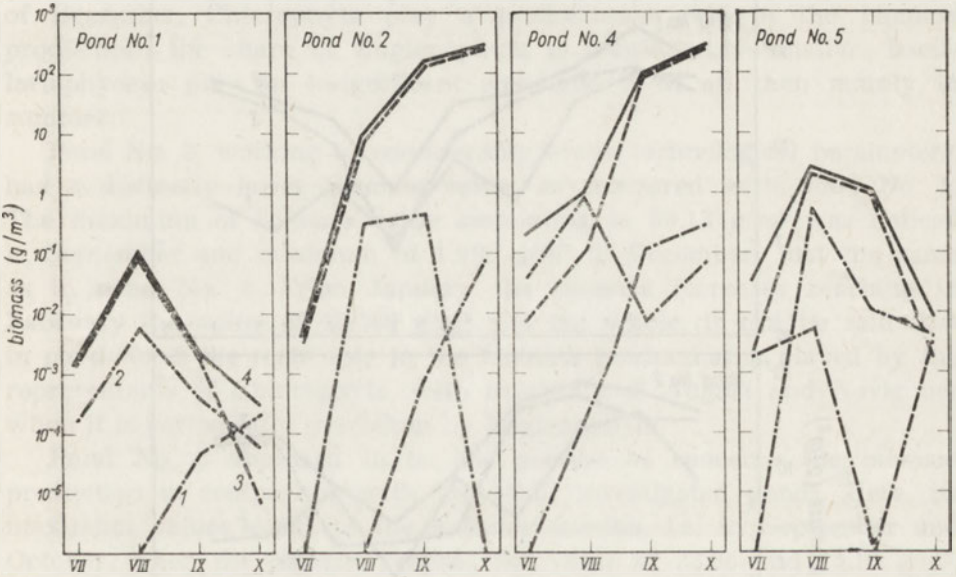

Fig. 4. Phytoplankton biomass in the ponds of the Sewage Treatment Plant in Kielce. 1 — Cyanophyta, 2 — Bacillariophyceae, 3 — Chlorophyta, 4 — total algae

from 0.23 g/m³ in July up to 225.33 g/m³ in October. Immediately after the inflow of sewage, in July and also in August, Bacillariophyceae were the main component of the biomass, whereas in September and October the Chlorophyta group was prevalent.

In pond No. 5, with the highest hydraulic loading and BOD_5 loading of pollution, the biomass increment at the beginning of investigations, amounting to 0.0377 g/m³, was similar to that in ponds No. 2 and 4. In August the biomass increment amounted to 2.471 g/m³ and was more than twice higher than in pond No. 4. As the time went on, not only there was no increase in the development of algae, but a marked decrease was noticed, instead. This may be explained by the fact that

already at the beginning of September there was a mass development of Cladocera (*Bosmina longirostris*) feeding on algae.

Summing up, it can be said that in July, after the inflow of sewage, in all the ponds there was a minimal increase in biomass, ranging from 0.0009 g/m³ in pond No. 3 to 0.232 g/m³ in pond No. 4, and the predominance of any group of algae was not noticed at that time. In August, however, Chlorophyta group prevailed in the biomass of all the ponds, except in pond No. 4. In September, the ponds were already differentiated into two distinct groups: one group was characterized by mass development of algae and high biomass value, the other by low biomass value.

The monthly mean biomass value of each group of algae is given in Fig. 5.

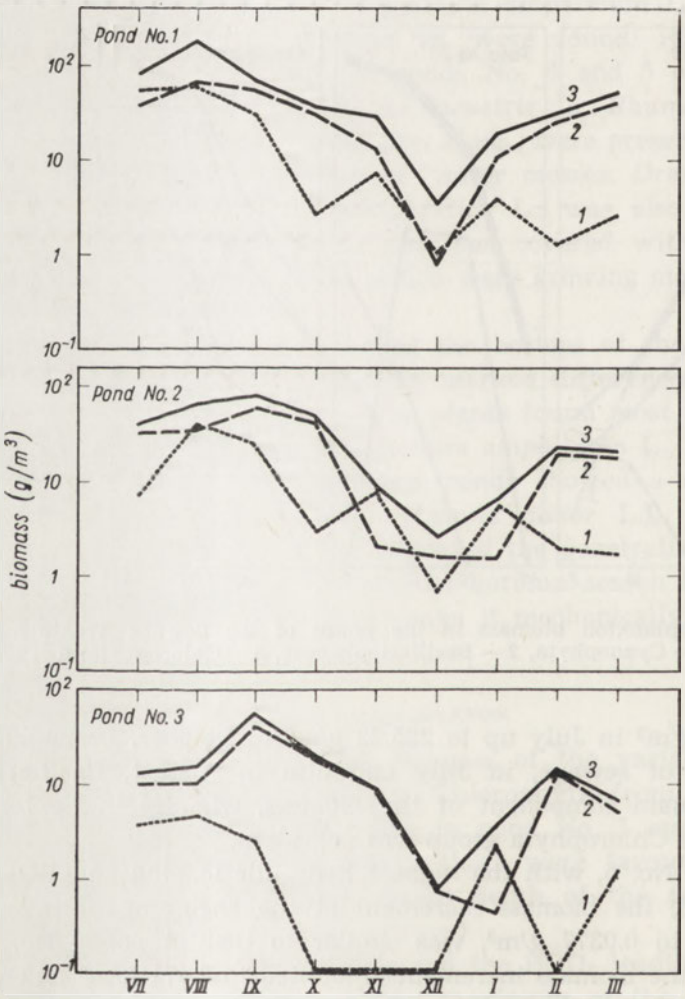

Fig. 5. Phytoplankton biomass in the ponds of the Experimental Sewage Treatment Plant in Częstochowa. 1 — Euglenophyta, 2 — Chlorophyta, 3 — total algae

On the whole, all the ponds differed considerably from one another in respect of the biomass value [1].

In July 1967, at the beginning of the investigations, the biomass value amounted to 87.78 g/m^3, in August — 109.25 g/m^3. Beginning from September, the biomass value was lowering gradually until it reached its minimum of 2.81 g/m^3 in December. Then, there was again an increase in the biomass value up to 14.68 g/m^3 in January, 22.49 g/m^3 in February, and 35.25 g/m^3 in March. Data obtained in the typical vegetative period, i.e. in July, show that Euglenophyta are the main biomass producer (48.5%), Chlorophyta are the second (32%) and Bacillariophyceae the third (19.5%). From August till March, with exception of December, Chlorophyta play a predominant role in the biomass production; the share of Euglenophyta is considerably smaller, Bacillariophyceae play an insignificant role, and if at all then mainly in summer.

Pond No. 2, working at considerably lower technological parameters, had a distinctly lower biomass value, as compared with pond No. 1. The maximum of biomass value amounting to 59.13 g/m^3 was noticed in September and minimum of 1.990 g/m^3 in December, just the same as in pond No. 1. From January the biomass increases reaching in February the value of 14.309 g/m^3. On the whole, it can be said that in pond No. 2 the main role in the biomass production is played by the representants of Chlorophyta, with exception of August and November when it is periodically overtaken by Euglenophyta.

Pond No. 3 appeared to be the poorest as concerns the biomass production in comparison with the other investigated ponds. Here, its maximum values occur in the autumn months, i.e. in September and October, when the biomass reaches the value of 33.66 and 15.10 g/m^3, respectively. A considerable decrease of biomass is observed in late autumn with the minimum value of 0.60 g/m^3 occurring in December. Then, there is again a gradual increase up to 2.09 and 11.830 g/m^3 in January and February, respectively, and once more a decrease to 6.66 g/m^3 in March. In the biomass production, as in other ponds, Chlorophyta are placed first, Euglenophyta — second, Cyanophyta and Bacillariophyceae are insignificant.

Maximal values of the biomass were noted in pond No. 1, considerably lower in pond No. 2, the lowest in pond No. 3. The phenomenon of the most dynamic development of the phytoplankton in pond No. 1 and the gradual lowering of the biomass value in the subsequent ponds suggest that the most favourable conditions for the development of algae were in pond No. 1. The gradual depletion of food

[1] The ponds have been filled with water since 1966, and the investigations started in July 1967.

substances suitable for algae was probably the limiting factor in their development in the subsequent ponds.

THE INTERDEPENDENCE OF TEMPERATURE, pH, CARBON DIOXIDE, AND DISSOLVED OXYGEN IN THE PONDS

In order to get a more comprehensive picture of the role of algae in the process of photosynthesis in correlation with the determined physico-chemical factors, the interdependence of algae and temperature, pH, free carbon dioxide, dissolved oxygen were analysed in 24 hr cycles (every 3 hrs).

The two most representative ponds in Kielce were taken under consideration, namely, pond No. 1 with a weak development of phytoplankton and pond No. 2 with an intensive one.

It was found that in the latter the dissolved oxygen content was much higher than in the former one (Fig. 6). The highest content of

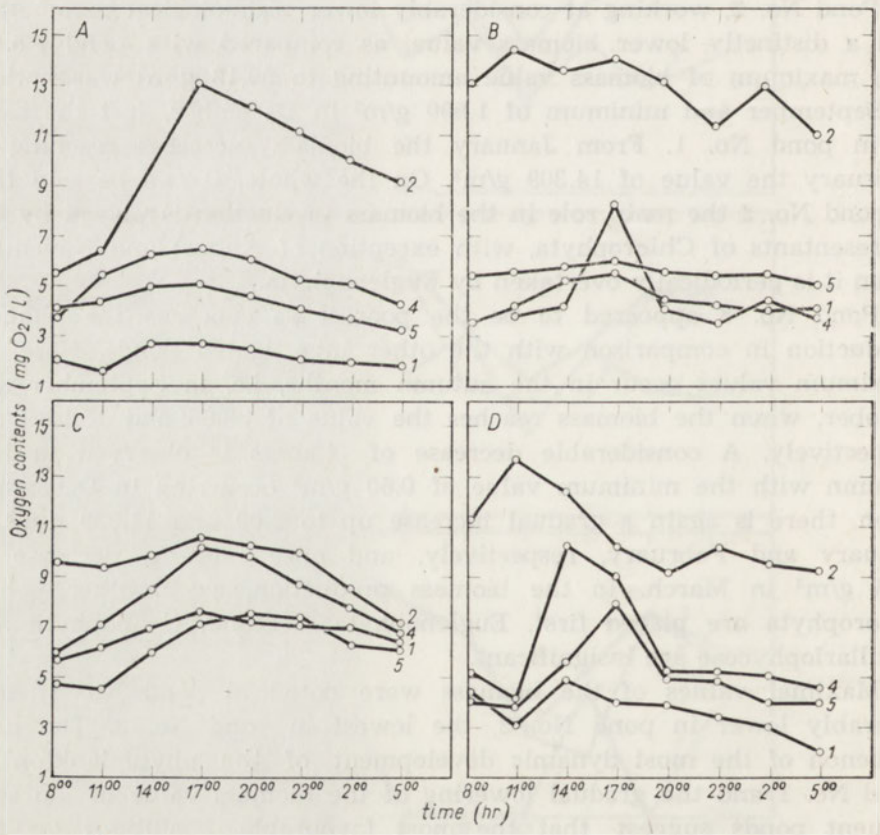

Fig. 6. Changes of dissolved oxygen contents during 24 hr records in Sewage Treatment Plant in Kielce. 1–5 — pond numbers. A — July 31–Aug. 1, B — Aug. 27–28, C — Sept. 28–29, D — Oct. 16–17

Table V. Changes of temperature, pH, free CO_2 during four 24-hr records in ponds No. 1 and 2 of the Sewage Treatment Plant in Kielce

	O'clock	July 31—Aug. 1			Aug. 27—28			Sept. 28—29			Jan. 16—17		
		Temp. (°C)	pH	CO_2 (mg/l)	Temp. (°C)	pH	CO_2 (mg/l)	Temp. (°C)	pH	CO_2 (mg/l)	Temp. (°C)	pH	CO_2 (mg/l)
Pond No. 1	8	23	7.95	9.5	15	7.30	9.0	13	8.85	10.5	11	7.8	9.8
	11	23	7.75	7.5	15	7.35	11.5	12	8.80	10.0	11	8.0	9.0
	14	24	7.70	9.5	17	7.35	9.5	14	7.70	3.5	12	8.0	4.5
	17	25	7.70	8.5	17	7.60	7.5	12	8.05	3.5	12	7.9	4.0
	20	23	7.55	10.0	17	7.60	7.5	12	8.25	4.5	12	7.8	4.2
	23	22	7.40	10.5	16	7.50	11.0	12	8.00	5.5	11	7.7	7.8
	2	22	7.32	13.5	15	7.30	11.5	12	8.00	9.0	11	7.7	9.0
	5	21	7.20	15.0	15	7.25	12.5	12	8.10	5.5	11	7.6	8.5
Pond No. 2	8	24	8.75	5.6	15	8.6	0	11	9.65	0	11	9.0	0
	11	24	9.00	0	16	9.0	0	11	9.60	0	11	9.0	0
	14	26	9.00	0	17	9.0	0	11	10.50	0	12	9.4	0
	17	27	9.00	0	17	9.0	0	10	10.30	0	12	9.4	0
	20	24	9.00	0	17	9.0	0	10	10.20	0	11	8.9	0
	23	23	9.00	0	16	9.0	0	10	9.75	0	11	8.8	0
	2	22	9.00	0	15	8.5	0	10	9.70	0	11	8.7	0
	5	22	9.00	0	15	8.5	0	10	9.40	0	11	8.7	0

Table VI. Analyses of oxygen conditions in the ponds in

Pond	Month	Mean water temp. (°C)	Average visible lt energy (cal/day)		Average sewage BOD_5 (mgO/l)	Alage conc. C_c (mg/l)	Yield of cell material Y_c (mg/l · day	Combustion heat H (cal · day)
			S per cm²	S' per 1				
Kielce								
Pond No. 1	July	23.4	336	48.0	5.2	0.00	0.00	0.00
(depth 70 cm,	Aug.	21.5	334	47.5	4.5	0.10	0.00	0.01
detention pe-	Sept.	14.7	250	35.5	6.9	0.00	0.00	0.00
riod 50 days)	Oct.	12.0	122	17.4	5.1	0.00	0.00	0.00
Pond No. 2	July	24.3	336	32.0	5.2	0.00	0.00	0.00
(depth 105 cm,	Aug.	22.5	334	31.8	4.5	7.65	0.33	2.00
detention pe-	Sept.	14.4	150	14.4	6.9	121.42	5.30	31.8
riod 23 days)	Oct.	11.0	122	11.7	5.1	257.53	11.20	67.0
Pond No. 4	July	23.1	336	43.0	5.2	0.23	0.02	0.14
(depth 78 cm,	Aug.	24.0	334	43.0	4.5	1.07	0.11	0.64
detention pe-	Sept.	14.7	150	19.2	6.9	87.34	8.73	52.3
riod 10 days)	Oct.	12.)	122	15.6	5.1	225.33	22.53	135.0
Pond No. 5	July	24.0	336	30.0	5.2	0.04	0.013	0.07
(depth 113 cm,	Aug.	22.5	334	29.6	4.5	2.47	0.82	4.94
detention pe-	Sept.	14.7	150	13.3	6.9	0.92	0.31	1.83
riod 3 days)	Oct.	12.0	122	10.8	5.1	0.01	0.00	0.02
Często-								
chowa	July	22.8	460	30.5	29.0	83.83	23.30	140.0
Pond No. 1	Aug.	21.4	373	24.7	37.5	109.26	30.40	182.0
(depth 151 cm,	Sept.	19.0	263	17.4	35.7	82.26	22.90	137.0
detention	Oct.	13.7	149	9.9	31.0	28.49	7.92	57.5
period 3.6 days	Nov.	6.9	75	4.9	24.3	19.52	5.43	32.6
July–Nov.,	Dec.	4.5	39	2.6	23.5	2.82	0.54	3.24
5.3 days	Jan.	3.8	51	3.4	22.8	14.68	2.77	16.6
Dec.–March)	Febr.	4.8	88	5.84	21.7	22.49	4.25	25.5
	March	6.2	230	15.2	21.8	35.26	6.65	40.0
Pond No. 2	July	24.2	460	31.3	20.6	29.27	8.62	51.7
(depth 147 cm,	Aug.	21.8	373	25.4	12.8	53.82	15.80	95.0
detention	Sept.	18.5	263	17.9	22.8	59.14	17.40	104.0
period: 3.4 days	Oct.	14.2	149	10.8	15.8	36.27	10.65	64.0
July–Nov.,	Nov.	6.2	75	5.1	13.5	9.94	2.92	17.5
5.2 days	Dec.	3.8	39	2.7	11.1	1.99	0.38	2.28
Dec.–March)	Jan.	2.8	51	3.5	13.9	6.86	1.32	7.90
	Febr.	4.5	88	6.0	13.3	14.31	2.76	16.6
	March	5.3	230	15.7	12.6	12.92	2.41	14.5
Pond No. 3	July	24.0	460	26.2	10.3	11.59	3.21	19.2
(depth 175 cm,	Aug.	21.5	373	21.3	12.7	12.85	3.57	21.4
detention	Sept.	18.5	263	15.0	18.0	33.66	9.35	56.0
period	Oct.	13.7	149	8.5	9.7	15.10	4.20	25.2
3.6 days	Nov.	6.5	75	4.3	12.0	4.85	1.34	8.05
July–Nov.,	Dec.	4.0	39	2.2	10.5	0.61	0.11	0.66
5.5 days	Jan.	3.5	51	2.9	12.1	2.09	0.38	2.28
Dec.–March)	Febr.	5.3	88	5.0	12.5	11.83	2.33	14.0
	March	6.0	230	13.1	11.0	6.62	1.20	7.20

relation with phytoplankton biomass and light energy

$F=H/S'$	d/D	L_1/S	F_C	Oxygen factor $P=F/F_C$	$W_O=27.1\ F\cdot S$ (kgO_2/ha · day)	Dissolved oxygen		BOD_5 load (kg/ha · day)		Reduction (%)
						mg O_2/l	kgO_2/ha	influx	outflow	
0.00	1.4	0.02	0.01	0.00	0.00	3.2	22.6	0.7	0.5	3.3
0.00	1.4	0.01	0.00	0.04	0.02	5.5	38.4	0.8	0.5	1.8
0.00	1.4	0.03	0.01	0.00	0.00	4.7	33.0	0.9	0.5	49.5
0.00	1.4	0.04	0.02	0.00	0.00	8.7	61.0	0.7	0.4	32.0
0.00	4.6	0.02	0.03	0.00	0.00	9.0	93.5	2.2	1.5	31.0
0.09	4.6	0.10	0.02	3.55	5.64	11.0	116.0	1.9	1.5	20.0
3.18	4.6	0.03	0.05	28.70	19.0	12.3	129.0	3.0	1.5	48.0
8.20	4.6	0.04	0.07	81.00	272.0	20.3	214.0	2.3	1.5	34.0
0.00	7.8	0.02	0.04	0.07	0.29	4.1	32.0	4.0	1.7	55.0
0.01	7.8	0.01	0.03	0.49	1.34	3.4	26.5	3.5	1.7	51.0
2.72	7.8	0.03	0.08	20.30	110.0	7.2	56.2	5.4	1.7	68.0
8.65	7.8	0.04	0.12	71.50	284.0	13.8	108.0	4.0	1.7	57.0
0.00	37.7	0.02	0.22	0.00	0.23	3.6	40.6	19.6	13.2	32.6
0.17	37.7	0.01	0.15	1.13	15.0	4.1	46.2	17.0	13.2	22.4
0.14	37.7	0.03	0.38	0.21	5.54	5.4	61.0	26.0	13.2	49.0
0.02	37.7	0.04	0.58	0.00	0.05	11.9	134.8	19.2	13.2	31.0
4.60	42.0	0.06	0.97	4.70	575.0	6.0	90.5	122.0	86.0	30.0
7.35	42.0	0.10	1.56	4.70	742.0	3.5	53.0	158.0	54.0	66.0
7.85	42.0	0.14	2.11	3.70	560.0	5.7	86.0	150.0	96.0	36.0
5.80	42.0	0.21	3.24	1.80	234.0	5.2	78.5	130.0	66.5	49.0
6.60	42.0	0.32	5.05	1.31	134.0	4.3	65.0	102.0	56.5	44.5
1.24	28.5	0.60	6.30	0.20	13.2	2.5	37.7	65.0	31.6	51.0
4.90	28.5	0.45	4.70	1.04	67.9	1.7	25.6	63.0	39.6	37.0
4.35	28.5	0.25	2.20	1.61	103.0	5.8	87.5	62.0	38.0	39.0
2.63	28.5	0.09	1.00	2.63	164.0	7.6	114.0	62.0	36.0	42.0
1.65	43.2	0.04	0.72	2.30	206.0	5.9	86.5	89.0	44.5	50.0
3.74	43.2	0.03	0.55	6.80	377.0	6.1	89.5	55.0	54.0	20.0
5.80	43.2	0.09	1.39	4.17	413.0	6.0	88.0	98.5	78.0	20.8
5.90	43.2	0.11	0.69	3.50	239.0	6.9	101.0	68.0	42.0	38.0
3.43	43.2	0.18	2.88	1.20	70.0	4.9	72.0	58.5	52.0	11.1
0.86	28.3	0.28	2.97	0.29	9.2	2.7	39.0	31.4	29.7	5.5
2.28	28.3	0.27	2.86	0.80	31.5	1.7	25.0	39.2	34.2	12.7
2.77	28.3	0.15	1.58	1.75	66.0	5.6	82.0	37.5	35.4	5.6
0.92	28.3	0.05	0.58	1.60	57.5	6.0	88.0	35.5	31.2	12.0
0.73	48.5	0.22	0.40	1.83	92.0	8.4	147.0	41.1	28.4	31.0
1.00	48.5	0.03	0.61	1.64	101.0	9.1	158.0	51.0	43.0	15.7
3.72	48.5	0.07	1.23	3.00	266.0	8.2	143.0	72.0	63.0	12.5
2.96	48.5	0.06	1.16	2.55	119.0	6.5	114.3	38.8	31.6	18.5
1.87	48.5	0.16	2.86	0.65	38.0	5.2	91.0	48.0	45.0	6.3
0.30	31.8	0.27	3.14	0.09	3.14	3.1	55.0	27.9	24.0	14.0
0.78	31.8	0.24	2.78	0.28	10.85	2.7	47.2	32.0	33.0	0.0
2.80	31.8	0.14	1.67	1.67	67.0	5.3	92.5	33.1	34.0	0.0
0.55	31.8	0.06	0.68	0.81	34.2	8.6	150.0	26.1	26.0	10.7

dissolved oxygen was noted between 2 and 5 p.m., the lowest between 2 and 8 a.m. Differences in oxygen content between the two ponds were considerable, throughout the 24 hr period, in pond No. 2 its value amounted to more than twice as much as in the other one. The relation of the oxygen and carbodioxide was inversely proportional (Table V). Likewise, the pH value increased with the decrease of CO_2 content. The very great difference in oxygen content in pond No. 1 and 2, should be especially emphasized. When in pond No. 1, with weak development of phytoplankton, the maximum content of dissolved oxygen in particular 24 hr cycles amounted to 2.75, 5.6, 7.5 and 4.5 mg O_2/l, then in the other pond abounding with phytoplankton, the corresponding values were much higher: 13.0, 14.4, 10.5 and 13.7 mg O_2/l. And the mean values from four 24 hr cycles were 4.26 and 10.7 mg O_2/l, respectively. Doubtlessly, this on an average 2.5-fold increase in the oxygen content is due to the greater number of algae. The next noteworthy instance is the observed convergence of fluctuations of the oxygen content and the temperature in the 24 hr cycle. In the first 24 hr cycle (July 31 — Aug. 1), in pond No. 2, there was noted a considerable temperature fluctuation: 22—27°C (a very sunny day) and also analogically great fluctuation of the dissolved oxygen content, ranging from 5.6 to 13.0 mg O_2/l. In pond No. 1, notwithstanding considerable temperature fluctuations no significant variations were observed in the oxygen content. During the subsequent 24 hr cycles, an increase in the oxygen content occurred regularly in pond No. 2, whereas its value in pond No. 1 remained pretty much the same. This fact may be taken as evidence of the important role of algae in the oxygen production through photosynthesis.

PHOTOSYNTHETIC EFFICIENCY OF ALGAE

Efficiency in oxygen production by plant organisms, in this case phytoplankton algae, depends on density of their population and the quantity of light energy per area unit.

It was assumed as the basis for the present considerations that the quantity of oxygen required for stabilization of polluting matters, expressed in BOD_5, derives from the process of photosynthesis only. The obvious fact of the supplementary enrichment of the environment in oxygen from reaeration was left out of account. Basing on that postulate it was necessary to calculate, on the one hand, the value of critical photosynthetic efficiency expressed in per cent (F_c) depending on BOD_5, light energy, depth, sewage detention time, and on the other, the actual photosynthetic efficiency (F), depending on the phytoplankton biomass and insolation. It was estimated that for production of 1 mg of algae as much energy is required as obtained from combustion of 1 mg of algae, i.e. 6 cal (Oswald et al. 1957). The F/F_c ratio characterizing

oxygen conditions in the environment is generally denominated as oxigenation factor (*P*).

For a better illustration of the oxygen conditions in the ponds of Kielce and Częstochowa oxygen values are given in mg O_2/l, they were obtained from direct chemical measurements and the oxygen values in kg/ha · day obtained from the expression (3). Results are given in Table VI.

The comparison of those values allows to state that on the whole the minimum oxygen values from direct measurements corresponding to its lowest values from the formula (3) and vice versa (e.g. in pond No. 2 in October, 20.3 mg O_2/l from chemical determination of the oxygen level can be conversed into 214 kg O_2/ha, comparable value computed from the formula amounts to 272 kg O_2/ha · day).

It has to be emphasized that at the low value of phytoplankton density the value of the oxygen level from direct measurements is in most cases higher than it would result from the actual photosynthetic efficiency calculated from the formula. On the contrary, at high value of phytoplankton density the dissolved oxygen values are slightly lower than it results from the calculations. On the basis of the investigations in Kielce it can be presumed that oxygen production in the process of photosynthesis starts to be decisive only then when the concentration of algae is slightly greater than 0.5 mg O_2/l · day.

In spite of charging the ponds with a reduced load of polluting matters the process of purification continued further on. The reduction of the load of waste matters in ponds No. 2 and 4 amounted on an average to 33.4 and 57.5%, respectively.

4. DISCUSSION

The analysis of the obtained results allows to declare that species composition of phytoplankton in the ponds of Częstochowa showed marked differences in comparison with those in Kielce. While in the latter Chlorophyta, in particular Chlorococcales, were decidedly predominant, in the former two groups of algae predominated: Chlorophyta (Chlorococcales, Volvocales), and Euglenophyta. Cyanophyta and Bacillariophyceae were present in insignificant numbers in the ponds of both places.

Scenedesmus genus dominated in the phytoplankton of the Kielce ponds. Representants of the genera Phacus, Euglena, Chlamydomonas and Chlorella were predominant in the Częstochowa ponds.

The species composition from both places was in accordance with data from the literature (Zakharov, Konstantinova 1929, Silva, Papenfuss 1953, Malchov-Meller et al. 1955, Bick,

Scholtysek 1960, Sivko, Sokolova 1964, Singh, Saxena 1969).

In the first period of operation of the Kielce ponds the daily production of algae was very low, in consequence the oxygenation factor (P) value was also low. With the biomass increase in the ponds the oxygen conditions improved.

Notwithstanding the longest detention time of sewage in pond No. 1 there was a very weak development of phytoplankton (the Y_c maximum value in August — 0.00196 mg/l. day) and therefore the oxygenation factor was very low.

Generally speaking, the best oxygen conditions were found in ponds No. 2 and 4, i. e. those in which the highest production of the phytoplankton was noted. They showed a fairly high oxygenation factor after a month of experiment already. This was confirmed by the chemical determination of the dissolved oxygen. The maximum daily increase in algae production occurred in October; average Y_c — 11.20 and 22.53 mg/l · day for ponds No. 2 and 4, respectively. The highest P values was also noted in October; 81.0 and 71.5, respectively.

Taking into account that the process of biological purification of sewage in the ponds has a normal course when P value is slightly greater than 1.0 the charge of the waste matters load could be much higher in the ponds characterized by intensive phytoplankton development.

The investigations of Częstochowa ponds showed the highest Y_c values in pond No. 1 — average 11.5, maximum — 30.4, minimum — respectively. In pond No. 3 they were the lowest — 2.086, 9.35, 0.54 mg/l · day. In pond No. 2 they were lower — 6.9, 17.4, 0.38 mg/l · day, 0.111 mg/l · day, respectively.

The above presented data show a distinct decrease in the biomass production in subsequent ponds. It can be inferred that in the course of the purification of sewage the environment in the subsequent ponds became more and more deficient in indispensable food components.

The actual photosynthetic efficiency (F) oscillated rather nearer to the lower limit of the potential efficiency. The F value averaged approximately 5% (oscillation range 1.24–7.85%), 3% (0.92–5.90%), 1.65% (0.297–3.72%) for the subsequent ponds 1, 2 and 3, respectively.

The critical photosynthetic efficiency (F_c), characterizing the given oxygen demand, averaged in pond No. 1 3.1% (ranging from 0.975 to 6.3%). This value was also nearer the bottom limit of the theoretical possibility (upper limit 12.5%). In pond No. 2 the F_c values were still smaller, averaging 1.7% (ranging from 0.55 to 2.97%). In pond No. 3 — 1.6% (0.40–3.14%).

The oxygenation factor value was $P > 1.0$. In pond No. 1, the mean P value was 3.1 (oscillation ranging from 0.197 to 4.7). A sharp defi-

ciency of oxygen occurred in December; in January there was an equilibrium between the requirement and the photosynthetic efficiency.

In pond No. 2, the mean P value averaged 2.5 (maximum 6.8, minimum 0.29). Similarly to pond No. 1, there was a deficiency in oxygen in December and January.

In pond No. 2, the mean P value averaged 2.5 (maximum 6.8, mi- in oxygen occurred in November, December and January. From the above presented values it is evident that the mean value of the oxygenation factor (P) decreases proportionally to the biomass in the subsequent ponds, from 3.1 in pond No. 1 to 1.4 in pond No. 3.

The control experiments on dissolved oxygen confirmed the accordance of the dissolved oxygen with the factor P. In pond No. 1 the lowest oxygen content was recorded in December and January (1.7—2.5 mg O_2/l), i. e. in those months when the value of the factor P was lowest. In ponds No. 2 and 3, likewise, the minimum values of dissolved oxygen and factor P occurred in the same months. Analogically, with exception of few cases which must be treated as non-typical, the maximum P values went along with the maximal values of dissolved oxygen.

The actual efficiency in oxygen production in kg/ha · day through photosynthesis (W_0) computed in Table VII is approximate to the value given in the Introduction. The average value for pond No. 1 throughout the experiment period amounted to 288 kg O_2/ha · day (oscillation from 13.2 kg O_2/ha · day in December to 742 kg O_2/ha · day in August). Analogically the values for pond No. 2 are considerably smaller — 163 (from 9.2 in December to 413 in September). The smallest values were noted in pond No. 3 — 91.5 (from 3.14 in December to 266 in September) respectively.

It should be also mentioned that in spite of relatively low load of waste matters in the Częstochowa ponds, expressed in kg BOD_5/ha · day, nevertheless, there was a further reduction, averaging 44% (range 30—66%), 19.5% (5.5—50%) and 12% (0—31%) for ponds No. 1, 2 and 3, respectively. The obtained reductions are not very high but one must remember that decomposition of those organic substances remaining after the biological purification is particularly difficult.

The results of working of the ponds, obtained on the basis of calculations from formulae (8) and (9), correspond exactly to the results from physico-chemical analyses. This fact confirms the theoretical postulates accepted in those formulae and at the same time proves that algae are the main producer of oxygen in the ponds.

Thus, knowing the characteristics of sewage and climatic conditions of a given region it is possible to design, manage and control the operating of the ponds.

Summing up, it should be mentioned that the degree of purification

of sewage discharged into the ponds has an effect on the species composition of phytoplankton. A lower degree of purification is favourable to development of Euglenophyta and Chlorophyta, particularly Volvocales and Chlorococcales. On the other hand, strong mineralization of sewage is followed by development of Chlorophyta — Chlorococcales.

Algae occurring in the ponds are characterized by dynamic increase in numbers. The maximum density of population is found in the summer-autumn season. In the Częstochowa ponds the highest phytoplankton increase in conversion into biomass amounted to 30.4 mg/l · day or 304 kg/ha · day. In the Kielce ponds the daily increase in biomass was lower.

At the increase in biomass greater than 0.5 mg/l · day algae, due to photosynthesis, were the main producer of oxygen in the ponds. Maximum oxygen content produced through photosynthesis, computed from the formula $W_o = 27.1 \cdot F \cdot S$, amounted to 742 kg O_2/ha · day (pond No. 1) in Częstochowa, 284 kg O_2/ha · day (pond No. 4) in Kielce. Oxygenation factor (P) characterizing oxygen conditions (ratio of actual and critical photosynthetic efficiency) was proportional to the biomass increase. At the biomass increase greater than 0.5 mg/l · day coefficient (P) was greater than 1.0.

6. SUMMARY

In the biological ponds in Kielce and Częstochowa, working at different technological parameters, complex biological and physico-chemical experiments were carried out, including meteorological data. The object of the study was to determine phytoplankton population in the biological ponds and its share in oxygen production.

On the basis of investigations, it has been established that:

The degree of sewage purification in the ponds has a selective effect on Euglenophyta and Chlorophyta, Volvocales and Chlorococcales, in particular. On the other hand the predominance of Chlorococcales went along with far-fetched mineralization.

The specific characteristics of the investigated ponds was the small number of represented species with a large number of individual organisms. Chlorophyta were predominant in the phytoplankton biomass, yet, in the ponds of Częstochowa, working at higher technological parameters, Euglenophyta had an important share. Cyanophyta and Bacillariophyceae played a secondary role in both groups of ponds. Algae were characterized by a dynamic increase in numbers. Their maximum density occured in the summer-autumn time, the minimum in winter. The highest daily rate of growth of algae amounted to 30.4 mg/l, corresponding to 304 kg/ha · day. Mass developing algae are the main producer of oxygen derived from photosynthesis. Oxygenation factor P (i.e. F/F_c), characterizing oxygen conditions in the ponds, was proportional to the biomass of algae.

The maximum quantity of oxygen produced through photosynthesis in the ponds of Kielce and Częstochowa amounted to 284 and 742 kg/ha · day, respectively.

The results of the present research allow to ascertain that algae living in the ponds in great concentration and with adequate insolation are the main producer of the oxygen obtained through photosynthesis. The quantity of oxygen derived from photosynthesis can be calculated for practical purposes with sufficient exactitude.

7. STRESZCZENIE

Przeprowadzono kompleksowe badania biologiczne i fizyczno-chemiczne, z uwzględnieniem danych meteorologicznych, w stawach biologicznych w Kielcach i Częstochowie, pracujących przy różnych parametrach technologicznych. Celem badań było określenie populacji fitoplanktonu w stawach biologicznych oraz jego udziału w produkcji tlenu.

Na podstawie badań stwierdzono, że:

Stopień oczyszczania ścieków dopływających do stawów wpływał różnicująco na fitoplankton. Niższy stopień oczyszczania ścieków sprzyjał rozwojowi Euglenophyta oraz Chlorophyta, szczególnie Volvocales i Chlorococcales. Natomiast daleko posuniętej mineralizacji towarzyszył rozwój głównie Chlorococcales.

Charakterystyczną cechą badanych stawów była niewielka liczba gatunków przy znacznej liczebności organizmów. Grupami dominującymi w biomasie fitoplanktonu były Chlorophyta, przy czym w stawach w Częstochowie, pracujących przy wyższych parametrach technologicznych, poważny udział przypadał również Euglenophyta. Cyanophyta i Bacillariophyceae zarówno w jednych jak i drugich stawach odegrały rolę raczej drugorzędną. Występujące glony charakteryzowały się dynamicznym zwiększeniem liczebności. Maksymalna ich liczebność przypadała na okres letnio-jesienny, a najniższa w okresie zimowym. Najwyższy uzyskany dzienny przyrost glonów wynosił 30.4 mg/l, co odpowiada 304 kg/ha · doba. Masowo rozwijające się w stawach glony były głównym producentem tlenu wytwarzanego w procesie fotosyntezy. Współczynnik natlenienia P (t.j. F/F_c) charakteryzujący stosunki tlenowe w stawach, kształtował się proporcjonalnie do biomasy glonów.

Maksymalna ilość tlenu wytworzonego w procesie fotosyntezy dla stawów w Kielcach wynosiła 284 kg/ha · doba, a dla stawów w Częstochowie — 742 kg/ha · doba.

Przeprowadzone badania pozwoliły stwierdzić, że glony występujące w stawach przy odpowiednim nasłonecznieniu i zagęszczeniu są głównym producentem tlenu wytwarzanego w procesie fotosyntezy. Ilości tlenu pochodzącego z fotosyntezy, mogą być dla celów praktycznych wyliczone z wystarczającą dokładnością.

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ROLE OF ALGAE IN SEWAGE PURIFICATION. PART II. NUTRIENT REMOVAL

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ABSTRACT

The participation of algae in the process of nitrogen and phosphorus removal from sewage has been determined. It has been established that algae eliminated nutrient components from sewage, and reduction of nitrogen corresponds roughly to its proportional share in the dry weight of algae, whereas reduction of phosphorus was higher. The higher reduction of phosphorus was due not only to the activity of algae but also to the physico-chemical processes occurring in the investigated environment.

1. INTRODUCTION

In clean natural waters concentrations of nitrogen and phosphorus are insignificant, much greater quantities of their compounds are found in polluted waters. Sewage is an important source of nitrogen and phosphorus, owing to its composition made up of organic and inorganic substances, either dissolved or in form of colloids and suspensions.

By using conventional methods in the process of biological sewage purification (activated sludge, trickling filters) according to Wuhrman (1965), 30–50% reduction of nitrogen and about 30% of phosphorus was obtained. The remaining part of nitrogen and phosphorus is drained off together with sewage directly into a reservoir. This, to a considerable degree, renders difficult the possibility of utilization of ground water for communal and industrial use, since the nutrient substances (nitrogen, phosphorus) contained in the biologically purified sewage promote excessive development of algae, and that may lead in turn to an incidental pollution of water reservoirs. Moreover, a high content of nitrogen compounds, particularly in form of non-ionized ammonia at the lowered content of oxygen dissolved in water and low pH, causes the toxicity of waters. The toxic effect of free ammonia occurs already at the concentration of 10 mg $N\text{-}NH_3/l$. To limit the fertility of ground waters it is often stipulated that the content of nitrogen should not exceed 4 mg/l (Gańczarczyk 1966). For that reason a great deal of attention is given, lately, to the problem of nitrogen and phosphorus removal from sewage after its biological purification.

Nitrogen and phosphorus compounds can be removed from sewage by physical, physico-chemical, and biological methods.

The use of physical methods for nitrogen removal from sewage through ionic conversion, adsorption, or thermic exhausting, is limited from economical and technological viewpoint. Those methods allow to remove only the organic and ammonia nitrogen, but they do not solve, at all, the problem of oxygen removal in oxidized form.

The removal of phosphorus from sewage by physico-chemical methods gives satisfactory results (Lea et al. 1954, Neil 1957, Vasseur 1962, Wuhrman 1964). Among those methods, the one with the use of coagulants, such as alumi-

nium and ferrum salts, is worthy notice. By using coagulants it is possible to obtain reduction of phosphorus amounting up to 99% (Lea et al. 1954).

Besides the two mentioned above, one can also use biological methods for nitrogen and phosphorus removal from the sewage.

The removal of nitrogen compounds can be accomplished through denitrification, during which at the oxygen scarcity nitrates, owing to the action of nitrifying bacteria, are reduced into nitrites, ammonium, or N_2. From the viewpoint of practical utilization this process solves the technological problems of municipal sewage purification (Bringmann et al. 1959, Wuhrman 1964). Experiments were also carried out on the possibility of making use of denitrification for the industrial wastes treatment. In the method used by Bringmann et al. (1959) and Wuhrman (1964), as well, sewage undergoes denitrification after the end of biological purification through nitrification.

The removal of nutrient substances by algae found practical application in the biological ponds as the so-called tertiary sewage treatment. Bush et al. (1961), Bogan (1961), Bożko et al. (1962, 1966) and Loehr, Stephenson (1964) declare that it is one of the simplest and least expensive methods of nutrient substances removal from the sewage.

Studies carried out in many countries on a large scale confirmed the usefulness of that method and potential possibility of its application in the treatment of municipal sewage, and even industrial wastes. Depending on local needs and conditions, ponds of that type are designed either as single units or in a complex of several units working in series or in parallel.

This paper presents the results of studies undertaken with a view to determine the role of algae in the removal of nutrient substances (nitrogen and phosphorus) from sewage. A thorough investigation of that problem in the operating conditions was rather difficult due to the coexistence of a series of environmental factors which could, in consequence, obscure the real picture, completely. Therefore, experiments were carried out in laboratory conditions.

2. MATERIAL AND METHODS

Experimental tests were performed on two kinds of sewage, mechanically or biologically purified, mixed together in 1 : 1 proportion. Samples were collected from the Experimental Sewage Treatment Plants of the Institute of Municipal Economy in Warsaw. The mechanically purified sewage was sampled from the primary settling tank, whereas the other one — from the secondary settling tank.

The physico-chemical characteristics of the examined sewage was as follows: sewage temperature 9°C, pH 6.5. There was a trace of dissolved oxygen and the content of free carbon dioxide amounted to 30 mg CO_2/l. The total suspended solids content was about 93.5 mg/l, BOD_5 — about 92.50 mg O_2/l. There was a considerable amount of ammonium nitrogen — 32 mg $N\text{-}NH_4$/l. The nitrite and nitrate nitrogen contents were 0.13 mg $N\text{-}NO_2$/l and 0.35 mg $N\text{-}NO_3$/l, respectively. Organic nitrogen — 13.0 mg N_{org}/l, and phosphorus — 9.0 mg P_2O_5/l.

Tests were performed in 10 l flat-bottom flasks, kept in the rack. Each flask contained 8 l of sewage. The test-rack was lit up from the sides and the bottom by normal electric bulbs. Incident light intensity was about 3000 lux. Lighting time — about 7 hr a day. In order to avoid an excessive heating of sewage, the regular temperature was maintained by fan cooling, operating at the same time as lighting. Twice a day the contents of each flask were stirred.

The basic physico-chemical determinations comprised measurements of sewage temperature, pH, dissolved oxygen, free carbon dioxide, BOD_5, ammonium, nitrite, nitrate, organic nitrogen and phosphorus.

The methodology of determination, just as in investigations in field conditions were adopted after Hermanowicz et al. (1967).

The scope of biological examinations was as follows: isolation of algae monocultures, quantitative analysis of algae and calculation of coefficient K (average daily growth rate of algae), examination of the degree of greenery on the Coli 5 membrane filters, calculation of dry weight of algae.

Quantitative determination of algae was performed in a plankton chamber of 1 ml volume. The numbers of organisms are given per 1 l of sewage.

The average daily increase in the number of cells (K) was calculated from Myers' formuła (1953), as follows:

$$K = \frac{N_0 \cdot t}{N}$$

where: N — number of cells at the time of measurements, N_0 — number of cells at the beginning of experiment, t — time (days).

The degree of greenery on the Coli 5 membrane filters was determined as follows: 10 ml sewage samples were strained through the Coli 5 membrane filters, by means of a filtration apparatus. Next, filters were dried in room temperature. Algae suspension caused a permanent colouring of the membrane filters.

Determination of the value of the algae dry weight required the use of a special procedure, due to the fact that sewage, after mechanical and biological purification, as well, contains a considerable quantity of suspended solids, which are composed of both organic and mineral compounds. Calculation of the increment of algae by direct determination of suspension by weighing method would not give a real picture of algae development. Therefore, the number of cells, in the monoculture grown in a liquid mineral medium, was determined with a microscope in 1 ml sample. Then, the exactly measured amount of monoculture (500 ml) was strained through Coli 5 membrane filters by using a Coli apparatus. Before filtration, the membrane filters were dried to a constant weight, at 105°C, during 3 hr. After filtration, filters with algae suspension were also dried and weighed. In each culture 3 parallel tests were carried out in order to obtain the mean value. Knowing the values of the number of the cells in the examined sample and the dry weight of algae, it was possible to make an adequate calculation which, consequently, allowed to determine the number of cells of each genus per 1 mg of dry mass volume.

With the use of that method, the results were as follows (million of cells per 1 mg of dry weight):

Chlorella vulgaris — 100.0
Scenedesmus quadricauda — 24.0
S. quadricauda var. alternans — 11.5
Scenedesmus obliquus — 50.0
Scenedesmus acuminatus — 32.5.

Thus, the density of algae cells served as starting point for the calculation of dry mass in laboratory experiments.

Samples were collected, on the average, every three days, at about 8 a.m., during the 33 day period of experiment. Before sampling the sewage was stirred thoroughly.

3. RESULTS

After the preparation of the testing material, sewage was inoculated with the following cultures of algae:

Chlorella vulgaris Beijerinck,
Scenedesmus quadricauda (Turp.) Breb.,
Scenedesmus quadricauda var. alternans G. M. Smith,
Scenedesmus obliquus (Turp.) Krüger,
Scenedesmus acuminatus (Lagerh.) Chodat,
Mixed culture,
Control (without algae).

After the inoculation of sewage, the density of population in each particular genus was approximately equal in all the cultures, amounting to: $148 \cdot 10^5$, $153 \cdot 10^5$, $146 \cdot 10^5$, $147 \cdot 10^5$, $145 \cdot 10^5$, $146 \cdot 10^5$ per 1 l, respectively. Their dry mass amounted to: 0.148, 0.638, 1.27, 0.294, 0.445, 0.547 mg/l, respectively. Thus, directly after inoculation the highest dry mass weight was noted in the culture of *Scenedesmus quadricauda var.*

Table I. Growth of alga species in sewage water

Culture	Estimations	Unit	Day									
			1	7	9	11	13	15	17	23	26	33
Chlorella vulgaris	Number of cells	No./l	$148 \cdot 10^5$	$2 \cdot 10^8$	$28 \cdot 10^7$	$64 \cdot 10^7$	$196 \cdot 10^6$	$3 \cdot 10^9$	$44 \cdot 10^8$	$7 \cdot 10^9$	$8 \cdot 10^9$	$18 \cdot 10^9$
	K	—		1.93	2.10	3.92	10.20	13.50	17.50	20.60	20.60	36.8
	Dry weight	mg/l	0.148	2.000	2.800	6.400	19.600	30.000	44.00	70.00	80.00	180.00
	Increment of dry wt.	mg/l	—	1.851	2.652	6.252	19.452	29.852	43.852	69.852	79.852	179.852
Scenedesmus quadricauda	Number of cells	No./l	$153 \cdot 10^4$	$76 \cdot 10^6$	$8 \cdot 10^7$	$192 \cdot 10^6$	$52 \cdot 10^7$	$52 \cdot 10^7$	$96 \cdot 10^7$	$136 \cdot 10^7$	$2 \cdot 10^9$	$4 \cdot 10^9$
	K	—		0.71	0.58	1.14	2.61	2.31	3.70	3.86	5.02	7.93
	Dry weight	mg/l	0.638	3.170	3.330	8.000	21.70	21.70	35.80	56.60	83.50	16.700
	Increment of dry wt.	mg/l	—	2.532	2.692	7.362	21.062	21.062	35.162	56.962	82.862	165.362
Scenedesmus quadricauda var. alternans	Number of cells	No./l	$146 \cdot 10^5$	$15 \cdot 10^7$	$16 \cdot 10^7$	$4 \cdot 10^8$	$44 \cdot 10^7$	$84 \cdot 10^8$	$124 \cdot 10^7$	$130 \cdot 10^7$	$140 \cdot 10^7$	$192 \cdot 10^7$
	K	—	—	1.47	1.21	2.50	2.32	3.66	5.00	3.90	3.70	4.00
	Dry weight	mg/l	1.27	13.00	13.90	34.80	38.20	69.50	108.00	113.00	121.50	167.00
	Increment of dry wt.	mg/l	—	11.73	12.62	33.53	36.93	68.23	105.73	111.73	120.23	166.362
Scenedesmus obliquus	Number of cells	No./l	$147 \cdot 10^5$	$96 \cdot 10^6$	$24 \cdot 10^7$	$7 \cdot 10^8$	$198 \cdot 10^7$	$2 \cdot 10^9$	$28 \cdot 10^8$	$32 \cdot 10^8$	$41 \cdot 10^8$	$44 \cdot 10^8$
	K	—	—	0.93	1.82	4.32	10.30	9.10	11.30	9.50	10.75	9.10
	Dry weight	mg/l	0.294	1.920	4.800	14.000	39.600	40.00	56.00	64.00	82.00	88.19
	Increment of dry wt.	mg/l	—	1.626	4.506	13.706	39.306	39.706	55.706	63.706	81.706	87.896
Scenedesmus acuminatus	Number of cells	No./l	$145 \cdot 10^5$	$14 \cdot 10^6$	$172 \cdot 10^6$	$3 \cdot 10^8$	$16 \cdot 10^6$	$21 \cdot 10^8$	$24 \cdot 10^8$	$38 \cdot 10^8$	$5 \cdot 10^9$	$5 \cdot 10^9$
	K	—	—	1.38	1.32	1.87	8.50	9.63	9.72	11.35	13.30	10.50
	Dry weight	mg/l	0.445	4.300	5.290	9.230	49.300	64.60	74.00	117.00	150.445	143.445
	Increment of dry wt.	mg/l	—	3.855	4.845	8.785	48.855	64.155	73.555	116.555	159.000	153.000
Mixed culture	Number of cells	No./l	$146 \cdot 10^5$	$192 \cdot 19^6$	$309 \cdot 10^5$	$116 \cdot 10^8$	$2168 \cdot 10^6$	$367 \cdot 10^7$	$362 \cdot 10^7$	$543 \cdot 10^7$	$718 \cdot 10^7$	$1515 \cdot 10^7$
	K	—	—	1.87	2.43	1.42	11.20	15.30	18.80	16.30	28.70	30.80
	Dry weight	mg/l	0.547	2.948	5.784	16.963	36.803	49.765	64.815	102.030	129.015	219.740
	Increment of dry wt.	mg/l	—	2.401	5.237	16.416	36.256	49.218	64.268	101.483	125.463	219.193

Table II. Growth of alga species in sewage water — the mixed culture

Culture	Estimations	Unit	Day									
			1	7	9	11	13	15	17	23	26	33
Chlorella vulgaris	Number of cells	No./l	$3 \cdot 10^6$	$16 \cdot 10^7$	$20 \cdot 10^7$	$1 \cdot 10^9$	$12 \cdot 10^8$	$2 \cdot 10^9$	$14 \cdot 10^8$	$4 \cdot 10^9$	$5 \cdot 10^9$	$12 \cdot 10^9$
	Dry weight	mg/l	0.03	1.60	2.0	10.00	12.00	20.00	24.00	40.00	50.00	120.00
	Increment of dry wt.	mg/l	—	1.57	1.97	9.97	11.97	18.97	23.97	39.97	49.97	119.97
Scenedesmus quadricauda	Number of cells	No./l	$32 \cdot 10^5$	$6 \cdot 10^6$	$7 \cdot 10^6$	$75 \cdot 10^5$	$8 \cdot 10^6$	$4 \cdot 10^7$	$4 \cdot 10^7$	$4 \cdot 10^7$	$4 \cdot 10^7$	$5 \cdot 10^7$
	Dry weight	mg/l	0.133	0.250	0.292	0.313	0.324	1.665	1.665	1.665	1.665	2.080
	Increment of dry wt.	mg/l	—	— 0.117	0.159	0.180	0.191	1.532	1.532	1.532	1.532	1.947
Scenedesmus quadricauda var. alternans	Number of cells	No./l	$28 \cdot 10^5$	$7 \cdot 10^6$	$12 \cdot 10^6$	$4 \cdot 10^7$	$48 \cdot 10^6$	$8 \cdot 10^7$	$2 \cdot 10^8$	$44 \cdot 10^7$	$44 \cdot 10^7$	$5 \cdot 10^8$
	Dry weight	mg/l	0.243	0.610	1.045	3.470	4.170	6.950	17.400	38.200	38.200	43.500
	Increment of dry wt.	mg/l	—	0.367	0.801	3.227	3.927	8.707	17.157	37.957	37.957	43.257
Scenedesmus obliquus	Number of cells	No./l	$29 \cdot 10^5$	$9 \cdot 10^6$	$3 \cdot 10^7$	$36 \cdot 10^6$	$72 \cdot 10^7$	$75 \cdot 10^7$	$78 \cdot 10^7$	$8 \cdot 10^7$	$15 \cdot 10^8$	$24 \cdot 10^8$
	Dry weight	mg/l	0.058	0.18	0.60	0.72	14.40	15.00	15.60	16.00	30.00	48.00
	Increment of dry wt.	mg/l	—	0.122	0.542	0.662	14.342	14.942	15.542	15.942	29.942	47.942
Scenedesmus acuminatum	Number of cells	No./l	$27 \cdot 10^5$	$1 \cdot 10^7$	$6 \cdot 10^7$	$80 \cdot 10^6$	$19 \cdot 10^7$	$2 \cdot 10^8$	$20 \cdot 20^7$	$20 \cdot 10^7$	$20 \cdot 10^7$	$20 \cdot 10^7$
	Dry weight	mg/l	0.083	0.308	1.850	2.460	5.85	6.15	6.15	6.15	6.15	6.15
	Increment of dry wt.	mg/l	—	0.225	1.767	2.377	5.767	6.067	6.067	6.067	6.067	6.067
Total species	Number of cells	No./l	$146 \cdot 10^5$	$192 \cdot 10^6$	$309 \cdot 10^6$	$1163 \cdot 10^6$	$2186 \cdot 10^6$	$367 \cdot 10^7$	$362 \cdot 10^7$	$548 \cdot 10^7$	$718 \cdot 10^7$	$1515 \cdot 10^7$
	Dry weight	mg/l	0.547	2.948	5.784	16.963	36.803	49.765	64.815	102.03	126.015	219.74
	Increment of dry wt.	mg/l	—	2.401	5.237	16.416	36.256	49.218	64.268	101.483	126.468	219.193

alternans, the lowest — *Chlorella vulgaris*. Algae development rate in particular cultures, the coefficient K, and dry weight values are presented in Tables I and II, and in Fig. 1.

After 7 days of culture an increase in cells of all the above mentioned groups of algae species was observed, expressed by coefficient K it

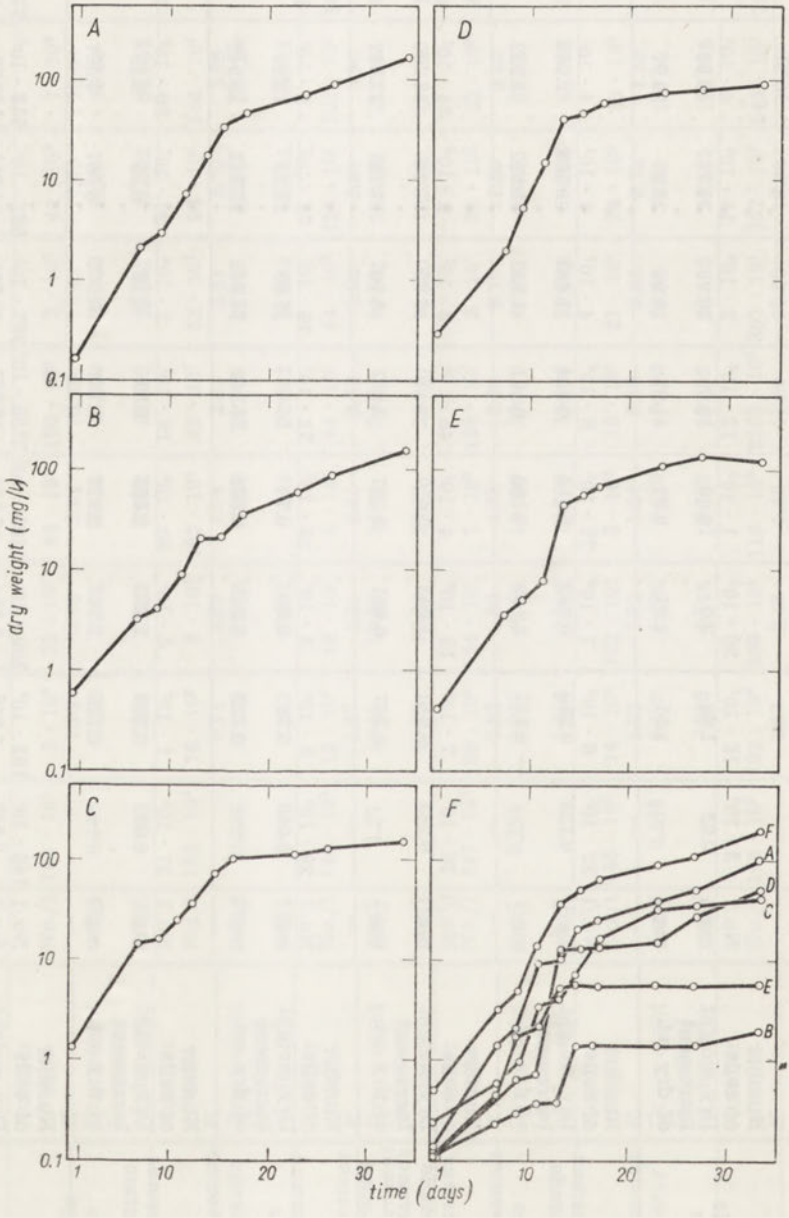

Fig. 1. Growth of algae on sewages in the laboratory. (semi-log scale). A — *Chlorella vulgaris*, B — *Scenedesmus quadricauda*, C — *Scenedesmus obliquus*, E — *Scenedesmus acuminatus*, F — mixed culture

amounted to: 1.93, 0.71, 1.47, 0.93, 1.38, 1.87, respectively. As can be seen, *Chlorella vulgaris* showed the highest development dynamics and *Scenedesmus quadricauda* — the lowest. In the course of the next period of experiment (after 9, 11, and 13 days of culture) a further development of algae was noticed, i.e. increase in cell number, dry weight, and the coefficient *K* value.

After 13 days, the mixed culture had the highest *K* value — 11.20. *Scenedesmus obliquus* — 10.30 and *Chlorella vulgaris* — 10.20 — followed closely. *Scenedesmus quadricauda var. alternans* — 2.32, i.e. the lowest *K* value. The highest dry weight value was reached by *Scenedesmus acuminatus* — 49.30 mg/l. Next comes *Scenedesmus obliquus* — 39.60 mg/l. *Scenedesmus quadricauda var. alternans* and mixed culture — 36.20 and 36.80 mg/l, respectively. The lowest values — 24.70 and 19.60 mg/l had *Scenedesmus quadricauda* and *Chlorella vulgaris*, respectively.

After 15 days, just as in the previous period the highest *K* values were found in mixed culture and *Chlorella vulgaris*, the lowest in *Scenedesmus quadricauda*.

After 23 days, the course of development in particular cultures was analogical. *Chlorella vulgaris* reached the highest *K* value (20.60) among all the groups. The coefficient *K* showed also an upward tendency in *Scenedesmus acuminatus* and *Scenedesmus quadricauda* cultures. On the other hand, it has decreased in the three remaining cultures, as compared with the previous period, thus: *Scenedesmus quadricauda var. alternans* — 22%, *Scenedesmus obliquus* — 15%, the mixed culture — 16%. At the same time, the highest dry weight value was noted in the cultures: *Scenedesmus quadricauda var. alternans* — 113.0, *Scenedesmus acuminatus* — 117.0, and mixed culture — 102.03 mg/l. Somewhat lower values — 70.0 and 64.0 mg/l were noted for *Chlorella vulgaris* and *Scenedesmus obliquus*, respectively, and the lowest one — 56.0 mg/l for *Scenedesmus quadricauda*.

After 26 days, there were further changes in the coefficient *K* value, in comparison with the foregoing period, as it increased in the cultures: the mixed one, *Scenedesmus acuminatus*, *Scenedesmus obliquus*, and *Scenedesmus quadricauda*, and decreased in *Scenedesmus quadricauda var. alternans*, remaining at the same level as previously in the *Chlorella vulgaris* culture.

After 33 days of algae culture in the sewage medium the maximum content of cells was observed, which in conversion into 1 l of sewage was for each particular species as follows: *Chlorella vulgaris* — $18 \cdot 10^9$, mixed culture — $1515 \cdot 10^7$, *Scenedesmus quadricauda* — $4 \cdot 10^9$, *Scenedesmus acuminatus* — $5 \cdot 10^9$, *Scenedesmus obliquus* — $44 \cdot 10^8$, *Scenedesmus quadricauda var. alternans* — $192 \cdot 10^7$ per 1 l. The highest value of the coefficient *K* was obtained in *Chlorella vulgaris* — 36.80 and somewhat lower in the mixed culture — 30.80. Among the algae of Scenedesmus

species the highest *K value* — 10.5 — was noted in *S. acuminatus*, slightly lower — 9.10 and 7.93 in *S. obliquus* and *S. quadricauda*, respectively, the lowest — 4.00 in *S. quadricauda var. alternans*. The highest dry weight value — 219.74 mg/l was found in the mixed culture, at the same time it should be mentioned that *Chlorella vulgaris* had the greatest share in the increase of dry mass in this particular culture (54.5%), *S. obliquus* a slightly smaller one (22%) and *S. quadricauda var. alternans* (20%). The share of *S. acuminatus* and *S. quadricauda* was very small — 2.5 and 1%, respectively. The dry mass value in the cultures was for *Chlorella vulgaris* — 180.0, *S. quadricauda* and *S. quadricauda var. alternans* — 167.0, *S. acuminatus* — 153.44, and *S. obliquus* — 88.19 mg/l.

The progressive development of algae was confirmed by examinations of the Coli 5 membrane filters. The range of gradation of colouring from grey-yellowish (colour of sewage) through all the shades from very light up to very bright green shows a very systematic increase in the density of the algae cells. The degree of the colouring of filters changed proportionally to the increase in dry mass of a particular algae species. Species with a higher dry weight value imbued the filters with a darker shade of green. Filters with control samples were almost to the end of experiment grey with yellowish hue, only in the last week they tinged with green due to the spontaneous self-development of algae.

REDUCTION OF PHOSPHORUS AND NITROGEN COMPOUNDS

In the initial stages of the experiment at a low level of oxygen (Table III) there was a considerable reduction of BOD_5 while no basic changes were observed in the content of the various forms of nitrogen (Table IV). They appeared, however, in the later stages of the investigations. Along with the increase in the development of algae, the content of soluble oxygen and the pH value were increasing while the content of carbon dioxide decreased. In result of photosynthesis the developing algae enriched the environment in oxygen at the expense of light energy and CO_2 which in turn created favourable conditions for the progress of nitrification.

The allover course of nitrogen conversion may be presented as follows: after 17 days of the experiment there was observed a fairly rapid decrease in the content of the ammonium nitrogen along with a high increase in nitrite nitrogen and an inconsiderable one in nitrate nitrogen. The degree of decrease in the former was higher than the increase in the latter two. This was followed by a systematic decrease in mineral nitrogen. It may be assumed that the relatively considerable loss of ammonium nitrogen could be an after effect of both the process of nitrification and fixation by algae.

For a better illustration it is worthwhile to present the course of mineral nitrogen reduction in *Chlorella vulgaris* culture, where nitrogen conversions, in the general outline, had a similar character to those observed in other cultures. On the 9th day of investigations the total content of mineral nitrogen was 37.28 mg N/l and from that day its value decreased slowly but systematically down to 23.58 mg/l (the 17th day). Then, after 23 days from the beginning of the experiment there was a rather sudden loss of that form of nitrogen (13.75 mg N/l). At that time ammonium nitrogen with its 5.62 mg $N\text{-}NH_4$/l value ceased to be preponderant overall and its role was overtaken by nitrite nitrogen (7.07 mg $N\text{-}NO_2$/l). As concerns organic nitrogen its value decreasing during the first 11 days increased distinctly in the following days.

In the initial period of the experiment the process of decomposition of organic nitrogen outweighed its increase in a form of algae, hence a loss of organic nitrogen and a slight increase in ammonium nitrogen. It may be assumed that that phase of conversion took its course without a decisive influence of algae. Next, with the gradual development of algae and increase in their biomass, the presence of algae in the environment began to bear effect on nitrogen cycle conversions. First of all, there was an increasing requirement for mineral nitrogen used in the formation of cells. This, in turn, determined not only the level of mineral nitrogen requisite by the increased demand but also of organic nitrogen as result of an intensified development of algae. Though it is quite certain that the organic nitrogen compounds underwent dissociation, but they gave priority to the processes of nitrogen fixation, in form of the increasing biomass of algae. Then, the apex of the dynamics of algae development was denoted, extensive saturation of the environment with oxygen, and intensified nitrification. In result, ammonium nitrogen content was reduced practically to naught, with simultaneous high increase in nitrite nitrogen (over 20 mg $N\text{-}NO_2$/l). The organic nitrogen content was also increased considerably. The high increase in the nitrite nitrogen value evindenced the intensity of the process of nitrification in its first phase, one can suppose that there was also a second phase of nitrification, but the produced nitrates were used by algae.

Nitrogen conversion in the control culture had a somewhat different course. During the first 15 days of the experiment the ammonium nitrogen content was increasing which proved that there was a preponderance of the process of decomposition of the organic substances containing nitrogen, but the process itself lasted much longer than in the cultures with algae (7—9 days). Then, in the further course of the test, while in the cultures with algae a striking loss of ammonium nitrogen was observed, its slow systematic decrease was only beginning in the control culture, which was probably due to the process of nitrification. It should be also mentioned that in the last period of the test, algae were self-

Table III. Physico-chemical changes in sewage inculated with various alga cultures

Culture	Estimations	Unit	Day 1	7	9	11	13	15	17	23	26	33
Chlorella vulgaris	Sewage temp.	°C	15	17	17	18	18	18	14	18	20	20
	pH		6.5	7.0	7.0	7.4	8.4	8.6	9.0	8.2	8.2	8.2
	Dissolved oxygen	mgO_2/l	0	1.0	3.1	5.1	7.9	7.8	9.5	6.8	6.8	6.4
	Carbon dioxide	$mgCO_2/l$	30.50	11.34	11.3	4.30	0	0	0	0	0	0
	BOD_5	mgO_2/l	97.8	60.1	—	—	55.1	—	—	48.2	—	40.1
Scenedesmus quadricauda	Sewage temp.	°C	15	17	17	18	18	18	14	18	20	20
	pH		6.5	7.0	7.0	8.3	7.8	8.5	9.0	8.8	8.8	8.8
	Dissolved oxygen	mgO_2/l	0	1.8	4.7	5.5	8.7	9.2	8.8	7.6	8.8	7.2
	Carbon dioxide	$mgCO_2/l$	31.0	13.96	9.7	0	0	0	0	0	0	0
	BOD_5	mgO_2/l	97.7	58.1	—	—	54.3	—	—	44.1	—	39.3
Scenedesmus quadricauda var. alternans	Sewage temp.	°C	15	17	17	18	18	18	14	18	20	20
	pH		6.5	7.0	8.6	8.6	8.8	9.5	10.3	9.1	9.1	9.1
	Dissolved oxygen	mgO_2/l	traces	2.7	5.0	7.3	10.9	8.8	9.8	9.8	6.4	6.8
	Carbon dioxide	$mgCO_2/l$	30.0	9.72	0	0	0	0	0	0	0	0
	BOD_5	mgO_2/l	97.5	60.5	—	—	51.1	—	—	35.0	—	28.7
Scenedesmus obliquus	Sewage temp.	°C	15	17	17	18	18	18	14	18	20	20
	pH		6.5	7.0	7.0	8.4	8.2	8.4	8.8	8.8	8.8	8.8
	Dissolved oxygen	mgO_2/l	traces	1.5	4.8	7.8	9.6	8.5	9.6	8.8	5.6	7.0
	Carbon dioxide	$mgCO_2/l$	29.0	11.34	2.75	0	0	0	0	0	0	0
	BOD_5	mgO_2/l	97.4	57.7	—	—	53.5	—	—	31.8	—	22.5

Scenedesmus acuminatus	Sewage temp.	°C	15	17	17	18	18	18	14	18	20	20
	pH		6.5	7.0	7.0	8.2	8.0	8.5	8.9	9.15	9.2	9.2
	Dissolved oxygen	mgO_2/l	traces	2.8	4.7	7.2	9.3	8.7	9.3	5.2	7.6	7.8
	Carbon dioxide	$mgCO_2/l$	28	9.72	8.0	0	0	0	0	0	0	0
	BOD_5	mgO_2/l	97.5	56.60	—	—	52.3	—	—	31.2	—	23.8
Mixed culture	Sewage temp.	°C	15	17	17	18	18	18	14	18	20	20
	pH		6.5	7.0	7.0	7.3	8.2	9.0	9.55	9.2	9.2	9.2
	Dissolved oxygen	mgO_2/l	traces	2.0	4.8	7.3	9.5	8.8	9.2	7.2	6.0	7.2
	Carbon dioxide	$mgCO_2/l$	30.0	9.72	11.3	11.34	0	0	0	0	0	0
	BOD_5	mgO_2/l	97.5	58.2	—	—	47.7	—	—	34.8	—	22.5
Control	Sewage temp.	°C	15	17	17	18	18	18	14	18	20	20
	pH		6.5	7.0	7.0	7.3	7.2	7.3	7.45	7.8	7.9	8.0
	Dissolved oxygen	mgO_2/l	traces	3.0	3.6	3.7	3.1	3.6	3.9	4.5	3.6	5.8
	Carbon dioxide	$mgCO_2/l$	30.0	9.72	17.8	38.88	11.34	16.2	9.72	8.5	8.1	6.4
	BOD_5	mgO_2/l	92.5	71.0	—	—	60.0	—	—	56.4	—	51.0

— No data.

Table IV. Changes in nitrogen and phosphorus contents in sewage water with algae

Culture	Estimations	Unit	Day									
			1	7	9	11	13	15	17	23	26	33
Chlorella vulgaris	Phosphorus*	mg P_2O_5/l	9.4	9.3	8.8	8.0	7.6	5.2	5.0	4.8	4.5	4.0
	Ammonia nitrogen	mg N_{NH_4}/l	32.2	38.72	36.80	35.95	33.11	23.43	20.66	5.62	0	0.89
	Nitrite nitrogen	mg N_{NO_2}/l	0.23	0.001	0.004	0.12	0.056	0.32	1.3	7.07	23.0	21.25
	Nitrate nitrogen	mg N_{NO_3}/l	0.25	0.40	0.48	0.48	0.28	0.98	1.12	1.06	1.04	1.44
	Organic nitrogen	mg N_{org}/l	14.4	6.04	8.46	8.12	8.62	17.36	18.92	8.85	18.93	18.42
	Organic nitrogen*	mg N_{org}/l	11.62	5.04	4.48	2.96	3.97	7.54	7.05	8.62	4.48	8.12
	Total nitrogen	mg N/l	46.68	45.16	45.74	44.65	42.07	42.15	42.50	42.60	49.97	42.00
	Total dissolved N	mg N/l	44.30	44.16	41.76	39.60	40.00	31.33	38.63	36.37	28.52	31.70
Scenedesmus quadricauda	Phosphorus*	mg P_2O_5/l	9.0	8.4	8.0	6.4	5.2	5.2	4.2	3.8	1.0	0.12
	Ammonia nitrogen	mg N_{NH_4}/l	30.1	28.22	32.81	31.80	29.51	29.73	18.62	7.61	0.89	0.89
	Nitrite nitrogen	mg N_{NO_2}/l	0.15	0.001	0.002	0.0032	0.056	0.40	1.6	9.97	21.0	15.0
	Nitrate nitrogen	mg N_{NO_3}/l	0.25	0.48	0.48	0.69	0.28	0	1.96	1.44	1.12	1.72
	Organic nitrogen	mg N_{org}/l	13.3	13.48	9.11	9.63	10.19	12.20	19.43	13.77	16.85	22.08
	Organic nitrogen*	mg N_{org}/l	11.0	11.18	5.54	1.90	7.36	6.04	5.54	3.47	4.48	6.12
	Total nitrogen	mg N/l	43.80	42.1	42.40	42.10	40.03	42.33	41.61	39.40	39.86	39.69
	Total dissolved N	mg N/l	40.5	39.80	38.83	34.37	37.20	38.17	27.72	33.0	27.49	27.73

Scenedesmus quadricauda var. alternans	Phosphorus*	mg P_2O_5/l	8.0	7.6	5.2	2.0	1.6	1.0	0.8	0.5	0.04	0
	Ammonia nitrogen	mg N_{NH_4}/l	31.5	28.72	31.30	28.72	27.16	25.59	21.50	17.36	10.19	11.20
	Nitrite nitrogen	mg N_{NO_2}/l	0.12	0.001	0.005	0.048	0.072	0.26	0.63	0.48	0.78	0.7
	Nitrate nitrogen	mg N_{NO_3}/l	0.25	0.40	0.48	0.60	0.28	0	1.74	1.11	0.6	0.8
	Organic nitrogen	mg N_{org}/l	12.74	11.20	12.20	10.19	16.85	18.42	20.52	22.00	—	26.95
	Organic nitrogen*	mg N_{org}/l	3.73	5.04	3.97	2.96	1.90	1.96	1.90	3.0	9.63	5.04
	Total nitrogen	mg N/l	44.61	40.32	43.38	39.55	44.36	44.27	44.39	41.90	—	38.86
	Total dissolved N	mg N/l	35.6	34.16	35.75	32.32	29.41	28.81	25.77	22.90	21.20	17.75
Scenedesmus obliquus	Phosphorus*	mg P_2O_5/l	8.3	8.0	8.0	6.4	6.4	4.4	3.2	2.0	0.5	0.28
	Ammonia nitrogen	mg N_{NH_4}/l	33.2	29.73	30.24	30.24	29.73	28.72	22.66	21.50	9.63	7.05
	Nitrite nitrogen	mg N_{NO_2}/l	0.13	0.001	0.004	0.003	0.116	0.26	2.4	1.63	6.08	8.9
	Nitrate nitrogen	mg N_{NO_3}/l	0.35	0.36	0.60	0.48	0.28	0	1.52	1.12	1.16	1.68
	Organic nitrogen	mg N_{org}/l	12.04	10.61	10.19	9.11	12.92	12.92	13.03	16.35	—	17.36
	Organic nitrogen*	mg N_{org}/l	3.2	3.97	4.48	3.97	3.47	7.05	6.55	4.29	6.55	6.09
	Total nitrogen	mg N/l	44.72	40.70	41.03	39.83	43.04	41.90	39.61	40.60	—	34.94
	Total dissolved N	mg N/l	36.0	35.70	35.32	34.69	33.59	36.03	28.51	28.51	28.01	23.72
Scenedesmus acuminatus	Phosphorus*	mg P_2O_5/l	8.0	7.6	6.4	5.4	4.8	1.2	0	0	0.02	0.24
	Ammonia nitrogen	mg N_{NH_4}/l	30.8	28.72	28.22	30.80	28.72	25.58	25.08	6.04	1.0	0
	Nitrite nitrogen	mg N_{NO_2}/l	0.13	0.001	0.001	0.032	0.108	0.58	2.50	12.57	21.0	21.0
	Nitrate nitrogen	mg N_{NO_3}/l	0.35	0.36	0.32	0.48	0.28	0.2	0.20	1.28	1.56	1.0
	Organic nitrogen	mg N_{org}/l	11.04	10.12	10.05	8.62	13.77	11.20	14.85	15.29	18.92	18.28

Culture	Estimations	Unit	Day									
			1	7	9	11	13	15	17	23	26	33
Scenedesmus acuminatus	Organic nitrogen*	mg N_{org}/l	4.30	6.04	1.90	3.47	6.55	6.55	5.61	6.0	3.47	1.19
	Total nitrogen	mg N/l	42.32	39.20	38.59	39.91	42.87	37.57	42.63	36.18	42.48	40.28
	Total dissolved N	mg N/l	35.58	35.12	34.00	32.76	30.65	32.92	33.39	26.89	27.03	23.19
Mixed culture	Phosphorus*	mg P_2O_5/l	8.0	7.6	7.0	3.4	6.0	3.3	2.1	0.8	0.42	1.28
	Ammonia nitrogen	mg N_{NH_4}/l	29.4	31.80	32.31	30.24	26.15	19.93	21.00	20.44	14.78	12.20
	Nitrite nitrogen	mg N_{NO_2}/l	0.11	0.001	0.002	0.012	0.056	0.24	1.8	0.41	6.66	12.5
	Nitrate nitrogen	mg N_{NO_3}/l	0.35	0.36	0.32	0.46	0.16	0	0.28	1.16	1.2	2.95
	Organic nitrogen	mg N_{org}/l	12.04	6.04	5.97	9.63	14.28	16.85	16.70	16.85	16.51	14.28
	Organic nitrogen*	mg N_{org}/l	8.82	6.04	1.40	1.89	6.04	7.61	6.55	8.12	4.48	4.48
	Total nitrogen	mg N/l	41.90	38.20	38.60	40.34	40.64	37.02	39.78	38.86	39.15	41.93
	Total dissolved N	mg N/l	38.69	38.20	34.03	32.60	32.40	27.78	29.63	30.13	22.12	20.13
Control	Phosphorus*	mg P_2O_5/l	9.4	8.8	8.8	8.0	8.8	8.6	7.0	6.8	7.1	6.0
	Ammonia nitrogen	mg N_{NH_4}/l	30.01	30.2	33.37	34.31	35.04	35.54	32.31	25.20	22.57	19.63
	Nitrite nitrogen	mg N_{NO_2}/l	0.25	0.40	0.70	0.35	0.28	0.28	0.88	1.12	1.12	1.42
	Nitrate nitrogen	mg N_{NO_3}/l	0.12	0.001	0.002	0.004	0.04	0.40	5.08	9.53	11.85	12.25
	Organic nitrogen	mg N_{org}/l	12.04	11.62	9.11	8.48	8.04	7.23	6.81	6.04	8.57	7.05
	Organic nitrogen*	mg N_{org}/l	—	11.50	8.72	7.12	6.20	5.42	5.54	4.48	4.02	3.97
	Total nitrogen	mg N/l	42.42	42.221	41.882	43.144	43.40	43.17	42.08	41.89	42.31	40.65
	Total dissolved N	mg N/l	—	42.121	42.492	41.784	41.56	41.36	40.81	40.33	39.76	37.57

* Nitrogen and phosphorus were estimated in the filtered samples.

-developing spontaneously and at the same time a slight increase in organic nitrogen and pH value was noticed together with a decrease in CO_2. The colouring of the membrane filters appeared simultaneously.

The content of soluble phosphorus at the time of the inoculation of algae in the sewage amounted to approximately 9.4 mg/l. In the course of experiment a systematic decrease of soluble phosphorus was observed in subsequent samplings from each particular culture. Its highest content was found in *Chlorella vulgaris* culture. After 33 days it amounted to 4.0 mg/l, while in other cultures it was much lower and did not exceed the value of 1 mg P_2O_5/l.

In subsequent samplings from the control culture its decrease was relatively insignificant. After 33 days its value was 6.0 mg P_2O_5/l.

4. DISCUSSION

Considering the dynamics of algae development in the sewage on the basis of results from the experiment, one can say that its highest value in respect of the number of cells and the increase in dry mass, as well, was shown by *Chlorella vulgaris*. The high value of the coefficient *K* indicates that *Chlorella vulgaris* cultures were characterized by an intensive and systematic increase in growth values during the whole experimental period, while e.g. *Scenedesmus quadricauda var. alternans* reached its maximal development after 17 days, already, and *Scenedesmus acuminatus* after 26 days.

It should be emphasized that the coefficient *K* values given by Myers et al. (1953) were distinctly divergent from those presented by Vladimirova et al. (1966), who had cultured *Chlorella vulgaris* in specific conditions (temperature and lighting) and obtained after 5 days, already, the *K* values ranging from 35 to 80.

Data on the increase in algae dry mass and changes in the level of the content of total soluble nitrogen (total sum of all particular forms of nitrogen, i.e. $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, soluble N_{org}) and soluble phosphorus, are given in Tables V and VI. The comparative analysis was performed for 7 day periods.

According to the data from literature the percentage of nitrogen content in the algae dry mass ranges from 6.5 to 10%, and from 2.2 to 7.65% for Chlorella (Gitelzon 1964) and Scenedesmus species (Krauss 1956), respectively.

The ratio of the nitrogen loss to the increase in *Chlorella vulgaris* dry mass ranged from 6.75% after 23 days to 10.8% after 13 days. Throughout the experiment the loss of nitrogen averaged 7% in relation to the increase in dry mass, so it is within the limits given in other papers (after 33 days total soluble nitrogen — 12.60 mg/l, the increase in dry weight — 179.85 mg/l).

Table V. Dry mass of algae, dissolved nitrogen and phosphorus contents during 33 days of algae growth in sewage water (mg/l)

Culture	After inoculation			After 7 days			After 13 days			After 23 days			After 33 days		
	Dry mass	Total N	P	Dry mass	Total N	P	Dry mass	Total N	P	Dry mass	Total N	P	Dry mass	Total N	P
Chlorella vulgaris	0.148	44.30	9.4	1.468	44.16	9.30	40.000	40.00	7.60	118.000	36.27	4.80	180.000	31.70	4.00
Scenedesmus quadricauda	0.638	41.50	9.0	3.170	39.80	8.40	21.698	38.20	5.20	56.598	34.00	3.80	166.998	29.75	0.20
Scenedesmus quadricauda var. alternans	1.27	35.60	8.0	3.050	34.16	7.60	38.400	29.41	5.38	86.900	19.90	0.5	166.970	19.75	0.00
Scenedesmus obliquus	0.294	36.01	8.3	1.925	35.70	8.00	39.324	33.66	6.40	64.000	28.51	3.2	88.194	23.72	0.80
Scenedesmus acuminatus	0.445	35.58	8.0	4.300	35.12	7.60	49.500	30.65	4.80	116.945	26.88	0	153.445	23.18	0.24
Mixed culture	0.574	38.68	8.0	2.948	38.20	7.60	36.803	32.40	6.00	102.030	28.13	0.8	219.740	20.13	0.80

Table VI. The balance of nitrogen and phosphorus contained in sewage water and consumed by algae

Culture	After 7 days					After 13 days				
	Dry mass increment (mg/l)	Loss (mg/l)		The share in dry mass (%)		Dry mass increment (mg/l)	Loss (mg/l)		The share in dry mass (%)	
		N	P	N	P		N	P	N	P
Chlorella vulgaris	1.352	0.14	0.1	10.4	7.35	39.852	4.30	1.8	10.8	4.5
Scenedesmus quadricauda	2.532	0.7	0.6	27.7	23.7	21.06	3.3	3.8	15.7	18.0
Scenedesmus quadricauda var. alternans	11.760	1. 4	0.4	12.2	3.25	37.13	6.19	6.4	16.6	17.2
Scenedesmus obliquus	1.631	0.31	0.3	19.0	18.2	39.03	2.42	1.9	8.35	78.0
Scenedesmus acuminutus	3.855	0.46	0.4	12.0	10.4	49.055	4.93	3.2	10.0	6.5
Mixed culture	2.401	0.48	0.4	20.0	16.5	36.256	6.28	2.0	17.3	5.5

Culture	After 23 days					Dry mass increment (mg/l)	After 33 days				Theoretical consumption after 33 days (mg/l)			
	Dry mass increment (mg/l)	Loss (mg/l)		The share in dry mass (%)			Loss (mg/l)		The share in dry mass (%)		N		P	
		N	P	N	P		N	P	N	P	min	max	min	max
Chlorella vulgaris	117.852	7.93	4.6	6.75	3.90	179.852	12.6	5.4	7.0	3.0	11.65	18.80	1.80	3.60
Scenedesmus quadricauda	55.96	7.50	5.2	13.4	9.30	166.36	10.75	8.88	6.45	5.32	3.68	12.80	1.66	3.32
Scenedesmus quadricauda var. alternans	85.73	15.7	7.5	18.2	8.75	165.7	17.85	8.0	10.8	4.84	3.32	12.70	1.66	3.32
Scenedesmus obliquus	63.706	7.50	5.1	11.70	8.0	87.9	12.29	8.02	13.9	9.10	1.94	6.80	0.88	1.76
Scenedesmus acuminatus	116.50	8.70	8.0	7.50	6.86	153.0	12.39	7.76	8.75	5.10	3.37	11.70	1.53	3.06
Mixed culture	101.483	8.55	7.2	8.4	7.10	219.193	18.55	7.8	8.40	3.35	4.85	17.0	2.2	4.4

In the *Scenedesmus quadricauda* culture oscillations in the percentage of the nitrogen loss in relation to the increase in dry mass was much higher. The lowest one was noted after 23 days (13.4%), the highest after 7 days (27.6%). As concerns the last value, doubtlessly, it could not come only as result of the presence of algae. The average loss in relation to the increase in algae dry mass in this culture was after 33 days 6.45%, so it is also within the range given in literature. The weight loss of the total soluble nitrogen was 10.75 mg/l.

Analogical data for *S. quadricauda var. alternans* are expressed in values ranging from 10.8 to 18.20%. After 33 days the ratio of nitrogen loss to dry mass increase was 10.8%, i.e. much more than the value given in literature (7.65%). The weight loss of nitrogen after 33 days was 17.85 mg/l.

The highest percentage of loss after 33 days was noted in *S. obliquus* culture — 13.90%. On the basis of this study it is not possible to explain that phenomenon explicitly but one can suppose that there must have been some other factors (bacterial flora) that were not taken under investigation and might have caused the supplementary reduction of nitrogen.

In the remaining cultures, i.e. *S. acuminatus* and mixed culture, the value of the percentage mentioned above was but slightly exceeded; for the former it amounted to 8.75%, for the latter 8.40%, as related to 7.65%. It should be emphasized that the overall reduction of nitrogen was rather considerable and for the greater part it exceeded the percentage rate presupposed on the basis of the value of the algae dry mass increase.

For comparison, in the control sample after 23 days some loss of total nitrogen was also noted — 1.79 mg/l, while at the same time in the samples with algae those values ranged from 7.50 to 15.7 mg/l. Similar results were obtained by Isaac, Lodge (1960). In respect of the control sample the last week of investigations was left out on account of spontaneous self-development of algae.

The greater reduction of nitrogen observed in the initial phase corresponds to the results obtained by Gerloff, Skoog 1954, 1958). They agree that this phenomenon does not come as result of the lack of nitrogen and phosphorus in the environment but is caused by the ageing process in the cultures, which absorb less and less of those compounds.

Data from the analysis of quantitative changes in soluble phosphorus are also given in Tables V and VI.

According to the data from literature the content of phosphorus in the dry mass of algae amounts to 1—2%.

Data obtained on the basis of investigations on the loss of phosphorus are significantly divergent from the values of the dry mass of algae.

The lowest loss of phosphorus in relation to dry mass was noted after 33 days in *Chlorella vulgaris* — 3%, the highest in *Scenedesmus obliquus* culture — 9.10%. The exceptional convergence of results of the highest loss of both, nitrogen and phosphorus, in the latter is worthy noticing. It seems that such a high percentage in the loss of phosphorus was caused, also among other factors, by the mass development of algae.

It is generally known that the intensive development of algae goes along with the increased pH value, which is likewise evident in the present study (Table III). The pH value has increased from 6.5 up to 9.5, and in some cases was even more than 10. The high pH value precipitates the slightly soluble phosphorus salts from the environment. This comes as result of PO_4 ion becoming an insoluble salt in alkalic environment. Probably among other reducing agents (e.g. consumption of phosphorus by nitrifying bacteria) this was the most important one, in producing the unusually high reduction of phosphorus. In this case algae played, so to say, an intermediary role.

The obtained results lead to the following conclusions:

The increase in the algae dry mass is associated with reduction of total soluble nitrogen and insoluble phosphorus.

This interrelation is not strictly connected with the increase in dry mass and has quite significant divergences in respect of the proportional nitrogen and phosphorus share in the dry mass increase,

There is a difference in the percentage value of the nitrogen and phosphorus loss in particular algae cultures,

Along with the proceeding of the experiment the percentage of the nitrogen and phosphorus decreased.

Results from laboratory examinations were compared with possible nitrogen and phosphorus reduction (after 33 days of experiment) basing on the data from papers by Krauss (1956) and Gitelzon (1964) (Table VII).

Table VII. The actual (according to the investigations) and possible (according to the literature data) reduction of phosphorus and nitrogen

Culture	N		P	
	Actual (%)	Possible (%)	Actual (%)	Possible (%)
Chlorella vulgaris	28.4	42.5	57.5	28.4
Scedenesmus quadricauda	26.0	30.8	99.0	37.0
Scedenesmus quadricauda var. alternans	50.0	35.6	99.2	41.5
Scenodesmus obliquus	34.0	19.0	96.5	21.2
Scenedesmus acuminatus	35.0	33.0	97.0	58.3
Mixed culture	50.0	46.5	97.5	55.0
Mean	37.0	34.6	91.1	38.0

The actual total reduction of soluble phosphorus after 33 days of the experiment averaged for 6 cultures 37.3%, i.e. only slightly exceeding the possible reduction of phosphorus requisite for cell formation (34.6%). Considerable differences, however, are observed in particular cultures as concern both the actual and the possible loss of phosphorus. The actual reduction of phosphorus is very high, averaging 91.1%, i.e. it is nearly 2.5-fold greater (38.5%) than it would result from the possible reduction of phosphorus required for cell formation.

5. SUMMARY

Experiments carried out in laboratory conditions were to determine the role of algae in the removal of nitrogen and phosphorus from sewage. The investigated sewage, consisting of a mixture of sewage samples, mechanically and biologically treated, was inoculated with algae commonly occurring in biological ponds, namely: *Chlorella vulgaris, Scenedesmus quadricauda, S. quadricauda var. alternans, S. obliquus, S. acuminatus,* and mixed cultures.

During the course of the experiment adequate physico-chemical measurements were performed, as concerns: temperature, pH, soluble oxygen, CO_2, BOD_5, ammonium, nitrite, nitrate and organic nitrogen, and phosphorus. The scope of biological examinations comprised studies on quantitative analysis, average daily increase, dry mass of algae, greenery of the membrane filters, and so on.

The obtained results indicated an intensive development of algae and reduction of nitrogen and phosphorus from the sewage. Differences in the degree of reduction of nitrogen and phosphorus in particular monocultures were estimated. The highest reduction of both components in proportion to the dry mass increase was noted in *Scenedesmus obliquus* culture. The lowest percentage of nitrogen reduction was found in *S. quadricauda,* of phosphorus — in *Chlorella vulgaris.* The value of nitrogen reduction approximated the proportional share of this component in the algae dry mass, whereas the reduction of phosphorus was higher.

At the beginning of the experiment the percentage reduction of both nitrogen and phosphorus was higher than at the time of its final period.

6. STRESZCZENIE

Prowadzono badania w warunkach laboratoryjnych nad określeniem roli glonów przy usuwaniu azotu i fosforu ze ścieków. Badane ścieki, stanowiące mieszaninę ścieków po mechanicznym i biologicznym oczyszczeniu, zaszczepiono glonami powszechnie występującymi w stawach biologicznych, a mianowicie: *Chlorella vulgaris, Scenedesmus quadricauda, Scenedesmus quadricauda var. alternans, Scenedesmus acuminatus* oraz kulturą mieszaną.

Podczas eksperymentu wykonywano odpowiednie oznaczenia fizyczno-chemiczne, takie jak: temperatura, odczyn pH, tlen rozpuszczony, CO_2, BZT_5, azot amonowy, azotynowy, azotanowy, organiczny i fosfór. Zakres badań biologicznych obejmował analizę ilościową glonów, średniodobowy przyrost glonów, badania zazielenienia na sączkach membranowych, suchej masy glonów.

Na podstawie wyników badań stwierdzono intensywny rozwój glonów oraz reukcję azotu i fosforu ze ścieków. Dla poszczególnych monokultur notowano różną redukcję azotu i fosforu. Najwyższą redukcję azotu i fosforu w stosunku do przyrostu suchej masy glonów stwierdzono w kulturze *Scenedesmus obliquus.* Najniższy procent redukcji azotu w stosunku do przyrostu suchej masy stwierdzono w kulturze *Scenedesmus quadricauda,* a fosforu w kulturze *Chlorella vulgaris.* Redukcja azotu odpowiadała w przybliżeniu procentowemu udziałowi tego składnika w suchej masie glonów, natomiast redukcja fosforu była wyższa.

Na początku eksperymentu procentowa redukcja azotu i fosforu była wyższa niż w okresie końcowym.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	20	3	435-441	1973

The presented below methodical paper by M. B. Ivanova "Some remarks on the method of production estimation" is to criticize the method of calculation of zooplankton production proposed by W. Zawiślak ("Production of crustacean zooplankton in Moty Bay, Lake Jeziorak. Part I. The method of production estimation". *Pol. Arch. Hydrobiol.*, 1972, 19, 179–191), which has been elaborated — according to the author's intention — to modify significantly the common accepted method by Winberg; the modification having to increase the credibility of the estimations.

The subject of calculation methods, i. e. the estimation of animal biomass and production in a water body, is still investigated and discussed, especially when there are concerned such animals as zooplanktonic ones, i. e. those the life cycle of which is very short, the numbers and age structure are continuously changing, and their recruitment is continuous. During the development of researches on organism productivity, the problem of calculation methods have been widely discussed; many specialistic elaborations, particularly in the Soviet literature, can be found. They offer many methods which are commonly used. The detailed exposition of various calculation methods, including those for zooplankton, was published in IBP Manual No. 17 (W. T. Edmondson, G. G. Winberg [Eds] "*A Mannual of Methods for the Assessment of Secondary Productivity in Fresh Waters*". 1971, Blackwell, Oxford. In spite of all the systems existing which set the methods in order and unified them, each investigator estimating zooplankton production meets some difficulties and doubts. The most important of them are connected with great changeability of planktonic animals populations on the one hand, and with ever insufficient sampling methods, thus limited information on basic parameters for production calculations (requirement, growth, individual size) on the other hand. Therefore there is the situation stimulating to search for different modifications of the existing methods to make them more detailed.

That is why the Editorial Board of *Polish Archives of Hydrobiology* decided to publish the papers by W. Zawiślak notwithstanding the autor did not assume an attitude towards the critical notices of the papers reviewer, Dr. A. Hillbricht-Ilkowska. At present, this is the subject of the detailed critical estimate in the paper by M. B. Ivanova, published below.

Both the papers: by W. Zawiślak and M. B. Ivanova, discuss very important and interesting problems for all the investigators of secondary productivity, not only those who study the zooplankton productivity.

We invite to discuss.

The Editors

M. B. IVANOVA

SOME REMARKS ON THE METHOD OF PRODUCTION ESTIMATION

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ABSTRACT

The paper comprises the polemics against the papers by W. Zawiślak "Production of crustacean zooplankton in Moty Bay, Lake Jeziorak. Part I. The

method of production estimation" (*Pol. Arch. Hydrobiol.*, 1972, 19, 179–191), in which the modification of the Winberg's method of production calculation was presented.

Zawiślak (1972 a, b) critisizes the methods of production estimation of the population with the prolonged reproductive period (with continuous recruitment). This method is based on the calculation of the population production on the basis of the mean daily weight increment or growth rate of the animals.

The proposed remarks and "corrections" resulted from the author's wrong understanding of the analysed method.

This method was presented by Petrovich et al. (1961) for the first time and then developed in the subsequent works of Pechen' Shushkina (1964), Winberg et al. (1965), Pechen' et al. (1968), Winberg (1971). According to this method, the production value of a separate age group at every given moment is obtained by multiplying the average growth rate (weight increment) (C_B) by biomass (B). C_B for a given age (stage) equals an average rate of growth of the separate animals $\overline{C}_w$, and it is defined according to the growth curve. In the first variation $\overline{C}_w$ was calculated on the basis of the differences between the average weights (Petrovich et al. 1961).

$$C_w = \left(\frac{\overline{W}_{i+1} - \overline{W}_i}{D_i}\right) : \overline{W}_i \tag{1}$$

where D_i stands for the length of the development of the i stage.

But later, the calculation of the mean daily growth rate was made more precise (Pechen', Shushkina 1964, Winberg et al. 1965) and the difference between the initial W_i and final W_{i+1} weights of a given stage was taken instead of the differences between the average weights, while the final weight of the stage is considered to be the initial one for the stage $i+1$.

$$C_w = \left(\frac{W_{i+1} - W_i}{D_i}\right) : W_i \tag{2}$$

Because if $B_i = N_i \cdot W_i$, then $P_i = \left(\frac{W_{+1} - W_i}{D_i}\right) : N_i$

The production of the animal population (P) at every given moment of time equals:

$$P = \sum_{i=1}^{n} P_i = \sum_{i=1}^{n} \left(\frac{W_{i+1} - W_i}{D_i}\right) N_i \tag{3}$$

where n stands for the number of age groups, and the eggs ought

to be considered, as the first group in the sequence (i=1). If the eggs' initial weight equals zero, then the egg production equals:

$$P_e = \frac{N_e}{D_e} \cdot W_e \tag{4}$$

As a rule, while calculating the production, a whole day is considered the time measuring unit. It is evident that the population production during a longer period of time will be considered as an integral value.

$$P_t = \int_{t_o}^{t_n} P\,(dt)\,t \tag{5}$$

For example, it has been determined that for the moments of time t_1, t_2, $t_3 \ldots t_n$, the following values of production P_1, P_2, $P_3 \ldots P_n$ were obtained (Fig. 1).

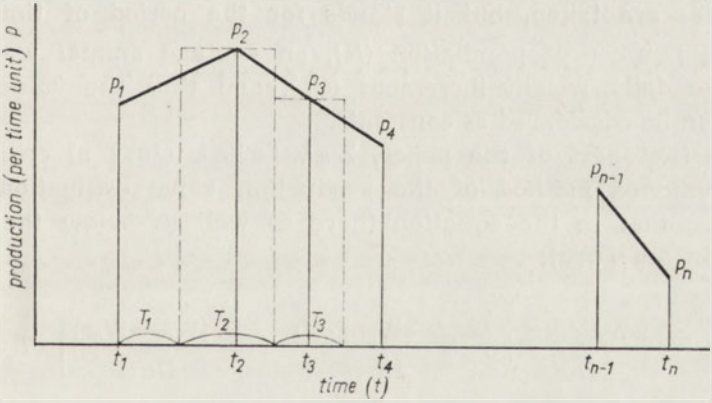

Fig. 1. Production estimation by means of the biomass increase. After the abscis: the length of time between the samplings; after the ordinate: the value of the population production (or of the age group) per time unit

The population production for the whole period of time is calculated on the basis of the area, limited by the curve of the daily production value. As a rule, the simplest method of integration, it means the calculation of the trapezium area, is used.

$$P_{t_1-t_n} = \frac{P_1+P_2}{2}(t_2-t_1) + \frac{P_2+P_3}{2}(t_3-t_2) + \ldots + \frac{P_{n-1}+P_n}{2}(t_n-t_{n-1}) \tag{6}$$

or:

$$P=P_1\left(\frac{t_2-t_1}{2}\right)+P_2\left(\frac{t_2-t_1}{2}+\frac{t_3-t_2}{2}\right)+\ \ldots\ +P_n\left(\frac{t_n-t_{n-1}}{2}\right)= \tag{7}$$

$$=P_1\frac{T_1}{2}+P_2T_2+\ \ldots\ P_n\frac{T_n}{2}$$

where T stands for the length of the period of time, for which we can assume that C_B is a constant. If the samples are taken at the same intervals of time, the formula looks as follows:

$$P = (P_2+P_3+\ \ldots\ +P_{n-1})\ T + (P_1+P_n)\frac{T}{2} \tag{8}$$

because $t_2-t_1=t_3-t_2=t_n-t_{n-1}=T$

Thus in formula

$$P_t=(Pe+Pn+Pc)T \tag{9}$$

where Pe, Pn and Pc stand for the production value of different age groups, T does not stand for the intervals of time between which the samples are taken, but it stands for the period of time during which the number of population (N), an average animal weight ($\overline{W}$), an average daily weight increment (C_B), and thus the value of production, can be considered as constants.

In the first part of the paper, Zawiślak (1972 a) critisizes the above mentioned method of the production value estimation, misunderstanding that in the equation (here as well as below the formula is given for Copepoda):

$$P_{TW}=\left[\frac{We\cdot Ne}{De}+\frac{(W_0c-W_0n)\cdot Nn}{Dn}+\frac{Nc(Wa-W_0c)}{Dc}\right]\cdot T \tag{10}$$

T stands for the length of time between the different production value estimations. Basing on this wrong presentation, i. e. that during the T average growth rate varies, the author suggests to introduce some corrections into the existing formula. His opinion is based on the assumption that during the period T the animals have changed their age group, so that their $\overline{C}_w$ also changes.

Zawiślak divides each age group into two classes (the method of division has not been described): the animals in which the age status has not changed during the T period belong to the first class, thus for the second class — the ones which will be transformed into the next age group. In the result the equation for calculation of the production looks as follows:

$$P_{TW} = \left[\left(\frac{Ne \cdot We}{De}\right) \cdot T + \left(\frac{PnT \cdot T}{2}\right) \cdot \frac{Ne}{De}\right] + \left[PnT \cdot NnCl_1 + \frac{PcT}{2} NcCl_2\right] + \left[PcT \cdot NcCl_1 + \frac{PcT}{2} NcCl_2\right] \quad (11)$$

The authors does not take into consideration the fact, as it is seen from equation (11), that during the intervals between the sampling not only individual animals change their age group but their number is changed because of elimination. As it is well known, the changes in number can be described by the exponential function:

$$N_t = N_0 e^{rT}; \quad r = \frac{\ln N_t - \ln N_0}{T} \quad (12)$$

where N_0 stands for the initial number, N_t stands for the final number, T — the interval of time between the calculations, i. e. $t_2 - t_1$ while using this method (equations 5, 6, 7 picture) or T according to the author's understanding of the articles being under discussion. Let us discuss this change in the number on the example of the egg production calculation (Fig. 2) representing the simplified picture from Zawiślak (1972).

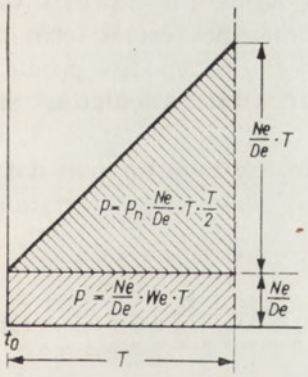

Fig. 2. The egg production estimation by means of Zawiślak's method (1972). After the absciss: the length of time between samplings; after the ordinate: the increase of the egg number per time unit (a day)

Zawiślak divides the whole egg production into the production of eggs ($Ne/De \cdot We$) and the production of those nauplii, which have hatched from the eggs laid by females during the investigated period of time. According to the author, on the basis of the daily egg production Ne/De the nauplii number hatching from the eggs equals $(Ne/De)T$, but the author ignores completely the nauplii decrease caused by the elimination.

But, it is clear, that in reality the nauplii number at the end of the T period will equals:

$$N_T = (Ne/De) \cdot T - \mu T \tag{13}$$

$$M_T = (Ne/De)_e^{-dT} \tag{14}$$

where M_T stands for the number of the eliminated animals, d — death rate.

Then the whole egg production (the first term of the equation 11) will be as follows:

$$Pe_T = \left(\frac{Ne \cdot We}{De}\right) \cdot T + \left[\left(\frac{Ne}{De} \cdot T - \frac{Ne}{De} \cdot e^{-dT}\right) \cdot PnT\right] \tag{15}$$

The value, calculated in such a way, will be lower than the one obtained by the author. Thus, while calculating the production, we take into account a very difficult for calculation value — the death rate of only those nauplii, which were hatched from the eggs laid by females during the period T. Therefore in order to calculate this value it was necessary to perform the detailed analysis of the nauplii age structure at the beginning and the end of the T period, and to estimate the nauplii growth exact to 24 hr which is very difficult.

Similar presentation of the constant animal number comprised the basis for the calculation of the nauplii production (the second term of the equation 11) as well as for the calculation of the copepodite production (the third term of the same equation). But, while calculating the production on the basis of the generally used method (equation 5, 6, 7, Fig. 1), changes in number, occurring between the investigation data, for which the production is being calculated, are taken into calculation.

And really,

$$P_1 = C_{B1} \cdot N_1 \cdot \overline{W}_1;\ P_2 = C_{B2} \cdot N_2 \cdot \overline{W}_2;\ P_T = \frac{P_1 + P_2}{2} \cdot T \tag{16}$$

And because of the fact, that the production calculated on the basis of the daily growth rate takes also into consideration the changes in number (the increase because of the recruitment and the decrease because of the elimination), therefore the population production, during the whole vegetative season, obtained on the basis of the biomass growth rate calculation equals the one, calculated after the elimination, only if the population number and its age structure is the same at the beginning and the end of the season.

The second part of the article contains some data concerning Jeziorak Lake production; the data have been obtained by means of formula offered by Winberg et al. (1965), modified by Zawiślak's (1972 a) interpretation as well as the author's own formulae. As it was expected, the general modifications concerning Copepodites with slower development were less significant, for Cladocera, that grow much quicker, the difference in the result appeared to be fairly significant.

The production value estimated by means of Zawiślak's equation (1972 a, b) is twice higher than the same value obtained by the means of an ordinary method. Most probable it indicates the considerable rate of the crustaceans elimination in this lake, that the author has not taken into consideration.

Summing up, it is necessary to note that it is impossible to agree with Zawiślak (1972 a, b) modifications that were suggested to be introduced into the formula of the production estimation on the basis of the rate of biomass increase.

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INVESTIGATIONS ON INFLUENCE OF HEATED WATERS FROM THE POWER STATION AT SKAWINA ON SKAWINKA AND VISTULA RIVERS

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ABSTRACT

Influence of heated waters discharged from the power station at Skawina on river biocenosis and chemical properties of water in Skawinka and Vistula rivers was investigated. In Vistula River, oxygen contents were decreasing and quantitative changes in biocenosis under the influence of discharges of heated waters were observed. The highest harmless temperature of heated water was established to be 30°C.

1. INTRODUCTION

The significance of the influence of heating the surface water by discharges from thermal power stations is ever increasing. Thermal power stations use considerable amounts of water for their cooling systems, thus heating it and bringing a negative influence on biological and chemical conditions of rivers. Up to now, any detailed data on destructiveness and on a permissible heating degree, causing no harmful effects are missing.

The subject of heated waters has been studied by several authors, especially since the early sixties. The first more extensive Polish investigation was on Vistula and San rivers (Możliwości... 1962). Kresser (1957) discussed heated waters and sewages. According to him, in polluted waters no definite relationship between increased temperature and self-purification was found. The Reports of US Committees (1962) are a quite comprehensive source of informations on chemical and biological conditions in artificially heated American waters. The papers by Trembley (1960, 1961) estimated an influence of heated waters on life of aquatic organisms in USA. In France the investigations of heated rivers were described by Goubet (1965), Appourchaux (1965) and Leynaud (1967). The present author described an influence of heating on chemism and biocenosis of surface waters in the Report for the European Commission for Economy of UNO in Geneva Turoboyski (1969 a, b) being a collective complex elaboration on the subject of heated waters.

The above mentioned papers offer a brief survey of investigations on heated waters. The aim of the present paper was to investigate an influence of heated waters on rivers and to elaborate some standards of harmless heating of water by a power station, by way of an example of Vistula and Skawinka rivers near Cracow, heated by the power station in Skawina.

2. TERRAIN DESCRIPTION

The mean water flow of Vistula River at Tyniec is about 80 m^3/sec. The drainage area of Skawinka River, a right-bank affluent of Vistula, is 364 km^2.

The Łączany-Skawinka Channel, 17 km long, leads water to the sluice at Borek Szlachecki and to power station at Skawina (Fig. 1).

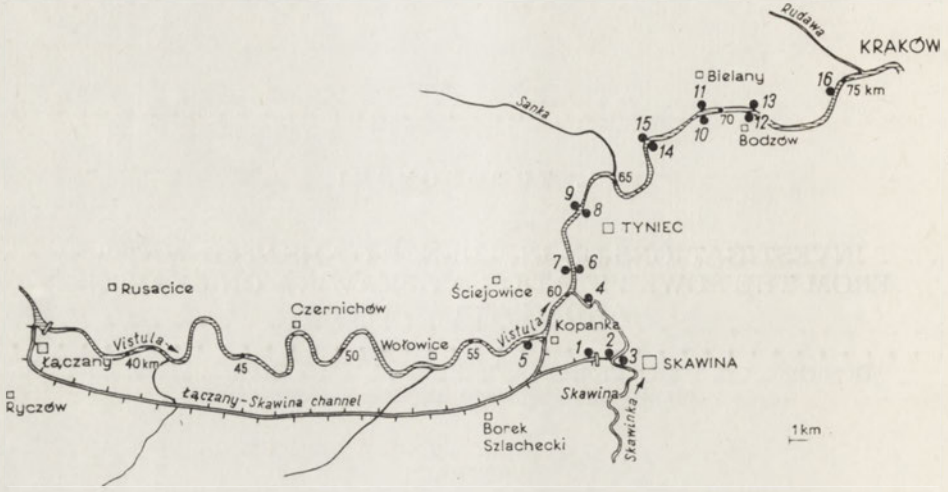

Fig. 1. The investigated sections of Vistula and Skawinka rivers. The numbered circles — sampling stations, arrows — directions of water flow

Water requirement of the power station is 16–24 m³/sec. The discharged water is heated in condensors of the power station and it falls into Skawinka River at its 3.3 rd km and further on into Vistula River. The water flow of Skawinka River (without the discharges from the power station) is 2.6 m³/sec. The water flow values are presented in Fig. 2.

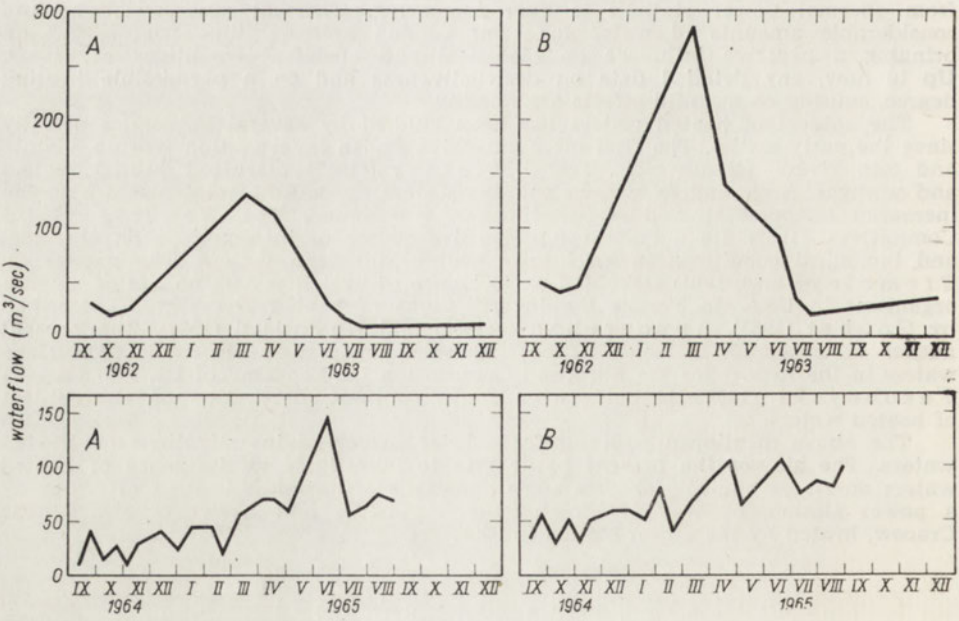

Fig. 2. The water flows in Vistula River in 1962–1963 and 1964–1965. A — upstream of the estuary of Skawinka River (km 57.5 th), B — at Bielany (km 69 th)

The main pollution sources of Vistula River are: trade wastes from Silesia carried by Przemsza and Upper Vistula, and trade wastes from the industrial regions of Trzebinia, Chrzanów and from the Chemical Works at Oświęcim. Trade wastes from Silesia contain coal powder, mineral oils, phenols, chlorides, sulphates, urban sewages and others. Besides, wastes from the Cellulose Factory at Klucze and several toxic organic compounds coming from the Chemical Works at Oświęcim are carried by Przemsza River; wastes from the petroleum rafinery at Trzebinia and others, e. g. heavy metals, come into Vistula River. Vistula River in the investigated section is dramatically polluted; oil smudges and various suspensions can often be seen in water; the bottom is covered with coal powder, sometimes water smells of phenols or petroleum sewages.

The investigations were carried on in the following stations (Fig. 1):

1. Łączany Channel; the water intake by the power station at Skawina,
2. The place of discharges of heated water of the power station to Skawinka River,
3. Skawinka River upstream of the discharges from the power station (km 3.5th),
4. Skawinka River upstream of its estuary to Vistula River (Vistula km 60.2nd),
5. Vistula River upstream of the estuary of Skawinka River (km 57.5th),
6. Vistula River downstream of the estuary of Skawinka River, right bank (km 61st),
7. Vistula River downstream of the estuary of Skawinka River, left bank (km 61st),
8. Vistula River at Tyniec, right bank (km 63.5th),
9. Vistula River at Tyniec, left bank (km 63.5th),
10. Vistula River at Bielany, right bank (km 69th),
11. Vistula River at Bielany, left bank (km 69th),
12. Vistula River at Bodzów, right bank (km 72nd),
13. Vistula River at Bodzów, left bank (km 72nd),
14. Vistula River upstream of Bielany, right bank (km 67.5th),
15. Vistula River upstream of Bielany, left bank (km 67.5th),
16. Vistula River at Cracow (km 74.5th),
17. Raba River at Książnice (km 37.4th) (investigated for comparison).

In 1962–1963 stations No. 1–13 were investigated, and in 1964–1965 — No. 4–7 and 14–17.

3. METHODS

The physico-chemical analyses were made according to Just, Hermanowicz (1964) and Przyłęcki (1954). The following estimations were made: water and air temperatures, water turbidity, colour, smell, pH, dissolved oxygen, air saturation BOD_5, chemical oxygen demand ($KMnO_4$), suphates, chlorides, total iron, manganese, ammonia nitrogen, total hardness, carbonate and non-carbonate hardness, total alkalinity, free and aggressive carbonate dioxide, dry residuals, suspensions, phenols. In total, 330 chemical samples were analysed.

Hydrobiological analyses were made in the following way:

In 1962–1963, the seston was filtered through the plankton net of silk gauze with meshes of 70 μ in diameter. 30 l of water were condensed up to 40 ml and the organisms were counted in a Kolkwitz's chamber after their previous more detailed determination on a slide. The results were recalculated per 1 l of water. A usual (not-inversed) Zeiss microscope was used. In total, 96 samples of seston were analysed.

Microbenthos from submerged stones was estimated according to a scale of five degrees: 1 — very few, 2 — few, 3 — many, 4 — very many, 5 — in masses. In total, 36 samples of microbenthos were analysed.

In 1964–1965 samples were taken from the so-called diatometers which had been placed in a river. A device is made of a polystyrene block connected with a microscope slide. Those diatoma that settled on the polystyrene blocks were counted and values were given in per cent, whereas the other organisms were determined on the basis of analyses of the populations settled on the slides and their densities were given per 1 cm^2 of a slide. As it happens, the diatoma grow specially well on polystyrene, and the other organisms — on slides. Periphyton was investigated by Pudo (1969) (320 samples).

Macrofauna was sampled from an area of 1 m^2 with a bottom scraper and the results were given per 1 m^2 of bottom. In total, 160 samples of macrofauna were taken (the analyses were made by mgr A. Kysela for a commission of the Institute of Water Economics Research).

The investigations lasted for 31 months and comprised 38 analysed series in total. Moreover, 15 series of simplified analyses of the Vistula profile at km 62nd in a single both banks section were made (Turoboyski, Humnicka 1965). In total, more than 1400 hydrobiological, chemical and bacteriological analyses were made, as well as 16 series of ichtiological investigations (Badania... 1967).

4. RESULTS

TEMPERATURE

An increase of water temperature in Vistula River caused by heating in the condensors of the power station was about 7°C with the maximum

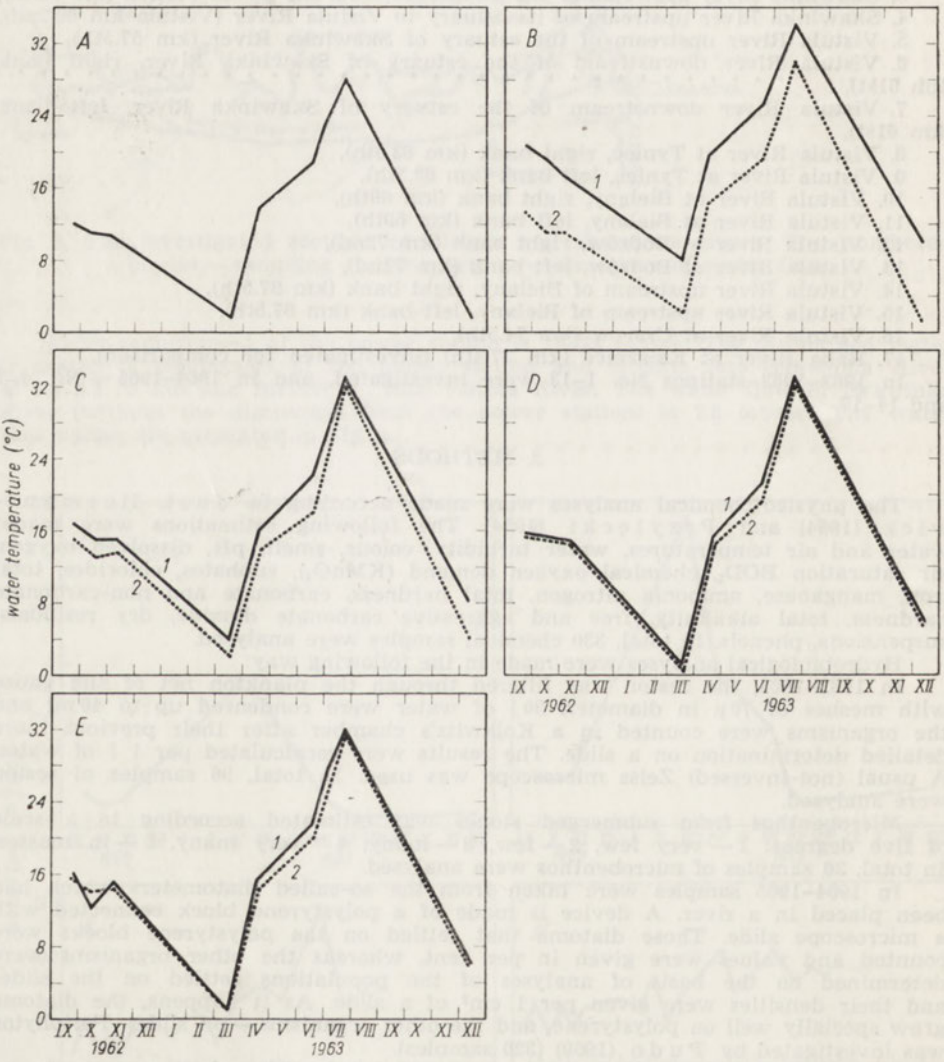

Fig. 3. Water temperature in Vistula River in 1962–1963. 1 — right bank, 2 — left bank. A — upstream of the estuary of Skawinka River (km 57.5 th), B — downstream of the estuary of Skawinka River (km 61 st), C — at Tyniec (km 63.5 th), D — at Bielany (km 69 th), E — at Bodzów (km 72 nd)

of 9.5°C. The maximum temperature of discharged water 35°C was recorded on July 26, 1963. The discharged waters were becoming slightly cooler already in Skawinka River; but only once (on March 19, 1963) it was by 3°C.

Maximum temperature of water in Vistula, heated with discharged water from the power station (34°C) was recorded on July 26, 1963, in the right heated current at km 61st (Fig. 3 B). On that day water temperature at Bielany (Fig. 3 D) and at Bodzów (Fig. 3 E) was 32°C. The differences in water temperatures of Vistula River between unheated water upstream of the estuary of Skawinka River (km 57.5th — Fig. 4 A)

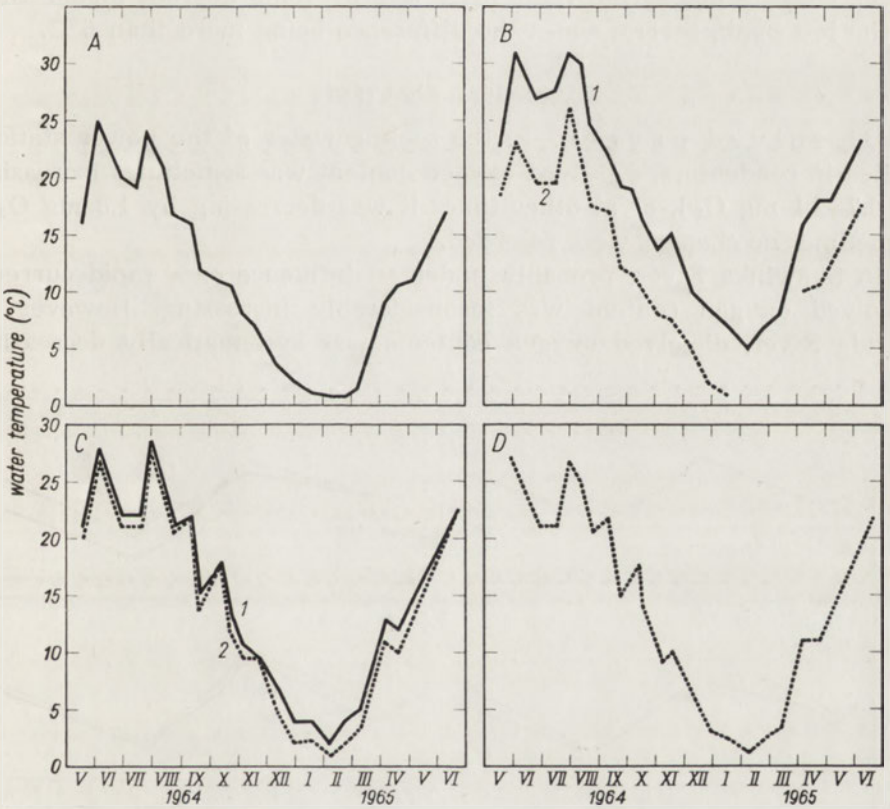

Fig. 4. Water temperature in Vistula River in 1964–1965. 1 — right bank, 2 — left bank. A — upstream of the estuary of Skawinka River (km 57.5 th), B — downstream of the estuary of Skawinka River (km 61 st), C — upstream of Bielany (km 67.5 th), D — at Cracow (km 74.5 th)

and heated water downstream of Skawinka River at right bank (km 61st — Fig. 4 B) were up to 9°C (e.g. in July 1964). Great differences in water temperatures were also downstream of the estuary of Skawinka River between the right and left current of Vistula (Fig. 3, 4). The right

current was as a rule warmer, since the heated waters from the power station flowing along Skawinka River were gradually mixing with water of Vistula and initially they were flowing near the right bank. In this way the water temperatures of the right and left current were gradually levelling and the temperature became even as far as at Bodzów (km 72nd) and sometimes at Bielany (km 69th). Water temperature at km 61st in the right current was never lower than 5°C.

In 1963 the additional investigations of water temperature in Vistula River were made at km 62nd at both banks during the exceptionally hot summer (Turoboyski, Humnicka 1965). Even then the heated water temperature at the right bank was by some degrees higher than at the left bank, several times the difference being more than 6°C.

CHEMICAL ANALYSES

Dissolved oxygen. In the cooling water of the power station, heated in condensors, dissolved oxygen content was sometimes increasing by 0.7–2.1 mg O_2/l, or at other times it was decreasing by 1.8 mg O_2/l. Sometimes no changes were recorded.

In Skawinka River, probably under an influence of a rapid current, dissolved oxygen content was inconsiderably increasing. However in Vistula River, dissolved oxygen contents was systematically decreasing

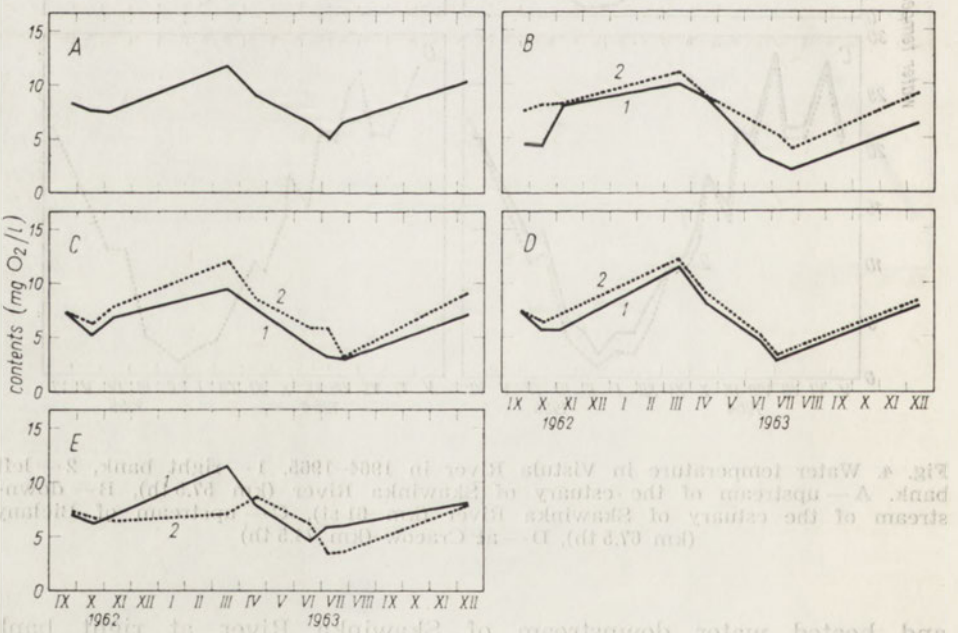

Fig. 5. Dissolved oxygen contents in water of Vistula River in 1962–1963. 1 — right bank, 2 — left bank. A — upstream of the estuary of Skawinka River (km 57.5 th), B — downstream of the estuary of Skawinka River (km 61 st), C — at Tyniec (km 63.5 th), D — at Bielany (km 69 th), E — at Bodzów (km 72 nd)

under an influence of heated waters flowing from the power station. It was lower by as much as 5 mg O_2/l at the right bank at km 61st on June 2, 1964; this is particularly injurious in conditions of the heavy polluted Vistula River. The minimum dissolved oxygen contents were recorded in the right current at km 61st on July 26, 1963, June 17, 1964, and July 29, 1964 — about 2 mg O_2/l (Fig. 6 B). Similar oxygen contents

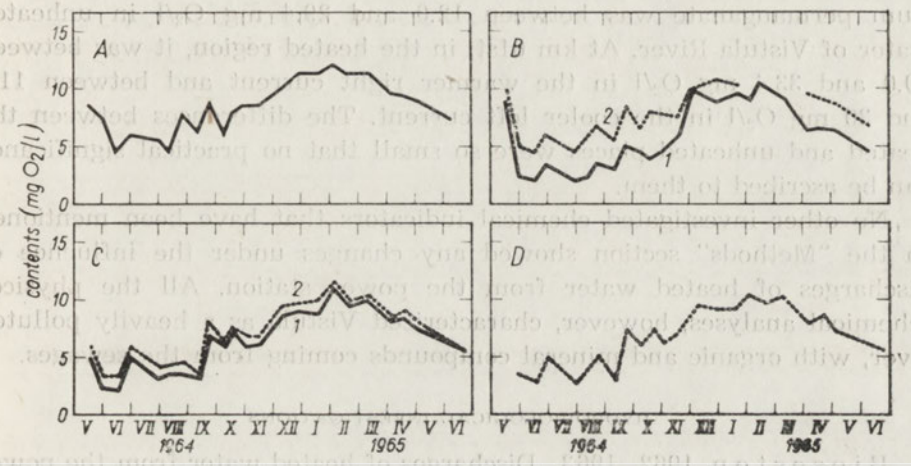

Fig. 6. Dissolved oxygen contents in water of Vistula River in 1964–1965. 1 — right bank, 2 — left bank. A — upstream of the estuary of Skawinka River (km 57.5 th), B — downstream of the estuary of Skawinka River (km 61 st), C — upstream of Bielany (km 67.5 th), D — at Cracov (km 74.5 th)

was found upstream of Bielany (km 67.5th) in June 1964 and then even at Cracow (km 74.5th) it was only 3 mg O_2/l. Also in the cooler left current, the dissolved oxygen contents were often low, e.g. it was as low as 3 mg O_2/l at Bielany (km 69th) on July 5, 1963 (Fig. 5). In general, per cent of saturation in Vistula River was decreasing under an influence of heated waters from the power station, especially in the right current at km 61st.

Biological oxygen demand. In Vistula River upstream of the estuary of Skawinka River (km 57.5th), BOD_5 was between 3.1 and 15.2 mg O_2/l in unheated water. Downstream of the estuary of Skawinka River, it was between 2.5 and 16.4 mg O_2/l in the heated right current and between 2.7 and 10 mg O_2/l in the less heated left current. The minimum values are not reliable as they must have been higher.

The water of Vistula River contains many toxic compounds which certainly influence the self-purification intensity being decreased through inhibition of bacteria activity; for these reasons BOD_5 is lower. Bio-

chemical oxygen demand is often somewhat higher in heated water than in unheated water upstream of the estuary of Skawinka River. However, on July 4, 1963, it was 5.3 mg O_2/l in unheated water and 9.3 mg O_2/l in heated water. But in general the differences were inconsiderable.

Chemical oxygen demand. Oxygen consumed from potassium permanganate was between 12.0 and 29.4 mg O_2/l in unheated water of Vistula River. At km 61st, in the heated region, it was between 10.0 and 33.4 mg O_2/l in the warmer right current and between 11.8 and 30 mg O_2/l in the cooler left current. The differences between the heated and unheated places were so small that no practical significance can be ascribed to them.

No other investigated chemical indicators that have been mentioned in the "Methods" section showed any changes under the influence of discharges of heated water from the power station. All the physico-chemical analyses, however, characterized Vistula as a heavily polluted river, with organic and mineral compounds coming from the sewages.

HYDROBIOLOGICAL INVESTIGATIONS

Bioseston 1962—1963. Discharges of heated water from the power station at Skawina have influenced neither the species composition nor the numbers of aquatic organisms around the place of discharge. The most numerous group was Bacillariophyceae.

In bioseston of Vistula River the most numerous were *Sphaerotilus natans* Kütz. bacteria, and Bacillariophyceae *Nitzschia palea* (Kütz.) W. Sm. and *Nitzschia acicularis* W. Sm. In summer, when water temperatures were between 28 and 34°C, *Nitzschia palea* was the most numerous species, *Nitzschia acicularis* W. Sm., and Chlorophyceae — *Eudorine elegans* Ehr. and *Scenedesmus quadricauda* (Turp.) Bréb. were also abundant. The most numerous animal species in summer was *Arcella vulgaris* Ehr. (Rhizopoda).

During very hot days the numbers of other organisms, such as *Sphaerotilus natans* and *Asterionella formosa* Hassal, were decreasing, both in the warmer regions of Vistula and in those not heated with cooling waters from the power station. Exceptionally high densities of bioseston were observed in the warm period (July 1963 — Fig. 7). The negative influence of heated water on the number of bioseston organisms could be observed in this period downstream of the estuary of Skawinka at km 61 st (Fig. 7 B — right bank). Their density there was lesser than upstream, at km 57.5 th (Fig. 7 A) where water was not heated.

In the warm period, as water became cooler downstream, numbers of bioseston organisms were gradually increasing (as compared with

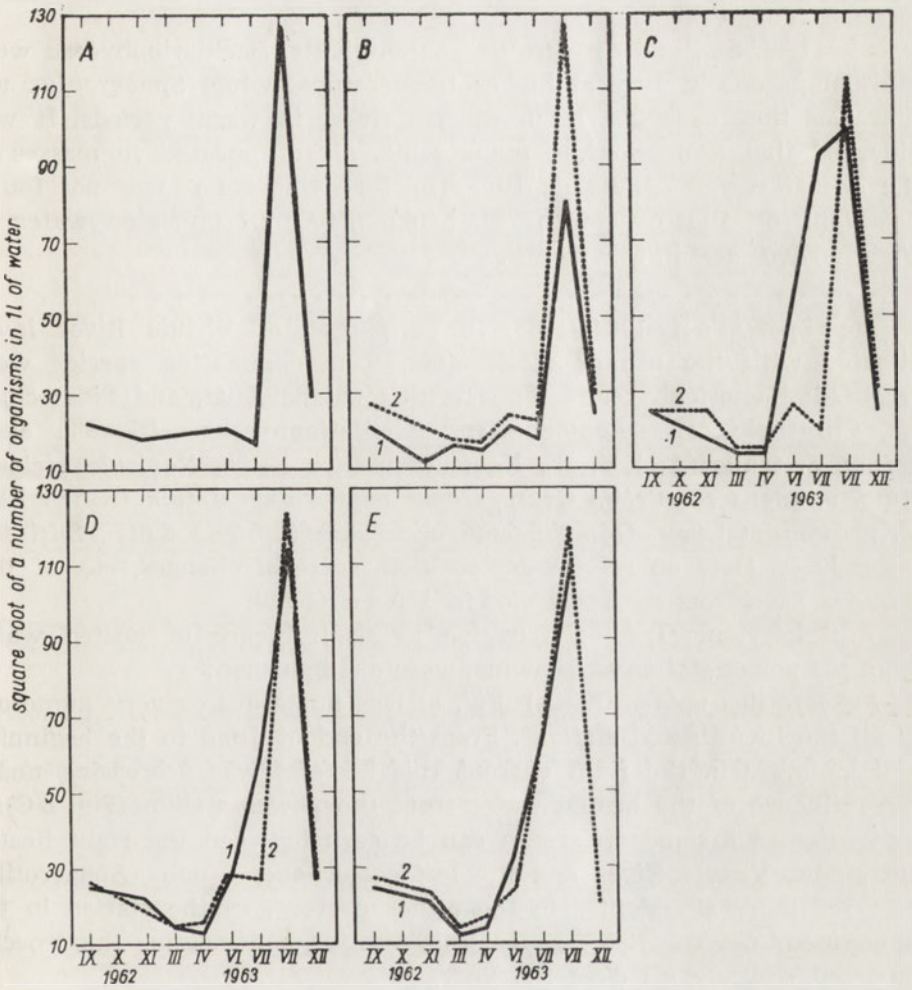

Fig. 7. The total numbers of microorganisms in the seston of Vistula River in 1962–1963. 1 — right bank, 2 — left bank. A — upstream of the estuary of Skawinka River (km 57.5 th), B — downstream of the estuary of Skawinka River (km 61 st), C — at Tyniec (km 63.5 th), D — at Bielany (km 69 th), E — at Bodzów (km 72 nd)

the station at km 61st) and at the end of the investigated section they were similar to those recorded upstream of the estuary of Skawinka River at km 57.5 th. In the warmer right current the organisms were less numerous than in the left one. To sum up, it can be stated that in the hot period the heated water brought about a decrease of algae, animal organisms and *Sphaerotilus natans* Kütz. bacteria, the most conspicuously when water temperature was above 30°C.

Microbenthos 1962–1963. In microbenthos communities, the most numerous organisms were *Nitzschia palea* (Kütz.) W. Sm., *Nitzschia*

acicularis W. Sm. and *Sphaerotilus natans* Kütz. Bacillariophyceae were most numerous in July at higher temperatures while *Sphaerotilus natans* was then decaying, as usually in rivers in warm periods. It was observed that *Stigeoclonium tenue* Kütz. algae appeared in masses in the heated region of Vistula River in July whereas it was not found upstream of the estuary of Skawinka River in unheated water of Vistula River also in this month.

Periphyton 1964—1965. In periphyton of Vistula River investigated with the use of diatometers, 9 predominating species were recorded: Bacteriophyceae — *Sphaerotilus natans* Kütz. and *Siderocopsa sp.*, Chrysophyceae — *Lagynion sp.*, Bacillariophyceae — *Diatoma elongatum V. tenue* (Ag.) V. H., *Navicula mutica* Kütz., *Navicula viridula* Kütz. *v. avenacea* (Bréb.) Grun., *Gomphonema angustatum* (Kütz.) Rbh. *V. productum* Grun. *Gomphonema olivaceum* (Lyngb.) Kütz., *Surirella ovata* Kütz. Data on species composition, seasonal changes, etc. of this group of organisms were compiled in Pudo (1969).

7 periphytonic species, sensitive to an influence of heated water from the power station at Skawina, were distinguished:

Sphaerotilus natans (Kütz.) (Fig. 8) was numerous or very numerous in all the investigated stations. From the end of June to the beginning of September in the right current this bacteria was decreasing under the influence of the heated water from the power station (Fig. 8 C).

In Figure 8 some difference can be seen between the right heated current of Vistula River and the left cooler one; usually Sphaerotilus occurred more abundantly in the cooler current. In the station in the unpolluted, natural Raba River, selected for comparison, this species occurred scarcely all over the year.

Siderocapsa sp. occurred in Vistula and Skawinka rivers only in summer, scarcely in Vistula River upstream of the estuary of Skawinka River and in masses in the heated section of Vistula. In Raba River it was not found.

Lagynion sp. occurred in masses in summer in all the investigated stations in Vistula and Skawinka rivers; in autumn it was less abundant as water got cooler and in February and March it disappeared completely in the left less heated current. In the right warmer current, it could be found, although in small amounts, all the winter. Thus it is a stenothermal species. In Raba River it occurred in small amounts in summer and autumn and since November it had disappeared.

Diatoma elongatum Lyngb. Ag. *V. tenue* (Ag.) V. H. occurred in small amounts in all the stations in summer and autumn, and in winter and spring it was more abundant. Under the influence of heated water from the power station, in Skawinka River and at km 61 st in the right current of Vistula River, considerable decreases of its numbers

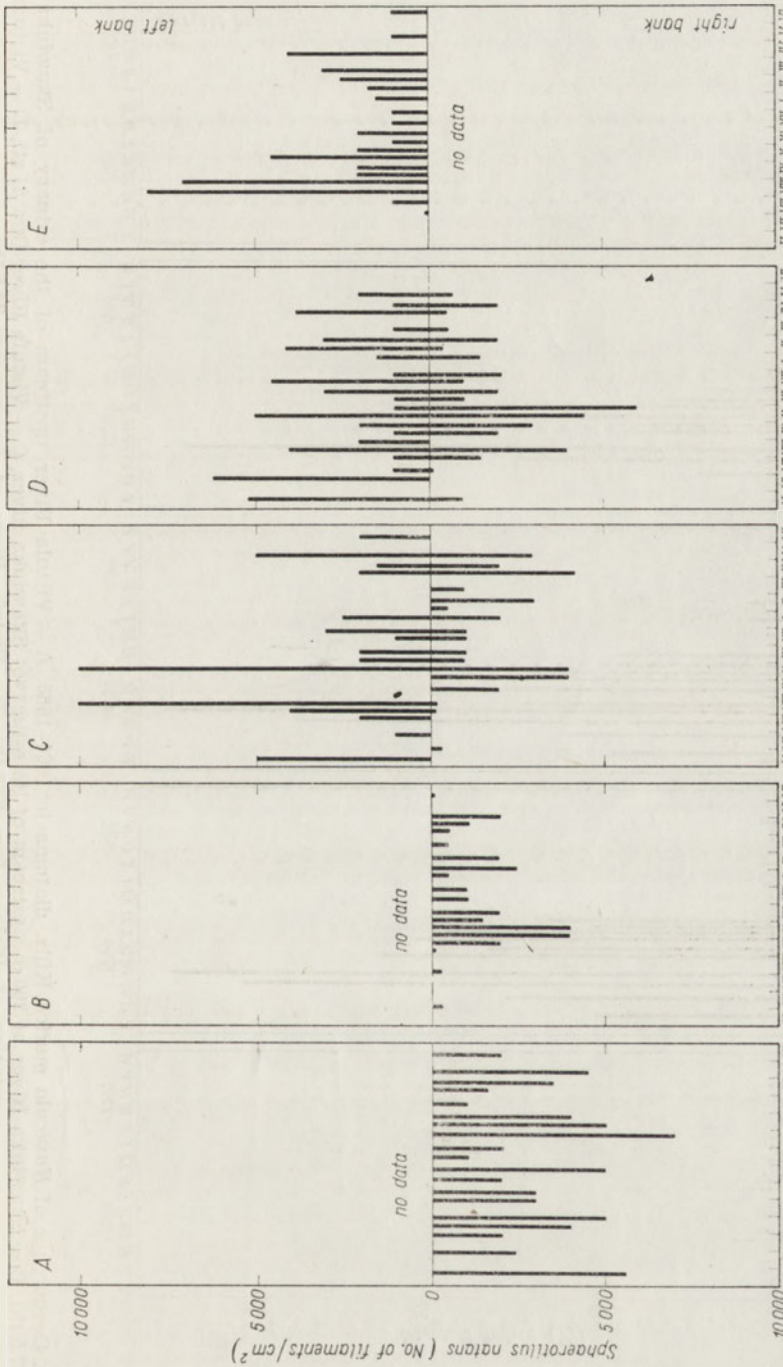

Fig. 8. Occurrence of *Sphaerotilus natans* Kütz. bacteria in 1964–1965 (the numbers of filaments per 1 cm² of a slide area). A — Vistula River upstream of the estuary of Skawinka River(km 57.5 th), B — Skawinka River at 300 m upstream of its estuary to Vistula River, C — Vistula River downstream of the estuary of A. — Vistula River upstream of the estuary of Skawinka River (km 67.5 th), E — Vistula River at Cracow (km 74.5 th)

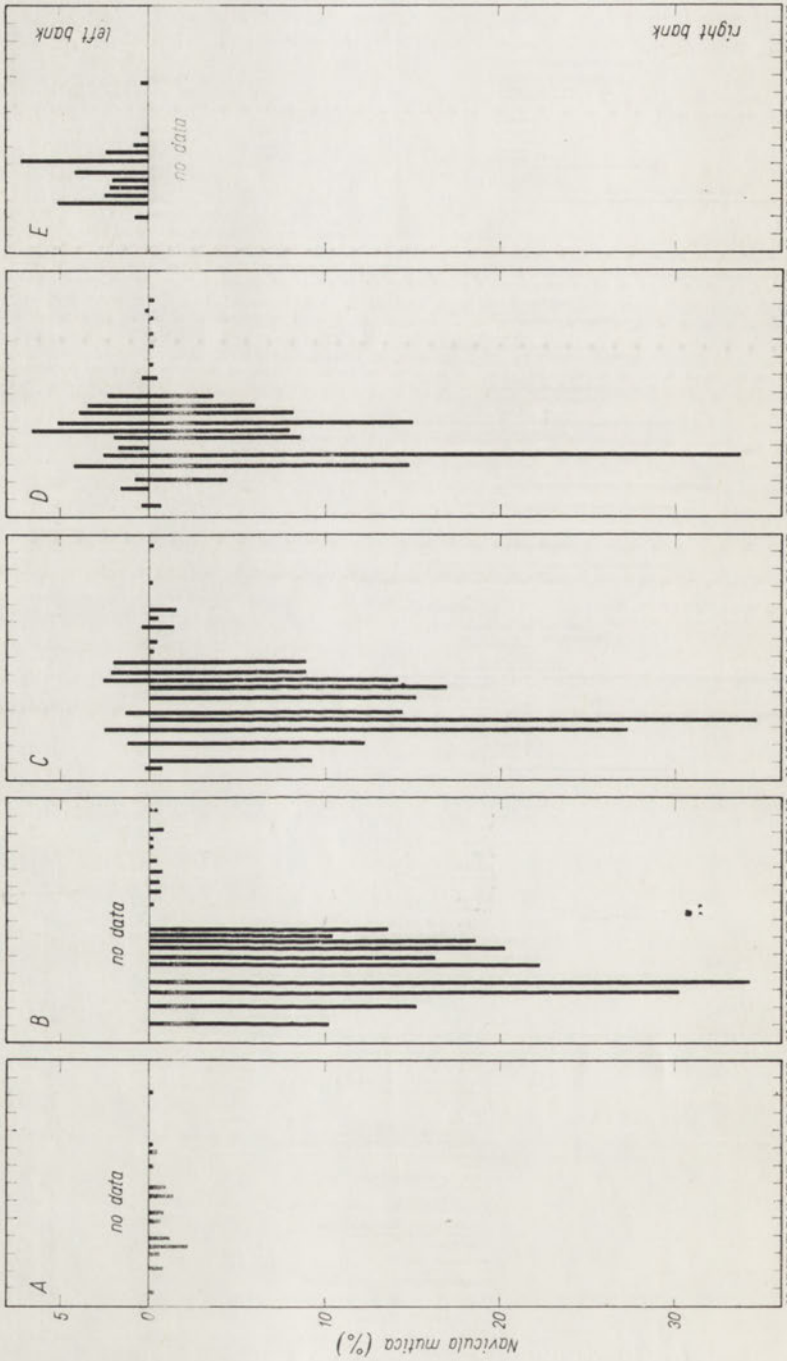

Fig. 9. Occurrence of *Navicula mutica* Kütz. diatoma in 1964–1965. A — Vistula River upstream of the estuary of Skawinka River (km 57.5 th), B — Skawinka River at 300 m upstream of its estuaryof Skawinka River, C — Vistula River (km 61 st), D — Vistula River upstream of Bielany (km 67.5 th), E — Vistula River at Cracow km 74.5 th)

were recorded. In Raba River, like in Vistula, the diatoma were not numerous in summer and autumn and abundant in winter and spring.

Navicula mutica Kütz. (Fig. 9) was remarkably reacting to the heated water from the power station; it was intensely developing in the heated region of Vistula River downstream of the estuary of Skawinka River while in the unheated region it was scarce. As water was cooler, e.g. by Cracow (km 74.5 th), it was less abundant than in the heated water. In Raba River it was not found at all.

Navicula viridula Kütz. *v. avenacea* (Bréb.) Grun. occurred all the year in Vistula and Skawinka rivers, but more abundantly in the heated current. In Raba River it was scarce.

Surirella ovata Kütz. occurred in Vistula and Skawinka rivers during the whole period of the investigations in the most heated places, i. e. in the right current of Vistula River at km 61 st and in Skawinka River upstream of its estuary to Vistula. In other stations it was most numerous from September to January. In Raba River it was scarce all the year.

Macrofauna 1964–1965. The investigations showed a nearly complete retreat of organisms sensible to pollutions, such as Ephemeroptera, Plecoptera and Trichoptera larvae, which had been abundant earlier (unpublished data of Kat. Ryb. UJ). It testifies to a catastrophic state of Vistula River; these organisms are still abundant in the unpolluted Raba River. The present analyses showed more intensive deve-

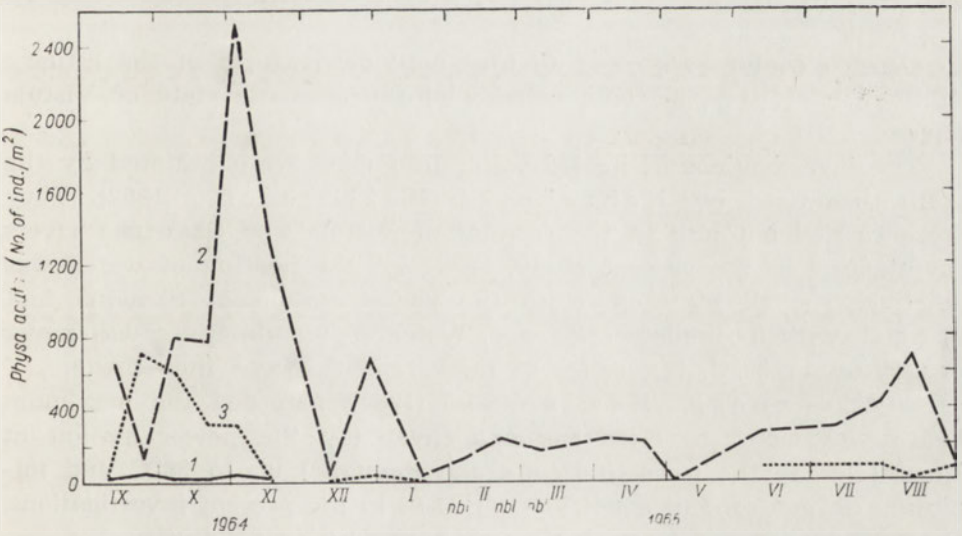

Fig. 10. Occurrence of *Physa acuta* Drap. snail in Vistula River in 1964–1965 (the numbers of individuals per 1 m² of bottom area). 1 — upstream of the estuary of Skawinka River (km 57.5 th), 2 — downstream of the estuary of Skawinka River (km 61 st), right bank, 3 — downstream of the estuary of Skawinka River (km 61 st), left bank

lopment of *Physa acuta* Drap. (Mollusca) in the heated region (Fig. 10). It was less and less abundant towards Cracow, as water became cooler.

5. DISCUSSION

To make the hydrochemical characteristics of Vistula River in its present state is by no means easy. Limnological criteria based on chemical spectral analyses for water under natural conditions cannot be used in the conditions of considerable and different pollutions.

This has not always been so. Starmach (1938) defined Vistula River near Bielany, according to Naumann spectra, as a mesotype river which was equivalent to a transition towards an eutrophic type.

Deterioration of water of Vistula during the recent years is unquestionable. In the present investigations, the considerable water salinity in Vistula River was recorded, up to 700 mg/l, with abundant sulphates which together with aggressive carbon dioxide constitute a harmful corrosive factor. The corroding impact was certainly intensified by heated water from the power station at Skawina. High turbidity of water (more than 100 mg SiO_2/l) inhibited photosynthesis by impairing the translucency. Skawinka River, beginning from the discharge place, can be characterized similarly. Frequent low oxygen content, caused by considerable organic pollutions as well as water toxicity, caused by abundance of such compounds as phenols and others, make the water of Vistula River to be qualified as unsuitable to any purposes. Coal powder covered the river bottom, thus mechanically destroying its biocenosis.

Oxygen content decrease, up to 2 mg/l downstream of the estuary of Skawinka River, is another index of the dramatic state of Vistula River.

The investigations of heated water in Poland were initiated by the team supervised by Nehrebecki (Możliwości ... 1962). They were carried out also in the sections of Vistula and Skawinka rivers investigated in the present study. However, the heating of water was relatively small by then since the power station at Skawina had operated with incomplete efficiency. Water of Vistula River was never heated up to 30°C. Six series of measurements were then made.

Stangenberg, Pawlaczyk (1960) recorded the maximum heating of water in Nysa Łużycka River (by the power station at Hirschfelde in the German Democratic Republic) up to 36°C and minimum oxygen content equal to 4 mg O_2/l. In the present investigations, the extreme values were 34°C and 2 mg O_2/l, respectively.

The investigations of American rivers in the USA (Trembley 1960, Report 1962) showed similar trends of changes as in Vistula River: an increase or decrease of oxygen content in water which just passed

through the condensors of power stations, a decrease of oxygen content and a small increase of BOD_5 in the rivers artificially heated by discharges from power stations.

The International Inquiry of the European Economical Committee of the UNO in Geneva (Turoboyski 1969 a, b) compiled a comprehensive comparable material on the effect of heated waters: In England, up to 30°C no harmful effects of heated water were stated as the chemical composition of water was unchanged. In France, decreases or increases of oxygen contents in water that passed through the condensors of the power stations were observed (similarly as at Skawina). Goubet (1965) described heated French rivers, and Appourchaux (1965) — the heated Seine River. The papers showed that water temperature in rivers should not be higher than 30°C. Oxygen content decreases were small or inconsiderable in general, while in Vistula they often raised to high values. In the German Federal Republic, water temperature in rivers in general did not exceed 28°C. It was suggested that heating of water might cause an increase of BOD_5. The Soviet reports on the subject tend to be similar to Polish ones.

The first Polish hydrobiological investigations of heated waters were described by Starmach (1962). The authors stated that in the heated waters of Skawinka and Vistula Rivers, numbers of algae cells in seston were decreasing but already at Tyniec in the warm current they were recovering. In the present investigations the results are similar. In Warta River, heated by wastes from the ironworks at Częstochowa town (Turoboyski 1968), no effect of heating on benthic and sestonic organisms was stated. Trembley (1960) recorded an increase of Cyanophyceae and some Bacillariophyceae as well as a decrease of Chlorophyceae and several Protozoa species in the heated Delaware River (USA). In the present investigations no visible increase of the numbers of Cyanophyta or decrease of Chlorophyta were found in Vistula and Skawinka rivers. Trembley (1960, 1961) recorded a decrease of numbers in the heated waters in the warm period. Appourchaux (1965) stated that in Seine, heated by the power station at Montereau, phyto- and zooplankton were still abundant, and in winter some plant and animal species could develop well owing to heated water. In the warm period, unicellular and filamentous algae of periphyton occurred more numerously in the unheated than in the heated places of Seine River. In the heated region of Vistula, some decreases in numbers of bioseston and periphyton were observed, some species of periphyton, however, were developing more intensely. Data on the influence of heated waters on rivers can also be found in the following papers: Kresser (1957), Dussart (1966), Leynaud (1967), Turoboyski (1969 a, b). It can be seen from these papers that water temperatures

in the range of 28—30°C can inhibit the development of different aquatic organisms.

Bacteriological investigations (S m y k et al. 1965) carried out simultaneously with those here presented, showed that the discharge of heated water into Skawinka and Vistula rivers diminished in some degree the microbiological activity of those rivers. The number of psychrophilic microorganisms was decreasing, photosynthesing bacteria disappeared, and Sphaerotilus-Leptothrix group was retreating. The bacteria assimilating nitrogen were retreating and the numbers of some sulphuric, nitrifying and cellulose decomposing bacteria were decreasing. Thus the decomposition of proteins, carbohydrates and aromatic hydrocarbons could be impeded. On the other hand, an increase of activity of other mesophilic bacteria was observed under the influence of heated water.

Ichthiological investigations of Vistula and Skawinka rivers (Badania ... 1967), also carried out by a commission of the Institute of Water Economic Research, simultaneously with our investigations, showed that the fish were gathering in the heated regions, especially in winter. Many times the changes in fish distribution could be observed, ranging up to 80—95% of their number in heated water while in unheated water there was 5—20%. No harmless effects of heating on fish were stated. A considerable tolerance of several species to increasing temperature, different pollutions and low oxygen contents was found.

Heating of water can also have positive effects, as it counteracts freezing and in this way it makes reaeration and navigation possible; however, in summer, on hot days, heating of water to more than 30°C can be harmful for industry, water intakes and biocenosis of Vistula River.

The investigations showed that to avoid the injuring effects of heating of Vistula River, a maximum permissible temperature of heating of the cooling water in the condensors of the power station at Skawina, in the place of discharges into Skawinka River, should be fixed at the 30°C level.

6. SUMMARY

Water temperature in Vistula River was increasing up to 34°C at km 61 st in the right current and up to 32°C at km 72nd under the influence of discharges of cooling water from the power station at Skawina. Maximum temperature of discharged water was 35°C. Oxygen content decrease was often considerable, the minimum level being 2 mg O_2/l at km 61st in the heated right current. As compared with the unheated station upstream of the estuary of Skawinka River, it could decrease even by 5 mg O_2/l in the heated region; thus the decrease was considerable. The other chemical parameters did not show changes of a practical significance.

In the bioseston of heated regions of Vistula River, the numbers of organisms were decreasing, as compared with the unheated region upstream of the estuary of Skawinka River. In the heated region, at km 61st, there was a double decrease of the amount of biosestonic organisms as compared with the unheated region.

In microbenthos, an increase of the number of *Stigeoclonium tenue* Kütz. was observed, and in periphyton the numbers of *Sphaerotilus natans* Kütz. bacteria and of some Bacillariophyceae were decreasing, while e. g., *Navicula mutica* Kütz. was more abundant. *Siderocapsa sp.* bacteria and Chrysophyceae *Lagynion sp.* were also more intensely developing in the periphyton of the heated region than in the unheated one.

In macrobenthos, number of *Physa acuta* Drap. (Mollusca) increased under an influence of heated waters.

The bacteriological investigations carried out simultaneously by the Department of Microbiology of Higher School of Agriculture in Cracow showed that heated water inhibited the activity of bacteria in Vistula River.

The ichthiological investigations carried out simultaneously by the Department of Fisheries of Higher School of Agriculture in Cracow showed that the fish were gathering in the heated water of Vistula and Skawinka rivers and no harmful effects of heated water on fish were found out.

7. STRESZCZENIE

Podgrzanie wody wiślanej przez zrzut chłodniczych wód z elektrowni w Skawinie dochodziło do 34°C na km 61 w prawej strudze, i do 32°C w rejonie km 72. Maksymalna temperatura wód zrzutowych z elektrowni wynosiła 35°C. Ubytki tlenu w Wiśle były niekiedy znaczne pod wpływem ocieplonych wód z elektrowni w Skawinie; najniższy poziom tego gazu wynosił 2 mg O_2/l na km 61 Wisły w prawym ogrzanym nurcie. W porównaniu zaś ze stanowiskiem powyżej Skawinki znajdującym się w miejscu niepodgrzanym Wisły, zawartość tlenu w strefie ogrzanej poniżej ujścia Skawinki mogła być mniejsza nawet o 5 mg O_2/l. Ubytek tlenu pod wpływem ciepłych wód z elektrowni mógł być zatem duży. Pozostałe parametry chemiczne nie wykazywały zmian o znaczeniu praktycznym.

W biosestonie Wisły ogrzanej notowano ubytki ilości organizmów w porównaniu do strefy niepodgrzanej powyżej Skawinki. W strefie ogrzanej zanotowano w okresie gorącym dwukrotnie mniejszą ilość organizmów w biosestonie (na km 61) niż w miejscu niepodgrzanym w Wiśle powyżej Skawinki.

W mikrobentosie zaobserwowano wzrost ilości glonu *Stigeoclonium tenue* Kütz., a w peryfitonie spadek bakterii *Sphaerotilus natans* Kütz. i pewnych gatunków Bacillariophyceae oraz wzrost innych jak np. *Navicula mutica* Kütz. Bakteria *Siderocopsa sp.* oraz spośród Chrysophyceae — *Lagynion sp.* także wyraźnie silniej się rozwijały w peryfitonie w strefie Wisły ogrzanej niż nieogrzanej.

W makrobentosie stwierdzono wzrost liczebności *Physa acuta* Drap. (Mollusca) pod wpływem ogrzanych wód.

Badania bakteriologiczne, przeprowadzone równolegle przez Katedrę Mikrobiologii W.S.R. w Krakowie wykazały, że podgrzanie hamuje aktywność flory bakteryjnej w Wiśle.

Z równolegle przeprowadzonych badań przez Katedrę Rybactwa W. S. R. w Krakowie wynikało, że ryby skupiają się w ciepłej wodzie w Wiśle i Skawince i nie stwierdzono ujemnych skutków podgrzania wody w stosunku do rybostanu.

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MARGINAL PLANT COMMUNITIES OF THE NESYT FISHPOND (SOUTH MORAVIA)

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ABSTRACT

Eight communities situated along the landward edge of the reed-belt of the Nesyt fishpond (South Moravia) were estimated for their seasonal maximum shoot biomass, shoot density and maximum shoot length at the peak of the 1971 growing season. Dry weight of standing dead material was estimated in some of the communities. The relatively wettest communities appeared to have the highest shoot production.

1. INTRODUCTION

Eight communities situated along the landward edge of the reed-belt of the Nesyt fishpond (South Moravia) were estimated for their seasonal maximum shoot biomass (W, kg/m^2), shoot density (d, n/m^2) and maximum shoot length (h, cm) at the peak of the growing season. The following average values of W, d, h were recorded, respectively: *Glycerietum aquaticae:* 0.99, 291, 191; *Phalaridetum arundinaceae*: 1.27, 422, 240; *Scirpo-Phragmitetum*, degraded, with *Mentha aquatica*: 0.71, 500, 110; *Bolboschoenetum martimi continentale:* 0.27, 536, 85; *Caricetum ripariae*: 1.01, 191, 201; *Caricetum otrubae* 0.54, 341, 113; *Atriplex hastata*-community: 0.89, 476, 201; *Cuscuto-convolvuletum pulicarietosum dysentericae* 0.99, 392, 122. Dry weight of standing dead material was estimated in some of the communities. The relatively wettest communities appeared to have the highest shoot production.

2. MATERIAL AND METHODS

In cooperation with the staff of the Institute of Geography, Czechoslovak Academy of Sciences, Brno, we obtained, with a camera situated on a low-flying aircraft model, a set of aerial photographs in colour, which recorded the pattern of Nesyt's littoral vegetation. Using these pictures, the surveying of the various plant communities was completed (see Husák, Šmíd in print), see Fig. 1. The releves of the phytocoenoses, together with the semi-quantitative estimates of the combined cover degree-abundance and sociability according to the scales of the Zürich-Montpellier School have been arranged in Table I (see also Hejný, Husák in print). Climatological characteristics of the area in question have been described in a paper by Šmid, Palát (in print). The soil pro-

Table I. Average values (with ranges) of the shoot density, maximum length, seasonal maximum shoot biomass, and standing dead material, by individual species, in 8 marginal plant communities (A–H) of the Nesyt fishpond. The last column (a-s) indicates combined cover degree-abundance and sociability according to Braun-Blanquet's scale

	Community	Density (n/m^2)	Maximum length (cm)	Biomass (g dry wt./m^2)	Standing dead (g dry wt./m^2)	a-s
A	*Glyceria aquatica*	281	191	983.0	356	5.5
	Mentha aquatica	7	33	2.8		1.1
	Persicaria amphibia	1	18	0.4		—0.1
	Galium palustre	2	42	0.2		+0.1
	Total	291 (209—403)	—	986.4 (858—1055)	356 (292—412)	
B	*Phalaris arundinacea*	344	240	1194.0	476	5.5
	Mentha aquatica	45	45	57.1		2.1
	Lycopus europaeus	21	38	11.4		1.1
	Persicaria amphibia	5	29	1.9		+0.1
	Hippuris vulgaris	3	18	0.9		+0.1
	Malva neglecta	1	22	0.8		—0.1
	Potentilla anserina	3	31	0.3		—0.1
	Total	422 (304—478)	—	1266.4 (775—1566)	476 (240—823)	
C	*Mentha aquatica*	307	103	624.8		5.5
	Hippuris vulgaris	77	55	36.0		2.1
	Polygonum lapathifolium	37	76	21.3		2.1
	Bidens tripartitus	3	110	16.2		1.1
	Persicaria mitis	15	54	10.3		1.1
	Veronica anagalis-aquatica	2	61	2.3		+0.1
	Agrostis alba	59	29	1.2		1.1
	Total	500 (360—664)	—	712.1 (509—1014)	—	
D	*Bolboschoenus maritimus*	526	85	266.8		5.5
	Agropyrum repens	10	53	0.9		+0.1
	Total	536 (304—660)	—	267.7 (226—315)	—	
E	*Carex riparia*	191	201	1008.0	652	5.5
F	*Carex otrubae*	87	113	231.0		3.2
	Potentilla anserina	192	32	275.0		5.5
	Hippuris vulgaris	62	17	33.0		+0.1
	Total	341 (229—388)	—	539.0 (207—940)	—	
G	*Atriplex hastata*	340	201	867.3		5.5
	Potentilla anserina	108	52	9.8		2.1
	Rumex maritimus	16	70	4.4		1.1
	Pulicaria dysenterica	4	49	2.2		+1.1

G	*Chenopodium glaucum*	2	21	0.7		+0.1
	Chenopodium rubrum	4	36	0.5		+0.1
	Carex riparia	2	42	0.2		−0.1
	Total	476 (208–760)	—	885.1 (572–1012)	—	
H	*Pulicaria dysenterica*	232	122	912.2		5.5
	Mentha aquatica	24	102	23.0		2.1
	Chenopodium rubrum	20	68	18.0		1.1
	Calystegia sepium	20	103	11.3		1.1
	Lycopus europaeus	8	106	10.4		+0.1
	Potentilla anserina	72	61	8.8		2.1
	Althaea officinalis	4	76	4.1		+0.1
	Carex hordeistichos	12	84	3.7		1.2
	Total	392 (332–444)	—	991.4 (656–1272)	—	

parties, together with the hydrochemical analyses were summarized by Úlehlová, Rejthar (in print). The sampling ot the biomass was carried out in the summer of 1971: on 28th June in A, B, and C, on 8th June in F, and on 28th August in C, D, G, and H. The sample plots measured 100 by 100 cm in the communities A, B, E, and F, 50 by 100 cm in D, and 50 by 50 cm in C, G, and H. In all cases, four parallel samples were analysed; the figures presented in Table I represent means of these 4 samples.

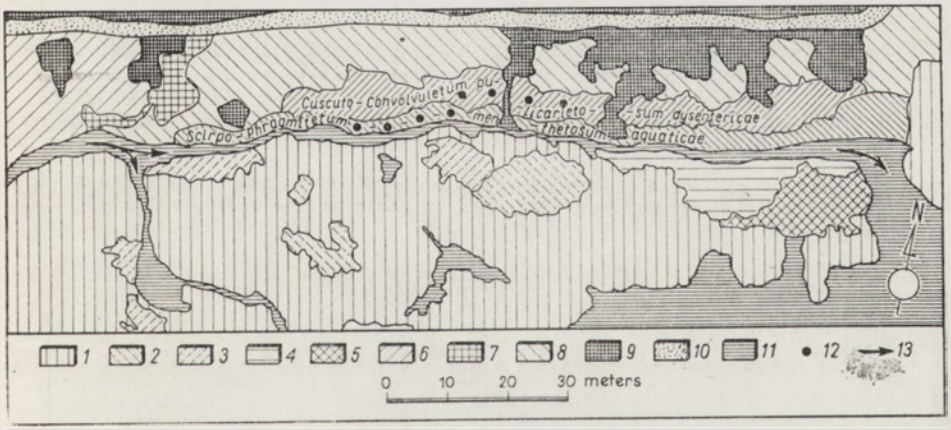

Fig. 1. Part of the vegetation map of the Nesyt fishpond showing the localities of two of the communities examined, i. e. C — *Scirpo-Phragmitetum* W. Koch 1926 *menthetosum aquaticae and* H — *Cuscuto-Convolvuletum* Tx. 1947 *pulicarietosum dysentericae*, and other communities shown in the map: 1 — mixed community of *Phragmites communis, Typha angustifolia* and *T. latifolia*, 2 — *Phragmites communis*, 3 — *Phalaris arundinacea*, 4 — *Glyceria maxima*, 5 — *Hippuris vulgaris*, 6 — *Potentilla anserina*, 7 — *Chenopodium glaucum*, 8 — mixed species from communities *Agropyro-Rumicion crispi* Nord. 1940, *Euarction* Tx. 1937 and *Sisymbrion officinalis* Tx. Lohm. et Preis. in Tx. 1950. 9 — sampling sites, 10 — buildings and deposits of village wastes, 11 — footpath, 12 — water, 13 — direction of water flow

3. RESULTS AND DISCUSSION

Besides the stands of *Phragmites communis* and *Typha angustifolia*, and other reedswamp communities of the alliance *Phragmition communis* W. Koch 1926 (marked A, B, C in the present paper), the shores of the Nesyt fishpond (see Hejný, Husák in print) are covered by other stands of littoral macrophytes which are interesting from both the phytocoenological and primary production point of view. On the one hand, they are represented by communities of helophytes (in the western and southern parts of the Sedlec Bay), among which the wide-spread littoral stands belong to the alliance *Scirpion maritimi* Dahl et Hadač 1941 (D). Landwards of the zone of the reedswamp stands, there are physiognomically conspicuous communities of tall sedges belonging to the alliance *Caricion gracilis* Neuhäusl 1959 (E), and communities of the alliance *Deschampsion caespitosae* Horvatić 1931 (F), which show considerable primary production. In the recent years, the artificially raised water level in Nesyt influenced the development of new phytocoenoses along the new littoral line. So far, these new stands did not achieve structural equilibrium and, consequently, it is difficult

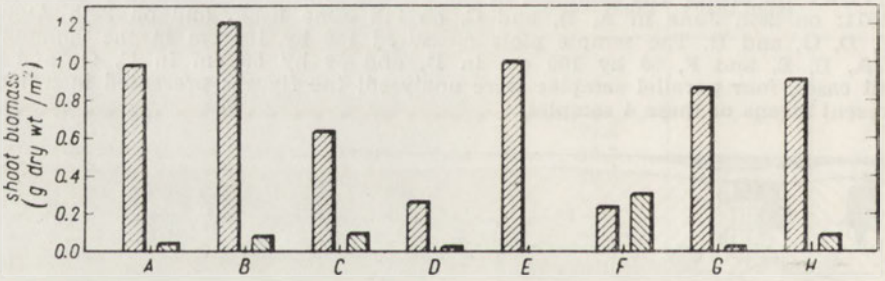

Fig. 2. Biomass of the dominant species (left columns) and the biomass of the other species (right columns) in the 8 communities examined (A–H)

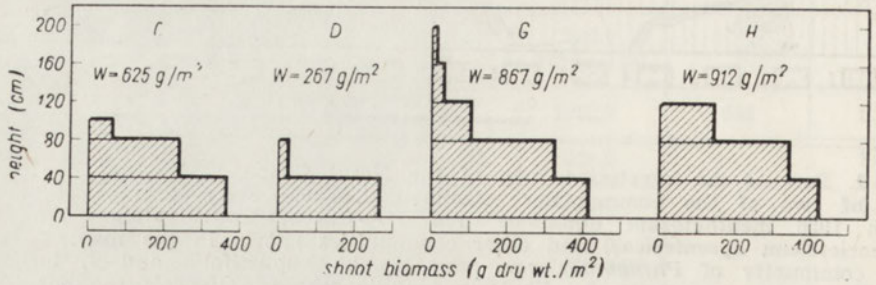

Fig. 3. Vertical distribution, by 40 cm layers, of shoot biomass of the dominant species in stands of the communities: C — *Mentha aquatica*, D — *Bolboschoenus maritimus ssp. compactus*, G — *Atriplex hastata* and H — *Pulicaria dysenterica*, assessed on Aug. 28. 1971. Values of total shoot biomass (*W*) of each of the dominats are also given

to identify their definitive position in the phytocoenological system. In a preliminary attempt, these communities were classed within the alliances *Bidention tripartiti* Nordhagen 1940 (G) and *Calystegion sepium* Tüxen 1947 (H). They can be found mainly in the northern part of the Sedlec Bay, and along the Southern margin of the fishpond.

Following are brief comments on the phytocoenoses analysed on the shores of the Nesyt fishpond (Fig. 2 and 3.).

A. *PHRAGMITION COMMUNIS* W. KOCH 1926, *GLYCERIETUM MAXIMAE* HUECK 1931

This phytocoenosis develops in the contact zone with *Phragmites communis*. In the Sedlec Bay western end of Nesyt), closed and compact stands cover the depressions flooded by water in the spring. In these transition stages, Glyceria achieves neither its highest aboveground biomass nor its maximum height, obviously, due to the predominating limosal ecophase. A similar phytocoenosis sampled on the Opatovický Fishpond in South Bohemia showed a density of 220 to 560 shoots and a biomass of 1390 to 2860 g/m^2 (Dykyjová 1971).

B. *PHRAGMITION COMMUNIS* W. KOCH 1926, *PHALARIDETUM ARUNDINACEAE* LIBBERT 1931

In the Sedlec Bay, this phytocoenosis appears to be a link between the reedswamp stands and, either initial stages of the succession, or, on the contrary, depleted patches of the stands belonging to the alliance *Agropyro-Rumicion crispi* and *Bidention tripartiti*. Among the marginal communities studied on the shores of Nesyt, this particular phytocoenosis showed the highest primary production.

C. *PHRAGMITION COMMUNIS* W. KOCH 1926, *SCIRPO-PHRAGMITETUM* W. KOCH 1926

A degraded stage with *Mentha aquatica* which forms a compact belt along the Sedlec Bay, just like the stands with predominating *Pulicaria dysenterica*. However, in the former stands the transition into a proper border community is not yet properly marked.

D. *SCIRPION MARITIMI* DAHL ET HADAČ 1941, *BOLBOSCHOENETUM MARITIMI CONTINENTALE* CSOÓ 1927/1957

A depleted form of this association had developed after ploughing on the southern shores of Nesyt early in the spring. The underground organs of *Bolboschoenus maritimus ssp. compactus* gave rise to a homogeneous stand. Though the ecophases were markedly retarded, the 20% of the shoots were fertile. The shoot production was, however, lowest among the analysed communities. Gradual rise of the water level in the fishpond shifts gradually this association towards the external border of the zone with predominating tall sedges (Magnocaricetea), as it was observed during the period of our studies. On the

other hand, the lowering of the water level pushes the Bolboschoenetum towards the zone of the reedswamp stands.

E. *CARICION GRACILIS* NEUHÄUSL 1959, *CARICETUM RIPARIAE* SOÓ 1928

The stands analysed in the Sedlec Bay show the predominance of the tall sedge *Carex riparia* which appears to develop polycormous patches. The habitat corresponds in its water conditions to that of the *Glycerietum maximae.*

F. *DESCHAMPSION CAESPITOSAE* HORVATIĆ 1931, *CARICETUM OTRUBAE PROV.*

A characteristic community in the Sedlec Bay, with relatively high species diversity. The fluctuating water level affects the spreading of *Potentilla anserina* which marks the limosal-terrestrial ecophase of the earlier period. *Hippuris vulgaris*, on the other hand, shows increased distribution following the littoral ecophase of the recent years. The stand appears to be fairly resistent to grazing by geese.

G. *BIDENTION TRIPARTITI* NORDHAGEN 1940, *ATRIPLEX HASTATA*-COMMUNITY

A phytocoenosis regularly developing on swampy margins of the Sedlec Bay, supported mainly by its favourable carpobiological features. The mature stands are relatively tall.

H. *CALYSTEGION SEPIUM* TÜXEN 1947, *CUSCUTO-CONVOLVULETUM* TX. 1947 *PULICARIETOSUM DYSENTERICAE*

A phytocoenosis regularly covering the wet margins of the Sedlec Bay, and alternating with the stands dominated by *Mentha aquatica.*

The pattern of the above described phytocoenoses can be readily seen in Fig. 2 and 3 which are derived from aerial photographs. The size of the areas covered by individual stands can be estimated according to the scale attached to the figures. The two last mentioned communities develop from *Scirpo-Phragmitetum atriplicetosum hastatae* Hejný 1970, and *Scirpo-Phragmitetum calystegietosum* in the process of suppressing the edificatory role of the reedswamp species, which results in the spreading of broad-leaved herbs possessing high primary production.

A comparison of phytocoenological releves with somewhat more quantitative methods of production ecology suggests that a combined approach using both methods results in a satisfactory description of the vegetation in its natural setting.

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D. ZIMAKOWSKA

EFFECTS OF 2,4-D-Na OF VARIOUS CONCENTRATIONS ON RESPIRATION OF AN AQUATIC CRUSTACEAN *ASELLUS AQUATICUS* L.

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ABSTRACT

Effects of 2,4-D-Na concentrations of 1.25, 2.50, 5.00 and 7.50 mM on oxygen consumption by *Asellus aquaticus* males after 6, 12 and 24 hr expositions in a given solution were investigated. It was found that effects of 2,4-D-Na concentrations on oxygen consumption in *Asellus aquaticus* were significant. A specially high increase of oxygen consumption (up to 142.1% of that of the control) was observed under the influence of 5.00 mM concentration. It seems to be the critical concentration for *Asellus aquaticus*. It can be supposed that 2,4-D-Na brings its influence on the respiration chain and causes oxidizing phosphorylation to be uncoupled.

1. INTRODUCTION

More and more intensive application of chemicals of the herbicide group in Polish agriculture, their significant toxicity and a possibility of contamination of drinking water supplies, are the reasons to search for any tests determining herbicide effects on an organism (Nowiński 1959, Braginskij 1962, Rola, Ostrowski 1964, Rusiecki 1964, Trzebiatowski 1966, Novakowa, Dinajewa 1970).

Effects of herbicides on a human organism have not been precisely known yet; neither have permissible contents of particular herbicides in water been established. Therefore it seems to be purposeful to investigate herbicide influence on physiological processes in organisms.

One of the coefficients defining the whole of internal processes in the system is oxygen consumption.

In the present paper an influence of 2,4-D-Na herbicide on respiration of a representant of aquatic animals, *Asellus aquaticus* (Crustacea), was investigated. This crustacean is a specially convenient object for such studies since, contrary to e.g. *Daphnia magna*, its receptivity is very high; its sensibility to several side-factors, both chemical (oxygen deficiency, electrolyte composition, accessory pollutions) and physical (mechanical injuries, shocks, temperature variations) as well as physiological (starvation) is low, and it is more long-lived (Cabejszek, Malaszewska 1970, Kamiński 1957, 1965).

2. MATERIAL AND METHODS

The formulated product of 2,4-D-Na contains several admixtures among which 2,4-chlorophenole (up to 3%) causes uncoupled oxidizing phosphorylation. Therefore the chemically pure, 2,4-D-Na preparate was used for investigations, obtained

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from the formulated product "Pielik" by purification with the method of Zvirgzds, Bālina (1969).

2,4-D-Na solutions were prepared in conditioned tap water, in the following concentrations: 1.25, 2.50, 5.00 and 7.50 mM.

Oxygen demand was determined in volumetric respirometers (Klekowski 1968) placed in a thermostat at 20°C.

The studied object was an aquatic crustacea *Asellus aquaticus* originating from our own aquarium culture. For experiments, male individuals identically pigmented and about 6 mm long were caught. They were acclimated for 2 weeks in a thermostat at 20°C and then put into crystallizers, 40 ml in capacity, 3 individuals in each, in a given 2,4-D-Na solution, 2.5 ml of volume per one individual. The animals were kept in the crystallizers for 6, 12 or 24 hr, and then their survival rates were estimated and their oxygen consumption was measured in volumetric respirometers at 20°C. Respiration measurements were made considering the daily rhythm of animals. In each experiment series, respiration was measured in 7 respirometers (2 controls with conditioned tap water and 5 repetitions with a given 2,4-D-Na solution), one individual in each. In total, respiration was measured in 230 individuals. After exposition, the animals were taken out of respiration chambers, dried on filter paper, and then weighed.

For estimation of significance of the differences between groups, U-criterion of non-parametric statistics of Wilkoxon-Mann-Whithney was used. Dispersion degree in groups was expressed by standard deviation (δ).

3. RESULTS

Effect of particular concentrations of 2,4-D-Na herbicide on oxygen demand in *Asellus aquaticus* can be seen in Fig. 1.

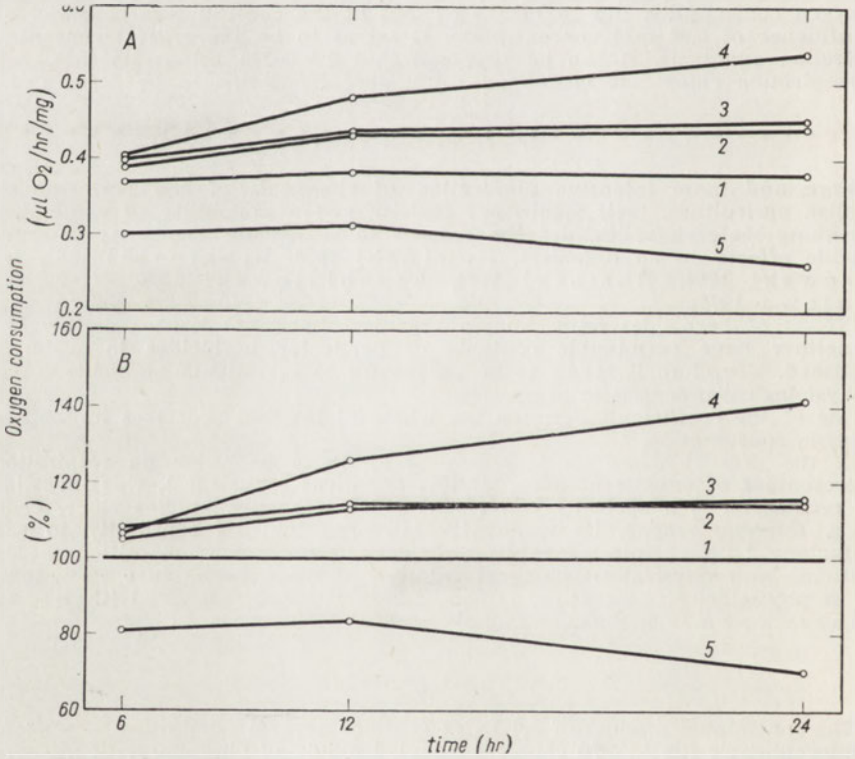

Fig. 1. Effects of different concentrations of 2,4-D-Na on oxygen consumption in *Asellus aquaticus* (means from 4 repetitions). Concentrations: 1 — 0 mM (control), 2 — 1.25 mM, 3 — 2.5 mM, 4 — 5.00 mM, 5 — 7.50 mM. A — oxygen consumption in weight units, B — oxygen consumption as per cent of the control

All the concentrations brought their visible effects on respiration of the experimental animals. The differences between the treated and control animals were statistically significant at $p<0.05$ level.

Figure 1 shows that oxygen consumption in *Asellus aquaticus* was similar in concentrations 1.25 and 2.50 mM (differences statistically insignificant at $p<0.05$ level). As the animals were kept longer in the herbicide, their oxygen consumption increased and after 24 hr it amounted to 0.45 µl O_2/hr/mg live weight.

However, in 5.00 mM concentration, oxygen consumption rapidly increased and after 24 hr it was 0.54 µl O_2/hr/mg live wt., i.e. as much as 142.1% of that of the control. In 7.50 mM concentration, animal

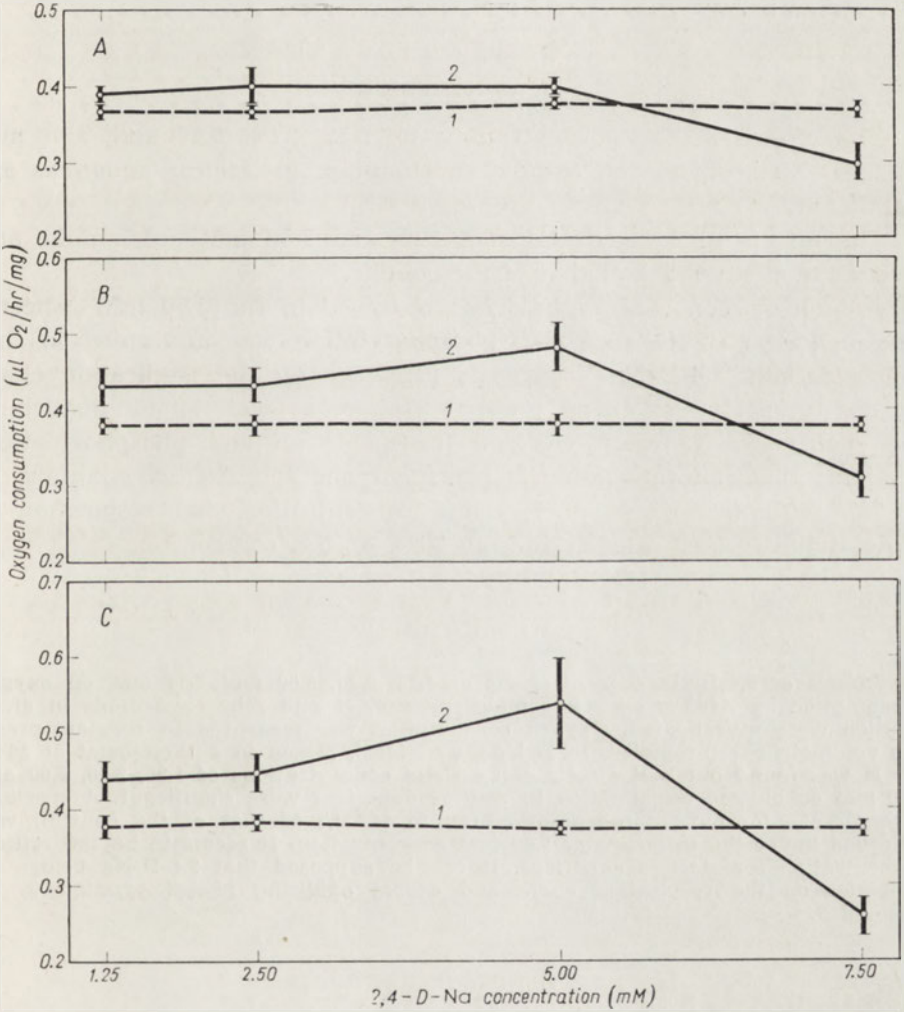

Fig. 2. Influence of exposition periods to different concentrations of 2,4-D-Na on oxygen consumption in *Asellus aquaticus* in comparison with the control. 1 — control animals, 2 — treated animals. A — 6 hr exposition, B — 12 hr exposition, C — 24 hr exposition

mortality was as high as 75%, and oxygen consumption of those which survived rapidly decreased, being 0.32 µl O_2/hr/mg live wt. after 6 hr exposition (i.e. 81.1% of that of the control), and 0.26 µl O_2/hr/mg live wt. (i. e. 70.3% of that of the control) after 24 hr exposition.

Effects of different concentrations of 2,4-D-Na on oxygen consumption in *Asellus aquaticus* after 6, 12 and 24 hr expositions in the herbicide are presented in Fig. 2. It can be seen that the animals exposed to the herbicide for 24 hr differ most from the control. In comparison with the control (0.38 µl O_2/hr/mg), respiration increased to 0.45 µl O_2/hr/mg in 1.25 and 2.50 mM concentrations, considerably increased to 0.54 µl O_2/hr/mg in 5.00 mM concentration, and significantly decreased to 0.25 µl O_2/hr/mg in 7.50 mM concentration.

4. CONCLUSIONS

The effects of the concentrations of 1.25, 2.50, 5.00 and 7.50 mM of 2,4-D-Na herbicide on oxygen consumption in *Asellus aquaticus* are significant.

The effects of concentrations of 1.25 and 2.50 mM are similar and amount to about 115% of that of the control.

A high oxygen consumption was observed in the 5.00 mM concentration. Probably the oxidizing phosphorylation was uncoupled in this concentration. 2,4-D-Na brings its influence on the respiration chain by disrupting the oxidizing phosphorylation, as well as by disturbing the connections between electron transportation and phosphorylation. It seems this concentration to be critical one for *Asellus aquaticus*.

75% of animals died in 7.50 mM concentration, and respiration of those that survived was as low as 70% of the control.

5. SUMMARY

Effects of 2,4-D-Na concentrations of 1.25, 2.50, 5.00 and 7.50 mM on oxygen consumption by *Asellus aquaticus* males after 6, 12 and 24hr expositions in given solution were investigated. Oxygen consumption was measured in constant-pressure volumetric respirometers (K l e k o w s k i 1968), placed in a thermostat at 20°C.

It was found out that effects of 2,4-D-Na concentrations of 1.25, 2.50, 5.00 and 7.50 mM on oxygen consumption in *Asellus aquaticus* were significant. A specially high increase of oxygen consumption (up to 142.1% of that of the control) was observed under the influence of 5.00 mM concentration. It seems to be the critical concentration for *Asellus aquaticus*. It can be supposed that 2,4-D-Na brings its influence on the respiration chain and causes oxidizing phosphorylation to be uncoupled.

6. STRESZCZENIE

Badano wpływ stężeń 1,25, 2,50, 5,00 i 7,50 mM 2,4-D-Na na zużycie tlenu przez samce *Asellus aquaticus* po 6, 12 i 24 godzinach ekspozycji w danych stężeniu herbicydu. Zużycie tlenu określano w stałociśnieniowych respirometrach wolumetrycznych (K l e k o w s k i 1968), umieszczonych w termostacie w temp. 20°C.

Stwierdzono, że 2,4-D-Na w stężeniach 1,25, 2,50, 5,00 i 7,50 mM wpływa w sposób istotny na zużycie tlenu przez *Asellus aquaticus*. Szczególnie duży wzrost zużycia tlenu (do 142,1% w stosunku do kontroli) występuje pod wpływem stężenia 5,00 mM. Wydaje się, że to właśnie stężenie 2,4-D-Na jest krytyczne dla *Asellus aquaticus*. Należy się spodziewać, że 2,4-D-Na wpływa na łańcuch oddechowy powodując rozprzężenie fosforylacji oksydatywnej.

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BIOLOGY AND RESPIRATION OF THE ANTARCTIC AMPHIPODA (*PARAMOERA WALKERI* STEBBING) IN THE SUMMER

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ABSTRACT

The present studies were carried out in the Antarctic Station Molodezhnaya in the summer time (December 1971 — March 1972). The sub-fast ice community in which *P. walkeri* is found during the winter undergoes destruction in summer and the animals move down to the sea bottom. In the investigated population of *P. walkeri* two generations were present — the younger one was in the intensive growth stage, the older one was dying out after breeding. Size distribution in the examined generations and interrelation between the length and weight of individuals have been analysed. *P. walkeri* metabolism has been studied experimentally at every two degree gradient of temperatures ranging from −1.2°C to 12.0°C and 29‰ salinity and also at 2.2°C and 15‰ salinity. Animals were acclimated to different temperatures. In summer the metabolic level was higher than in winter and the increase in temperature increased the oxygen consumption only slightly in contrast to winter. A decrease in salinity caused an increase in oxygen consumption.

1. INTRODUCTION

The initial studies on biology and metabolic processes of the Antarctic species of Amphipoda — *Paramoera walkeri* Stebbing were undertaken at the USSR Station Molodezhnaya in winter 1969 (Rakusa-Suszczewski 1972 b, Klekowski et al. 1973). At that time it has been found that *P. walkeri* population lives in the littoral zone. In the autumn, end of May, the species population migrates from the bottom up to beneath the ice where it stays during the winter being the main component of the forming sub-ice community. In the beginning of June females lay eggs and in October and November the new generation is hatched. The sexual maturity is reached in 19 months.

The respiration measurements carried out in winter 1969 have shown a low level of metabolism in the examined *P. walkeri* population and the oxygen requirement averaging 0.72 µl/ind./hr per mean 20.5 mg wet weight of individuals, at −1.9°C and 34.4‰ salinity, which corresponds to environmental conditions. At the experimentally increased temperature to those values to the species encounters within a year cycle a rapid increase in respiration was observed which calculated for the whole population gave a high coefficient value of Q_{10}=15.9.

A second stay at Molodezhnaya in the 1972/1973 summer season allowed to enlarge the scope of observations. Excepting the studies by Thurston (1968, 1970) and Bone (1972) concerning *Bovallia gigantea* the biology of the Antarc-

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tic species of Amphipoda is unknown. Environmental conditions in the littoral zone, fate of the sub-ice community, distribution of *P. walkeri*, its age structure and individual growth of the species were observed. Except the studies on metabolism of *Orchomonella chilensis* Heller (Armitage 1962), *Euphausia superba* Dana (McWhinnie, Marciniak 1964) and *Paramoera walkeri* Stebbing (Klekowski et al. 1973) there is a lack of information about the effect of temperature on the respiration of the bottom Crustacea. Thus it seems to be particularly interesting to undertake experiments on respiration both at the temperatures and salinities actually prevailing in the environment in summer and also at higher temperatures to which the animals were gradually acclimated. Results obtained in that manner allowed to make the comparison of metabolic processes of both seasons, winter and summer as well.

2. METHODS

Studies were carried out at Molodezhnaya (67°40′S, 45°50′E) in the period from December 22, 1971 to March 5, 1972. The assigned area for observations and sample collecting was at the "Opasnaya" bay situated near to the Thala Hills oasis.

Measurements of water temperature were performed with a reversible thermometer. Salinity was determined with a densitometer. Oxygen content in sea water was measured using Winkler method. Samples from the underside of the ice were collected by means of an "umbrella" (cf. Rakusa-Suszczewski 1972 b). Samples from the bottom were laddled out with an Ekman-type dipper. The material was transported to the laboratory in a thermos jug. The length of *P. walkeri* was measured from rostrum to the end of telson exact to 0.5 mm, and its wet weight was weighed exact to 0.5 mg. In January the hemolymph was collected from 25 individuals of length ranging from 10 to 15 mm long. Single capillaries with hemolymph were immediately sealed and in a frozen state brought back to the laboratory in Warsaw. The osmotic concentration of the body fluid was measured by means of a Ramsay-type microcryoscope modified by Klekowski (1963).

Measurements of oxygen consumption of *P. walkeri* were performed with the method of closed respirometric vessels (Fig. 1) of 220 ml volume. The oxygen concentration was determined with the Winkler method. In experiments at low temperatures at most 25 individuals of similar size were placed in a container

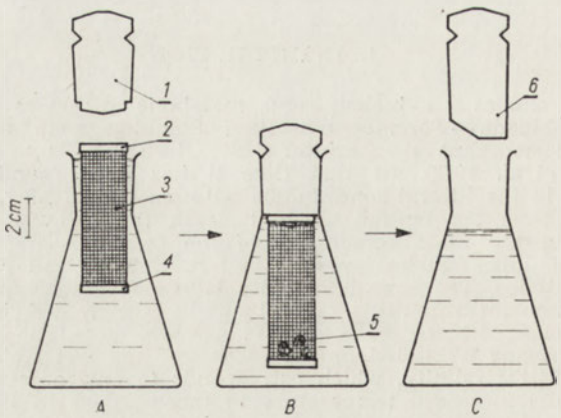

Fig. 1. Respirometric vessel. 1 — plexiglass stopper with a device at its lower end to fasten the net with animals, 2 — net made of a loosely woven bolting-cloth, 3 — plexiglass ring, 4 — plexiglass bottom cover, 5 — animals under examination, 6 — plexiglass stopper with bevelled lower end. A — vessel prepared to put in collected animals, 2 — vessel with animals in a plastic net during exposure time, C — vessel after the animals were taken out before fixation

of a loosely-woven bolting-cloth, at higher temperatures (above 6°C) only a few or several. The oxygen consumption was determined in relation to the control vessels. Respirometric vessels were placed in thermostats which maintained temperature exact to 0.1°C. Experiments were carried out at temperatures of: −1.2°C, 0.1°C, 2.2°C, 4.0°C, 6.0°C, 8.0°C, 10.0°C, 12.0°C and salinity of about 29‰. A series of experiments were also performed at 2.2°C and 15‰ salinity. The animals in which metabolism was measured at −1.2°C were not acclimated longer than 24 hours. For tests at other temperatures animals were fed with detritus and acclimated by gradual transfer to higher temperatures. The acclimation time for respective temperatures averaged in hours: 122(0.1°C), 206(2.2°C), 222(4.0°C), 141(6.0°C), 135(8.0°C), 205(10.0°C) and 284(12.0°C). In the experiment at 15‰ salinity the acclimation time to the temperature of 2.2°C was 152 hours. The time of exposure of animals before the measurements of oxygen consumption ranged between 5 and 6 hours. The experiments were started on January 6 and finished on February 19. Altogether 89 results were obtained. At the temperature of 12.0°C only one experiment was performed due to the small number of the acclimated animals. The single result is the mean value of the consumed oxygen converted into mean wet weight of the individuals used in the experiment. A high mortality rate in older individuals in the course of the long-lasting culture caused a decrease in the mean weight value of the animals used in the experiments at higher temperatures.

3. RESULTS

ENVIRONMENTAL CONDITIONS

Hydrological and ice conditions prevailing in the coastal region of "Opasnaya" Bay during the summer are illustrated in Fig. 2. Throughout the whole period of our studies the ice held on at the edge of the mainland

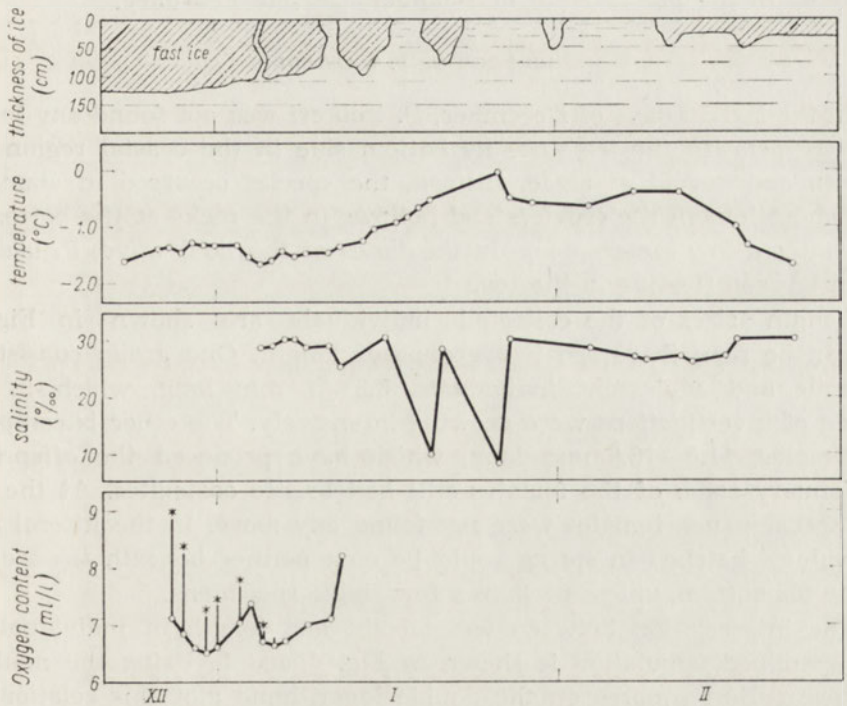

Fig. 2. Hydrologic and ice conditions at the littoral of the "Opasnaya" Bay (Alasheyev Bight) * — directly beneath the ice

and was not brought out to the sea. The thickness of ice in the littoral was on Dec. 22, 1971, between 125 and 132 cm thick. On Jan. 4, 1972, due to heavy waves was broken over a several hundred meters large distance from the shores. Together with progressive crumbling of ice in result of the tidal surge and the appearance of open water the temperature of the sea water began to rise from −1.6°C measured beneath the ice on Dec. 24, 1971, up to the maximum of −0.05°C measured at the surface on Jan. 24, 1972. On Feb. 21 water temperature dropped to −1.6°C. A fresh ice-cover 20–30 cm thick appeared again on the bay waters. Salinity in the early January and February remained at the same level of 26–29‰. Over the last three weeks of January salinity of the surface water decreased several times below 8‰ in result of the thawing of ice and diplacement of the fresh waters flowing into the sea from the oasis area in the coastal zone. The oxygen content in water examined at the beginning of investigations showed marked differences ranging from less than 7 ml/l at the depth of 2–3 m deep to 9 ml/l directly beneath the thawing ice.

Hydrologic conditions observed in the littoral and sub-fast ice zone during the summer are characterized by slight fluctuations in temperature and marked fluctuation in salinity and oxygen content in water. In the region under investigation the greatest environmental changes occurred in the period from mid-January to mid-February.

BIOLOGY OF *P. WALKERI*

In the last 10 days of December, *P. walkeri* was not found any longer directly beneath the ice since its bottom side in the coastal region was smooth and devoid of algae. Instead, the species occurred in abundant agglomerations in the crevices and hollows in the rocks at the bottom of the water a few meters deep. In the times of the tidal wave, *P. walkeri* could be seen floating in the sea.

Length sizes of the collected individuals are shown in Fig. 3. As can be seen there are two groups of length. One group consists of juvenile and adolescent individuals, 5.5–11 mm long, which in the period of investigations were growing intensively. The other is composed of females, 11.5—16.0 mm long, which have produced the offsprings. In January some of the females still had bristle oostegites. At the end of February such females were not found any more. In the littoral zone individuals hatched in spring could be seen neither beneath the ice nor at the sea bottom, unless perhaps a few single specimens.

The interrelation between wet weight and length of individuals of the examined population is shown in Fig. 4 and 5. Using the methods of the smallest squares on the double logarithmic plot this relationship can be expressed as: $W = a \cdot L^b$, where W — wet weight (mg), L — length (mm), a and b — regression coefficients. At the beginning of

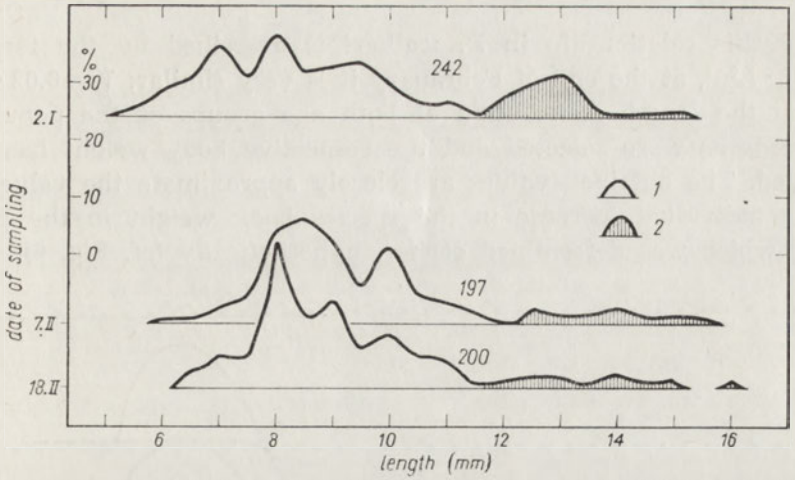

Fig. 3. Length distribution of *P. walkeri* population. 1 — juveniles and adolescents, 2 — females. Figures indicate the number of the measured animals

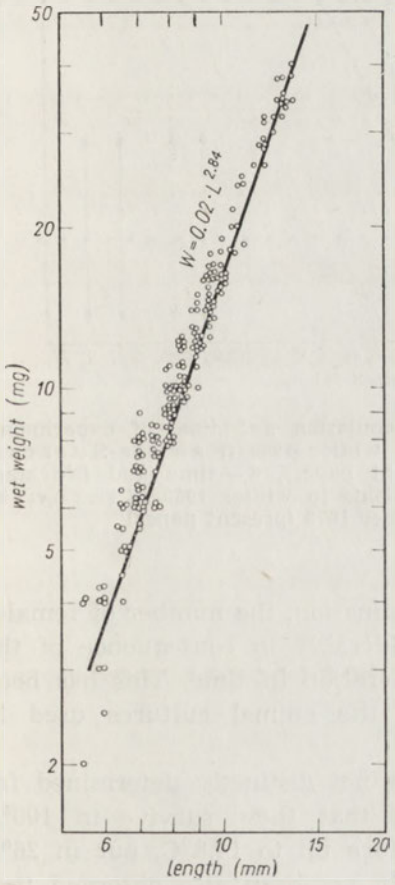

Fig. 4. Relation between wet weight and lenght of *P. walkeri* juveniles, adolescents, and females. 100 individuals were collected and measured on Jan. 2, 1972

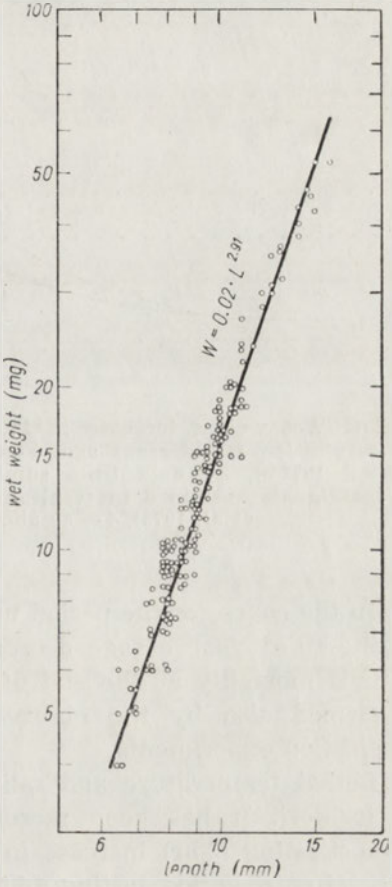

Fig. 5. Relation between wet weight and length of *P. walkeri* juveniles, adolescents and females. 100 individuals were collected and measured on Feb. 18, 1972

January this relationship in *P. walkeri* is described by the formula: $W = 0.02 \cdot L^{2.84}$, at the end of Febrauary it is very similar: $W = 0.02 \cdot L^{2.91}$.

From the length distribution of both age groups of the population modal lengths were selected and the respective body weight has been calculated. The obtained values are closely approximate the values expressing individual increase in the species body weight in the period of time which was determined earlier, hypothetically (cf. Fig. 6).

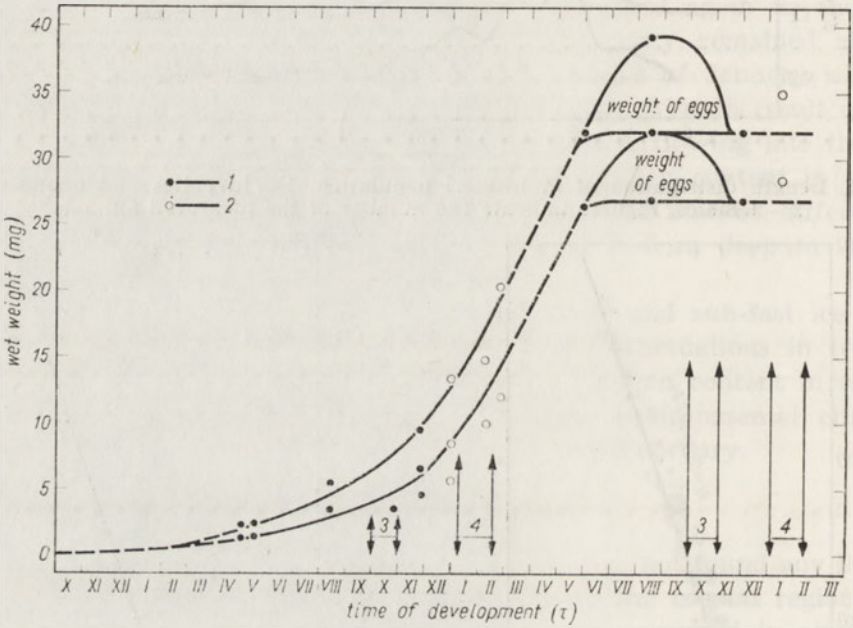

Fig. 6. Wet weight increase in *P. walkeri* population and time of experiments on respiration at different age. 1 — data from winter 1969 (Rakusa-Suszczewski) 1972 b), 2 — data from summer (present paper), 3 — time and life stage of individuals examined in respiration experiments in winter 1969 (Klekowski et al. 1973), 4 — as above in summer 1972 (present paper)

In the course of the period under examination, the number of females in the third year of age decreases considerably in consequence of the natural mortality at the end of that generation lifetime. This has been confirmed also by the observations of the animal cultures used in respiration experiments.

Lethal temperature and salinity were not distinctly determined for *P. walkeri*. It has been merely stated that they survive in 100% a shortlasting (3 hr) increase in temperature up to 14.8°C, but in 26% only after a period lasting 14.5 hours. It has been also observed that reduced salinity from 29 down to 15‰ caused an increase in locomotor activity and speed of limb movement.

Analysis of *P. walkeri* hemolymph showed the mean Δ°C value =

$= -2.06$ (S. E. ± 0.04) which proves its hyperosmotic concentration in relation to sea water salinity of 29‰.

RESPIRATION OF *P. WALKERI*

Results from the experiments on *P. walkeri* respiration in relation to temperature and salinity are shown in Table I. A slight increase in the metabolic rate of *P. walkeri* caused by an increase in temperature ranging from -1.2 to 0.1°C, i.e. a range that species encounters in the environment. At temperature of 2.2°C the rate of respiration decreases. Its lowest values were noted at 4.0°C. In the ranges of 4–6°C and 8–10°C the respiration rate has increased markedly. Between 6°C and 8°C there was almost no change at all. At 12°C respiration values in *P. walkeri* were lower than in individuals of similar weight examined at 10°C. Results from studies on *P. walkeri* respiration at 2.2°C and salinity 15‰ are also shown in Table I and as can be they have a marked effect on the increase in oxygen consumption.

Table I. Respiration of *P. walkeri* at various temperatures and salinity 29‰, and coefficients *a* and *b* values from the formula $R = a \cdot W^b$ (R — respiration, W — wet weight)

Temp. (°C)	Number of experiments	Number of animals used in experiments	Mean wet weight of 1 ind. (mg)	Respiration µl O_2/ind.hr	*a*	*b*
−1.2	15	290	11.0—25.0 20.7	1.87	0.27	0.65
0.1	13	274	11.0—25.0 20.4	1.98	0.34	0.61
2.2	9	156	12.0—25.0 27.7	2.40	0.22	0.73
2.2*	8	74	6.6—38.6 19.8	3.80	0.70	0.58
4.0	8	130	6.7—33.0 15.7	1.20	0.13	0.77
6.0	10	109	6.2—30.0 14.3	2.60	0.52	0.60
8.0	15	133	5.0—31.8 14.9	2.8	0.47	0.66
10.0	10	45	8.5—16.5 11.7	6.90	1.27	0.69
12.0	1	2	11.2	5.20	—	—

* Salinity 15‰.

The mean respiration values in *P. walkeri* (cf. Table I) at various temperatures and salinities are obviously influenced also by the factor of weight of animals used in the experiments. At higher temperatures the *b* values of the mean wet weight of individuals was lower in result of a higher mortality of older individuals during the longlasting culture period. Despite the limited range of body weight and a small number of results, we have decided to calculate the regression of oxygen consumption in relation to wet weight with the method of the smallest squares, it gave: $R = a \cdot W^b$, where R — oxygen consumption in O_2 µl/hr, W — wet weight (mg), a and b — regression coefficients (their value is given in Table I). Regression values computed for each temperature allowed to make the calculation of the respiration of individuals of the same wet weight and a comparison with the results obtained in winter (Fig. 7).

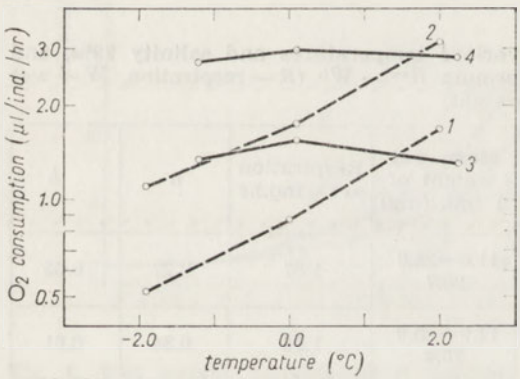

Fig. 7. Respiration of *P. walkeri* during winter (K l e k o w s k i et al. 1973) and summer (present paper) in the range of temperatures such as in natural conditions. 1 — individuals of 12 mg wet weight during winter, 2 — individuals of 35 mg wet weight during winter, 3 — individuals of 12 mg wet weight during summer, 4 — individuals of 35 mg wet weight during summer

4. DISCUSSION

In winter the temperature of sea water in the littoral and sub-fast ice zone remains at the same level of about −1.8°C, oxygen content is about 7.4 ml/l, salinity averages about 34.4‰ (cf. R a k u s a - S u s z c z e w s k i 1972 a). The observed in summer changes in temperature, salinity, and oxygen content, are striking though shortlasting. In the region under investigation those changes occurred within a period of time from mid-January to mid-February. Their occurrence may change from year to year and differ locally but they confirm nonetheless the typical character of hydrologic phenomena which are in the summer characterized by slight fluctuations in temperature and considerable fluctuations in salinity (cf. R a k u s a - S u s z c z e w s k i 1972 b).

The fate of the sub-ice community, including *P. walkeri* which is the main component of the crustacean fauna, in winter, is associated with the ice thawing process. As it was observed in 1969, the underside

of the ice was overgrown with a thick layer of micro-algae still at the beginning of December. The presence of three generations of *P. walkeri* population beneath the ice has been stated at that time. The present observations started in the last the days of January 1971 have shown that all the algae and *P. walkeri* population likewise went down to the bottom and the sub-ice community was destroyed. At the sea bottom two generations were found in the *P. walkeri* population occurring simultaneously. The younger group consisted of individuals in the second year of life passing through a phase of intensive growth, the other group was in the dying-out phase and was composed of the post-breeding females in the third year of age. It seems that some females are able to survive the summer and produce the offsprings once more, in the spring, similarly to that what has been observed in *Bovalia gigantea* (Thurston 1968, Bone 1972). Individuals representing the youngest *P. walkeri* generation hatched in the spring have not been catched this time and it can be only suspected that they might be present in the depths of sea waters farther from the shores. Reactions of animals inhabiting the Antarctic coastal waters, where temperatures are permanently below 0°C with extreme fluctuations not beyond 2°C, and the thermal lethal limits of those animals is a matter of great interest so much more that the knowledge of that subject is as yet very slight. The lethal temperature for the Antarctic Amphipoda *O. chilensis* averaged 15°C, as reported by Armitage (1962). *P. walkeri*, as observed in the cultures, survives 12°C and at a shortlasting time of exposure resists even to higher temperatures.

Relation of the range of survived temperatures to the range of fluctuations encounters by this species in the environment is many times higher than in the Arctic Crustacea which, as reported by Scholander et al. (1953) encountering throughout the year temperature fluctuations ranging from −2.0° to 9.4° died at 25°C. Krog (1954) has demonstrated the seasonal acclimation influence on thermal tolerance. Resistance to high temperatures can be also influenced by light (Hoar 1956), a longer day increases tolerance to higher temperatures. Studies on interrelated influence of salinity and temperature involve a more complex situation. Increase in salinity may increase the survival time at high temperatures (Kinne 1958) though according to Wiesepape et al. (1972) low salinity "are a better preparation for heat resistance at any salinity". When animals are exposed to the fluctuations of temperature and salinity their effect may be modified by the age of animals, as the younger, intensively growing, individuals are more resistant (Kinne 1960). In our results attention is called to a high value of the freezing point during the summer $\Delta°C = -2.06$ in *P. walkeri* in which hemolymph concentration is hyperosmotic in relation to the environment. This values is higher than that found by

Scholander et al. (1957) in the Arctic Crustacea and approximate to the value observed in three Antarctic fish species living in sub-ice zone in which $\Delta°C = -1.98$, -2.01 and -2.07 (De Vries, Somero 1970). Sensitivity of metabolic responses to temperature under experimental conditions are controlled by series of factors, among others also the solubility of gases in sea water, as may be expected.

The coefficient of the influence of temperature on metabolic changes is expressed by the Q_{10} value. The Q_{10} value may differ markedly within population or within one species as the effect of temperature influence on reactions activity is affected by other factors, such as weight of individuals under examination (Rao, Bullock 1954), their age (Vernberg, Vernberg 1969), acclimation temperature (Buffington 1969) and acclimation time (Ivleva 1972). Metabolic temperature sensitivity varies and the Q_{10} value is different for the same animals in measurements of their standard, routine or active metabolism (cf. Halcrow, Boyd 1967, Newell, Northcroft 1967, Roux 1972) which, however, is questioned by some authors (Tribe, Bowler 1968). As results from our respiration measurements (Klekowski et al. 1973) performed on *P. walkeri* in winter 1969 after 24-hr acclimation the Q_{10} value, in the range of temperatures -1.9—$0.0°C$, 0.0—$2.0°C$ and -1.9—$2.0°C$ for the whole population, amounted to 15.9, 4.6, and 8.6, respectively. It differed markedly in juvenil individuals and females with eggs. In experiments on *P. walkeri* performed in summer after many-day acclimation in -1.2 to $0.1°C$ temperature range, only a slight increase in metabolism was found. A similarly weak reaction on temperature as in *P. walkeri* was reported by Armitage (1962) in his acute experiments also performed in summer on *O. chilensis* respiration. As results from his study the temperature of sea water inhabited by *O. chilensis* was $-1.8°C$ and yearly fluctuations in the investigated region did not exceed $0.2°C$ (Littlepage 1965), the depths in which the animal lived excludes dramatic changes in salinity. Thus, it seems that differences between the reaction on temperature of *P. walkeri* population examined in winter and *O. chilensis* and *P. walkeri* population examined in summer are caused by different level of the metabolic processes. The influence of seasonal temperature changes seems to be of less importance here in relation to other factors, such as feeding conditions, light, activity, stage of life cycle. So, the steady state (Grainger 1956) is different in population in which the applied in *P. walkeri* in summer experiments acclimation was not of neglegible importance as can be seen from the similarity of results obtained by Armitage (1962) in his studies on response to temperature in *O. chilensis* which were not acclimated. It is very interesting, though, that in *O. chilensis* and *P. walkeri*, as well, a high degree of metabolic regulation was observed at the temperature between 6—8°C which

according to Armitage (1962) may come as result of "the genetic history of population that previously lived in water with wider fluctuation of temperature". In that way we would have in the Antarctic Amphipoda two zones of relative thermal independence. One, "contemporary" ranging at about 0°C and slightly below, and the other "historical" in 6—8°C range, however, this seems to be rather unlikely. In the Antarctic fish metabolic rate drops at temperatures above 0°C (Wohlschlag 1960) similarly as in Amphipod, but temperature of 6°C is already the lethal limit for the whole organism.

In the studies on correlation between body size and metabolic rate, which in double log. scale may be described as: $R = a \cdot W^b$, much attention was paid to the b coefficient. Generalizing observations on Crustacea, Sushchenya (1972) has stated that relation between metabolism and body weight at temperature of 20°C is expressed by the formula $R = 0.125 \cdot W^{0.759}$ and for Amphipoda as: $R = 0.142 \cdot W^{0.79}$. As results from various studies (Vernberg, Vernberg 1969, Buffington 1969, Buikema 1972 and others), the b value depends on temperature and salinity and is not stable for all species, it varies with geographically separated populations of the same species. It is affected also by stage of life cycle, body weight, light intensity and acclimation time. Measurements of maintenance and active metabolism (Newell, Northcroft 1967) or standard and routine (Halcrow, Boyd 1967, Bayne 1971) influence the coefficient b value in the formula presented above. In his studies on correlation between sizes and metabolism in the Antarctic Amphipoda, Armitage (1962) has examined a wide range of *O. chilensis* individuals of different weight, i.e. of various age, the obtained coefficient values were relatively low (0.455—0.665). The range of weight in the examined *P. walkeri* individuals was less diversified and was variable at different temperatures. The determined b coefficient values were higher than those in *O. chilensis* as well in summer (cf. Table I) as in winter (Klekowski et al. 1973). With an increase in metabolism the b value decreases, also then when the increase was cused by reduced salinity. With a decrease in metabolic processes at 4°C, as observed in *P. walkeri* and in *O. chilensis* as well, the b value increases. At low temperatures, slow growth and "discrete" weight and length within the population (cf. Rakusa-Suszczewski 1972 b) coming as effect of breeding onceayear, differentiation of the reaction of individuals of the same population may be greater than in populations growing faster and having a shorter life cycle. Results obtained for individuals of various weight and various stage of development were analysed in the course of investigations on relationship of weight and metabolism in Antarctic Amphipoda. Oxygen consumption by females at the end of their life time may be nearer their standard metabolism

while in smaller individuals growing more intensively it is closer to their routine metabolism.

Both, reaction on temperature increase in respiration rate of the whole population and correlation between body weight and metabolism observed in *P. walkeri* are distinctively differing between the summer and the winter. The level of metabolic processes in the whole population is also diverse, and what is worthy of notice it is higher in summer than in winter.

Acknowledgements

We are very grateful to dr. E. Styczyńska-Jurewicz for their cryoscope measurements.

5. SUMMARY

Studies were carried out in the summer from December 22 1971 till the end of February 1972 at the USSR station Molodezhnaya at the shores of the Alasheyev Bight (67°40′S, 45°50′E). They were undertaken in continuation of the research on biology and metabolism of Amphipoda — *Paramoera walkeri* Stebbing initiated over there in 1969. This species is in winter the main component of the community forming beneath the ice in the littoral zone. In summer, the environmental conditions of that regions are undergoing considerable changes. Water temperature fluctuates in the range from −1.6° to −0.5°C. Oxygen content shows differences ranging from 9 ml/l at the water surface to 7 ml/l at the bottom in a depth of several metres. Those differences come as result of reduced salinity of 26–29‰ and its shortlasting decreases at the surface to as low as below 8‰. The sub-ice community undergoes destruction with progression of a rapid thawing of ice. Algae overgrowing the underside of the ice drop to the bottom. *P. walkeri* goes down to the bottom, likewise. In the population of that species jevenile and immature individuals were found in their second year of life and a stage of intensive growth and females that have produced offsprings and were in their third year of age, i.e. at the end of their life-time. Correlation between wet weight and length of *P. walkeri* in January is expressed by formula: $W=0.02 \cdot L^{2.84}$, at the end of February as $W=0.02 \cdot L^{2.91}$. The present observations have confirmed the character of individual growth and the life cycle of the species described previously. The analysis of hemolymph indicated its hyper osmotic concentration in relation to sea water, as $\Delta t=-2.06$, and surviving +12.0°C in the culture. Respiration of *P. walkeri* was tested in experiments performed at every 2.0°C gradient of temperature ranging from −1.2 to +12.0°C and 29‰ salinity. Animals were fed and gradually acclimated during a period of about 5 to 10 days at subsequent temperatures. *P. walkeri* respiration was also measured at temperature of 2.2°C and 15‰ salinity. Analyses of the oxygen consumption dependence on the body weight of the examined species indicated that the coefficients from the formula $R=a \cdot W^b$ were changing respectively with the test temperatures and salinity (a from 0.13 to 1.27, b from 0.58 to 0.77). The obtained results were compared with earlier observations carried out in winter. Increase in temperature in the ranges close to those the animal encounters in the environment increases oxygen requirement in summer only to slight degree in contrast to the winter. Above 10°C metabolism decreases with impending lethal temperature. With decrease in salinity oxygen consumption increased. Basic differences in *P. walkeri* metabolic reaction on temperature between summer and winter seasons are due, as it seems, to various steady states of the examined population.

6. STRESZCZENIE

Badania prowadzono w okresie letnim od 22.XII.1971 do końca lutego 1972 na radzieckiej stacji Molodezhnaya położonej u brzegów Zalewu Alasheyeva

(67°40'S, 45°50'E). Były one kontynuacją prac rozpoczętych tu zimą 1969 r. i dotyczyły biologii i metabolizmu Amphipoda — *Paramoera walkeri* Stebbing. Gatunek ten w okresie zimowym jest głównym komponentem zespołu formującego się pod lodem, w strefie przybrzeżnej. Latem warunki środowiskowe ulegają w tej strefie znacznym zmianom. Temperatura wody waha się w granicach od −1.6 do −0.5°C. Zawartość tlenu wykazuje różnice od 9 ml/l na powierzchni do 7 ml/l przy dnie na głębokości paru metrów. Różnice te są wynikiem obniżonego zasolenia do 26–29‰ i krótkotrwałych jego spadków na powierzchni do poniżej 8‰. Zespół podlodowy ulega destrukcji wraz z postępującym gwałtownym topnieniem lodu. Diatomea porastające spodnią warstwę lodu opadają na dno. Na dno opada również *P. walkeri*. W populacji tego gatunku stwierdzono osobniki juvenilne i niedojrzałe będące w drugim roku życia w fazie intensywnego wzrostu, oraz samice, które wydały potomstwo i będące w trzecim roku u kresu życia. Zależność pomiędzy mokrą masą i długością *P. walkeri* w styczniu opisuje wzór $W=0.02 \cdot L^{2.84}$, z końcem lutego zaś $W=0.02 \cdot L^{2.91}$. Obserwacje obecne potwierdziły charakter wzrostu osobniczego i cykl życiowy gatunku opisany wcześniej. Analiza hemolimfy wykazała jej hyperosmotyczną koncentrację w stosunku do wody morskiej $\Delta t=-2{,}06$ oraz przeżywanie w hodowli temperatury +12,0°C. Oddychanie *P. walkeri* badano w eksperymentach przy temperaturze od —1,2 do +12,0°C co 2,2°C i w zasoleniu 29‰, karmiąc i aklimatyzując zwierzęta stopniowo przez okres od około 5 do 10 dni w poszczególnych temperaturach. Mierzono również oddychanie *P. walkeri* przy temperaturze 2,2°C i zasoleniu 15‰. Analizowano zależność zużycia tlenu od masy ciała badanego gatunku, stwierdzając że w temperaturach i zasoleniu prowadzonych eksperymentów współczynniki a i b we wzorze $R=a \cdot W^b$ zmieniają się odpowiednio (0,13–1,27 i 0,58–0,77). Wyniki porównano z wcześniejszymi obserwacjami prowadzonymi zimą. Latem poziom metabolizmu *P. walkeri* był dwukrotnie wyższy. Wzrost temperatury w przedziale bliskim temperaturom z jakimi zwierzę styka się w środowisku, tylko w niewielkim stopniu zwiększał zapotrzebowanie tlenowe, w przeciwieństwie do okresu zimowego. Powyżej 10°C metabolizm spadał wraz ze zbliżaniem się do temperatury letalnej. Spadek zasolenia wywoływał wzrost zużycia tlenu. Zasadniczego różnice w reakcjach metabolicznych *P. walkeri* na temperaturę w okresie zimowym i letnim są, jak się wydaje, wynikiem innego "steady state" badanej populacji, przy której stosowana w eksperymentach aklimatyzacja nie odegrała większego znaczenia.

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CALORIFIC VALUES AND METABOLISM OF *GLYPTOTENDIPES POLYTOMUS* KIEFF. (CHIRONOMIDAE) IN EARLY SPRING

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ABSTRACT

It was found that the spring temperature rise above 10°C causes important changes in the physiological state of *G. polytomus* larvae. The wet and dry weight increases more intensively with increase in length, the calorific value of the dry mass and of organic matter increases and so does the oxygen consumption rate measured under aerobic conditions. Preliminary measurements of oxygen consumption, *RQ* and lactic acid content under hypoxic conditions seem to indicate a higher contribution of anaerobic processes under these circumstances.

1. INTRODUCTION

Larvae of Chironomidae are found in most types of water bodies both in high-mountain streams and in waste stabilization ponds. Sometimes they constitute the dominating group in the benthic macrofauna. Their abundance and biomass may reach high values, for instance Jónasson (1969) reports for *Chironomus anthracinus* in the eutropic Lake Esrom in April a density of 5000 individuals/m² and a biomass of about 61 g/m². Frequently Chironomidae are multivoltine species, therefore, their annual production may attain high values, Kimerle, Anderson (1971) report 459 kcal/m² for *Glyptotendipes barbipes* in a waste stabilization pond.

Owing to these properties, Chironomidae are an important link in energy transfer in aquatic ecosystems. Their significances as an important component of fish food is widely known. At present, however, their role in energy removal from water environments polluted owing to the activity of man seems no less important. Owing to their relatively large dimensions and their occurrence in large numbers, short life cycle, and, particularly, ability of life at low oxygen content, Chironomidae seem to be most interesting from this point of view. Kimerle, Anderson (1971) report that the annual energy removal by *G. barbipes* from a waste stabilization pond amounted to 184 kcal/m² by emergence and 730 kcal/m² by respiration.

The role of Chironomidae, however, is probably not limited to energy removal which may be evaluated by measurement of emergence and respiration. They also take part in organic matter degradation. Moreover, some species are capable of acquiring energy by anaerobic pathways. Such a conclusion has been

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reached by Platzer-Schultz (1968 a) in her investigations. Even under conditions of high oxygen content, energy loss in respiration by *Chironomus dorsalis* larvae measured by Kashirskaya, Lyukshina (1970) constituted only 70–90% of their total energy loss. The authors suppose that at low oxygen content these differences would be still wider. Kashirskaya (1970) measured the rate of glycogen consumption by *C. dorsalis* larvae starved for 24 hr at 20°C. From the data of this author it results that, under conditions of unreduced oxygen content, the glycogen level fell by about 39%, at an oxygen content of 2.11 mg O_2/l (i.e. 23% air saturation) by 45%, and at a content of 0.8 mg O_2/l (i.e. 9% air saturation), by as much as 75%. The author concludes that it is at this oxygen level that mainly anaerobic processes occur.

The investigated *G. polytomus* larvae in the present study were very abundant on the bottom of the waste stabilization pond. Czeczuga (1962) mentions that this species has an exceptionally high hemoglobin content (30% of dry weight on the average) and that the glycogen content in *G. polytomus* (18.6% of dry weight on the average) was the highest as compared with the other seven species of Chironomidae studied. He claims that this species is exceptionally well adapted to life in an environment with low oxygen content.

The present study was undertaken in order to establish the character of growth, calorific value and respiration (oxygen consumption and *RQ*) of *G. polytomus* larvae in conditions of high oxygen content. The investigations were performed before and after the beginning of the vegetation season, in order to asecrtain whether the above mentioned elements are characterized by constant values in *G. polytomus* or whether they change in dependence on the life conditions of the larvae. Preliminary measurements of oxygen consumption, *RQ* and lactic acid content were also carried out in larvae bred in conditions of low oxygen content in order to observe their mode of adaptation to life under hypoxic conditions.

2. MATERIAL AND METHODS

The larvae of *G. polytomus* were collected in ponds in which wastes from the yeast factory at Józefów near Błonie are sedimented. Larvae were very abundant there and dominated in the fauna. Samples were taken on 6 and 20 March, and 4, 15 and 22 April, 1972. They were brought to the laboratory in thermoses with mud. Transport lasted about 3 hr. In the laboratory the animals were divided into three groups.

The first one was placed on enamelled trays with water and mud at room temperature and covered with a net. It proved that all the emerged imagines belonged to one species — *Glyptotendipes polytomus* Kieff.

The second group was preserved in 4% formalin solution. This material served for length and weight and calorific value measurements in all samples except the one from April 15.

The length of larvae was determined with an accuracy up to 1 mm. Earlier preliminary measurements performed in January 1972 on larvae of a related species *Chironomus plumosus* collected from ponds in Gołysz, Cieszyn District showed that preservation in formalin does not change the length of larvae. The mean length of 40 live larvae was 20.6 mm (95% confidence limits 19.5–21.7). On the other hand, their mean length after 13 days preservation in formalin was 20.1 mm (limits 19.0–21.0).

As another parameter of body dimensions the breadth of the head was tentatively used. Preliminary measurements were performed on the above mentioned 40 larvae of *Ch. plumosus*. The relation between wet weight and body length and the relation between wet weight and head breadth were established. These relations may be expressed by the formuła $W = a \cdot L^b$, where W is wet weight (mg), L is length (mm) ora breadth of head (in mm, measured under the microscope with an accuracy up to $1 \cdot 10^{-4}$ mm), and a and b are coefficients. For the range of weights of *Ch. plumosus* larvae from 3 to 40 mg these coefficients have the following values:

for the dependence of weight on	body length	head breadth
regression coefficient a	0.0108	62.46
regression coefficient $b \pm SE$	2.56 ± 0.138	2.89 ± 0.244

As seen, the variation of the weight-head breadth relation is much wider. This partly results from the error in head breadth measurement. The measured

larvae had to be then weighted and their calorific value had to be determined, therefore, whole (intact) larvae had to be placed under the microscope, and then the head was not so well visible. Moreover, in contrast to body length and weight, the head breadth is a discrete instar-specific criterion for midges (Czeczuga 1962, Edmondson, Winberg 1971). Since in the present study on *G. polytomus*, the age structure was not taken into account, only the size of the larvae, no other measurements except those of body length and weight were taken into account.

Wet weight of the animals dried with filter paper, and dry weight after previous drying at 50° over NaOH to constant weight were determined. The accuracy of weighing was ±0.01 mg.

For measurement of calorific value of 1 mg dry weight, larvae, were chosen with in a narrow range of lengths, 13–16 mm. A microbomb calorimeter (Phillipson 1964) modified by Klekowski, Bęczkowski (in print) was used, and the technique recommended by Prus (1968) was applied. Calibration and determination, of exactness of the calorimeter was performed directly before the determinations. Twenty five benzoic acid pellets were burnt. Heat sensitivity of the apparatus was 0.6724 cal/mm of galvanometre deflection, the coefficient of variation was 4%. The calorific value of 1 mg of dry organic matter was calcu- Ash content was determined by successive burning of subsamples in a muffle lated on the basis of the calorific value of 1 mg of dry weight and ash content. furnace up to constant weight.

The third group of animals was placed in vessels with mud. The water layer above the mud was continuously aerated. The vessel was placed in a water bath at 8.0±0.1°.

Oxygen consumption by the animals was determined in all samples with the exception of the one from 6 March, under conditions of oxygen content close to air saturation. The measurements were carried out on the same water bath. Constant-pressure volumetric respirometers described by Klekowski (1968) were used. Measurements in the number of 56 were performed on animals taken directly from the mud in laboratory culture, the remaining 26 measurements, on animals which were previously kept 24 hr in a vessel with pure aerated water. The animals were placed in glass respirometric chambers of about 4 ml volume. The total air volume in the system was about 12 ml. Good oxygen access to the water was ensured by the small amount of water in the chambers (0.3 ml). Accuracy of the readings was ±0.1 µl. On the basis of these measurements the relation between respiration, and both live weight and season was established. *RQ* was measured with full oxygen access on the same respirometres by the procedure described by Klekowski (1968). In these measurements pH was maintained within 6.2–6.9 (mean 6.6).

Several preliminary measurements of oxygen consumption, *RQ*, and lactic acid content were performed under conditions of low oxygen supply. Larvae weighing jointly 500 mg (about 50 of them) were taken from the mud and placed in bottles with ground glass stoppers. The volume of the bottles was determined with an accuracy up to 0.01 ml and amounted to 55–60 ml. Oxygen content in the water was reduced by nitrogen bubbling, water pH was 6.2–6.7. The animals were kept in these conditions for 18 hr at 8°.

Attempts of application of volumetric respirometres for oxygen consumption measurements, when the oxygen content was low and controlled were unsuccessful; therefore determinations were performed by the closed-bottle method. Oxygen content was determined after Winkler in initial control bottles, final control bottles and in experimental bottles containing animals. The results are means from two parallel measurements. Although the results obtained by the closed-bottles method are burdened with an error resulting from the enhanced oxygen consumption (overshoot) at the beginning of measurements (Kamler 1969), however, in this case the application of this method seems permissible since the time of exposure was long and always the same, and the oxygen content was lower than 3 per cent air saturation. Platzer-Schultz (1968 a, b) noted overshoot only at higher oxygen content (above ca. 14% air saturation) in *Chironomus strenzkei*, at lower oxygen values this phenomenon did not appear.

For measurement of *RQ*, CO_2 content was determined in control and experimental bottles. The Van Slyke method was used in Rodier's modification (Rodier 1960).

Lactic acid content was determined after Barker, Summerson (1941) in tissues of larvae taken directly from the mud used in laboratory culture, and larvae which after removal from the mud were kept for 18 h in water with

low oxygen content. Lactic acid content was also determined in water with larvae and without. No differences were found between the values obtained for water. Only further investigations may elucidate whether *G. polytomus* larvae actually do not excrete lactic acid into water, or whether the amounts removed were too small to be detected in the given experimental system.

3. RESULTS

The course of temperature in the investigated period is shown in Fig. 1. In the period between the collection of the last two samples (4—22 Apr.) the first warmer weather occurred, air temperature exceeded 10°C.

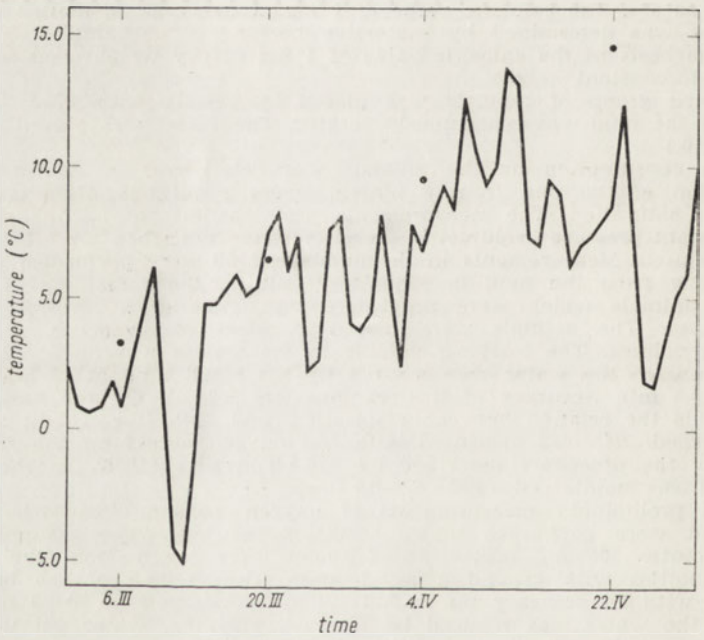

Fig. 1. Temperature course in spring 1972. Line — mean daily air temperature at Niepokalanów meteorological station (ca. 20 km from sampling places, data from PIHM). Dots — water temperature in ponds

The dependence of wet weight (range 0.8—25 mg) on the length (5—18 mm) in four samples of *G. polytomus* is shown in Fig. 2. In each sample one linear relationship on a double logarithmic scale can be found. In the very early spring period (samples collected from 6 March to 4 Apr., Fig. 2 A—C), the regression coefficients *b* are much below 3 what means that the shape of a growing larva changes to a more elongated form, whereas in the sample collected on Apr. 22 the same coefficient is very close to 3. This indicates that in this period some factor appeared compensating the changes towards body elonga-

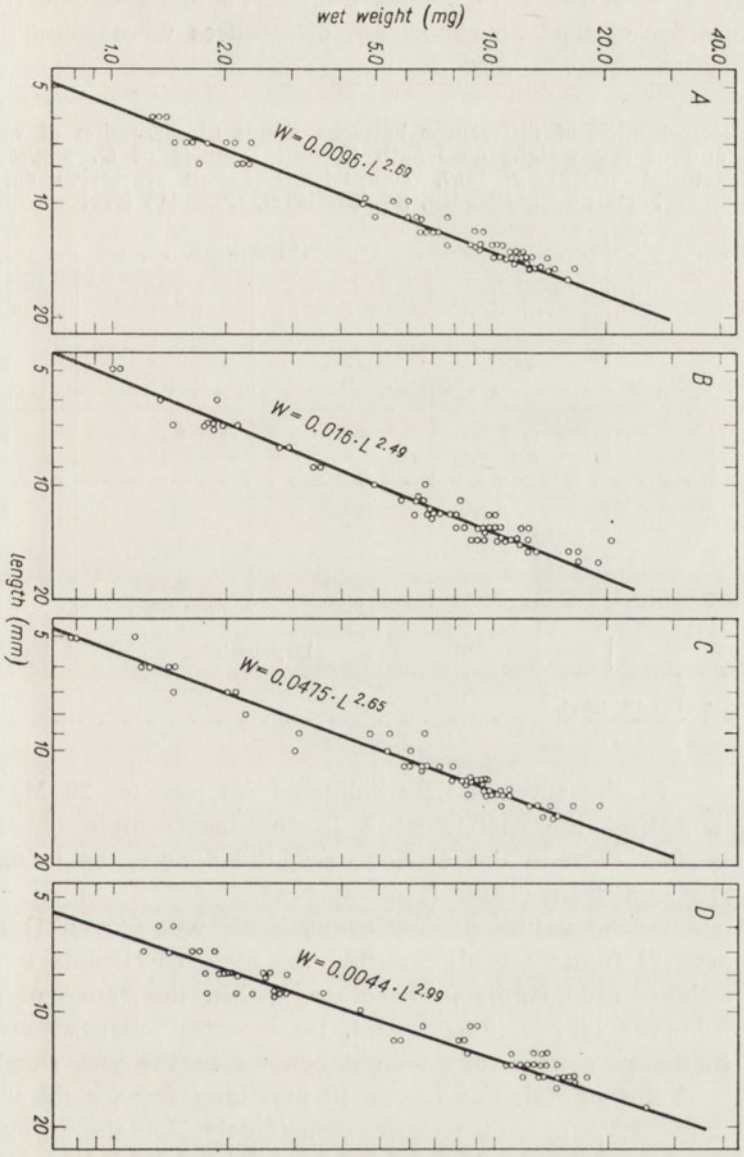

Fig. 2. Wet weight (W, mg) as a function of body length (L, mm) for *G. polytomus*
A — larvae collected on 6 March; B — 20 March; C — 4 April; D – 22 April

tion. Table I (upper right side) summarizes the results of calculation of the significance of differences between the regression coefficients b. As seen, the coefficient b found for the sample of Apr. 22 (Table I, last right column) differs significantly from those found for earlier collected samples.

All these regularities may also be observed when we consider the

dependence of dry weight on length (Fig. 3, Table I lower left side). It should be stressed that no significant differences were found between

Table I. Determination of differences between slopes of regression of wet weight on length and of dry weight on length in four samples of *G. polytomus*. The figures denote *d* (Bailey 1957, comparison of two regression coefficients). NS — no significance, S — 5% level, HS — 1% level

	Wet weight			
Day of sampling	6 March $n = 58$	20 March $n = 60$	4 Apr. $n = 54$	22 Apr. $n = 58$
6 March	X	2.30 S	0.34 NS	2.57 S
20 March	1.02 NS	X	1.36 NS	3.54 HS
4 April	O NS	0.85 NS	X	2.41 S
22 April	3.62 HS*	4.60 HS*	3.12 · HS	X
	Dry weight			

* Better, than 0.1% level.

coefficients *b* in the three earlier collected samples (6, 20 March and 4 April), and that the coefficient *b* in the last sample (22 Apr.) is markedly higher than in the earlier ones, the differences being statistically highly significant (Table I, lowest row).

Fig. 4 shows the values of coefficient *b* for wet (curve 1) and dry weight (curve 2) found for all samples. As seen, coefficents *b* for wet and dry weights are similar. Therefore, within the range of sizes of the *G. polytomus* larvae investigated, no essential changes are to be expected in the percentual dry weight content in the wet weight with the growth of the animal. For larvae 10 mm long dry weight was 18.0, 14.7, 17.3 and 15.2% of wet weight, respectively, for the samples collected on 6 and 20 March, 4 and 22 April. The mean ash content was 3.35% of dry weight (4 determinations, one from each sample).

Changes in the calorific value for larvae 13–16 mm long are shown in Fig. 5. In the earlier samples (6 and 20 March, 4 April) the calorific values are low and no significant differences between them were noted (Table II). In the last sample (of 22 April) the calorific values are higher (Fig. 5). As seen in Table II (for dry matter last right column, for organic matter lowest row), these differences are significant. Only the differences between the samples of 6 March and 22 April are not significant.

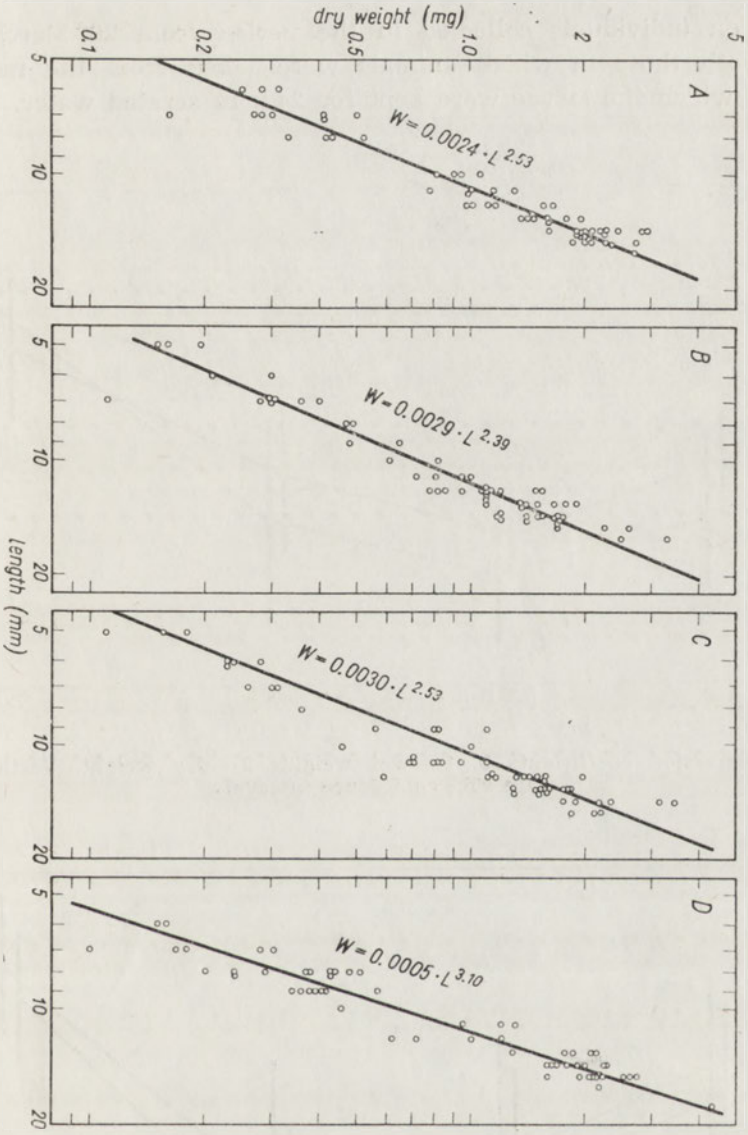

Fig. 3. Dry weight (W, mg) as a function of body length (L, mm) for *G. polytomus*. A — D as in Fig. 1

The relation of oxygen consumption (R, µl O_2/ind. · hr) to weight (W, mg wet weight) was established on the basis of 82 measurements performed with oxygen access in volumetric respirometres at 8° (Fig. 6).

This dependence (as calculated by least squares method) is expressed by the formula: $\log R = (-0.5240 \pm 0.0725) + (0.6718 \pm 0.0876) \cdot \log W$; the 95% confidence intervals for the parametres are shown here. Thus, $R = 0.3 \cdot W^{0.67}$. For calculating this relation all measurements were per-

formed on individuals collected in the period from 20 March to 22 April, both those in which animals were taken from the mud, and those in which the larvae were kept for 24 h in aerated water. It could

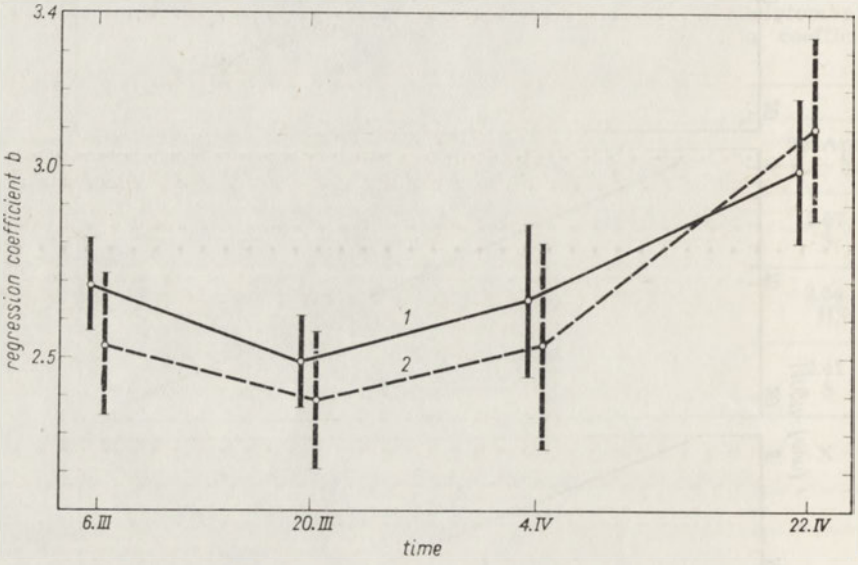

Fig. 4. Regression coefficients *b*. 1 — wet weight; 2 — dry weight. Vertical lines are 95% confidence intervals

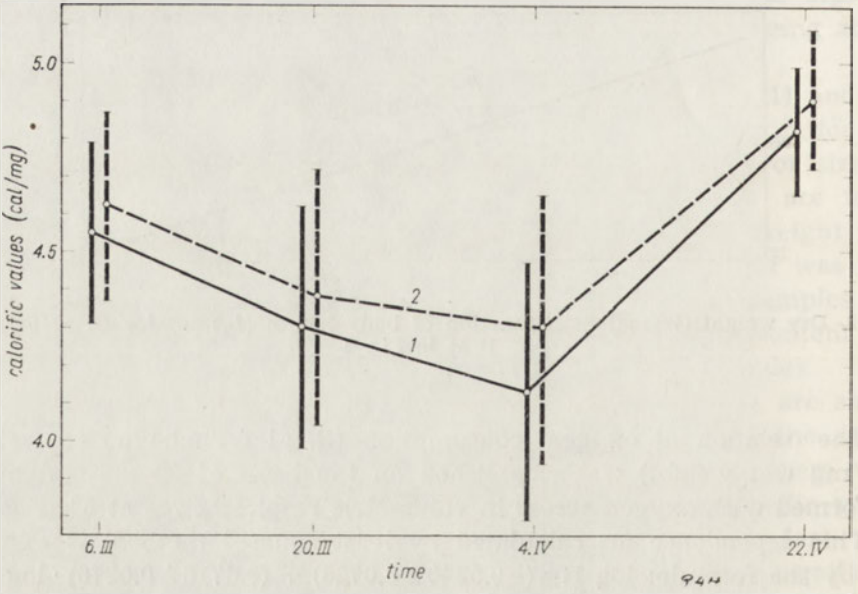

Fig. 5. Calorific values of *G. polytomus* (body length 13–16 mm). 1 — calorific value of 1 mg dry weight, 2 — calorific value of 1 mg organic matter. Vertical lines are 95% confidence intervals

Table II. Determination of differences between calorific values found in four *G. polytomus* samples. Figures denote *t* (Bailey 1959, comparison of means of two small samples). NS – no significance, S – 5% level, HS — 1% level

	Calories/mg dry weight			
Day of sampling	6 March $n = 8$	20 March $n = 6$	4 April $n = 6$	22 April $n = 7$
6 March	X	0.63 NS	1.03 NS	0.89 NS
20 March	0.58 NS	X	0.37 NS	2.70 S
4 April	0.78 NS	0.18 NS	X	3.41 HS
22 April	0.89 NS	2.55 S	2.89 S	X
Calories/mg organic matter				

have been expected that the larvae from mud lived before the measurements in worse oxygen conditions than those taken from water. It was necessary therefore, to ascertain whether the regression coefficient in the above given formula ($a = 0.3$) is not burdened with an error

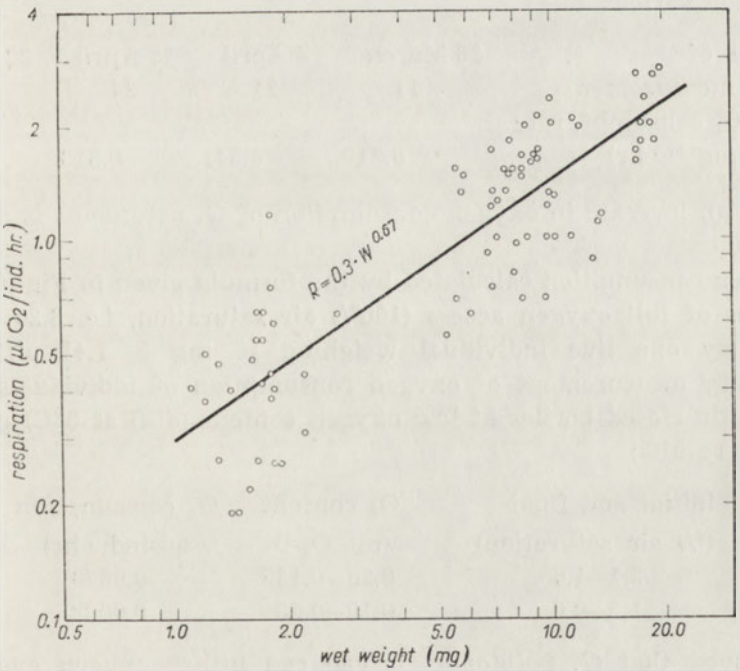

Fig. 6. Oxygen consumption (R, µl/ind. · hr) at 8°C as a function of wet weight (W; mg) for *G. polytomus*

resulting from these differences in the method, and whether oxygen consumption of the larvae from mud is not markedly higher (repayment of oxygen debt) than that of larvae kept in water. Since in all samples individuals of various weights were present (Fig. 2 and 3), and since the regression coefficient ($b=0.67$) differs significantly from 1.0, comparable units of body size (K l e i b e r 1961) had to be used for comparing oxygen consumption of the individuals taken from mud (56 measurements) and those from water (26 measurements), that is oxygen consumption rates per $mg^{0.67}$. Mean oxygen consumption of individuals taken from mud was 0.321 µl $O_2/mg^{0.67} \cdot hr$, and of those from water 0.323 µl $O_2/mg^{0.67} \cdot hr$. For evaluation of the significance of this difference, the criterion of nonparamatric U-test was used (W i l c o x o n-M a n n-W h i t n e y, in G u b l e r, G e n k i n 1969). U_{exp} calculated was 678. Since the table of G u b l e r, G e n k i n (1969) does not contain U values for $n>20$, the theoretical U value was calculated according to L u k' y a n e n k o (1967). For a 5% level $U_{theor}=531$, $U_{exp} > U_{theor}$, the difference is nonsignificant. Thus, on the basis of oxygen consumption solely, repayment of the oxygen debt by *G. polytomus* larvae kept in mud before measurements could not be ascertained. There is, therefore, no mistake in considering together the results of oxygen consumption for both these groups.

The mean oxygen consumption rates were compared for individuals collected at various dates

Sample of	20 March	4 April	15 April	22 April
No. of measurements	11	21	24	14
Mean O_2 consumpt. rate ($\mu l\ O_2/mg^{0.67} \cdot hr$)	0.219	0.341	0.313	0.369

Thus, an increase in oxygen consumption of *G. polytomus* is observed in spring.

Oxygen consumption calculated by the formula given in Fig. 6, under conditions of full oxygen access (100% air saturation, i.e. 8.26 ml O_2/l at 8°C) by one live individual weighing 10 mg is 1.41 µl/ind. · hr. Preliminary measurement of oxygen consumption of individuals of the same size in closed bottles at low oxygen content (also at 8°C) gave the following results:

initial and final (% air saturation)	O_2 content (ml O_2/l)	O_2 consumption (µl/ind. · hr)
3.1—1.6	0.26—0.135	0.0070
1.3—0.6	0.10—0.05	0.0032

It proved that *G. polytomus* larvae can utilize oxygen even from water with a very low oxygen content. Oxygen consumption under these conditions is several hundred times lower than when there is full

access of oxygen. Mortality of larvae kept in these conditions for 18 hr was not observed.

The mean respiratory quotient from 31 measurements performed with full oxygen access in volumetric respirometres was 0.55, thus it was very low what might be an indication of repayment of the oxygen debt. On the other hand, *RQ* measured at a low oxygen content (0.1—0.05 ml O_2/l, i.e. 1.3—0.6% air saturation) was exceptionally high reaching 44.4. Since the measurements were not replicated, this last result should be treated as an approximate evaluation of the order of magnitude.

Lactic acid content in *G. polytomus* tissues was determined preliminarily. Individuals taken from mud over which water was intensively aerated were examined as well as those taken from mud and kept for 18 hr in water with a low oxygen content (Table III). In the tissues

Table. III. Lactic acid content in tissues of *G. polytomus* larvae with mean weight 10 mg: temperature 8°C. — not determined

Individuals from mud	Individuals kept in water for 18 hr		Mean lactic acid accumulation rate (µg/ind. hr)
Lactic acid content (µg/ind.)	O_2 (% air sat.)	lactic acid content (µg/ind.)	
2.83	—	—	—
3.66	ca. 29.0	5.10 5.09	0.0797
4.59	3.1—1.6	7.55 7.47	0.1622

of the animals taken from mud, lactic acid was found, this indicating that they satisfied their energetic requirements partially on anaerobic pathways. In the tissues of individuals kept in water with low oxygen content a higher lactic acid content was found. This substance was detected in increasing amounts as the oxygen content decreased. It could not be elucidated whether the larvae excrete lactic acid into the environment or not.

4. DISCUSSION

The coefficient of regression *b* for the relation of weight and body length in the same *G. polytomus* population was not constant. In March and the beginning of April it was distinctly lower than 3, and reached this value on 22 April. At this time development of larvae, previously

inhibited, started, the thoracic segments became markedly swollen at the sites of future wing muscles in prepupal stage. Moreover, as demonstrated by Arabina, Gavrilov (1969), coefficient *b* is not only dependent on the nature of growth of the given species, but also on the environmental conditions in which growth occurs. For *Chironomus f.l. plumosus* Arabina, Gavrilov (1969) report widely differing coefficients *b*, for instance 2.2 in the Lake Narocz, 2.9 in ponds. In the present paper (preliminary measurements) $b=2.56$ was determined for *Ch. plumosus* (Gołysz ponds, January).

Below the calorific values (cal/mg dry wt.) are listed for Chironomidae larvae according to various authors.

Tubb, Dorris (1965)	*Harnischia tenuicaudata*	5.34	season not mentioned
	Tanypus stellatus	5.61	season not mentioned
	Chironomus plumosus	5.84	season not mentioned
Wissing, Hasler (1968)*	*Glyptotendipes sp.*	4.70–5.60 *	Aug.–Nov.
Kashirskaya, Lyukshina (1970)	*Chironomus dorsalis*	4.90–5.00	season not mentioned
Kimerle, Anderson (1971)	*Glyptotendipes barbipes*	4.98	the year round, but most summer samples
Wissing, Hasler (1971)	Chironomidae, mostly Glyptotendipes	4.90–6.41	Jun.–Sep.
Malikova (1971)*	*Chironomus plumosus*	4.94 **	season not mentioned

If we compare these data probably mostly obtained in the summer months, with the results of the present study (Fig. 5, curve 1), it is apparent that the calorific values for *G. polytomus* in the period 6 March–4 April were markedly lower than those in the above quoted literature. It is only on 22 April that the calorific values (4.65–4.99 cal/mg dry wt.) become similar to the lower range of values quoted for summer.

Seasonal changes in caloricity have been reported many a time, for instance Stroganov (1954) for sturgeons, Makhmudov (1966) for marine zoobenthos. Blažka (1966) gives the chemical composition

* Calculated from the ash-free values and per cent of ash given by Wissing, Hasler (1968).

** Calculated from chemical composition given by Malikova (1971) using calorific equivalents for carbohydrates, proteins and fat according to Winberg et al. (1971).

of *Chironomus plumosus* larvae from ponds. After conversion it appears that the calorific value on 12 Feb. was 5.24, and on 20 April 5.40 cal/mg org. matter, that is by 0.16 cal/mg org. matter more. In the present study an increase in calorific value of *G. polytomus* larvae was found in the period 6 March—22 April amounting to 0.28 cal/mg org. matter. Jónasson (1965) mentions the percentual nitrogen and fat content in *Chironomus anthracinus* in the eutrophic Lake Esrom. On the basis of these data and the percent of ash in dry weight found in the present determinations, it was calculated that between 14 and 22 April the calorific value of *Ch. anthracinus* increased by 0.37 cal/mg dry wt. This result is very close to that evaluated for the same period in the present work (0.40 cal/mg dry wt. — Fig. 5). Jónasson (1965) noted in this period a distinct increase in fats (from 10 to 18% of dry wt.) and believes that in this period larvae store fat as a fuel for the imago.

Oxygen consumption of *G. polytomus* measured under high oxygen content proved to be proportional to the body surface area (regression coefficient b for respiration/wet weight relationship was 0.67). Different results were obtained by Edwards (1958) who in *Chironomus riparius* found $b=0.83$. Uniform results cannot be expected in the entire taxonomic group of Chironomidae, as shown by Kasatkina (1960) who determined the relation between respiration and weight at 20°C in seven species of Chironomidae. For two species she found a relation quite different from the one here presented ($R=0.312 \cdot W^{0.91}$), whereas for the remaining four species (among them *Glyptotendipes gripekoveni*) the relation could be expressed by the formula $R=1.30 \cdot W^{0.73}$. Coefficient $b=0.73$ lies within the confidence interval obtained in the present study (0.58—0.77). When the value 1.30 is converted from 20° to 8° after Winberg (1956) (on the basis of Krogh's "normal curve"), we get $1.30 : 3.48=0.37$. This figure is only slightly higher than the upper 95% confidence limit obtained in the present work (0.36). It could have been expected that the respiratory values measured by Kasatkina (1960) are somewhat too high since she used a Warburg respirometre performing 100 shakings per minute. The proportionality of respiration under high oxygen content to the body surface area was observed by Konstantinov (1971) and Kashirskaya (1972) in a number of Chironomidae species.

Seasonal changes in the oxygen consumption rate have been pointed out among others by Edwards (1958) who found that this rate in *Chironomus riparius* is lower in winter than in summer. The change occurs in spring. This is confirmed also by the results of measurement of respiration of *Hexagenia recurvata* (Ephemeroptera) carried out by Morgan, Wilder (1936). Berg et al. (1958) found that the respiration rate of the snail *Ancylus fluviatilis* increases in March—April, the spring maximum is about 1.3 to nearly twice that of the consumption

noted in winter. A similar, about 1.6-fold increase in respiration rate in *Ephemera simulans* (Ephemeroptera) was reported by Eriksen (1964) in the second half of April when the temperature of the environment exceeded 10°C. The agreement of the results of the present study and of the quoted paper is extraordinary in view of the geographical and taxonomic distances separating them.

The *G. polytomus* larvae examined were collected from waste stabilization ponds of a yeast factory. Yeast is considered to be good food for Chironomidae (Czeczuga 1962, Platzer-Schultz 1968a, Burbanck, Mozley 1969). It appeared, however, that in winter, owing to low temperature and low oxygen content and lack of phytoplankton admixture in the food, the animals cannot utilize it, the larvae are practically starved. The winter depression of the regression coefficients *b* for the weight-length relationship, of calorific values and of respiration may be regarded as evidence of this as well as of an arrested development in the overwintering generation which remains at the larval stage from late autumn until late April. The consistent increase in all these parametres in the second half of April is connected with the improvement of conditions, and physiological changes preparing the initiation of reproductive activity.

Data concerning oxygen consumption by larvae of various species of Chironomidae in dependence on oxygen content in water are reported in papers of numerous authors (Walshe 1948, Berg et al. 1962, Jónasson 1964, 1965, 1969, Jónasson, Kristiansen 1967, Platzer-Schultz 1968a, Konstantinov 1971 and Kashirskaya 1972). It results from these papers that, in general, species living in environments poor in oxygen are of regulator type. When the oxygen values fall, they maintain their oxygen consumption at a rather stable level up to a critical oxygen pressure, below which consumption falls more abruptly. The highest oxygen pressure at which the animal is not capable of taking up oxygen from the environment is the residual oxygen pressure (Blažka, Kopecký 1961). According to the data of Berg, Jónasson (1965), the oxygen content 1—2 cm above the bottom of the eutropic Lake Esrom was 0.1—0.3 ml O_2/l at 9—11°C. These conditions were considered as close to natural for *G. polytomus*. Larvae under these conditions were still capable of oxygen take-up from water, its consumption was, however, markedly diminished thus, such an oxygen content lies within the critical and residual values for *G. polytomus*. Blažka, Kopecký (1961) give the residual pressure for *Carassius carassius* as 5 mm Hg. In the conditions of the present experiments the values 0.1 ml O_2/l corresponded to 1.9 mm Hg. This means that for *G. polytomus* residual oxygen pressure < 1.9 mm Hg, thus it is exceptionally low.

When the larvae of *G. polytomus* were transferred from hypoxic

conditions to water rich in oxygen, no enhanced oxygen consumption was noted (repayment of oxygen debt) as compared with that of the animals kept for a long time in high oxygen content. Platzer-Schultz (1968 b) found that *Chironomus strenzeki* larvae partially paid the oxygen debt. Konstantinov (1969, 1971) claims that the apparent payment of the oxygen debt measured in animals transferred from low to high oxygen content results from their enhanced motor activity. In larvae of *Chironomus plumosus, Ch. dorsalis* and *Ch. cingulatus* immobilized by anesthetics no increased oxygen consumption could be observed. This increase after a period spent in hypoxic conditions is characteristic rather for animals which find themselves for a short time in an environment with low oxygen content (e.g. Trutta acc. to Blažka 1958) than for animals which, as for instance *G. polytomus* studied here, live continuously in hypoxic conditions.

It was found that 10-mg larvae of *G. polytomus* taken from mud (conditions similar to natural) contained 2.83—4.59 μg of lactic acid/ind., that is, if we assume the mean value of 17% of dry weight in wet weight 1.64—2.71 mg/g dry wt. Augenfeld (1967) in *Ch. plumosus* larvae taken from a lake found very similar values, namely, 0.38—2.13 mg/g dry wt. This author after keeping the larvae for 12 days at 8°C under anoxic conditions (unfortunately he does not give the oxygen content) found an increase in lactic acid content to 4.64 mg/g dry wt. Almost identical lactic acid values after 18 h at the same temperature and at 3.1—1.6% air saturation were noted in the present study in *G. polytomus* — 4.44 mg/g dry wt. Augenfeld (1967), while trying to elucidate the question of lactic acid excretion under anaerobic conditions, also met with difficulties. His results seem to indicate that excretion does occur. After reintroduction of oxygen he found a decrease in lactic acid in animal tissues, thus, this substance may also be removed by active metabolic processes. In favour of such an interpretation would also speak the exceptionally low value of *RQ* (0.55) found in the present work in larvae kept in a medium with high oxygen content.

G. polytomus larvae are thus capable of living under conditions of exceedingly low oxygen content in the environment. In these conditions anaerobic processes, known to be energetically about 20 times less efficient than aerobic ones, prevail in their metabolism. Therefore, *G. polytomus* larvae living in great abundance on the bottom of waste stabilization ponds seem to be important for the degradation of the organic matter accumulated there in excess.

5. SUMMARY

In the period between 6 March and 22 April the length, wet and dry weight, calorific value and oxygen consumption were measured in *G. polytomus* larvae

in a medium with high oxygen content. The rise of temperature in spring above 10°C was found to produce important changes in the physiological state of the larvae. Wet and dry weight increased more intensively with increase in length, the calorific value of dry mass and organic matter increased and so did the intensity of oxygen consumption.

Metabolism was measured at various oxygen contents. Under hypoxic conditions respiration of *G. polytomus* was greatly reduced. The larvae are capable of taking up oxygen even from an environment very poor in this gas. The respiratory quotient of larvae under hypoxic conditions is very high, whereas in high oxygen content it is very low. Lactic acid accumulates in the tissues of the larvae in increasing amounts as the oxygen content is reduced. It would seem, therefore, that one of the forms of adaptation of these animals to life under hypoxic conditions is an increased contribution of anerobic processes to their metabolism.

6. STRESZCZENIE

W okresie od 6 marca do 22 kwietnia mierzono długość, żywy i suchy ciężar, wartość kaloryczną i zużycie tlenu w dobrych warunkach tlenowych u larw *G. polytomus*. Stwierdzono, że wiosenny wzrost temperatury powyżej 10°C spowodował ważne zmiany w stanie fizjologicznym larw. Żywy i suchy ciężar wzrastają intensywniej ze wzrostem długości, wzrasta wartość kaloryczna suchej masy i materii organicznej oraz wzrasta intensywność zużycia tlenu.

Przeprowadzono pomiary metabolizmu w różnej zawartości tlenu. W obniżonej zawartości tlenu respiracja *G. polytomus* znacznie się obniża, larwy mają zdolność wyczerpywać tlen nawet ze środowiska bardzo ubogiego w ten gaz. Współczynnik oddechowy (*RQ*) larw w obniżonych warunkach tlenowych jest bardzo wysoki, zaś w dobrych — bardzo niski. W tkankach larw gromadzi się kwas mlekowy w ilościach wzrastających wraz ze spadkiem zawartości tlenu. Można więc przypuszczać, że jedną z adaptacji tych zwierząt do życia w środowiskach ubogich w tlen jest wzrost udziału procesów anaerobowych w ich metabolizmie w tych warunkach.

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W. JĘDRYCZKOWSKI and Z. FISCHER

PRELIMINARY REPORT ON THE METABOLISM OF THE SILVER EEL (*ANGUILLA ANGUILLA* L.)

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ABSTRACT

Metabolism of silver eel was studied from the point of view of its adaptations in view of an economical expenditure of its energetic reserves. As compared with other fish, considerably low oxygen consumption and both carbon dioxide and nitrogen excretions were found. It is supposed that in the first place lipids serve as the main energetical material for this fish. The analyses of lipid and carbohydrate content in the body and liver of the investigated silver eels were made.

1. INTRODUCTION

It is commonly known that the silver eel, a typical fish of two habitats, makes long migrations connected with its reproduction. In Polish waters, it becomes mature at the age of 4–8 years (Sakowicz 1930). It then stops feeding and is ready to start migration to its spawning grounds. According to Piątek (1970), the weight of males amounts by then to about 90 g, and of females — to about 500 g.

It seemed interesting to search for the physiological mechanisms which make it possible for unfeeding silver eels to cover such long distances to reach the Sargasso Sea, where spawning takes place. Investigations of migrating silver eels are rather difficult; however, it is possible to observe them in estuaries of rivers when they begin their migrations.

Thus, it seems desirable to investigate oxygen consumption and carbon dioxide excretion in unfeeding silver eel, as well as to observe losses in its body weight during several months. After the experiments the fish were killed and dry weight and ash, lipid and carbohydrate contents were determined in their bodies and livers.

2. MATERIAL AND METHODS

Five mature individuals of silver eel (*Anguilla anguilla* L.) were used for investigations. They were caught in lakes near Olsztyn in the autumn of 1970. Their body length was 30 cm, and body weight — 70–100 g. According to the criteria recommended by Piątek (1970), they were recognized to be males. At the beginning of the experiments, live food (Turbificidae) was administe-

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red, but since the fish did not eat, no more food was given. The experiments lasted for 54 days (January 11 to March 5, 1971). During this period 85 measurements of oxygen consumption, 72 measurements of carbon dioxide excretion and 59 measurements of nitrogen excretion were made. The measurements were made between 11 a.m. and 1 p.m. Each day every fish was measured for only one parameter.

To check the gas exchange in the night, measurements of oxygen consumption and of both carbon dioxide and nitrogen excretion were made twice, at 10 p. m. and at midnight.

During the experiments the fish were kept in 10 l respiration chambers placed in a thermostat at 18±0.1°C (Fig. 1). Water in the respiration chamber was intensively aerated and renewed every four days. The fish were weighed on the first and last day of the experiment.

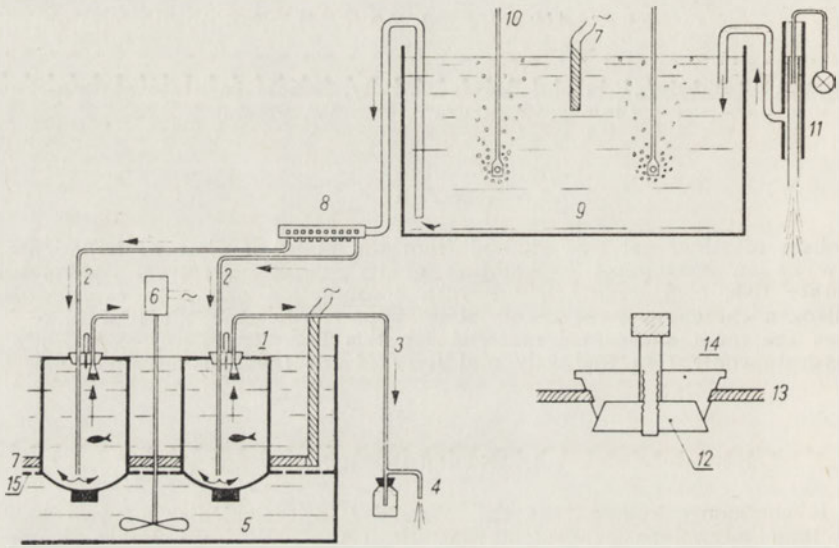

Fig. 1. Scheme of the experiment. 1 — the jar with fish, 2 — water inflow, 3 — water outflow, 4 — water sampling, 5 — thermostat, 6 — agitator, 7 — heater, 8 — dividers, 9 — water reservoir, 10 — air diffuser, 11 — regulator of water level, 12 — solid part of stopper, 13 — rim of jar, 14 — rubber part of stopper, 15 — stand of respiration chambers

Measurements of gas exchange (oxygen and carbon dioxide) were taken in a flowing water respirometer (Fig. 1) described in detail by Fischer (1970). One jar served as control and did not contain any fish. Oxygen, carbon dioxide and nitrogen contents in the water of the tanks with fish were compared with those of control water.

Oxygen consumption by fish was measured by determination of oxygen content in water outflowing from the respiration chamber, according to Winkler's method modified by Just, Hermanowicz (1964) and Carrit, Carpenter (1966). Carbon dioxide content in water was determined according to the Van Slyke method modified by Rodier (1960).

Ammonia nitrogen was determined according to Prochaskova (1964). The remaining non-protein nitrogen content was calculated for all the fish together by subtracting the ammonia nitrogen from total non-protein nitrogen estimated according to Kjeldahl's method. Probably the main component of the total non-protein nitrogen is urea nitrogen (Wood 1958, Altman 1961).

After the experiments the fish were killed and their livers were taken out. The bodies and the livers were homogenized and then analysed. The percentual water content was calculated from the difference in weight between fresh and homogenate dried to constant weight at 60°C. Ash content was determined by

combustion to constant weight in a muffle furnace at 550°C. Total carbohydrate content in dry weight of both the bodies and the livers was estimated by the anthrome method (Trevelyan, Harrison 1952), and lipids — according to Stern, Shapiro (1953).

3. RESULTS

Loss of body weight after 54 days of experiment is presented in Table I. It can be seen that the loss of weight in the fish not feeding for quite a long time was rather small (mean 3.77%) as compared with

Table I. Loss of body weight after 54-days experiment

Fish No.	Initial weight (g)	Final weight (g)	Weight Loss	
			g	%
1	72.25	68.70	3.55	4.68
2	105.92	101.04	4.88	4.40
3	66.55	66.05	0.50	0.74
4	82.80	77.60	5.20	5.90
5	86.40	83.60	2.80	3.13
			Mean	3.77

the data of Vieweger (1928 a, b) (about 10%). Losses of weight were similar in all fish except one, No. 3, which, for unknown reasons, showed hardly any loss. If this fish were not taken into consideration when calculating the mean value, the mean would increase from 3.77

Table II. Mean values of oxygen consumed and both carbon dioxide and ammonia nitrogen excreted

Fish No.	Consumption (µl O_2/hr/ind.±.SE.)	Excretion	
		µl CO_2/hr/ind.±S.E.	µg $N-NH_3$/hr/ind.±S.E.
1	1939±470	3736±404	48.8± 9.1
2	2074±456	2411±226	27.2± 8.0
3	1370±413	2293±302	16.8± 5.7
4	2570±850	3400±544	51.2± 15.8
5	1680±428	2836±355	44.9± 8.2

to 4.03% which is not a significant difference in so few samples. It could be supposed that during the experiment some changes in water content could have occurred in this fish; it was proved, however, after the experiment that the dry weight of this fish was 45%, being similar to that of the other fish (45.5—47.1%, Table III).

The mean values of oxygen consumption and carbon dioxide and ammonia nitrogen excretion are presented in Table II. Excreted nitrogen was determined on the basis of measurements made in daytime.

The mean oxygen demand of silver eel of the size under study was about 1926 µl O_2/hr/ind. Mean carbon dioxide excretion was about 2900 µl CO_2/hr/ind. Ammonia nitrogen was excreted in daytime only (night experiments gave no results). Mean ammonia nitrogen excretion was 37.7 µl N-NH_3/hr/ind. Mean remaining non-protein nitrogen was 24.5 µg N/hr/ind., and mean total non-protein nitrogen — 62.2 µg N/hr/ind.

The chemical compositions of the bodies and livers of the investigated fish are presented in Tables III and IV.

Table III. Chemical composition of silver eel body

Fish No.	Total wet weight after killing (g)	Dry weight (%)	Lipids in dry wt. (%)	Carbohydrates in dry wt. (%)	Ash in dry wt. (%)
1	68.9	45.5	55.1	0.18	6.4
2	99.1	47.1	49.7	0.18	3.3
3	62.8	45.6	49.9	0.18	6 3
4	78.7	46.1	44.2	0.19	7.5
5	81.4	46.0	53.2	0.14	7.6

Table IV. Chemical composition of silver eel liver

Fish No.	Wet weight (mg)	Dry weight (%)	Percentage of liver body weight (%)		Lipids (%)		Carbohydrates (%)	
			dry wt.	wet wt.	dry wt.	wet wt.	dry wt.	wet wt.
1	520	25.0	0.43	0.75	17.6	4.4	1.47	0.36
2	940	32.0	0.94	0.95	19.9	6.6	13.8	4.60
3	520	30.8	0.93	0.87	28.4	8.7	6.5	2.00
4	750	37.4	0.74	0.95	26.3	9.7	7.9	2.92
5	870	24.2	0.57	1.07	12.7	3.2	13.6	3.30

The values presented in Table III for chemical composition of the silver eel body are similar to those given by Piątek (1970) for this fish.

The results presented in Table IV point to a relatively small liver weight. Piątek (1970) found that under natural conditions a liver of a silver eel amounted to more than 2%, or even more than 4% of wet weight of the whole body, whereas in our experiments this value was close to 1% at its maximum. Yasuo Inui, Yasuo Ohshima (1966) gave values between 0.8 and 1.5%, depending on the starvation period (experiments carried out at 28°C). Undoubtedly this value is influenced by the duration of the fasting period.

Lipid content in the livers of the investigated fish were similar to those recorded by Yasuo Inui, Yasuo Ohshima (1966) at high temperatures (28°C). They recorded maximum mean values of

about 6‰, whereas in our experiments the mean value was 6.5‰. The ranges of the results are also similar. Yasuo Inui, Yasuo Ohshima (1966) found that the carbohydrate content in the liver decreased as the starvation period lasted longer. At the beginning of the experiment carried on at 28°C, it amounted to 6‰ and already on the 15th day it was lower than 1‰. However, in the experiment carried out at 12°C, carbohydrate content in the liver was higher (about 3‰), and its decrease, as the starvation period lasted, was slower: it was only on the 90th day that carbohydrate content reached 4‰. Our experiments were carried out at 18°C and probably this was why the carbohydrate content in the liver was between 0.3 and 4.6‰, i. e. considerably lower than that obtained by Yasuo Inui, Yasuo Ohshima (1966) at 28°C, but higher than at 12°C.

4. DISCUSSION

During the experiments, the investigated silver eels were hardly active. They did not change their positions for days, and even on nights no increase in their activity could be observed. In consequence, their metabolism could be considered only as resting metabolism. It is a striking example of the influence of laboratory culture, since under natural conditions this should have been a period of long and energy--consuming migration to their spawning grounds.

Admittedly, the study of the energy balance of the silver eel was not the aim of the present paper, it seems, however, that on the basis of the results obtained some suggestions can be made as to eel energetics. When dealing with an organism which does not feed or grow and excretes only few faeces, the commonly accepted formula of energy balance $C=P+R+F+U$ (where C — consumption, P — production, R — respiration, F — faeces, U — urinary) can be transformed in the following way: $0=P+R+0+0$, thus $R+(-P)=0$. The situation is similar to that occurring in energetic transformations of insect pupae. A detailed analysis of such a system in which negative production is equal to metabolism was given for *Tribolium castaneum* by Klekowski et al. (1967). According to these authors, during pupation the calorific value of an individual decreases at the cost of diminishing lipids, and respiration is considerably decreased. Thus, it would seem that it is mainly lipids which are oxidized in the respiration process.

On the basis of oxygen consumption measurements and carbon dioxide and nitrogen excretion, the calculated *RQ* coefficient was always higher than 1. Perhaps this indicates excessive oxidation of carbohydrates. Maybe, in silver eel not only fats are directly oxidized, but also proteins are transformed into lipids and the latter become oxidized, too. Another explanation of the high *RQ* can be anaerobic respiration.

However, it seems hardly probable in our experiments, as water was fully aerated. It can be mentioned in favour of the hypothesis of proteins being transformed to lipids, that the alimentary tract of fasting silver eels is resorbed.

In our experiments, the silver eels consumed about 1900 µl O_2/hr/ind. on the average, and the mean data for a feeding eel (Nicol 1960, Precht 1961) are about 3000 µl O_2/hr/ind. An analogy can be drawn between oxygen consumption in the silver eel against feeding eel, and respiration in pupae against larvae of *Tribolium castaneum*. Oxygen consumption in pupae is considerably lower than in larvae (Klekowski et al. 1967), similarly as oxygen consumption in silver eel in our experiment is considerably lower than that in naturally feeding eel investigated by Nicol (1960) and Precht (1961). Boetius, Boetius (1967) obtained also approximate low values when investigating silver eels in the experiments similar to ours. Oxygen consumption values of silver eels obtained in the present paper as well as by Boetius, Boetius (1967) are surprisingly low. They are about three times as low as those obtained for other fish (Privolnev 1945, Ivlev 1954, Fry 1957, Gerking 1967, Fischer 1970, 1972 a, b). It is possible that the main reason is the reduced activity of the fish, however, it should be mentioned that the experiments on grass carp by Fischer (1970, 1972 a, b) were made by the same methods, in chambers of the same capacity, and thus grass carp and silver eel had the same mobility conditions. Nevertheless, the levels of activity of those fish were quite different.

The negative growth of the investigated fish is surprisingly low being on the average only 3.7% after 54 days, i. e. only 0.08% per day. The possible explanation can be also the small activity of the fish. In similar experiments on feeding eels which were starved, Vieweger (1928 a, b) obtained 0.14% daily loss of the initial weight, and Yasuo Inui, Yasuo Ohshima (1966) — 0.1%. It can be calculated from data of Tables I and III, assuming regular loss of all body components, that our silver eels were losing about 70 mg of wet weight per day thus about 215 cal per day. The mean oxygen consumption (1900 µl O_2/hr/ind., thus 46 ml O_2/24 hr/ind.) can be converted to 220 cal per day. In this way surprisingly similar calorific values resulted. To obtain an approximate energetic value of respiration, an oxy-calorific coefficient was adopted (Ivlev 1939, Harrow, Mazur 1958), amounting to 4.84 cal for all the animals. Since *RQ* value was higher than 1, indirect calorimetry was not used. A possible error resulting from the approximation should not affect the results which are anyway only preliminary. According to Kamler (1972), the possible error is not higher than 7%. Similarity of the results in calories of both respiration and

negative growth was obtained when the mean values were taken into consideration, whereas for the particular fish they were as in Table V.

Table V. Comparison between silver eel respiration and loss in calorific value of its body

Fish No.	*R*	$-P$
	cal/ind./24 hr	
1	221	195
2	230	270
3	163	23
4	297	288
5	196	153

It can be seen in Table V that in 4 cases out of 5, respiration is approximate to negative growth, whereas in fish No. 3, the smallest one, the negative growth was much lesser than respiration. Apparently the fish, sampled for experiments at random, were not in the same physiological state. While in most of our fish all the body components were uniformly oxidized, in fish No. 3 it involved mainly lipids. This suggests the possibility of utilizing different body components to satisfy the organism's requirements. Indeed, as it was mentioned, the experimental fish were little active, whereas under natural conditions fish at this stage start a long migration to their spawning grounds and cover a distance of a dozen or several dozen kilometers per day (Gajewski 1960). Thus, under natural conditions their energy expenditure is considerably higher, possibly even double (more than 400 cal).

On the basis of the measurements of excreted nitrogen (Table II) it can be supposed that oxidation of proteins in the respiration process of silver eel is but minimal. This is testified by remarkably small amounts of the excreted nitrogen, being four times lower than in other fish (Smith 1929, Ivlev 1939, Blažka 1966, Fischer 1972 b). Yasuo Inui, Yasuo Ohshima (1966) had starved feeding eels weighing about 100 g. They found that nitrogen excretion after 50 days of starvation was about 6 mg/24 hr/ind. It can be calculated that a similar fish from our experiments excreted about 0.8 mg of nitrogen per day (considering that night excretion had not been observed). Comparison of these values clearly suggests an economical protein regime in silver eel.

Assuming a double increase of metabolism of migrating silver eels as compared with those of our experiments, a silver eel of 80 g should use up all its lipid reserves in one year. This time is quite sufficient to reach the spawning grounds, assuming (Szmidt 1950, Gajewski 1960) the mean rate of migration to be 30—50 km per 24 hr. It seems that migration time is a physiological period when a fish may not lose its

energy in the search for food. From an energetic point of view, release of the energy accumulated in an organism is more advantageous. Therefore, the regime of a silver eel organism is fully adapted to the release of lipid energy. The lack of observable differences between day and night activity of silver eel (which are so distinct in feeding eel) suggests that the physiology of its organism is strongly directed towards uninterrupted migration. Owing to such an adaptation, the fish, though not a very fast swimmer, can cover immense distances in a relatively short time.

The present paper is a preliminary report. The results are based on too small a number of individuals and do not allow to elaborate the subject more completely. Further studies will be undertaken in the near future.

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5. SUMMARY

Metabolism of silver eel was studied. Loss of weight after 54 days of the experiment was found to be 3.7% of initial fish weight. The investigated fish used about 1.9 ml O_2/hr/ind.

Analyses of chemical composition of both body and liver were made. On the basis of the results it is supposed that the main energetic material for silver eel are lipids, and its whole metabolism is adapted to an economical energy expenditure due to which silver eel can migrate long distances to its spawning grounds, using fats stored in its organism.

6. STRESZCZENIE

Prowadzono badania nad metabolizmem węgorza schodzącego. Badano spadek ciężaru ciała po 54 dniach doświadczenia, który wynosił 3,7% w stosunku do początkowego ciężaru ryb. Badane ryby zużywały około 1,9 ml O_2/godz./ind.

Przeprowadzono również analizę składu chemicznego ciała i wątroby. Na podstawie otrzymanych wyników autorzy wysuwają wnioski, że głównym materiałem energetycznym dla węgorza schodzącego są lipidy. Cały zaś metabolizm jest dostosowany do oszczędnej gospodarki energetycznej, dzięki czemu węgorz może bez odżywiania pokonać długą drogę na tarlisko korzystając z nagromadzonego tłuszczu.

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CHROMOSOMES OF EUROPEAN EEL (*ANGUILLA ANGUILLA*) AS RELATED TO IN VIVO SEX DETERMINATION

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ABSTRACT

The problem of sex determination in the eel was studied with the use of karyological methods. In this species sex differentiation is difficult on account of the identical morphology of females and males. In the present investigation the karyotype of the eel was established. The diploid number of chromosomes of the eel is $2n=38$, including 36 autosomes and 2 heterochromosomes. The results permitted development of karyological method for in vivo sex identification in the eel, this being of importance for experimental studies of the culture of this fish under natural conditions.

1. INTRODUCTION

The subject of study involves the karyology of the eel and the problem of sex differentiation at the early and prematural stages of ontogenesis in this species. The present study deals with a new method for in vivo sex determination.

No extensive investigations on the karyology of the eel have as yet been performed. According to the available literature, diploidal number $2n=38$ has so far been found for this species (Makino 1951, Sick et al. 1962, Chiarelli et al. 1968). The latter paper mentions also a heteromorphic pair of chromosomes which the authors consider to be heterochromosomes.

As early as in 1874, in adult eels flowing from lakes and rivers down to the sea the so-called "Syrski's organ" has been detected, to be subsequently identified as immature testes by Siebold and Freud, with the use of histological methods. Attempts have been made to bring about further development of the gonads by injection of various hormone preparations (Boetius et al. 1962, Boetius 1967), and the induction of the complete male sexual cycle was thus attained. However, all the above mentioned methods rule out further experimentation with the fishes treated, as they have to be sacrified.

Possibly earliest in vivo sex determination in the eel is of great importance on the genetic and physiological mechanisms underlying differentiation in the size of fishes flowing down to the sea, as well as for studies of their energy balance carried out at the Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Polish Academy of Sciences (Jędryczkowski, Fischer 1973).

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The aims of the present investigation comprised:

1. Determination of the karyotype characteristics of both sexes of the eel. The results of this part of the study were the starting point and control for its second part dealing with:

2. Development of a method for in vivo sex determination.

2. MATERIAL AND METHODS

Eels imported for culture purposes were brought by plane from France. They were cultured in aquariums. The fishes studied were 5–30 cm long.

THE KARYOTYPE OF THE EEL

The procedure used in this series of experiments for obtaining karyological preparations involved injection of about 50 µl of a 0.025% solution of colchicine into the dorsal muscles of eels about 2 hr prior to sectioning (McPhail, Jones 1966). After the fish had been sacrified, gill epithelium was removed and cut with scissors; fragments of this tissue were kept in distilled water for 3–4 hr to reduce cell turgor and facilitate preparation of so-called "squashes". The material was then fixed with a 50% acetic acid solution during 20 min, whereupon "squashes" were prepared by placing the fixed tissue between a glass slide coated with adhesive and a cover glass, and subsequently compressing the glasses at a pressure up to 15 kg/cm² in a press with flat steel jaws. After the cover glass had been removed by dipping in 70% ethanol, the preparation was stained with alkaline fuchsine in phenol solution and differentiated in 96% ethanol. Finally, the preparations were mounted in Canada balsam (Matthey 1952, Jordan 1959).

The well spread metaphases were photographed and karyotypes were established by arranging the different elements according to structure and size.

IN VIVO SEX DETERMINATION

The second series of experiments was designed to obtain in vivo mitotic divisions, in order to identify the heterochromosomes characteristic of this species.

In eels placed on a wet surface, epithelium from the region of the tail was scrapped off with a sharp scalpel, whereupon the culture of the treated fishes in aquariums was continued. After 3–4 days the regenerating epithelium was once more scrapped off from the same place. Thus obtained tissue fragments were treated for 2 hr with a 0.4% solution of colchicine on a watch glass, and then were transferred to a hypotonic 0.9% solution of sodium citrate for 30 min, whereupon preparations were obtained according to the described procedure.

3. RESULTS

On the grounds of the data obtained in both series of experiments the karyotype of the eel was established. The karyotypes of both sexes of this species obtained in the first series of experiments (after colchicine injection) are recorded in Fig. 1. No picture of the karyotype determined by the in vivo method is presented, since there are no important differences in the structure and shape of chromosomes obtained by both methods.

The diploid number for the eel is $2n=38$, including 36 autosomes and 2 heterochromosomes. Autosomes exhibit marked differentiation in size and structure. They comprise 10 pairs of metacentric or submetacentric chromosomes and 8 pairs of distinctly acrocentric chromosomes.

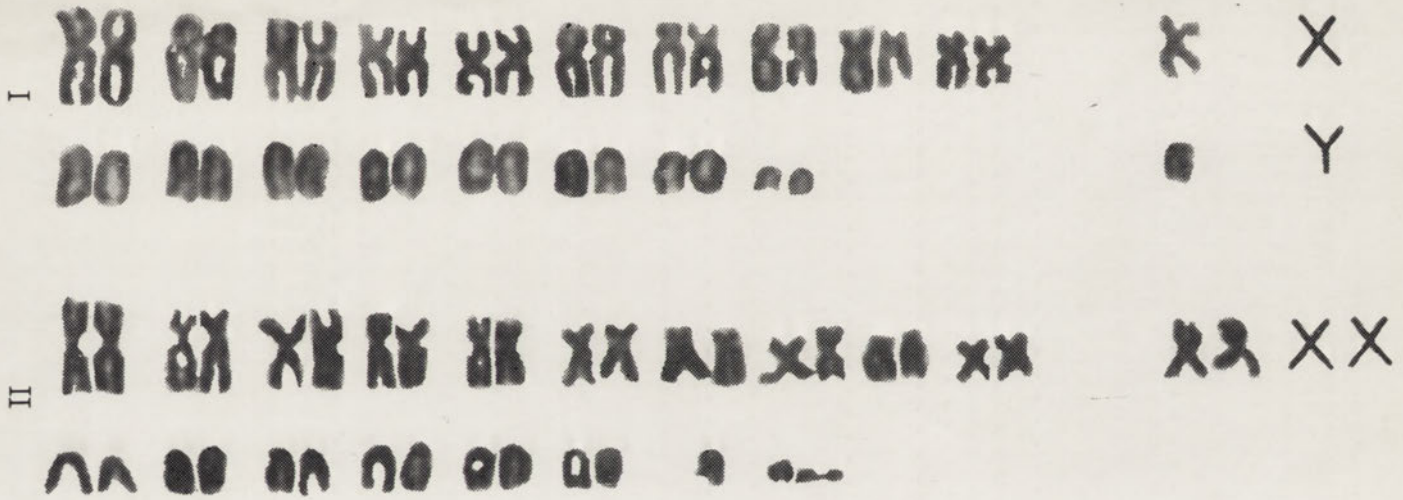
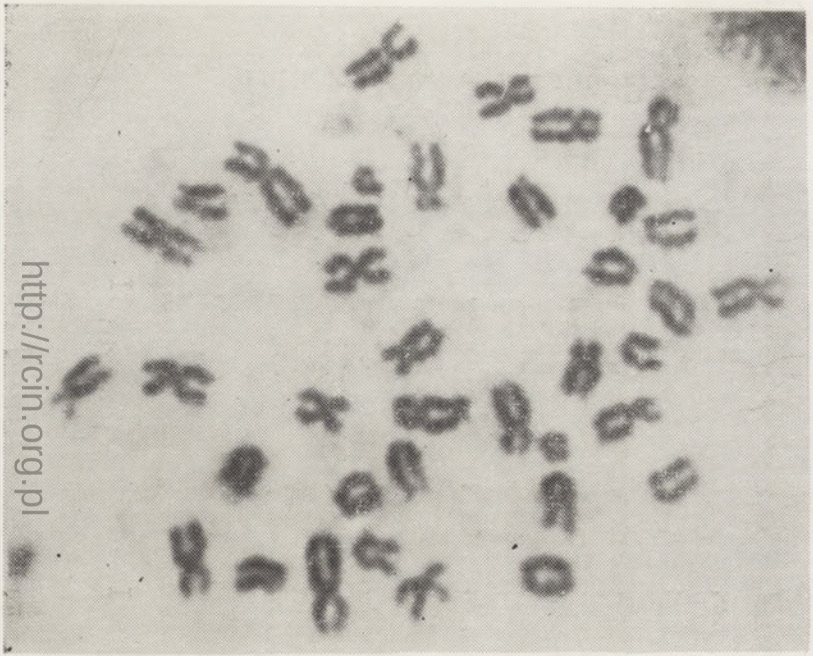


Fig. 1. Kariotypes of eels. Heterochromosomes X and Y can be seen on the right side of the plate



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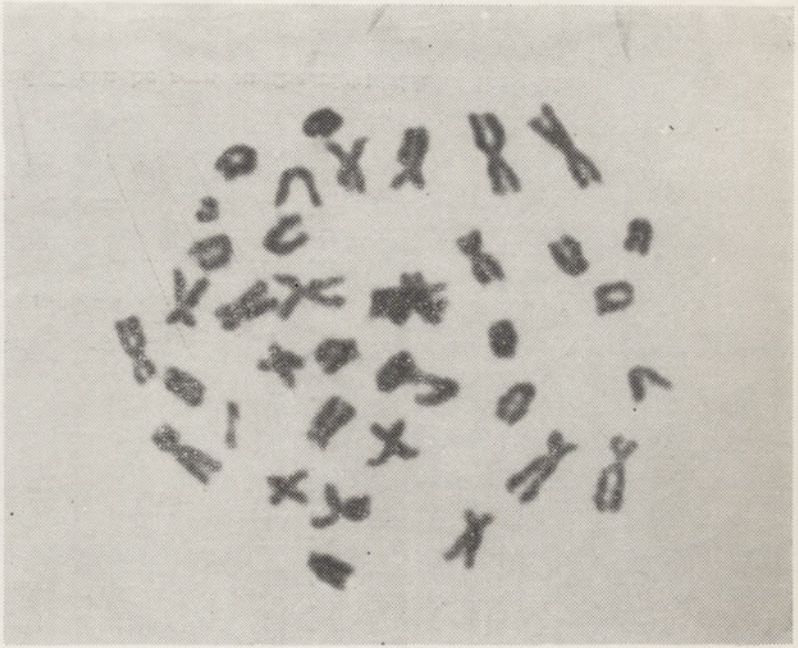

II

Fig. 2. Mitotic plates of eels

Heterochromosomes show a characteristic structure. Chromosome X (Fig. 1 and 2) is metacentric, and its size is similar to that of the 8th pair of autosomes. Heterochromosome Y (Fig. 2) is markedly smaller than Y; its structure is submetacentric, and the size is intermediate between that of the 7th and 8th pair of acrocentric autosomes.

The present results establish the karyotype of the eel; moreover, they permit the development of a method for in vivo sex identification in this species. The latter fact has already been mentioned to be of consequence with respect to investigations on the differences in size, nutrition, respiration and metabolic energy balance between both sexes of the eel. Subsequent studies in this series will be designed to identify the different autosomes and the heterochromosome pair by methods used in karyosystematics.

Since the present paper was finished new data on chromosomes of *Anguilla rostrata* have been published (Ohno et al. 1973). In view of the fact that the sex of the specimens studied here was net established, the next paper will be devoted to a further explanation of the sex determination problem.

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