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PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF THE BALTIC SEA. PART III. PRIMARY PRODUCTION IN THE SOUTHERN PART OF THE BALTIC

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ABSTRACT

The results of the primary production measurements carried out in the southern part of the Baltic Sea in 1970–1971 are presented in this paper. The highest primary production per day (1068 mg C/m² · day) was observed in the open sea in July 1970, while its mean value in the summer months was about 400 mg C/m² · · day. The calculated annual primary production of the Gdańsk Deep in 1970 and 1971 amounted to 117.5 g C/m² and 72.9 g C/m², respectively. It has been estimated that the annual primary production in the Bornholm Deep area and in the Arkona Sea is lower as compared with that of the Gdańsk Deep.

1. INTRODUCTION

The Steemann-Nielsen (1951, 1952) method of the estimation of primary production has brought about an growing interest in the studies on the productivity of water ecosystems. This method proved to be very useful especially as the sea is concerned, where the primary production is rather small. Due to its sensitivity it is of inestimable service there (Sorokin 1957, 1962).

Twenty years of research in this field has brought scores of scientific descriptions and critical analyses that resulted in a consistent picture of the World Ocean productivity, though it still needs some complementing (Yentsch 1963, Ryther 1962). As shown in numerous studies (Steemann-Nielsen, Aabye-Jensen 1957, Ryther 1962) the greater part of the ocean and also of some lakes (Talling 1965) has productivities relatively stable in time and they can be described by means of semi-empirical mathematical formulae (Steele 1965, Vollenweider 1965). On the contrary, the Baltic Sea environment exhibits strong variability in respect of salinity, temperature, etc. (Głowińska 1963, Fonselius 1969) and also in respect of some specific conditions such as e.g. stratification (Nehring et al. 1969, Piechura 1970). Variability of the Baltic environment is caused, in the first place, by influxes of salt water from the North Sea that take place with changing intensities as depending on meteorological conditions, the volume of the run-off waters into the Baltic basin, and, at last but not least, on the distance of the area under consideration from the entrance to the Baltic proper (Wyrtki 1954). In that situation one can expect the different regions of the Baltic to differ from one another in respect of productivity. Anyhow, this is evident from numerous papers dealing with the distribution of the zooplankton (Mańkowski 1959) or phytoplankton (Renk 1971, 1972, 1973, Ringer 1971), and also from studies on the primary production

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that were carried out in the southern Baltic (Rochon 1966, 1968), though these were not frequent enough. As it is, the only way to come to a conclusion about the global annual primary production one had to infer indirectly from the measurements conducted as well in the Transition Area (Steemann-Nielsen 1965), as in the central part of the Baltic (Fonselius 1971), in the Gulf of Finland (Bagge, Lehmusluoto 1971, Bagge, Niemi 1971), and in the Gulf of Bothnia (Fonselius 1971).

The results obtained from measurements of the annual primary production in the above mentioned regions of the Baltic have shown very great differences ranging from 30 to 200 g C/m². Therefore, to get some more information about that matter research was carried on during 1970 and 1971 in the southern Baltic and the results are presented in this paper. These results allow us to get better acquainted with the "nutritive chain" in the sea and also to draw conclusions as to the fisheries productivity, and at the same time they may be used for the evaluation of the eutrophication rate taking place in the Baltic Sea.

2. MATERIAL AND METHODS

The material for this study consists in measurement and observations made during research cruises on board the R. V. "Birkut" to the southern Baltic in 1970 and 1971. Measurements were made at stations the positions of which are given in the map (Fig. 1). Routine stations are marked with dots (many years'

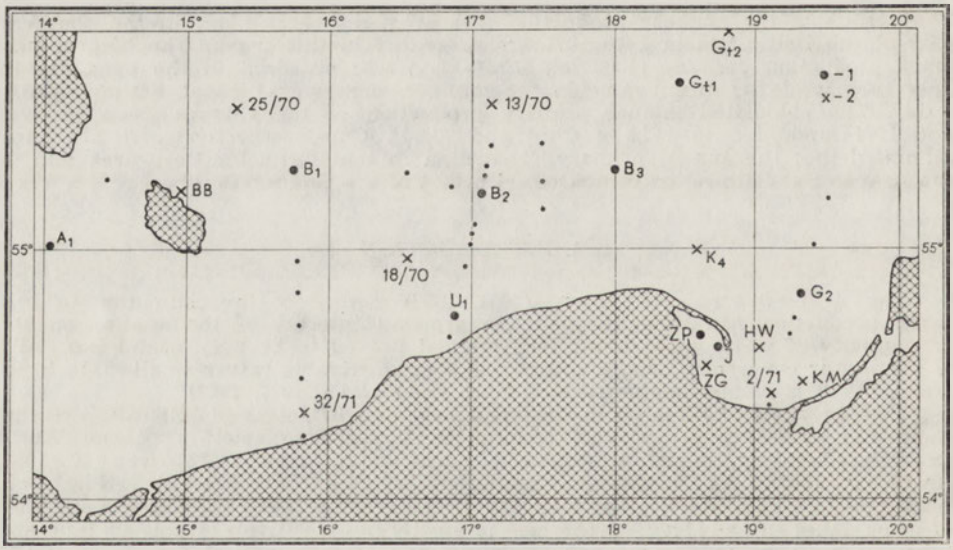

Fig. 1. Positions of stations where the measurements of primary production were carried out. 1 — routine stations, 2 — temporary stations (cf. Table I)

studies have been carried out there), whereas crosses denote temporary stations where measurements of the primary production were made during one year only, or sometimes just once. Position of each station and depths at which the measurements were taken are shown in Table I.

The measurements of primary production were taken using the radioisotope method (Steemann-Nielsen 1952, 1962) at 0.5, 5, 10, 15 and 20 m depth and, now and then, also additionally at 7.5 and 30 m depth. For the two upper depths two or three light bottles, and for the lower levels — one or two bottles were used. Incubation of bottles containing phytoplankton was conducted in situ during a half of a sunny day, each time from dawn to noon. For the computation of primary production the coefficient 1.06 was used as correction for the isotopic effect (Doty, Oguri 1959, Thomas 1964, Vollenweider 1969). Inor-

ganic carbon dissolved in water was determined using the Anderson and Robinson method (1946) described in detail also by the Strickland, Parsons (1968), but after its adjustation to the Baltic hydrological conditions (Torbicki, Renk 1972). Measurements of the insolation intensity at the sea surface

Table I. Characteristics of the stations investigated in 1970–1971

Station	Position	Region	Depth(m)	Notes
A_1	14°01′E 55°02′N	Arkona Deep	50	Routine stations
B_1	15°45′E 55°20′N	Bornholm Deep	98	
B_2	17°00′E 55°13′N	Słupsk Furrow	91	
U_1	16°50′E 54°46′N	Ustka district	34	
B_3	18°00′E 55°20′N	Słupsk Furrow (eastern part)	78	
G_2	19°20′E 54°50′N	Gdańsk Deep	116	
Gt_1	18°28′E 55°40′N	Gotland Deep (southern part)	98	
J	18°45′E 54°35′N	Hel district	55	
ZP	18°37′E 54°37′N	Puck Bay	28	
ZG	18°39′E 54°29′N	Gdańsk Bay	15	Temporary stations
13/70	17°10′E 55°35′N	Middle Shelf	30	
18/70	16°35′E 54°53′N	Słupsk Shelf	20	
25/70	15°19′E 55°36′N	Bornholm district	74	
HW	19°09′E 54°36′N	Hel district (eastern part)	80	
Gt_2	18°46′E 56°00′N	Gotland Deep	116	
KM	19°20′E 54°25′N	Krynica Morska district	40	
BB	14°54′E 55°16′N	Bornholm district (eastern part)	37	
2/71	19°08′E 54°23′N	Gdańsk Bay	30	
K_4	18°35′E 55°00′N	Gdańsk Deep	81	
34/71	15°48′E 54°23′N	Kołobrzeg district		

during the incubation time were made with a solarimeter on the top of a special mast rising from the deck of the vessel. All the details of the measurement method were described in other papers (Renk et al. 1972, Renk, Torbicki 1972, Renk 1972, 1973).

3. RESULTS

The measurement results showed that the photosynthetic processes in the Baltic are most intense in the top water layer down to 10 m depth. The dependence of the primary production and chlorophyll concentrations upon the depth at stations G_2, B_1, A_1, Gt_1, B_2 and B_3 in 1970 is shown in Fig. 2 and 3. For the year 1971 at stations G_2, K_4, Gt_1 it is shown in Fig. 4. At stations B_3, B_2, B_1 and A_1 the dependence upon the depth is presented in Fig. 5, and at the coastal stations 2, U, J and ZP — in Fig. 6.

As can be seen, in the Gdańsk Deep the highest primary production in the spring seasons of 1970 and 1971 was observed just at the sea surface. It was decreasing with increasing depth (cf. Fig. 2, station G_2, May 30, 1970 and Fig. 4, station G_2, March 28 and April 7, 1971). A similar dependence of primary production upon the depth during springtime was found in the Gulf of Gdańsk (cf. Fig. 6, station 2, April 8 and

June 1, 1971). On the other hand, the areas of the Bornholm Deep and the Słupsk Furrow showed in the spring the highest primary production, as a rule, at 5—10 m depth (cf. Fig. 2, station B_1, May 27, 1970 and June 10, 1971, Fig. 3, station B_3, April 2, 1971, station B_2, April 1 and June 1, 1971, station B_1, May 28, 1971). In summer and autumn the dependence of the intensity of photosynthesis upon the depth shows but insignificant differences between different areas ranging from the Born-

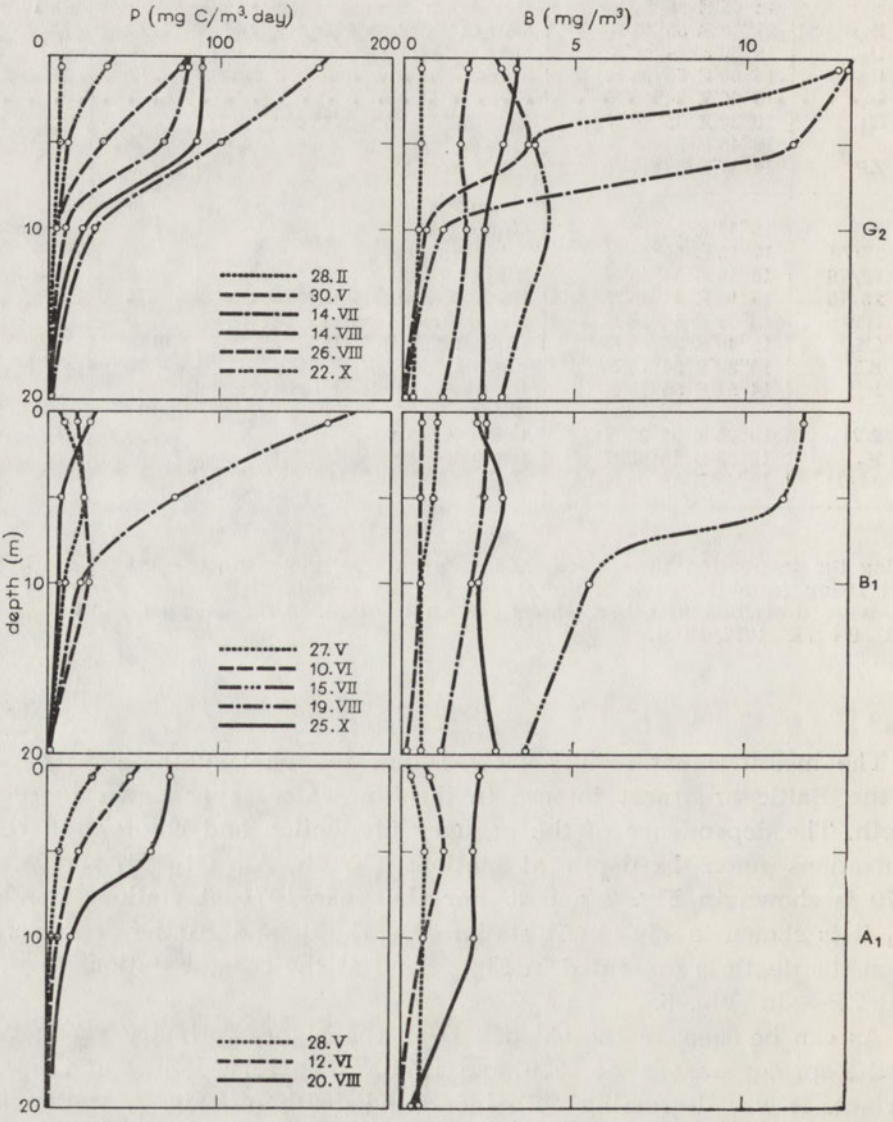

Fig. 2. Dependence of the primary production (P) and chlorophyll-a concentration (B) on the depth at stations G_2, B_1 and A_1 in 1970

holm Deep to the Gdańsk Deep. It may be said that in those two seasons the primary production within the top water layer down to 5 m depth was most frequently nearly homogenous, whereas at depths grea-

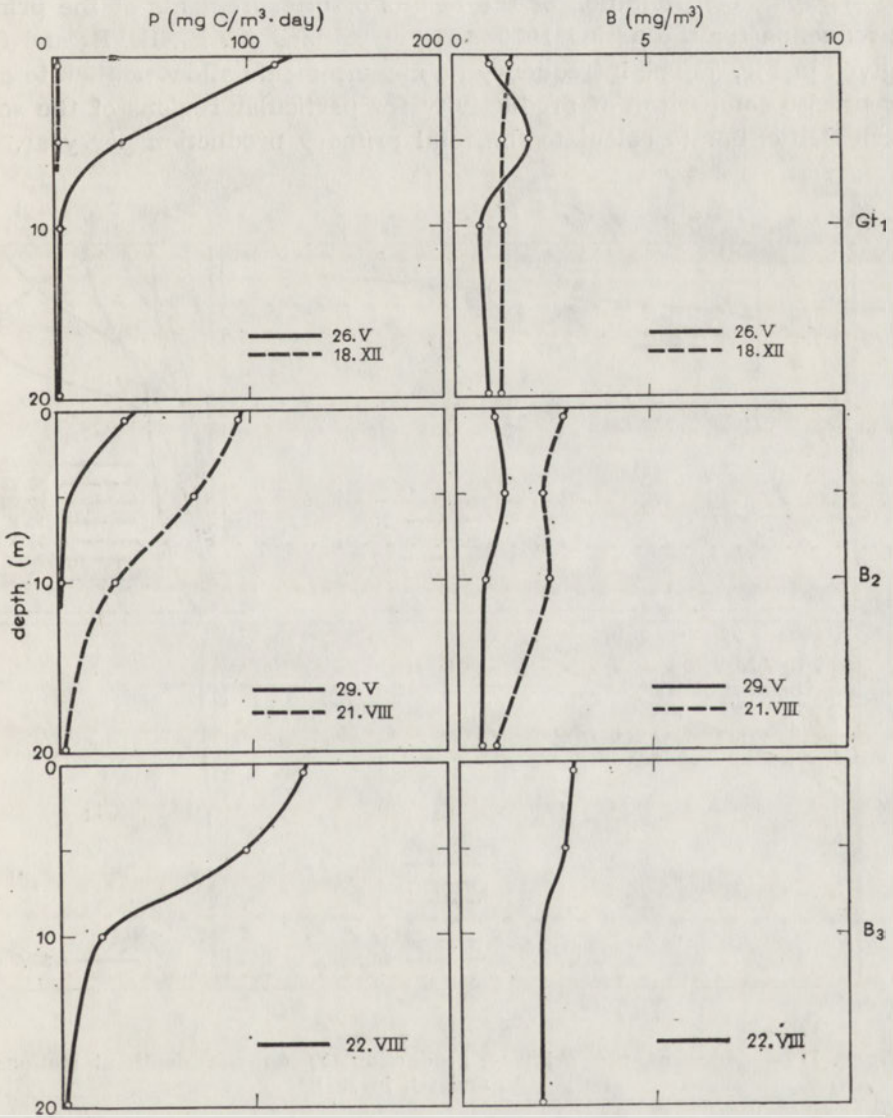

Fig 3. Dependence of the primary production (P) and chlorophyll-a concentration (B) on the depth at stations Gt_1, B_2 and B_3 in 1970

ter than 5 m it was decreasing gradually. In winter months a monotonic decrease in primary production was observed with increasing depth.

The total daily primary production under 1 m² of the sea surface was obtained by graphic integration of the curves expressing the dependence of the primary production upon the depth throughout the whole thickness of the euphotic layer.

Graphic confrontation of the results of measurements of the primary production for the period 1970–1971 at stations A_1, B_1, B_2, B_3 and G_2 is given in Fig. 7. The infrequency of measurements allow neither to make a precise comparison of productivity for particular regions of the southern Baltic, nor to calculate the total primary production per year.

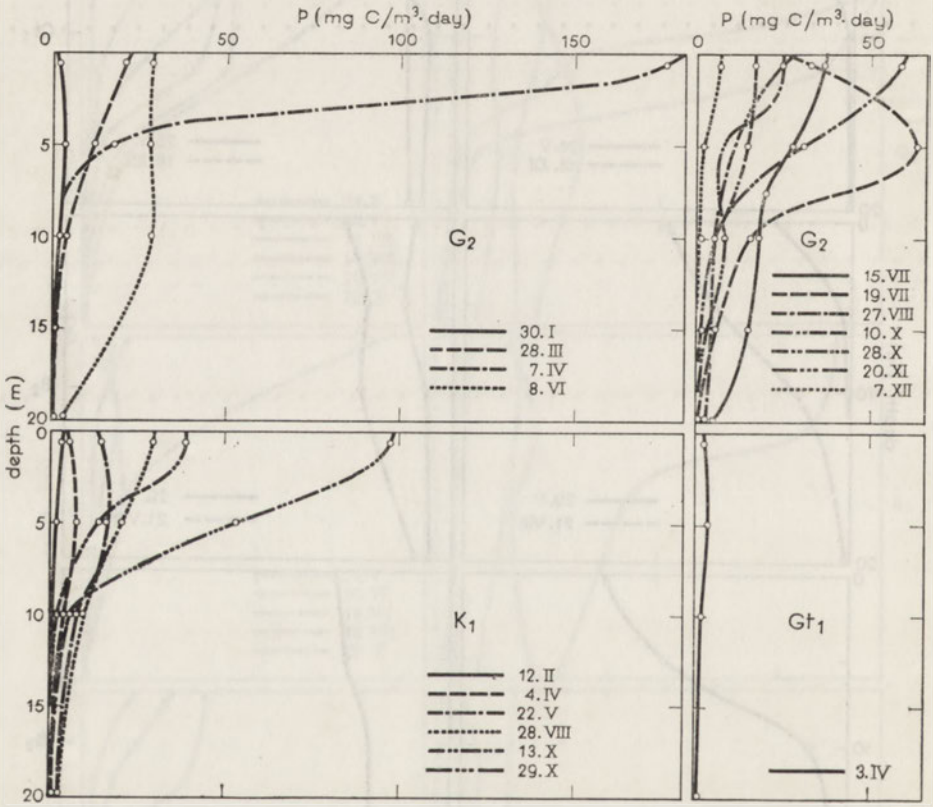

Fig. 4. Dependence of the primary production (P) on the depth at stations G_2, K_4 and Gt_1 in 1971

Nevertheless, Fig. 7 makes it possible to draw two conclusions:

1. The primary production in the annual cycle was probably greater in 1970 than in 1971;

2. In the spring season the primary production under 1 m² of the sea surface in the Gdańsk Deep was greater than in other areas of the southern Baltic.

The latter conclusion finds its strong support in the chronological list of the measurement results from the period 1970—1971, shown in Table II. Thus, for instance, in the period June 3 to 8, 1971 (Table II)

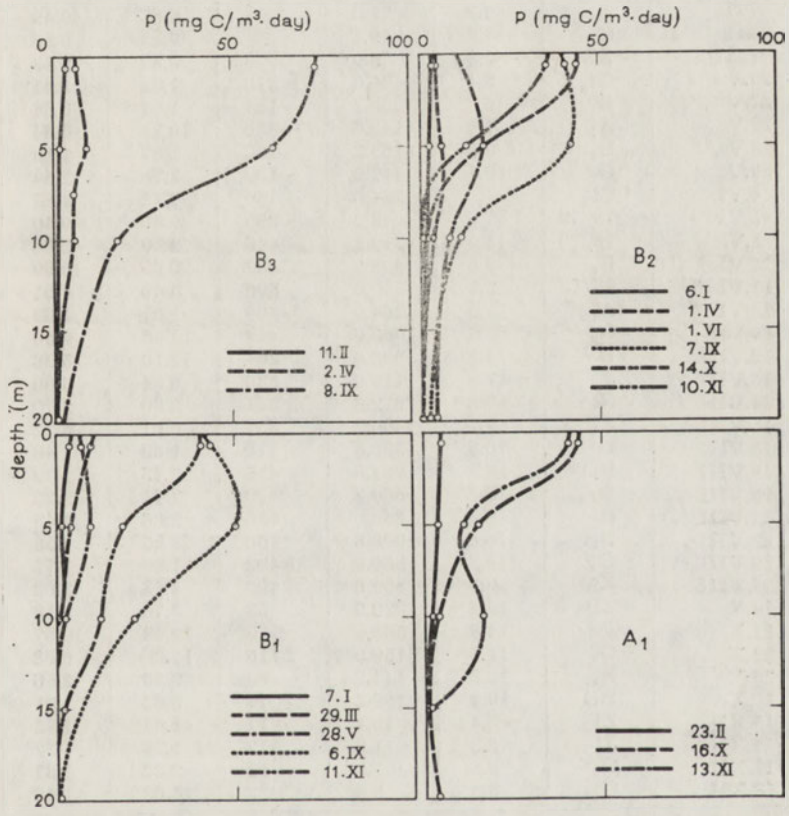

Fig. 5. Dependence of the primary production (P) on the depth at stations B_3, B_2, B_1 and A_1 in 1971

the insolation was approximately the same all over the southern Baltic, whereas the primary production in the Gdańsk Deep was at the same time three times as great as in the Słupsk Furrow. On the other hand, in August 1970 (Table II) by the almost uniform insolation throughout the whole area from the Gdańsk Deep in the East to the Bornholm Deep in the West the highest primary production was found in the West. Moreover, the results from Table II clearly show that in the period from March 28 to April 7, 1971 the temperature of the surface water in the Gdańsk Deep increased from 1.84 to 3.53°C and simultaneously the primary production increased from 122.5 to 500 mg C/m² · · day. At the same time a tenfold increase in the chlorophyll content

Table II. The results of measurements in 1970 and 1971. P — primary production at 0.5 m depth, B_{10} — mean chlorophyll concentration of

	Date	Station	Temp. (°C)	P (mg C/m² · · day)	E (cal/cm² · · day)	B_0 (mg/m³)	B_{10} (mg/m³)	P/E
1970	27.II	J	—0.3	72.0		0.67	0.49	
	28.II	G_2	—0.2	59.0		0.55	0.53	
	1.III	ZG	—0.3	76.0		0.39	0.43	
	26.V	Gt_1	5.4	486.0	~310	1.02	1.41	~1.57
	27.V	B_1	6.0	223.2	~134	1.04	0.94	~1.61
	28.V	A_1	7.6	142.6	~320	0.31	0.64	~0.45
	29.V	B_2	6.3	153.2	~100	1.07	1.10	~1.50
	30.V	G_2	10.7	412.0	~180	2.75	2.34	~2.29
	4.VI	Ct_2		264.0	~160	7.12	4.87	~1.65
	5.VI	13/79		49.0	~390	0.60	0.40	~0.13
	9.VI	18/70	11.0	319.2	446	2.30	2.08	0.71
	10.VI	B_1	9.5	308.0	482	0.68	0.69	0.64
	11.VI	25/70	9.2	352.0	596	5.46	4.51	0.59
	12.VI	A_1	11.0	264.0	404	1.01	1.03	0.65
	14.VII	G_2		1068.0	414	13.55	9.50	2.58
	12.VIII	HW	18.3	998.0	264	12.10	5.28	3.77
	13.VIII	J	~17	1418.0	580	6.34	6.00	2.49
	14.VIII	G_2	16.8	812.6	584	2.90	2.70	1.39
	15.VIII	ZP	19.1	1400.0	357	6.51	6.12	3.95
	18.VIII	U	16.8	398.6	170	1.80	2.48	2.35
	19.VIII	B_1	16.5	964.8	456	2.43	2.39	2.11
	20.VIII	A_1	16.2	569.2	426	2.41	2.22	1.33
	21.VIII	B_2	16.3	860	456	2.86	2.45	1.88
	22.VIII	B_3	16.8	908.8	460	2.85	2.55	1.89
	26.VIII	G_2	16.3	588.6	492	1.89	1.72	1.19
	27.VIII	KM	19.3	580.6	490	3.13	3.08	1.18
	19.X	ZP	12.4	328.0	53	8.70	9.18	6.07
	21.X	J	11.8	508,8	70	10.43	10.57	7.27
	22.X	G_2	11.6	150.0	116	13.20	6.08	1.29
	25.X	B_1	10.8	141.2	80	2.20	2.56	1.77
	26.X	BB	10.5	185.2	79	2.62	2.91	2.34
	15.XII	ZP	~5.4	10.1	11	1.34	1.45	0.91
	16.XII	J	5.5	11.8	12	1.72	1.32	1
	17.XII	G_2	5.8	2.8	13	2.22	1.87	0.23
	18.XII	Gt_1	5.3	5.4	22	1.63	1.43	0.25
1971	6.I	B_2	3.50	33.9	56	0.52	0.53	0.62
	7.I	B_1	3.21	17.4	50	0.79	0.71	0.35
	8.I	U	2.85	27	20	0.72	0.75	1.35
	9.I	G_2	3.57	23.5	36	0.63	0.62	0.65
	26.I	B_1	3.08	19.5	68	0.52	0.58	0.29
	28.I	A_1	2.69	11.5	12	1.10	0.82	0.96
	29.I	B_3	3.00	14.5	38	0.46	0.44	0.38
	30.I	G_2	2.84	20.5	58	0.87	0.88	0.35
	5.II	2/71	2.62	47.0	143	0.79	0.86	0.33
	6.II	G_2	3.07	28.0	75	0.61	0.60	0.37
	10.II	Gt_1	2.87	14.7	47	0.38	0.38	0.31
	11.II	B_3	2.51	21.1	67	0.70	0.73	0.32
	12.II	K_4	2.65	18.5	67	0.44	0.28	0.28
	17.II	U	2.37	52	55	1.24	1.26	0.96

of the surface water (at 0.5 m depth) was observed and this implied a phytoplankton spring bloom to have occurred. In spite of that, at a relatively weak insolation on April 7 (117 cal/cm²), a rather high primary

under 1 m² of the sea surface, E — daily insolation, B_o — chlorophyll concentration the water layer from the sea surface down to 10 m depth

	Date	Station	Temp. (°C)	P (mg C/m² · · day)	E (cal/cm² · · day)	B_0 (mg/m³)	B_{10} (mg/m³)	P/E
1971	18.II	B_2	2.46	15.5	33	0.70	0.63	0.47
	21.II	B_1	2.63	23.6	49	0.58	0.58	0.48
	23.II	A_1	2.57	54.7	103	0.98	0.91	0.53
	2.III	U	1.54	29.0	125	1.91	2.31	0.23
	28.III	G_2	1.84	122.5	187	1.86	2.02	0.66
	29.III	B_1	1.53	43.2	121	1.30	1.31	0.36
	30.III	A_1	1.60	30.7	75	1.98	1.85	0.41
	1.IV	B_2	1.75	66.7	395	1.49	1.49	0.17
	2.IV	B_3	2.00	87.2	315	0.85	0.93	0.28
	3.IV	Gt_1	1.92	36.3	307	0.63	0.56	0.12
	4.IV	K_4	2.20	74.5	390	0.79	0.82	0.19
	7.IV	G_2	3.57	506.0	117	20.5	18.0	4.33
	8.IV	2/71	3.20	200	87	3.58	1.9	2.30
	22.V	K_4		177.5	240	3.01	2.69	0.74
	21.V	G_2	11.41	134	357	2.13	2.04	0.38
	27.V	A_1	8.21	112	325	2.83	3.09	0.35
	28.V	B_1	6.06	71	595	2.76	2.60	0.12
	1.VI	2/71	18.63	393	700	6.42	3.46	0.56
	3.VI	B_3	11.2	142.5	640	1.34	1.45	0.22
	4.VI	B_2	11.55	212	624	0.50	1.28	0.34
	8.VI	G_2	14.05	425	640	1.37	1.71	0.66
	15.VII	G_2	15.62	351	357	1.39	1.34	0.98
	16.VII	ZP	16.52	302	401	3.33	3.20	0.75
	18.VII	J	16.52	446		4.13	3.96	
	19.VII	G_2	13.75	485.8	471	2.22	2.29	1.03
	27.VIII	G_2	17.47	167.2	160	1.72	1.67	1.04
	28.VIII	K_4	17.36	231.2	227	1.49	1.63	1.01
	1.IX	J	16.26	971	467	3.54	3.51	2.07
	2.IX	2/71	16.55	622	305	5.78	4.79	2.04
	6.IX	B_1	15.25	508	473	1.40	1.36	1.18
	7.IX	B_2	15.66	412.5	450	1.87	1.83	0.92
	8.IX	B_3	15.43	639.5	398	2.12	2.19	1.60
	10.X	G_2	12.56	149	156	3.40	3.43	0.95
	11.X	ZP	12.72	380	159	3.43	3.51	2.49
	12.X	J	12.76	340	98	5.08	4.59	3.46
	13.X	K_4	12.43	181	118	2.96	3.25	1.53
	14.X	B_2	12.20	220	148	2.62	2.54	1.49
	16.X	A_1	12.22	240	94	2.01	1.99	2.56
	28.X	G_2	10.86	326.7	141	3.76	4.40	2.31
	29.X	K_4	10.98	560.0	189	4.87	4.88	2.96
	3.XI	ZP	10.42	319.0	130	5.00	4.78	2.44
	10.XI	B_2	9.40	175.0	92	2.88	2.97	1.90
	11.XI	B_1	9.43	272	95	2.09	2.23	2.86
	13.XI	A_1	9.67	255	70	2.60	2.66	3.63
	15.XI	32/71	9.31	32	50	3.57	3.13	0.64
	20.XI	G_2	8.41	143.1	—	5.12	5.29	—
	7.XII	G_2	7.38	24	29.5	2.19	2.31	0.81
	15.XII	J	5.00	27.7	22.5	1.10	1.1	1.23
	16.XII	J		26.0	31.0	1.10	1.2	0.84

production was noted. Hence, it is evident that the spring increase in water temperature plays an important role in initiating the spring bloom of phytoplankton.

Table III. Energy efficiency in primary production

Date of invstigations	G_2	K_4	B_2	B_2	B_1	BB	A_1
26–30.I.1971	0.35		0.38		0.29		0.96
6–12.II.1971	0.33	0.28	0.32				
18–23.II.1971				0.47	0.48		0.53
28.III–7.IV.1971	0.66–4.33	0.19	0.28	0.17	0.36		0.41
21–28.V.1971	0.38	0.74			0.12		0.35
3–8.VI.1971	0.66		0.22	0.34			
10–12.VI.1970	~2				0.64	0.59	0.65
15–19.VII.1971	0.98–1.03						
14–26.VIII.1970	1.39–1.19		1.89	1.88	2.11		1.33
27.VIII–8.IX.1971	1.04	1.01	1.60	0.92	1.18		
10–16.X.1971	0.95	1.53		1.49			1.56
28–29.X.1971	2.31	2.96					
22–26.X.1970	1.29				1.77	2.34	
10–20.XI.1971				1.90	2.86		3.63
7.XII.1971	0.84						
17.XII.1971	0.23						

BB — Bornholm area.

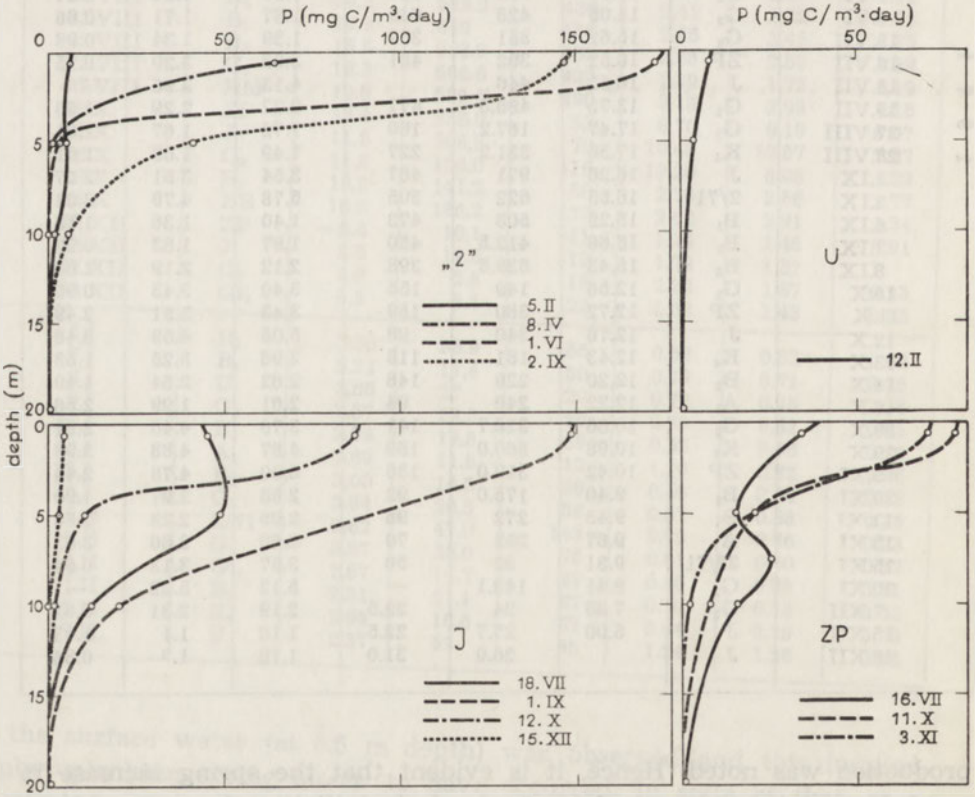

Fig. 6. Dependence of the primary production (P) on the depth at stations "2", U, J and ZP in 1971

Table III showing the coefficients of the energy efficiency in primary production in various seasons of 1970—1971 makes possible to trace thoroughly the differences in the productivity of particular regions of the

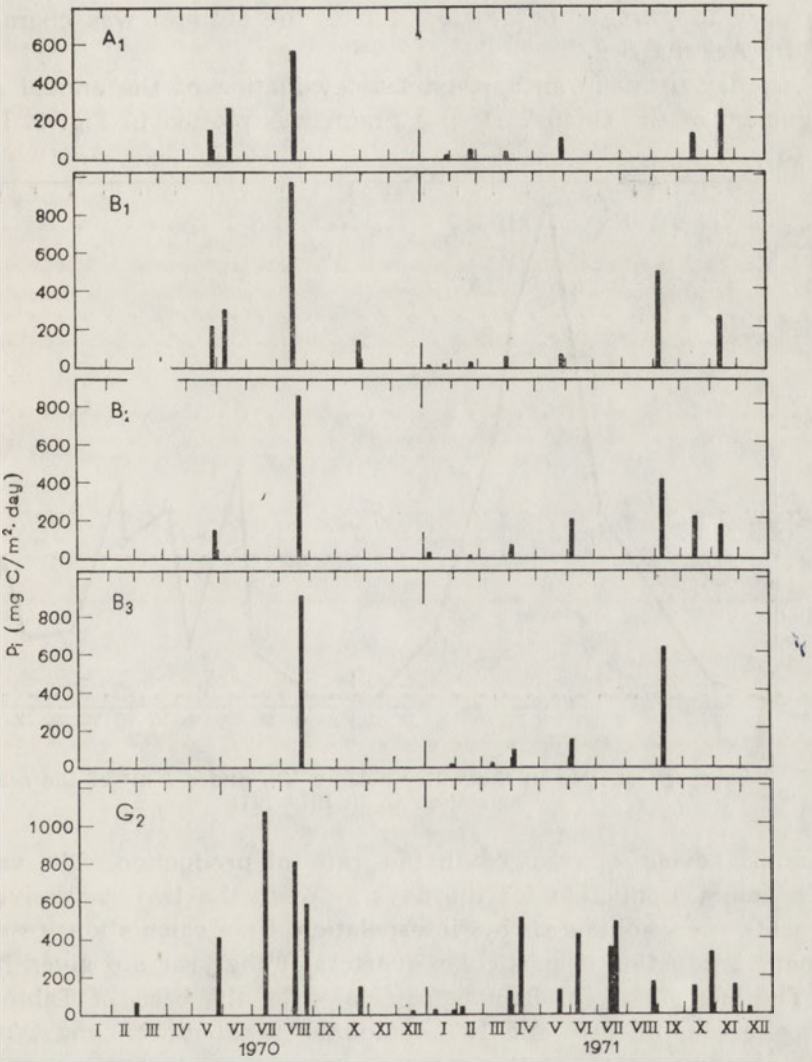

Fig. 7. Primary production (P_i) in the water column under 1 m² of the sea surface at stations A_1, B_1, B_2, B_3 and G_2 in 1970–1971

southern Baltic. During the winter seasons the rate of primary production of the Baltic is low. There are, then, no significant differences in the productivity of particular regions, the higher energy efficiency in primary production being merely noted in the area of the Arkona Sea. In the spring months the highest photosynthetic productivity was observed in the Gdańsk Deep, the lowest one — in the area of the Słupsk

Furrow and of the Bornholm Deep. In the summer, it was higher in the latter areas as compared with the Gdańsk Deep. The high energy efficiency in primary production in the Bornholm Deep continued to be so through the autumn months. Yet, the highest level of the energy efficiency in primary production during the autumn was characteristic for the Arkona Sea.

In order to make an approximate evaluation of the annual primary production of the Gdańsk Deep a graph was plotted in Fig. 8. It shows

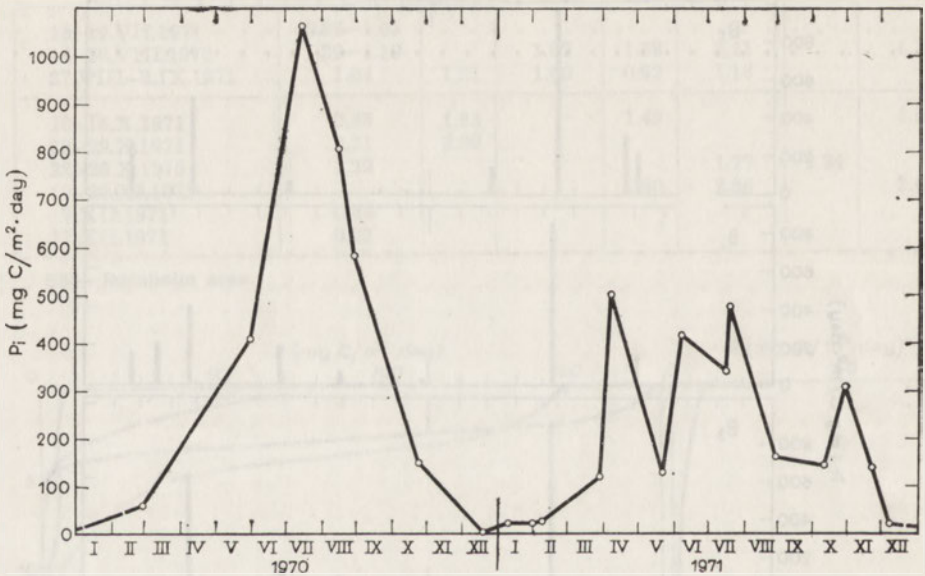

Fig. 8. Primary production in the water column (P_i) under 1 m^2 of the sea surface at station G_2 in 1970–1971

an annual cycle of changes in the rate of production. The values of the primary production for the days between the two successive measurements were obtained by interpolation. The calculation results for primary production in particular quarters of the year are given in Table IV. The mean daily production calculated on the basis of Table IV for spring seasons of 1970 and 1971 amounts to about 350 mg C/m^2 · day, In the summer of 1970 the mean daily primary production was about 760 mg C/m^2 · day.

Table IV. Primary production of the Gdańsk Deep calculated for every quarter of the years 1970 and 1971

Year	Quarter				Annual production (g C/m^2 · year)
	I	II	III	IV	
1970	5.8	37.2	67.1	7.4	117.5
1971	5.0	30.4	24.4	13.1	72.9

4. DISCUSSION

To demonstrate further differences in the primary production in the waters of different parts of the southern Baltic, the mean values of primary production for the period 1966—1971 have been calculated for the consecutive months of the year. The average monthly primary production was calculated as an arithmetic average from all the measurements taken in the corresponding months of the years 1966—1971. The measurement results obtained by Rochon (1966, 1968) at Sea Fisheries Institute, Gdynia, were turned to account in our calculations. Changes in the mean monthly primary production during an annual cycle are

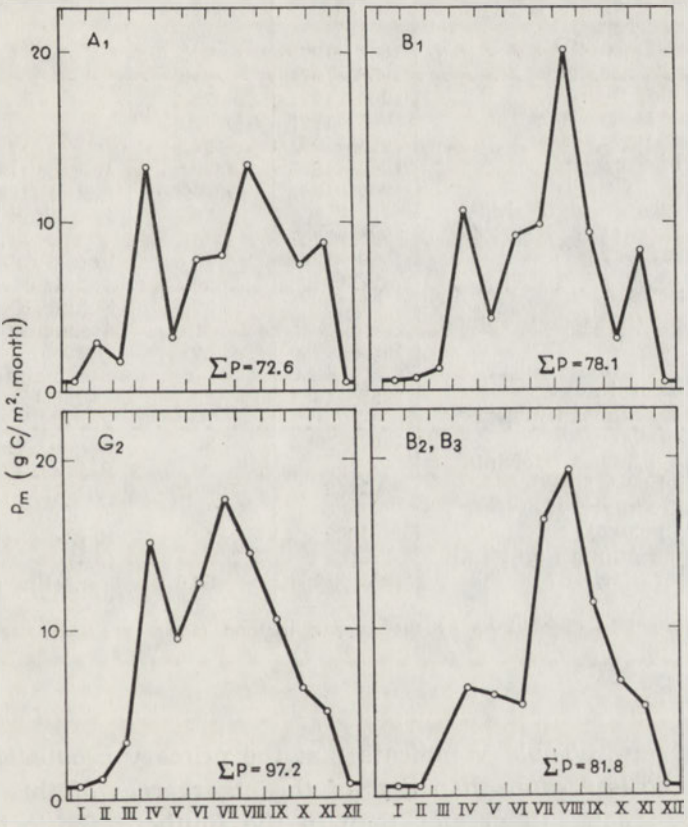

Fig. 9. Mean monthly primary production in the water column under 1 m² of the sea surface in the period of 1966–1971 at the following stations: A_1 — Arkona Deep, B_1 — Bornholm Deep, G_2 — Gdańsk Deep, B_2, B_3 — Słupsk Furrow

presented in Fig. 9, and the compilations of the calculated mean values of the annual primary production for particular stations are to be found in Table V, items 3—7.

The results of calculations presented in Table V, in spite of their approximate character, reveal differences between primary production of various regions. The annual primary production of the Gdańsk Deep is by 25% higher than that of the Arkona Sea. Other items in Table V allow a comparison between the primary production of the southern Baltic waters and of those in other areas of this sea.

Table V. Annual primary production of the Baltic Sea

No.	Region	Period (year)	P ($gC/m^2 \cdot year$)	Author
1	Gdańsk Deep G_2	1970	117.5	
2	Gdańsk Deep G_2	1971	72.9	
3	Gdańsk Deep G_2	1967—1971	97.2	
4	Słupsk Furrow	1966—1971	81.8	
5	Bornholm Deep	1966 - 1971	78.1	
6	Arkona Deep	1966—1971	72.6	
7	Gdańsk Bay	1965—1971	104.1	
8	Firth of Szczecin	1964—1966	380*	Wiktor (1971)
9	Rügen	1960—1962	90—900	Hübel (1966)
10	Baltic proper (light ship Havringe)	1963—1967	78	Fonselius (1971)
11	Baltic proper	1969—1970	30	Sen Gupta (1972)
12	Gotland Deep	1969	59.4	Schulz, Keiser (1971)
13	Gotland Deep	1970	37.9	Schulz, Keiser (1971)
14	Kattegat (light ship Anhold Nord)	1954—1960	67	Steemann-Nielsen (1965)
15	Great Belt (light ship Halsskov Rev)	1953—1957	59	Steemann-Nielsen (1965)
16	Gulf of Finland (Loviisa Archipelago)	1967—1968	30	Bagge, Niemi (1971)
17	Gulf of Finland (Helsinki Region)	1968	150—200	Bagge, Lehmusluoto (1971)
18	Gulf of Finland (Loviisa Archipelago)	1969	40	Bagge, Niemi (1971)
19	Gulf of Bothnia (light ship Finngrundet)	1961—1968	56.6	Fonselius (1971)

* Primary production determined by the oxygen method (after Wiktor 1971 — 1.02 kg O_2/m^2).

Results given in Table V indicate that the primary production of the southern Baltic is greater than that of the other areas of this sea. The primary production still greater than in the southern Baltic is known only for the eutrophic regions of the Gulf of Finland (Bagge, Lehmusluoto 1971, Bagge, Niemi 1971), in the basins of Rügen Insland (Hübel 1966), and in the Firth of Szczecin (Wiktor 1971).

The fact that the phytoplankton production of the southern Baltic is greater than in its central part finds its confirmation also in measurements made by Nehring, Francke (1971 a, b) and substantiated by the measurements of chlorophyll concentration from 1971

showing a much higher chlorophyll content of waters of the southern Baltic than that of the central areas of this sea (Renk 1973).

Assuming the values of primary production determined at different stations to be representative for the whole area represented by these stations we tried to determine the approximate value of the annual primary production in particular regions of the Baltic Sea. The latitude of 56°N has been accepted as the boundary of the southern Baltic, the boundaries of other sea regions being denoted after Segestrale (1957). The results of calculations of the approximate total annual primary production for each particular region of the Baltic are given in Table VI.

Table VI. Approximate annual production of phytoplankton in the Baltic Sea

Region	Annual primary production per 1 m^2 ($gC/m^2 \cdot$ · year)	Area (km^2)	Total annual production (milion ton)		
			Organic carbon	Oxygen	Net dry mass of phyto-plankton
Southern Baltic					
Arkona Sea	72.6	18,000	1.35	3.6	4.0
Bornholm Basin	78.1	32,900	2.56	6.8	7.6
Słupsk Furrow – Gdańsk Deep	89.5 *	28,200	2.52	6.7	7.4
Southern part of Gdańsk Bay	104.1	4,900	0.51	1.4	1.5
Southern Baltic		84,800	6.94	18.5	20.5
Baltic proper	50	135,000	6.75	18.0	20.0
Gulf of Finland	40	31,200	1.25	3.3	3.7
Gulf of Bothnia	57	118,000	6.74	18.0	20.0
Gulf of Riga		16,200	0.91	2.4	2.7
Baltic Sea			22.59	61.7	66.9

* Mean value for stations B_3 and G_2 taken from Table V.

In Table VI there are also shown the data on the annual primary production of the areas where no measurements were performed as well as the annual primary production for the whole area of the Baltic. In the latter case the following mean annual values of primary production per 1 m^2 of the sea surface were adopted: for the central part of the Baltic — 50 g $C/m^2 \cdot$ year (Table V), for the Gulf of Finland — 40 g $C/m^2 \cdot$ year and for the Gulf of Bothnia — 57 g $C/m^2 \cdot$ year (Table V). The corresponding data for the Gulf of Riga are not available. Yet, taking into account the fact that the area of that Gulf makes up only 4% of the total Baltic area, its share in the total primary production is rather insignificant. Thus, the error (deviation from the virtual value) in the evaluation of the total annual primary production of the Gulf of Riga will quite insignificantly affect the accuracy of calculations con-

cerning the total production of the Baltic. Therefore in the rough estimation of the total annual primary production of the Baltic Sea the author has accepted that the primary production under 1 m² of the sea surface in the Gulf of Riga is the same as in the neighbouring Gotland Deep. As results from those calculation the annual primary production for the whole southern Baltic amounts to about 6.9 million ton of organic carbon and for the whole of the Baltic area it amounts to 22.6 million ton.

Oxygen essential for life to exist is generated mainly in the processes of photosynthesis. Table VI shows also the approximate quantities of oxygen produced by photosynthesis in the euphotic layer of particular Baltic regions. In the area of the southern Baltic the oxygen production amounts to nearly 18.5 million ton per year, and in the total area of the Baltic — 61.7 million ton per year.

The last column of Table VI contains net values of the primary production per year in particular regions of the sea expressed by the dry phytoplankton mass. In our calculations we have accepted the equivalent of 1 g of carbon corresponding to approximately 3.3 g of dry phytoplankton mass (Winberg 1960, Hagmeier 1961). Before the values in the last column were calculated, the value of primary production expressed in form of carbon had been reduced by 10%, i.e., by the assumed losses because of phytoplankton respiration (Steeman-Nielsen, Aabye Jensen 1957, Ryther 1956). The value of the total annual primary production of the southern Baltic amounts to approximately 20.5 million ton of organic dry mass, whereas that of the whole Baltic Sea is evaluated to be about 66.9 million ton.

Acknowledgements

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5. SUMMARY

Measurements of primary production carried out with the radioisotope method in the southern Baltic Sea in the period 1970–1971 indicate the highest rate of photosynthesis to occur in the top water layer down to about 10 m depth. In an annual cycle the primary production of the water column under 1 m² of the sea surface is lowest in winter months when it amounts to about 10 mg C/m² · day. The mean primary production in the southern part of the Baltic Sea during summer months is about 400 mg C/m² · day, whereas the greatest production of the open waters of the Baltic was found in July 1970 to be of 1068 mg C/m² · day. The annual primary production at station G_2 which is representative of the Gdańsk Deep area was estimated for 1970 and 1971 as amounting to 117.5 and 72.9 g C/m², respectively.

The results of the measurements indicate that in spring the intensity of photosynthesis is higher in the Gdańsk Deep than in the Bornholm Deep and Arkona Sea, while in summer the primary production of the Bornholm Deep is higher than that of the Gdańsk Deep. After our calculations the annual primary production is the highest in the Gdańsk Deep and decreases gradually along the Słupsk Furrow and Bornholm Deep towards the Arkona Sea (Fig. 9).

The total annual primary production of the southern Baltic limited towards the North by the latitute of 56°N was estimated to be as high as 6.9 million ton of carbon or — if expressed as the net production of dry phytoplankton mass — 20.5 million ton.

6. STRESZCZENIE

Pomiary produkcji pierwotnej wykonane metodą radioizotopową w latach 1970–1971 na obszarze południowego Bałtyku wskazują, że największe tempo fotosyntezy zachodzi w powierzchniowej warstwie wody do około 10 m. W cyklu rocznym produkcja pierwotna w słupie wody pod powierzchnią 1 m² w miesiącach zimowych jest najmniejsza i wynosi około 10 mg C/m² · d. Przeciętna produkcja pierwotna na obszarze południowego Bałtyku w miesiącach letnich wynosi około 400 mg C/m² · d, natomiast największą produkcję wód otwartego Bałtyku zanotowano w lipcu 1970 r., wynosiła ona 1068 mg C/m² · d. Roczną produkcję pierwotną dla stacji położonej na Głębi Gdańskiej skalkulowano na 117,5 g C/m² w roku 1970 oraz 72,9 g C/m² w roku 1971. Przeprowadzone pomiary wykazują, że w okresie wiosennym intensywność fotosyntezy na Głębi Gdańskiej jest większa aniżeli w Basenie Bornholmskim i Arkońskim, natomiast w okresie lata produkcja pierwotna Basenu Bornholmskiego przewyższa produkcję pierwotną Głębi Gdańskiej. Z przeprowadzonych kalkulacji wynika, że roczna produkcja pierwotna Głębi Gdańskiej jest największa i stopniowo maleje przesuwając się przez Rynnę Słupską, Głębię Bornholmską do Głębi Arkońskiej (Fig. 9). Całkowitą roczną produkcję pierwotną południowego Bałtyku (do 56°N) skalkulowano na 6,9 milionów ton węgla, lub produkcję netto suchej masy fitoplanktonu 12,4 milionów ton.

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VERTICAL DISTRIBUTION OF ALGAE COMMUNITIES IN MALJOVICA STREAM (RILA — BULGARIA)

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ABSTRACT

The qualitative composition of algae in the stream Maljovica and in the Černi Iskâr and Iskâr was examined and the number of algae was estimated. Basing upon the dominating species three zones were distinguished corresponding with analogous ones in the streams of the High Tatra.

1. INTRODUCTION

The algae populating the high mountain streams form differentiated communities along their courses. Algological investigations carried during several years in the streams of the Polish part of the High Tatra (Kawecka 1971) indicated to the fact that algae communities develop in zones. In zone I (about 1560–1780 m above sea level) communities of crusty blue-green algae dominated. in zone II (about 890–1550 m above sea level) *Hydrurus foetidus, Homoetrix janthina* and diatoms, in zone III (540–890 m above sea level) diatoms were the dominating species. The present observations on the distribution of algae communities in the streams of the mountains Rila were carried with the aim of performing comparisons.

2. TERRAIN DESCRIPTION, MATERIAL AND METHODS

Rila mountains form the highest range on the Balkans Peninsula (Musała peak, 2925 m above sea level). They are part of the Rodope massif which was lifted during the Alpine orogenesis. The highest parts of those mountains aequited the Alpine form with many lakes as a result of glacial action. Rila mountains are built from gneiss and granite while calcium is hardly present. The forest zone area reaches the level of 1900–2000 m above sea level, and dwarf mountain pine area reaches the level 2600 m a.s.l. (Horvat et al. 1937). The longest river which the whole flows on the Bulgaria territory is the river Iskâr (375 km long) which joins the Danube. It carries the water of the streams: Černi, Levi and Bieli Iskâr, which take the origin in the Rila mountains. Numerous streams of the south slopes of the mountains form the Černi, Iskâr. One of those streams which drains lakes (Maljoviski and Elenski ezera) is called Maljovica.

The algological material was collected in August 1969, from: Maljovica stream (outflow from the Lake Elenski ezero) and from Černi Iskâr and Iskâr up to Samokov. Materials were collected at the eight stations, at height of about 2600–1000 m above sea level (Fig. 1).

From each station at least 10 or more samples were collected from the diversity habitats as stones, slime, moss. Samples were preserved on the spot with a 4% solution of formalin. A part of the material for the investigation of

diatoms was macerated at laboratory during 24–48 hours with a mixture of the sulfuric acid and saturated solution of the potassium bichromate in the ratio 3 : 1. Then it was washed with distilled water on a contrifuge. The diatom frustules without inner content were preserved in distillated water. The slides were embedded in synthetic resin "Pleurax".

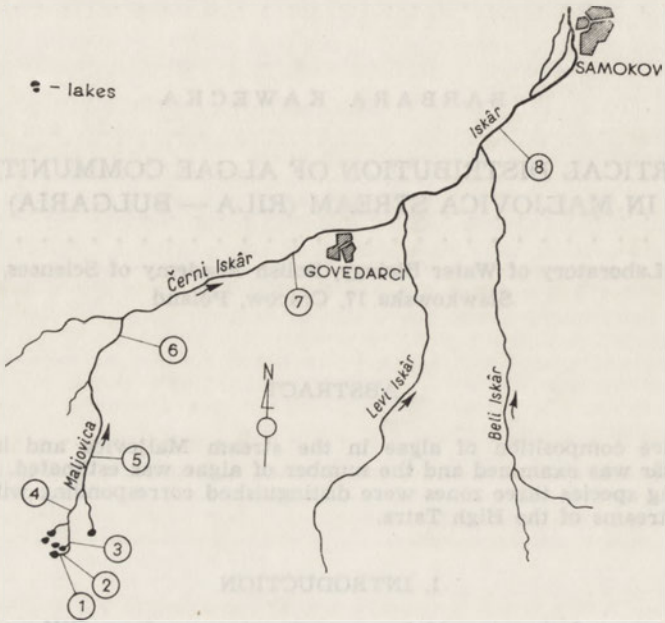


Fig. 1. Localization of sampling stations

The quantitative relations were evaluated by the estimation method. On a section of the stream of about 16 m² regarded as typical of the locality, the size of the area occupied by macroscopic concentration of algae such as: *Hydrurus foetidus, Homoeothrix janthina, Lemanea fluviatilies*, thali of Bacillariophyceae was estimated according to the scale of covering:

1. species occurring sporingly,
2. covering less than 25% of the area,
3. covering 25–50% of the area,
4. covering 50–75% of the area,
5. covering 75–100% of the area.

The quantitative analysis of microscopic algae was carried out according to the Starmach's method (Starmach 1962, Kawecka 1964, Bucka 1966, Wasylik 1971).

After the identification of algae the quantity of species was estimated according to the 6 grade scale of amount:

+. very rare, 1–6 specimens in 3 examined slides,
1. single, 1–6 specimens in one slide, about 10 individuals in 3 slides,
2. few, 7–16 specimens in one slide, about 50 individuals in 3 slides,
3. moderately, 1–3 specimens in almost all microscopic fields, about 100–150 individuals in 3 slides,
4. many, 4–5 individuals in almost all microscopic fields, about 250 specimens in 3 slides,
5. very many, the organism predominates, and it appears in a number greater than 5 individuals in each microscopic field, in sum total more than 250 specimens in 3 slides.

Further calculations were applied only for the group of diatoms.

To estimate the coverage degree of the particular diatom species at first the size estimation of specimens was introducted according to the scale presented in Table I.

Table I. Scale of size

Scale value	Average length of organism (μ)	Calculation coefficient
5	350	16
4	250	11
3	150	7
2	70	3
1	22	1
+	2	0.1

Comment: The size is estimated on the base of the part of field of view in a microscope, which is occupied by the perpendicular projection of the given specimen and it is conventionally accepted to express this surface area by an average length of cell. Because successive symbols in the size scale are not proportional to the average length of the organisms, therefore the calculation coefficient was applied here. This gives the correct proportions between all the degree values in the scale, from the lowest up to the highest one.

The obtained calculation coefficient for every species was then multiplied by the quantity of species determined according to the accepted scale to give so called the coverage index. This number accentuates the importance of a given species in a community, and it more or less corresponds to a number characterizing the quantity of species in a sociology of higher plants.

In a synthetic approach the coefficient of coverage for every species occurring in the all samples collected on the station was calculated according to the formula $P=s \cdot 100/n$, where s designates the sum of the coverage indices of species, n a number of the samples taken for examination.

3. RESULTS

OBSERVATIONS ON SOME SPECIES

Table II gives the list of 178 species and varieties found in the examinated area. It gives as well the characteristic of each stand and their approximate altitude.

Cyanophanon mirabile Geitl. (Fig. 2A). Cells 62.5–75×1.25 μ; the epiphitic form on the filaments of *Calotrix gypsophila* (Kütz.) Thuret. The Cyanophanales order is formed maybe temporary because it is not clear whether those organisms belong to Cyanophyta or Bacteria (Starmach 1966).

Caloneis silicula (Ehr.) Cl. *var. alpina* Cl. (Fig. 2 B). Cells 23.7–40× ×6.25–7.5 μ, 18 striae in 10 μ. It appeared only on the highest part of Maljovica stream. Siemińska (1964) raports that this variety is a typical Alpine form.

Cymbella cistula (Hemp.) Grun. (Fig. 2 C). Cells 37.5–10×11–13.75 μ, 9–10 striae in 10 μ, the 2 to 5 middle striaes of the ventral side have separate points at their ends. The breadth of these specimens in less than that reported by Hustedt (1930), Proshkina-Lavrenko (1949/50), Siemińska (1964) of 15–36 μ. The observed breadth corresponds to the one reported by Cleve-Euler (1953) of 8–21 μ, Van Eygen (1959) of 14–15 μ, Schoeman (1970) of 8–21 μ. The

Table II. List of species with their mean numbers evaluated according to the amount scale and covering scale of species from all samples from the station. * denotes algae species forming macroscopic concentrations — "flock"

Station characteristics	Maljovica stream						Cerni Iskâr	Iskâr
	1	2	3	4	5	6	7	8
	Outflow from the Lake Elenski ezero	Slope	Terrace	Above the Torist House	Above the Maljovica Tourist Complex	Lower part of the valley	Above the Govederci village	Above the Samokov town
Approx. altitude (m)	2600	2500	2400	2100	1750	1300	1150	1000
Water temp. (°C)	8.5	8	6	7.5	10	8.5	19.5	21.5
pH	6.2	6.2	6.2	6.2	6.2	6.2	7.2	7
Insolation	very good				good	poor	very good	
Plant zone	mauntain-pine				forest		meadow, field	
Species	Station							
	1	2	3	4	5	6	7	8
Chamaesiphon fuscus (Rostaf.) Hansgirg					+	+		
C. incrustans Grunov	2			2	3			
C. polonicus (Rostaf.) Hansgirg	2*	1*	1*	2*	1*	1*	1*	+
Cyanophanon mirabile Geitl.	2							
Scopulonema polonicum (Racib.) Geitl.	2*	1*						
Lyngbya kützingii (Kütz.) Schmidle	1			1				
Phormidium corium (Agardh) Gomont	1							
P. favosum (Bory) Gomont	1*			1*	1*	1*	2*	1*
Nostoc edaphicum Kondratieva	1							
Calothrix gypsophila (Kütz.) Thuret	3*							
Homoeothrix janthina (Bornet et Flahault) Starmach	2*	1*	1	1	3*	3*	+	1
Tolypothrix distorta (Fl. Dan.) Kütz.				1*	1			
Hydrurus foetidus Kirchn.	+	4*	+	3*	2*	2*		
Melosira distans (Ehr.) Kütz. var. *alpigena* Grun.		+	1					
M. distans var. pfaffiana (Reinsch) Grun.			2					
M. italica (Ehr.) Kütz. var. *valida* (Grun.) Hust.	1	1						
M. roseana Rabh		+						
Tabellaria flocculosa (Roth.) Küztz.	1	+	2	+	+	+	+	+
Meridion circulare Ag.	+	+	+	+	+	+	+	+

Table II continued

Species	Station							
	1	2	3	4	5	6	7	8
M. circulare var. constricta (Ralfs) V. H.	+	+	+	+			+	+
Diatoma hiemale (Lyngb.) Heib.	1	4	3	2	2	2	+	+
D. hiemale var. mesodon (Ehr.) Grun.								
D. vulgare Bory			+					
Fragilaria capucina Desm.	1	2	2	1	1	2	2	2
F. construens (Ehr.) Grun.	+		+					
F. pinnata Ehr.	1	+	2	+	+	+	+	
Ceratoneis arcus (Ehr.) Kütz.	2	4	2	2	3	3	4	4
C. arcus var. amphioxys (Rabh.) Grun.		+	+	+				
Synedra rumpens Kütz.	+		+	+	+	+	1	+
S. ulna (Nitzsch) Ehr.					+	+	2	1
S. vaucheriae Kütz.								+
Eunotia arcus Ehr.			+					
E. diodon Ehr.	+		1					
E. diodon forma?	+	+						
E. exigua (Bréb.) Rabh.	+		+					
E. fallax Cl.			+					
E. monodon Ehr. *var. maior* (W. Sm.) Hust.	+		+					
E. lunaris (Ehr.) Grun. *var capitata* Grun.			+					
E. parallela Ehr.		+						
E. pectinalis (Dillw.? Kütz.) Rabh.		+	+	+	+			
E. pectinalis (Dillw.? Kütz.) Rabh. *var. minor* (Kütz.) Rabh.	+	+	1	+	+	+	+	+
E. praerupta Ehr.	+	+	1	+	+	+		
E. robusta Ralfs				+				
E. robusta var. tetraodon (Ehr.) Ralfs			1					
E. trinacria Krasske			+					
E. sp.			+	+	+			
Cocconeis diminuta Pant.								+
C. placentula Ehr.								+
C. placentula var. euglypta (Ehr.) Cl.	+	+		+	+	+	2	1
C. placentula var. intermedia (Herib. et Perag.) Cl.					+	+		
C. placentula var. klinoraphis Geit.								+
C. pediculus Ehr.	+						+	
Achnanthes flexella (Kütz.) Brun.	+	+		+	+	+		+
A. lanceolata (Bréb.) Grun.					+	+	1	1
A. lanceolata var. capitata O. Müll.							+	+
A. lanceolata var. ventricosa Hust.							+	+
A. lapidosa Krasske			+					
A. lapponica Hust.			+				1	+
Achnanthes linearis (W. Sm.) Grun.								+
A. microcephala (Kütz.) Grun.	+	+	+	1	1	1	2	+
A. minutissima Kütz.	2	2	2	2	2	1	2	1

Table II continued

Species	Station							
	1	2	3	4	5	6	7	8
Frustulia rhomboides (Ehr.) De Toni	+			+	+			
F. rhomboides var. saxonica (Rabh.) De Toni	1	+	2	+			+	
F. rhomboides f. capitata (Mayer) Hust.				+	+			
F. rhomboides f. undulata Hust.				+				
F. vulgaris (Thw.) De Toni	+		+				+	
Anomoeoneis serians (Bréb.) Cl. *var. brachysira* (Bréb.) Hust.	+	+	+	+	+	+		+
A. cerians var. brachysira f. thermalis (Grun.) Hust.	+	+	+					
Stauroneis anceps Ehr.	+	+	+	+	+			+
S. phoenicentron Ehr.	+	+						
S. smithii Grun.							+	+
Navicula cocconeiformis Greg.								+
N. contenta Grun.	+	+						
N. contenta var. biceps Arn.			+					
N. cryptocephala Kütz.	+		+	+		+	1	1
N. cryptocephala var. intermedia Grun.	+	+		+	+		1	1
N. dicephala (Ehr.) W. Sm.								+
N. exigua (Greg.) O. Müll.	+	+			+		+	1
N. gracilis Ehr.						+		
N. gregaria Donk.							+	+
N. hungarica Grun. *var. capitata* (Ehr.) Cl.	+	+		+				+
N. menisculus Schum.	+							+
N. mutica Kütz.			+					
N. perpusilla Grun.	+	+	+	+	+		+	+
N. pseudoscutiformis Hust.	+	+			+			
N. pupula Kütz	1	+					+	
N. pupula var. rectangularis (Greg.) Grun.			+					
N. radiosa Kütz.	+				+		+	+
N. rhynchocephala Kütz.			+				+	+
N. rotaeana (Rabh.) Grun.	1	1	2	1	+	+	1	+
N. viridula Kütz.							+	
Pinnularia appendiculata (Ag.) Cl.			+					
P. borealis Grun.	+	+	+	+	+	+		+
P. braunii (Grun.) Cl. *var. amphicephala* (Mayer.) Hust.	2	1	+			+		
P. dactylus Ehr.					+			
P. divergens W. Sm.	+							
P. gibba Ehr.	1							
P. hemiptera (Kütz.) Cl.								+
Pinnularia lata (Bréb.) W. Sm.		+						
P. microstauron (Ehr.) Cl.	2	1	1	+	+		+	+
P. subcapitata Greg. *var. hilseana* (Janisch) O. Müll.							+	
P. viridis (Nitzsch.) Ehr.			+		+			
P. viridis var.sudetica (Hilse) Hust.			+	+				+

Table II continued

Species	Station							
	1	2	3	4	5	6	7	8
Neidium affine (Ehr.) Cl.	1	1						
N. affine var. amphirhynchus (Ehr.) Cl.			+					
N. bisulcatum (Lagerst.) Cl.	1		+					
N. dubium (Ehr.) Cl.	+	+	+					
N. iridis (Ehr.) Cl.	+		+	+				
Caloneis silicula (Ehr.) Cl. *var. alpina* Cl.	1	1						
C. silicula var. gibberula (Kütz.) Grun.	+							
Amphora ovalis Kütz.	+	+					+	+
A. ovalis var. pediculus Kütz.			+				+	+
Cymbella aequalis W. Sm.			+					
C. affinis Kütz.					+		1	2
C. cistula (Hem.) Grun.	1	+						
C. gracilis (Rabh.) Cl.			1					
C. helvetica Kütz.						+	+	1
C. hebridica (Greg.) Grun.			+					
C. lanceolata (Ehr.) V. H.	+							
C. naviculiformis Auersw.	2	+	1	+	+		+	+
C. perpusilla Cl.		+	+					
C. sinuata Greg.						+	2	1
C. ventricosa Kütz.	2	2	2	2	1	1	3	2
Gomphonema acuminatum Ehr.		+						
G. acuminatum var. coronatum (Ehr.) W. Sm.								+
G. angustatum (Kütz.) Rabh.	3	2	+	1	+	+	+	+
G. angustatum var. productum Grun.					+		+	+
G. constrictum Ehr.	+							
G. gracile Ehr.	1	+	+	+				
G. intricatum Kütz.					1	+	1	
G. intricatum var. pumilum Grun.				+		+	+	+
G. longiceps Ehr. *var. montanum* (Schum.) Cl.	1	+	+	+	+			
G. olivaceum (Lyngb.) Kütz.							+	+
G. olivaceum var. calcareum Cl.								+
G. parvulum (Kütz.) Grun.	+							
G. parvulum var. micropus (Kütz.) Cl.						+		
Epithemia zebra (Ehr.) Kütz.						+		
Hantzschia amphioxys (Ehr.) Grun.							+	+
Nitzschia acicularis W. Sm.	+	+		+				+
N. dissipata (Kütz.) Grun.							+	1
N. hantzschiana Rabh.			+	+	+	+	+	+
N. linearis W. Sm.				+			+	1
N. palea (Kütz.) W. Sm.							1	+
N. recta Hantzsch.	+							
N. sublinearis Hust.								+
Surirella angustata Kütz.	1	1					+	+
S. linearis W. Sm.	+	+	+					
S. ovata Kütz.	+						+	+
S. spiralis Kütz.								+
Ulothrix zonata Kütz.					+	+	2*	1*

Table II continued

Species	Station							
	1	2	3	4	5	6	7	8
Chlorhormidium rivulare Kütz.			+	1*	+			
Microspora sp.				+				
Draparnaldia plumosa (Vauch.) Agardh.					1*			
Spirogyra sp.				1*	1*	1*	1*	1*
Penium cylindrus (Ehr.) Bréb.			+					
Closterium leibleinii Kütz.			+	+				
Cosmarium caelatum Ralfs	+	+	+	+			+	
C. crenatum Ralfs *f. boldtiana* (Gutw.) W. et G. S. West			+	+	+	+		
C. curtum (Bréb.) Ralfs	+			1				
C. decedens (Reinsch.) Racib. *var. minutum* Krieger et Gerloff				+				
C. einarteilingii Krieger et Gerloff			+					
C. impressulum Elfv.		1	1	1	+	+		
C. margaritaum (Lund) Roy et Biss. *var. margaritatum* forma Růžička			+	+				
C. subspeciosum Nordst. *var. transiens* Messik.	+		+	2			2	
C. subcrenatum Hantzsch.		+						
C. quadratum Ralfs				+				
Staurastrum orbiculare Ralfs. *var. hibernicum* W. West et G. S. West.							+	
S. punctulatum (Bréb.)	1		+	2	+	2	1	+
S. punctulatum var. pygmaeum (Bréb.) W. et G. S. West				2		2	+	+
S. turgescens De Not				+		+		
Euastrum affine Ralfs		+	+					
E. elegans (Bréb.) Kütz.		+	+					
E. montanum W. et G. West.			+					
E. subalpinum Messik. *var. crassum.* Messik.			+					
Tetmemorus laevis (Kütz.) Ralfs	+		1	+				
T. laevis var. intermedius (Woron.) Růžička			1					
Micrasterias truncata (Corda) Bréb.					+			
Lemanea fluviatilis C. Ag.						2*	1*	1*

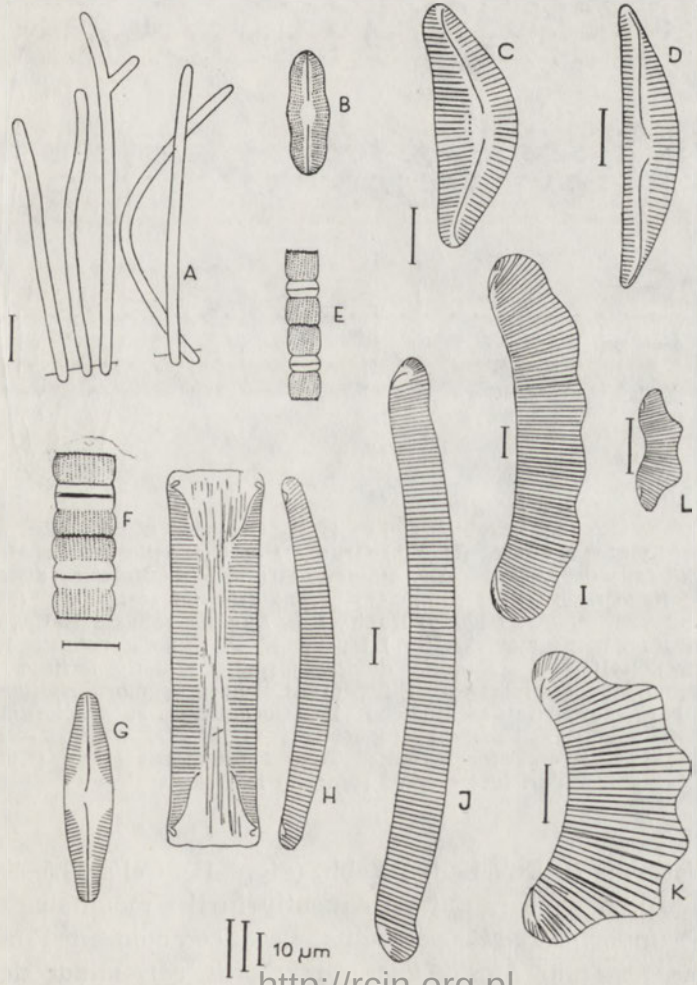
similar species called *Cymbella sp.* was noticed in Wielki Staw in the Valley of Five Polish Lakes in High Tatra Mts. in the lake plankton (Kawecka 1970) and in the bottom sediments (Wasylik 1965). These cells were even more narrow, their breadth was 9.6–11.4 μ.

Cymbella gracilis (Rabh.) Cl. (Fig. 2 D). Cells 27–53.75×4.2–10 μ, 9–11 striae in 10 μ. The cells are even more narrow than those in Alps Mts. streams (Kawecka in prep.; breadth 5.5–10 μ). Their breadth

is far from the one reported as 7—10 μ by Hustedt (1930), Proshkina-Lavrenko 1949/50), Siemińska (1964) and deviated less from breadth of 5—11 μ reported by Cleve-Euler (1955).

Eunotia diodon form? (Fig. 2 L). Cells 18.75×7.5 μ, 14 striae in 10 μ. The similar specimens were observed in High Tatra Mts. (Kawecka 1971; 21.25—22.5×7.5 μ, 15—17 striae in 10 μ), and in Finstertaler stream in the Austrian Alps (Kawecka in prep.; 18.7–28.7×6.6–7.7 μ, 12 striae in 10 μ).

Eunotia monodon Ehr. *var. maior* (W. Sm.) Hust. (Fig. 2 J). Cells 131.25—150×12.5—16.25 μ, 10—11 striae in 10 μ. Some cells of this variety were a little too broad according to the accepted diagnosis of Hustedt (1930, 1932), Proshkina-Lavrenko (1949/50), Cleve-Euler (1953), Siemińska (1964), which report as an upper limit of breadth 15 μ.



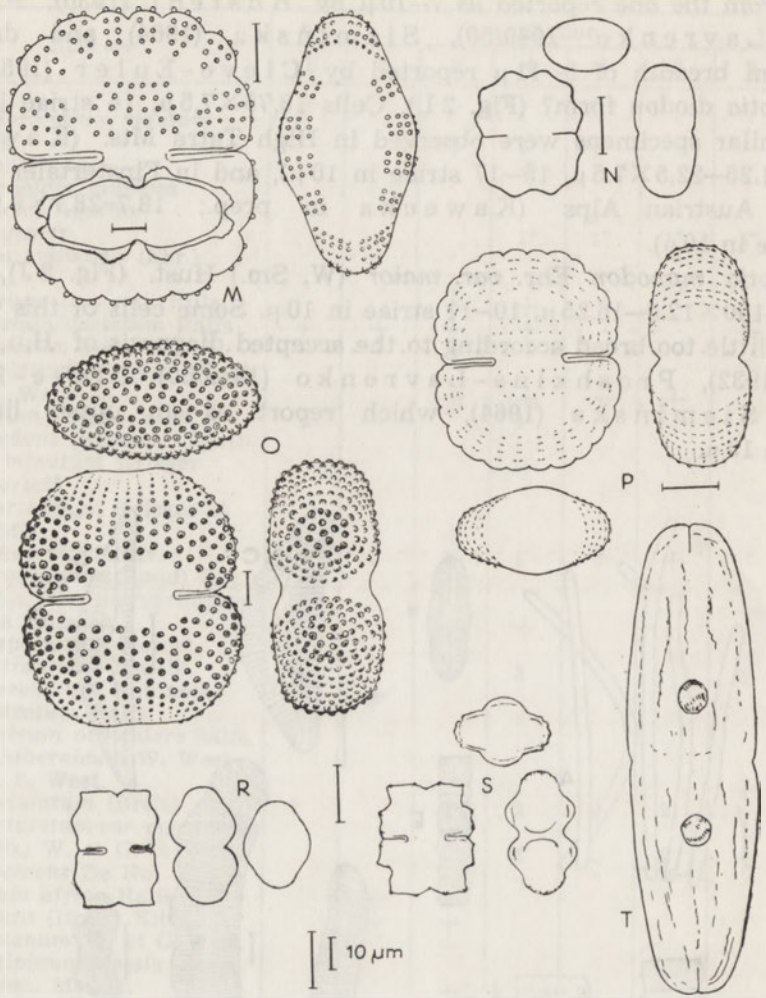


Fig. 2. A — *Cyanophanon mirabile* Geitl., B — *Caloneis silicula* (Ehr.) Cl. *var. alpina* Cl., C — *Cymbella cistula* (Hemp.) Grun., D — *Cymbella gracilis* (Rabh.) Cl., E — *Melosira distans* (Ehr.) Kütz. *var. alpigena* Grun., F — *Melosira distans* (Ehr). Kütz. *var. pfafiana* (Reinsch) Grun., G — *Pinnularia microstauron* (Ehr.) Cl., H — *Eunotia pectinalis* (Dillw? Kütz.) Rabh., I — *Eunotia robusta* Ralfs., J — *Eunotia monodon* Ehr. *var maior* (W. Sm.) Hust., K — *Eunotia robusta* Ralfs *var. tetraodon* (Ehr.) Ralfs, L — *Eunotia diodon form?*, M — *Cosmarium caelatum* Ralfs., N — *Cosmarium impressulum* Elfr., O — *Cosmarium margaritatum* (Lund.) Roy et Biss. *var. margaritatum* Růžička, P — *Cosmarium subspeciosum* Nordst. *var. transiens* Messik., R — *Euastrum montanum* W. et. G. West, S — *Euastrum subalpinum* Messik. *var. crassum* Messik., T — *Tetmemorus laevis* (Kütz.) Ralfs *var. intermedius* (Woron.) Růžička

Eunotia pectinalis (Dillw? Kütz.) Rabh. (Fig. 2 H). Cells 52.5–95.5×5 µ, 10 striae in 10 µ. It appears quite frequently in the mountains together with variety minor. *Eunotia pectinatis* were common in Finstertaler stream in the Austrian Alps. *Eunotia pectinalis var. minor* developed

aboundantly in the stream Rybi Potok (High Tatra Mts.) polluted with organic materials coming from the Tourist House in Morskie Oko Lake.

Eunotia robusta Ralfs. (Fig. 2 I). Cells 91.25×18.75 μ, 8–10 striae in 10 μ. This species belong to north-Alpine algae (Siemińska 1964).

Eunotia robusta Ralfs *var. tetraodon* (Ehr.) Ralfs. (Fig. 2 K). Cells 50–56.25×17.5–25 μ, 7 striae in 10 μ. Some cells were too broad and too long according to the description of Hustedt (1930, 1932), Proshkina-Lavrenko (1949/50)), Siemińska (1964) — the length of cells riches 50 μ, breadth 13–20 μ.

Melosira distans (Ehr.) Kütz. *var. Pfaffiana* (Reinsch) Grun. (Fig. 2 F). Diameter of valve 11.25 μ, height 3.75–6 μ, 13 longitudinal rows of punctae in 10 μ. It is rather rare species from the north and west Europe (Siemińska 1964). It was found in peloreophil habitat together with *Melosira distans* (Ehr.) Kütz. *var. alpigena* Grun. (Fig. 2 E), diameter of value 5.5 μ, height 7.5 μ, 16 longitudinal rows of punctae in 10 μ.

Pinnularia microstauron (Ehr.) Cl. (Fig. 2 G). Cells 25–37.5×6.25–7.5 μ, 12–13 striae in 10 μ. The cells are too narrow as compared with the following reports: Hustedt (1930), Proshkina-Lavrenko (1949/50), Siemińska (1964). The breadth of cells 7–11 μ, Cleve-Euler (1953) — 8.5–14 μ. The cells of breadth 6.25 μ were found also in Morskie Oko Lake in High Tatra Mts.

Cosmarium caelatum Ralfs. (Fig. 2 M). Cells 42–47.5×37.5–42.5 μ, isth. 12.5 μ, crass. 20–22.5 μ. According to Růžička (1964) it is a common species on humid rock in High Tatra Mts. In High Tatra streams is rare, only few specimens were found in Roztoka stream (Kawecka 1965).

Cosmarium impressulum Elfr. (Fig. 2 N). Cells 22.5–25×12.5–18.75 μ, isth. 6.25–7.5 μ, crass. 9–12.5 μ. One of the most frequently occurring species of genus Cosmarium in Maljovica stream.

Cosmarium margaritatum (Lund.) Roy et Biss. *var. margaritatum* Růžička (Fig. 2 O). Cells 62.5–68.75×47.5–53 μ, isth. 15–18.75 μ, crass. 25–31.25 μ. Růžička (1964) found this form in High Tatra Mts. in the humid rock habitat. In Tatra streams it was not observed yet.

Cosmarium subspeciosum Nardst. *var. transiens* Messik. (Fig. 2 P). Cells 32.5–37.5×25–30 μ, isth. 10–15 μ, cross. 15–20 μ. The variety found for the first time in Alps (Messikommer 1942). As reported (Wasylik 1971) it appears in the West Tatras in the upper part of Chochołowski and Kościeliski streams (altitude 1100–1600 m a.s.l.).

Euastrum montanum W. et G. West (Fig. 2 R). Cells 21×15 μ, isth. 4 μ, cross. 12 μ. It appeared in High Tatra in Roztoka valley on humid rocks (Gutwiński 1909), and in Roztoka stream (Kawecka 1965).

Euastrum subalpinum Messik. *var. crassum* Messik. (Fig. 2 S). Cells 18×15 μ, isth. 4.5 μ, cross. 13.5 μ. It appears rarely in Europe, and only in the high mountains (Růžička 1964).

Tetmemorus laevis (Kütz.) Ralfs *var. intermedius* (Woron.) Růžička (Fig. 2 T). Cells 70–81.25×20–25 μ, 1–3 pyrenoids in each half a cell, the cell membrane with fine points or without. Růžička (1959) distinguished this variety, which differs from the main species in the number of pyrenoids in half a cell (the species has 3–6 pyrenoids in each half cell, and variety 1–2–3). He thinks, however, that it is not a definitive criterium for a systematic classification. Forster (1970) stresses this point and considers the name of the variety as synonymous with the name of species.

ALGAE COMMUNITIES

Considering appearance of the dominating species which formed macroscopic conglomerations, and diatoms species with the highest coefficient of coverage in the limited sections of waters in streams, we can distinguish three zones (Fig. 3, Table III):

Zone I. It includes over the short part of stream below the outflow from the Elenski Lake (station 1); approximate altitude 2600 m above sea level, water temperature 8.5°C, the pH 6.2; the level of water was low, average depth 5 cm; the bottom was covered with moss. Communities of Cyanophyta with dark-brown thalli were dominating. They were composed mainly of *Calothrix gypsophila, Chamaesiphon polonicus, Scopulonema polonicum, Homeothrix janthina. Calothrix gypsophila* filament was covered with *Cyanophanon mirabile, Chamaesiphon incrustans* and *Lyngbya kützingii.* Diatom communities are rich in taxons. They did not occur in large numbers. In communities of moss, slime in braids of threads Cyanophyta the highest coefficient of coverage had: *Ceratoneis arcus, Fragilaria capucina, Gomphonema angustatum, Frustulia rhomboides var. saxonica, Gomphonema longiceps var. montanum, Pinnularia braunii var. amphicephala.* The small amounts of northern Alpine forms were encount there; the most frequent among them were: *Caloneis silicula var. alpina* (Table III).

Zone II. It includes the middle part of stream down to the place where it left the valley (stations 2–6, approximate altitude 2500–1200 m above sea level, water temperature 6–10°C, pH 6.2). This part of stream showed the great variety. Lotic biotop was distinct on slopes (stations 2, 4, 5, 6), and lenitic, which was encounted not only near the bank, but was dominating on the large terrace (station 3). There was a great differentiation in the insolation changing from very good above the upper timber line, to poor and sometimes very shadowy in the forest zone.

Next to the lithoreophilous and pelorheophilous algae communities, the diatoms community living on moss was of a great importance. Moss

formed the thick turf in the upper part of stream (station 2), and its amount decreased with decreasing altitude.

In lotic habitat *Hydrurus foetidus, Homeothrix janthina* and diatoms were dominated. *Hydrurus foetidus* prevailed in the upper part of zone,

Table III. Vertical distribution of diatom communities. Coefficients of coverage: A — 100–200, B — 200–400, C — 400–600, D — 600–800, E — 800–1000, F — >1000

Species	Zone							
	I	II					III	
	Station							
	1	2	3	4	5	6	7	8
Caloneis silicula var. alpina	A							
Melosira italica var. valida	A							
Cymbella naviculiformis	A							
Cymbella cistula	A							
Pinnularia gibba	A							
Surirella angustata	A							
Pinnularia microstauron	A							
Pinnularia braunii var. amphicephala	B							
Gomphonema longiceps var. montanum	B							
Neidium affine	A	A						
Frustulia rhomboides var. saxonica	B		C					
Eunotia robusta var. tetraodon			A					
Fragilaria pinnata			A					
Tabellaria flocculosa			A					
Gomphonema angustatum	B	A		A				
Diatoma hiemale / *Diatoma hiemale var. mesodon*	A	C	B	B	A	A		
Achnanthes microcephala						A	A	
Fragilaria capucina	B	C	C	B	B	C	C	D
Cymbella ventricosa	B	B	B	A	A	A	B	B
Ceratoneis arcus	B	F	C	D	E	F	F	F
Achnanthes minutissima	A	A	A	B	A		B	
Synedra ulna							C	C
Cocconeis placentula var. euglypta							A	A
Cymbella sinuata							B	A
Gomphonema intricatum							A	
Nitzchia linearis								C
Cymbella helvetica								B
Cymbella affinis								B
Nitzschia dissipata								A

and was particularly developed at the station 2. The thick moss turf and a rich growth of *Hydrurus foetidus* indicate that there are many springs. Below, on the same slope next to the stretch of snow still lying there, the stones were covered with *Chamaesiphon polonicus* and *Scopulonema polonicum*, and the amount of moss was much smaller. Further down on the station 4 diatoms formed the separate communities

next to *Hydrurus foetidus*. There were also *Phormidium favosum, Tolypothrix distorta, Chlorhormidium rivulare* and *Spirogyra sp.* Still further down *Homeothrix janthina* and *Lemanea fluviatilis* were rich; the later one formed the specially thick conglomerations at the station 6. Among the diatoms the highest coefficient of coverage had: *Ceratoneis arcus, Fragilaria capucina, Diatoma hiemale, D. hiemale var. mesodon, Achnanthes minutissima, Cymbella ventricosa* (Table III).

Algae community consisting mainly of diatoms and desmids was developing in lenitic habitat. *Frustulia rhomboides var. saxonica, Fragilaria capucina, Ceratoneis arcus, Diatoma hiemale, D. hiemale var. mesodon* from diatoms had the highest coefficient of coverage. *Cosmarium impressulum, Tetnemorus laevis* and *T. laevis var. intermedius* from desmids were often.

Zone III. It includes the lowest part of water system — rivers Černi Iskâr and Iskâr down to Samokov (stations 7, 8); approximate altitude 1000—1200 m above sea level, water temperature 20°C, pH 7. *Hydrurus foetidus* was completely extined, and *Homeothrix janthina* was encounted only in a very small quantities. Diatoms communities were dominating and among them *Ceratoneis arcus, Fragilaris capucina, Synedra ulna, Nitzschia linearis, Cymbella helvetica, Cymbella affinis, Cymbella ventricosa* reached the highest coefficient of coverage (Table III). Among the Chlorophyta, the *Ulothrix zonata* and *Spirogyra sp.* were occurring frequently, and among the Cyanophyta — *Phormidium favosum.*

4. DISCUSSION

Previous to the investigations in the Rila Mts. similar work has been done in the High Tatra Mts., and zonal distribution of the algae communities was observed along the streams (Kawecka 1971).

Similar algae communities develop within the same zone in both mountain ranges. It is possible therefore to suggest some conclusions. Figure 3 shows the distribution of algae communities during the summer period along the Maljovica stream in Mila Mts., and along the Sucha Woda, one of the main streams in the High Tatra Mts.

The first zone is formed below the stream outflow from the lake and only when this stream is poor in water in such a degree that humid environment is predominating. Cyanophyta form the dominating communities in both mountain ranges. The *Chamaesiphon polonicus* and *Scopulonema polonicum* are predominating, and they are met together with *Ammatoidea normanii, Coelodesmium wrangelli, Calothrix braunii* in Tatra, and *Calothrix gypsophila* in Rila. *Chamaesiphon polonicus* develops as well frequently in the lower parts of the streams, which get periodicaly dry. Kann (1966) found that *Chamaesiphon polonicus* is characteristic for humid environment of the Alpine streams.

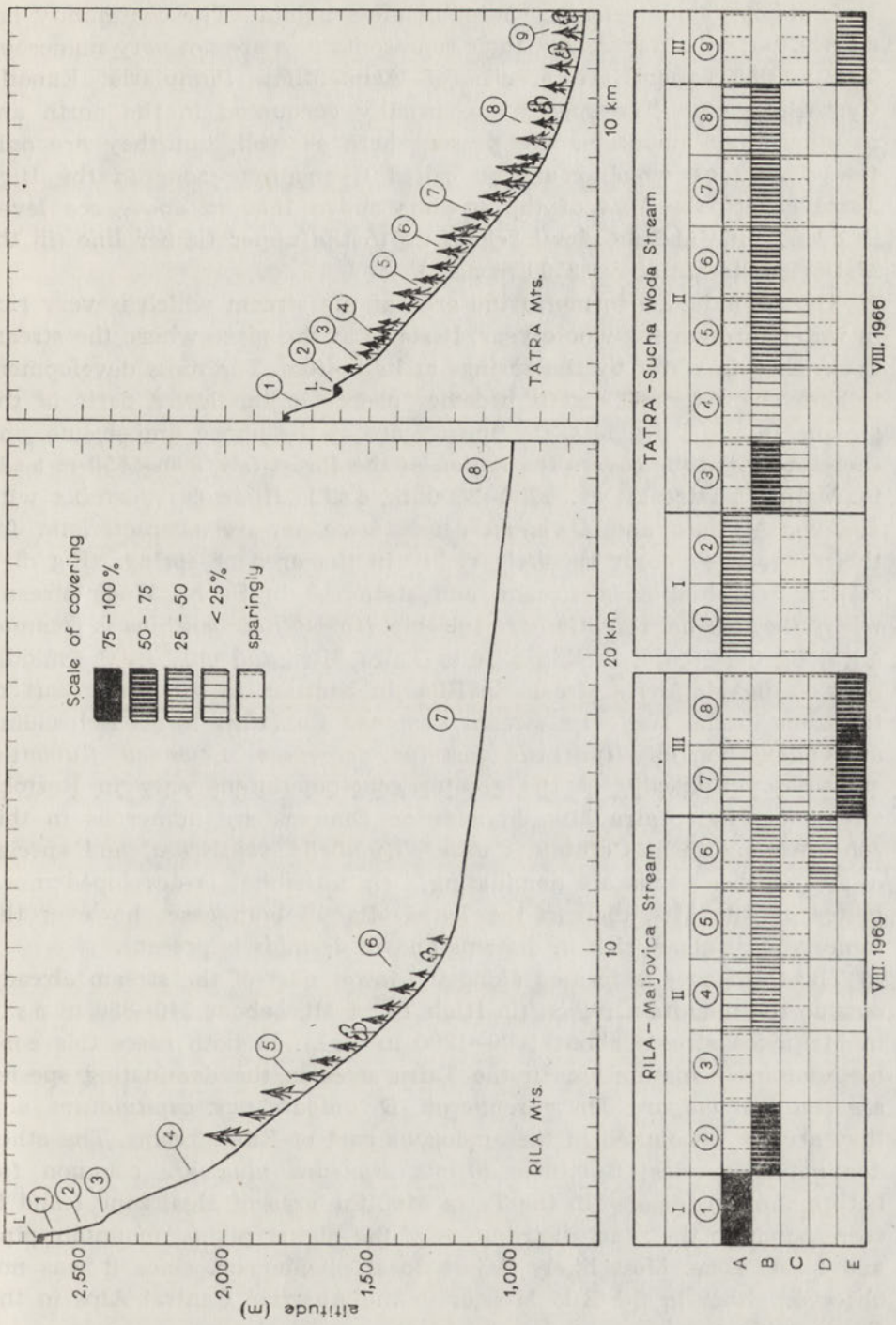


Fig. 3. Vertical distribution of algae communities forming macroscopic concentrations in Maljovica and Sucha Woda streams. A — Cyanophyta, B — *Hydrurus foetidus*, *Homoethrix janthina* and Bacillariophyceae, C — Chlorophyta, D — Rhodophyta, E — Bacillariophyceae. L — lakes, I–III — zones, 1–9 — sampling stations.

Diatoms populate mainly aerophil moss habitat. The community has a great variety of taxons but their representatives are not very numerous. The most frequent are species of Achnanthes, Pinnularia, Eunotia, Cymbella genus. The species are usually encounted in the north and in other high mountains are present here as well, but they are only few. This zone which could be called Cyanophyta zone in the High Tatra covers the area of the streams above 1550 m above sea level, in dwarf pine and meadows region up to the upper timber line (in the Maljovica stream above 2500 m a.s.l.).

The second zone forms in the area of the stream which is very rich in water through the whole year. It starts at the place where the stream is enriched in water by the springs at its bottom. The moss development is here characteristic and it becomes poorer in the lower parts of the stream. In the Tatra Mts. the springs are at the upper timber line, and therefore this zone covers the region of the forest (alt. 890—1550 m a.s.l.; in Maljovica stream alt. 1200—2500 m a.s.l.). *Hydrurus foetidus* with *Diatoma hiemale* and *D. hiemale var. mesodon* are characteristic for this zone. They occur specially richly in the area of springs (Fig. 3 — station 2 in Maljovica stream, and station 3 in Sucha Woda stream) where the thermal condition are suitable. *Homeothrix janthina* is common all along the stream in this zone in Tatra Mts., and only in the middle part of the Maljovica stream in Rila. In addition in the lower part of this zone in the Maljovica stream *Lemanea fluviatilis* forms rich clump and population of *Hydrurus foetidus* decreases. *Lemanea fluviatilis* appeared periodically in the greater conglomerations only in Roztoka stream in High Tatra Mts. In addition Diatoms are numerous in this zone. Among them *Ceratoneis arcus, Cymbella ventricosa,* and species of Achnanthes genus are dominating. Lenitic habitat is developed much better in Rila Mts. then in the Tatra Mts. In both cases however the same type of algae, rich in diatoms and in desmids is present.

The third zone is formed along the lower part of the stream already outside the mountain region (in High Tatra Mts. about 540—890 m a.s.l.; in Maljovica stream about 1000—1200 m a.s.l.). In both cases this zone has diatoms communities. In the Tatra streams the dominating species are *Diatoma vulgare var. ehrenbergii, D. vulgare var. capitulatum,* and they are not encounted in the analogous part of Rila streams. The other dominating species: *Cymbella affinis, Synedra ulna* are common for both mountain regions. In the Tatra Mts. the area of algal zone I and II corresponds to the zonal distribution of the higher plants: mountain-pine and forest zone. Most likely it is a local phenomena, since it was not observed either in the Rila Mts. or in the Austrian Central Alps in the Finstertaler stream.

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5. SUMMARY

The vertical distribution of algae communities in Maljovica stream (Rila Mts. in Balkan Peninsula) was described and compared with their distribution in the High Tatra streams (Kawecka 1971). On the ground of the domination of species three zones were distinguished.

Zone I (alt. about 2600 m). Cyanophyta communities with *Chamaesiphon polonicus, Calothrix gypsophila, Scopulonema polonicum, Homoeothrix janthina* are predominating. Diatom communities are rich in taxons and among them north-Alpine forms are accounted.

Zone II (alt. about 1200–2500 m). *Hydrurus foetidus* in the upper part and *Homeothrix janthina* and *Lemanea fluviatilis* in middle and lower parts are dominating. In diatom communities the most frequent are: *Ceratoneis arcus, Fragilaria capucina, Diatoma hiemale, D. hiemale var. mesodon.*

Zone III (alt. about 1000–1200 m). Diatoms are predominating and the most frequent species are: *Ceratoneis arcus, Fragilaria capucina, Synedra ulna, Nitzschia linearis, Cymbella affinis.*

Previous investigation (Kawecka 1971) showed that in the High Tatra Mts. streams the algae communities form three zones as well, although they differ in altitude (I — altitude about 1550–1780 m, II — altitude 890–1550 m, III — 540–890 m). In each zone of both mountain ranges the analogous type of algae communities develops (Fig. 3). In the High Tatra zone I and II correspond accordingly to the zone of mountain-pine and forest. In the Rila Mts. this correspondence has not been observed.

6. STRESZCZENIE

Przedstawiono pionowe rozmieszczenie zbiorowisk glonów w potoku Maljovica i porównano z rozmieszczeniem ich w potokach północnych stoków Tatr Wysokich (Kawecka 1971). W oparciu o gatunki dominujące wyróżniono 3 strefy.

Strefa I (wysokość około 2600 m. n.p.m.). Dominują tu zbiorowiska sinic *(Chamaesiphon polonicus, Calothrix gypsophila, Scopulonema polonicum, Homoeothrix janthina).* Zbiorowisko okrzemek jest zróżnicowane w gatunki, wśród których spotyka się formy północno-alpejskie.

Strefa II (wysokość około 1200–2500 m n.p.m.). W górnej partii tej strefy dominuje *Hydrurus foetidus,* a w środkowej i dolnej *Homoeothrix janthina* i *Lemanea fluviatilis.* W zbiorowisku okrzemek przeważa *Ceratoneis arcus, Fragilaria capucina, Diatoma hiemale* z odmianą mesodon.

Strefa III (wysokość około 1000–1200 m n.p.m.). Dominują tu okrzemki, z których najczęściej występuje *Ceratoneis arcus, Fragilaria capucina, Synedra ulna, Nitschia linearis, Cymbella affinis.*

Wcześniejsze badania (Kawecka 1971) wykazały, że w potokach Tatr Wysokich zbiorowiska glonów różnicują się także w trzech strefach, jakkolwiek różnice są w wysokości (I — wysokość około 1550–1780 m n.p.m., II — około 890–1550 m n.p.m., III — około 540–890 m n.p.m.). W każdej ze stref obu wysokogórskich obszarów rozwija się analogiczny typ zbiorowisk glonów. W Tatrach Wysokich przebieg strefy I i II koreluje się z piętrami roślin wyższych: kosodrzewiny i reglowego lasu świerkowego. W górach Riła prawidłowości tej nie obserwowano.

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WATER CONTENT IN LEAVES OF HELOPHYTES

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ABSTRACT

Changes in water content in leaves of *Phragmites communis*, *Glyceria aquatica*, *Typha latifolia*, *T. angustifolia*, and *Acorus calamus*, as well as in shoots of *Schoenoplectus lacustris*, occurring at different times of day and during the season, were investigated. Also changes in hydration of leaves of these helophytes in accordance to their location on the stem were determined. Water content in leaves changes at different times of day and during the vegetative season. Upper leaves contain less water than the lower ones. The decrease in hydration of leaves by the end of the vegetative season is probably related to the beginning of wilting of helophyte leaves.

1. INTRODUCTION

The water balance of plants has been investigated by a number of authors. Dastur (1925) has observed a clear-cut dependence of photosynthesis upon water content in the leaves of plants, and Taylor (1970) has found that water content in leaves varies as a function of their age. In the course of evolution, plants became adapted to adverse environmental conditions, among others, by way of modification of their water balance, as shown by Alekseev, Gusev (1950). Nakhutsrishvili (1971) has noted a decrease in water content in the leaves of wheat by the end of the vegetative season. A correlation between the water balance and photosynthesis has been presented by Kinzel (1971).

The above authors have mostly studied terrestrial plants taking up water from an environment, in which the available water occurs in varying amounts, mainly depending on climatic factors. Aquatic plants take up water through the whole body surface, if submerged, or from the bottom of water reservoirs. In both these cases, the water supply is appropriate.

The major part of investigations on the water balance of aquatic plants deal with evapo-transpiration processes Alekseev, Gusev 1950, Bejdeman 1956, Antipov 1961, Khashes, Bobro 1971).

As a part of investigations on the water balance of helophytes, measurements of transpiration in *Phragmites communis* (Królikowska 1971) and studies of the effect of reed parasites and pests on its water balance (Durska 1972) have previously been performed. The present investigation, being a continuation of these studies, was aimed at the determination of water content in the leaves of several helophyte species.

2. MATERIAL AND METHODS

The material comprised six following species of helophytes: *Phragmites communis* Trin., *Glyceria aquatica* (L.) Wahlb., *Typha latifolia* L., *Typha angustifolia* L., *Acorus calamus* L., *Schoenoplectus lacustris* (L.) Palla. These plants are common in lake littoral, occurring in pure or mixed communities.

Water content was determined in helophytes growing in littoral of Lake Tałty situated in the Mazurian Lakeland. It is a lake of moderate trophy, greatly exposed to wind (Olszewski, Paschalski 1959). The plant material was collected at a sampling station situated in a shallow bay with sandy bottom, located in the eastern part of the lake. Reed was the dominating species, whereas the remaining ones occurred in loose communities or singly.

The leaves of helophytes were the object of study, since they are the main photosynthesizing and transpiring organ, being decisive of plant productivity. Only in the case of Schoenoplectus, shoots were used instead of leaves, as the leaves of this species are greatly reduced.

Investigations were performed during the vegetative season of 1972, between June and August. No analyses were carried out throughout the whole vegetative season, as the present studies were only preliminary, preceding next year's investigations on the water balance of helophytes relative to their phenology. Plants to be examined were selected at random within the reach of the sampling station. Only in the case of Phragmites, plants growing as a dense reed-belt and those occurring singly were taken separately.

Determination was made of the daily and seasonal changes in hydration. Three highest growing, well developed leaves were collected. In the case of Schoenoplectus, three shoots of different height were taken. As a rule, collection was made of three top leaves; since for each plant these leaves were youngest and located at a similar distance from water level, they could be considered triplicates.

Water content in leaves according to their location on the stem was determined by analysis of the successive leaves from top downwards the plant. Determinations were carried out once in July and once more in August, by way of analysing three times on the same day leaves from three plants of every species studied. The difference between the maximally and minimally hydrated leaves (Table IV) was expressed as percentage of the maximal hydration, the number of grams of water per 1 g of dry substance of leaves being taken as 100%.

Changes in the hydration of leaves at different times of day were estimated once in July and once more in August. On the day of tests, water content in leaves was determined every 2 hours, between 6 a.m. and 8 p.m., i.e. at the time when plants phothosynthesize and transpiration is most intense.

Seasonal changes in the hydration of leaves were determined monthly by way of analyses carried out from June, till August, between 12 a.m. and 1 p.m.

Water content was determined as follows: immediately after collection, the leaves were weighed on a torsion balance, whereupon samples were dried to constant weight at 105°C and weighed once more. The difference between the fresh and dry weight represented the water content in the plant material. The amount of water contained in helophyte leaves and Schoenoplectus shoots, respectively, was expressed as percentage against the fresh weight.

On the basis of the behavior of water content in leaves between 6 a.m. and 8 p.m., the water deficit at different times of day was calculated by Vassiljev's method (1931) applied for calculation of Water Saturation Deficit, using the formula:

$$D = \frac{M - m}{M} \cdot 100$$

where: M — maximal water content (g/g dry weight), m — minimal water content (g/g dry weight), D — deficit (% of maximal content).

For calculation of the water deficit use was made of the hydration of leaves during bright hours of the day, but not in the night-time.

The present results represent the means of nine independent analyses (three leaves × three plants, and nine Schoenoplectus shoots, respectively).

3. RESULTS

Analysis of water content pointed to differences in the hydration of helophyte leaves. Mean daily water content in leaves was lowest for *Phragmites communis* (61%). *Typha latifolia* and *Acorus calamus* showed nearly similar hydration levels (77%. Hydration of *Schoenoplectus lacustris* shoots was relatively high (81%) (Table I).

Table I. Water content in leaves of helophytes (as percentage of fresh weight, mean during the day)

Species	Water content (%)	
	July	August
Phragmites communis	63.1	59.2
Glyceria aquatica	74.2	72.7
Typha latifolia	75.9	78.9
Typha angustifolia	73.1	74.8
Acorus calamus	76.8	78.1
Schoenoplectus lacustris (shoots)	82.0	80.7

Mean daily hydration of Phragmites, Glyceria and Schoenoplectus was higher in July, and that of the remaining plants — in August. The differences were relatively small.

CHANGES IN THE HYDRATION OF LEAVES AT DIFFERENT TIMES OF DAY

Water content in leaves of helophytes exhibited changes in the course of day.

During morning hours, with ambient temperature being lowest and the relative humidity of air highest (Table II), the major part of helophytes exhibited a high water content. Vice versa, during noon hours, with higher temperature and lower relative humidity, hydration was lower (Table II, Fig. 1). The results indicate that in the major part of helophytes the fluctuations in leaves hydration proceeded in like manner. However, differences were observed between the behavior of hydration at different times of day in July and August.

Table II. Climatic data on the days of water content determinations (t — ambient temperature, h — relative humidity of air)

Hour	July 9th		August 15th	
	t (°C)	h (%)	t (°C)	h (%)
6 a. m.	14.6	83	16.8	76
8 a. m.	17.3	77	20.5	65
10 a. m.	19.8	75	24.1	60
12 a. m.	21.3	62	25.1	55
2 p. m.	22.6	59	26.1	55
4 p. m.	23.2	57	25.8	57
6 p. m.	22.8	61	24.5	62
8 p. m.	20.0	72	20.4	73
Mean	20.2	68.2	22.9	62.8

Greatest fluctuations in hydration and highest water deficit were exhibited by leaves of *Typha latifolia* both in July and August; these fluctuations were smallest for Glyceria leaves in July and Schoenoplectus shoots in August (Table III).

Helophytes showing in July higher mean daily hydration than in August, in July exhibited also greater fluctuations in water content and a higher water deficit.

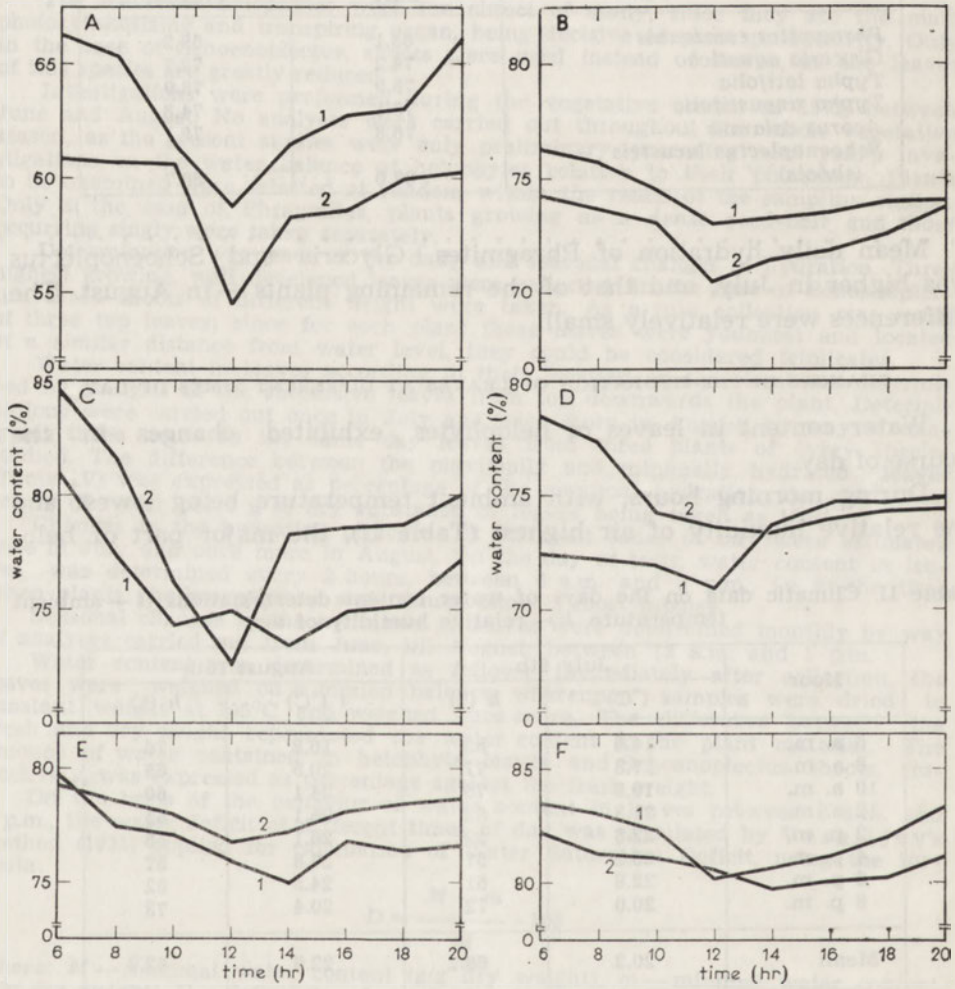

Fig. 1. Changes in water content in leaves of helophytes during the day (as percentage of fresh weight). A — *Phragmites communis*, B — *Glyceria aquatica*, C — *Typha latifolia*, D — *Typha angustifolia*, E — *Acorus calamus*, F — *Schoenoplectus lacustris* (water content in shoots). 1 — July, 2 — August

CHANGES IN THE WATER CONTENT IN LEAVES DURING THE SEASON

The results of analyses performed within three months of the vegetative season indicate that water content in leaves of helophytes changes not only at different times of day, but also during the season. Hydration of leaves was highest in June, and lowest in August (Fig. 2). Schoenoplectus shoots showed lowest fluctuations in water content, and leaves of Acorus — the highest ones.

Table III. Daily maximum (M) and daily minimum (m) water content, and water deficit (D) in leaves of helophytes

Species	July			August		
	Water content (% of fresh weight)		D (%)	Water content (% of fresh weight)		D (%)
	M	m		M	m	
Phragmites communis	66.4	58.7	20.4	61.0	54.5	10.8
Glyceria aquatica	76.2	73.0	15.8	74.8	70.5	17.8
Typha latifolia	80.9	73.4	35.4	81.6	72.6	50.5
Typha angustifolia	74.9	70.9	18.5	78.5	73.3	24.4
Acorus calamus	79.8	75.0	23.5	79.4	76.7	16.5
Schoenoplectus lacustris (shoots)	83.5	80.2	19.6	82.2	79.8	14.8

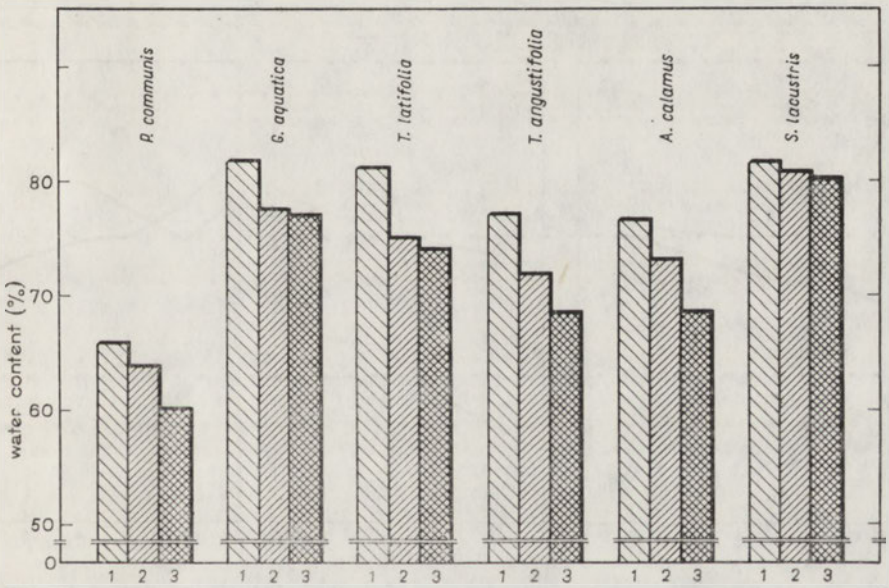

Fig. 2. Seasonal changes in water content in leaves of helophytes (as percentage of fresh weight). 1 — June, 2 — July, 3 — August

WATER CONTENT IN LEAVES ACCORDING TO THEIR LOCATION

The water content in a leaf depends on its location on the stem. Changes in water content in successive leaves of helophytes showed a different behavior in July, as compared with August (Fig. 3). In July lowest hydration was exhibited by leaves situated in the central part of the stem, and in August — by the upper leaves. Only in *Acorus calamus* (Fig. 3 E), in August the upper leaves showed a highest water content.

The differences in water content between the maximally and minimally hydrated leaves of an individual plant are recorded in Table IV. The results indicate that only in the case of *Typha latifolia* the differen-

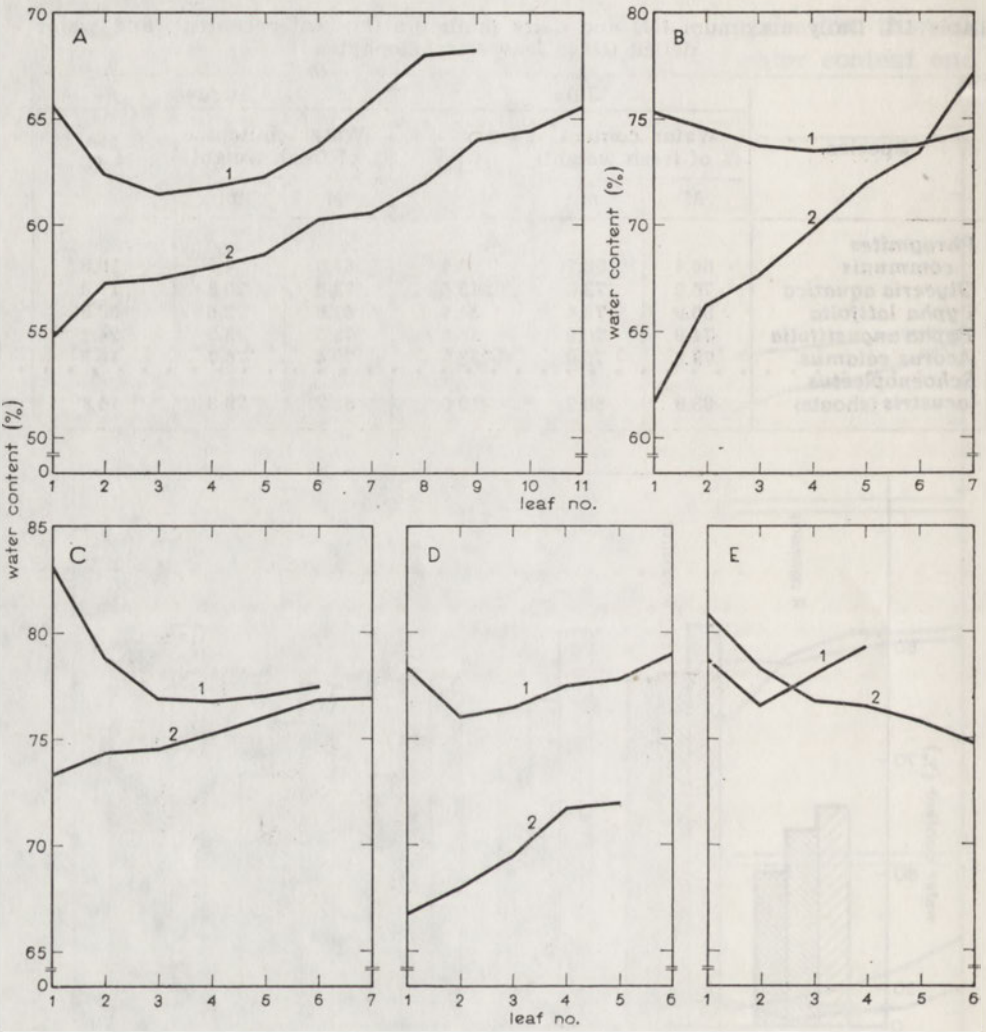

Fig. 3. Water content in successive leaves of helophytes (from upper to lower leaves; water content as percentage of fresh weight). A — *Phragmites communis*, B — *Glyceria aquatica*, C — *Typha latifolia*, D — *Typha angustifolia*, E — *Acorus calamus*. 1 — July, 2 — August.

ce between maximally and minimally hydrated leaves was greater in July than in August. For the remaining species the differences in leaf hydration were greater in August.

Differences in leaf water content were found not only between the various species of helophytes and as a function of leaf location on the stem within each individual species, but also — within one species — between individual plants growing under different conditions (Fig. 4 A). In the instance of *Phragmites communis* occurring in dense reed-belt and growing singly, respectively, the successive leaves were found to differ in size, biomass and water content. Leaves of reed growing as

Table IV. Water content in maximally (*M*) and minimally (*m*) hydrated leaves of helophytes

Species	July			August		
	Water content (% of fresh weight)		Difference (%)	Water content (% of fresh weight)		Difference (%)
	M	*m*		*M*	*m*	
Phragmites communis	68.2	61.4	26.4	65.6	54.8	36.1
Glyceria aquatica	75.2	74.3	12.3	77.0	61.7	50.0
Typha latifolia	83.0	76.7	34.5	76.8	73.3	16.3
Typha angustifolia	78.9	78.3	15.5	71.9	66.7	21.6
Acorus calamus	79.3	76.6	14.6	80.8	74.8	29.7

a dense reed-belt were characterized by smaller biomass and surface. The distribution of the biomass of leaves depended on their location on the stem (Fig. 4 B). In this case maximal biomass was found in leaf 9th (counting from top), whereas in reed growing singly the biomass of leaf 7th was maximal.

In spite of the differences in the biomass of leaves, being related to their surface, no dissimilarities in water content were observed between leaves situated in the central part of the stalk. The leaves situated both highest and lowest were more hydrated in densely growing reed, as compared with plants occurring singly. According to the curves presented in Fig. 4, when going from top of the stalk downwards, the biomass of leaves initially augmented, attained a maximum at the level of the

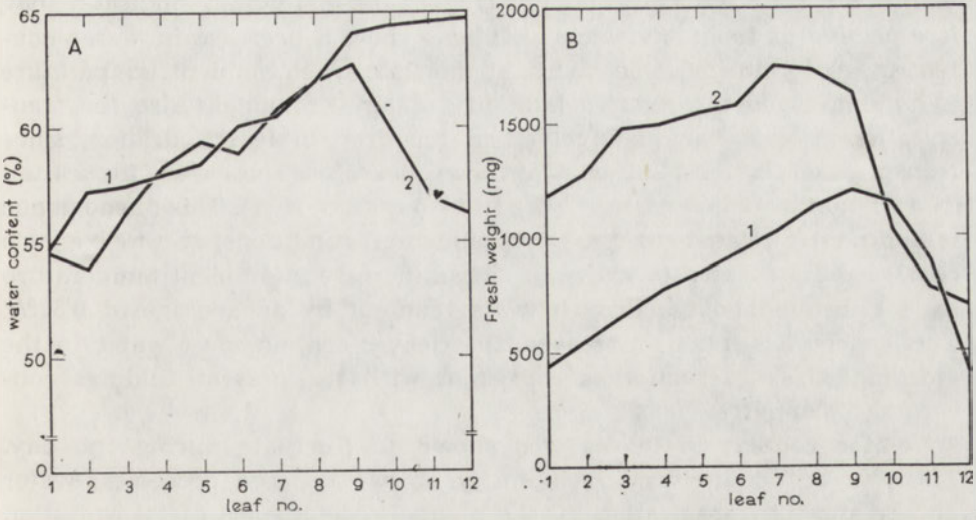

Fig. 4. Water content (A) and biomass (B) of successive leaves of *Phragmites communis* (from upper to lower leaves). 1 — leaves of reed from dense reed-belt, 2 — leaves of singly growing plant

7 th—9th leaf and then decreased. The behavior of water content in successive leaves was similar; in densely growing reed, successive leaves contained the more water, the lower they were located.

The present results prove that in the determination of water content in leaves of helophytes it is advisable to collect for testing only leaves located at the same level or to report the mean of the results obtained for all leaves.

4. DISCUSSION

Mean water content in leaves of helophytes varied within the range of 57—82% of their fresh weight. Water content was highest in leaves of *Typha latifolia* and shoots of *Schoenoplectus lacustris*. These plant species contain colloids of high hydrophily, this reflecting adaptation to life under conditions of reduced water content in the tissues (Antipov 1964).

The differences in water content in leaves can be due to the unlike intensity of plant transpiration. The latter phenomenon could directly result from dissimilarities in plant resistance to adverse environmental conditions, as developed in the process of evolution (Alekseev, Gusev 1950). Analysis of the changes in water content in helophyte leaves and Schoenoplectus shoots, respectively, taking place during the day shows that water content is highest during morning hours and lowest at noon. A similar pattern of changes, though in other plants, has been observed by a number of authors. A drop in water hydration at noon has been reported for savage grass by Rychnowská et al. (1972), winter wheat by Kryukova (1971), and high-mountain gramineous plants by Nakhutsrishvili (1971). These findings indicate that, irrespective of their environment, plants show a decrease in water content in leaves during noon hours, at the time when ambient temperature is maximal, and the relative humidity of air — minimal. Also the transpiration process can be involved in the drop in leaf hydration, since transpiration is most intense at noon hours, as observed for cattail (Novikova 1963) and reed (Królikowska 1971). This phenomenon is indirectly related to the environmental conditions. Strebeyko (1957) has shown that in leaves of oat an increase in ambient temperature by 1°C brings about a drop in water content by an average of 0.51%. This author has found, moreover, that leaves contain most water in the morning; this observation is consistent with the present findings concerning helophytes.

Water content in leaves was shown to fluctuate during the day. This can be due to varying intensity of two opposed processes: water uptake and its transpiration by the plant. Predominance of transpiration over water uptake could contribute to the occurrence of water deficit in the leaves, amounting in *Typha latifolia*, in an extreme case, to 50%.

There is an interrelation between transpiration and water deficit, both these phenomena being dependent on external factors and on the structure of leaves. Water deficit of leaves can be due also to excessively slow water flow in the plant's stem. Therefore, the loss of hygroscopic and capillary water during the day can probably add to increasing water deficit, though it is not bound to simultaneously cause a drop in transpiration (Rychnovsk'a et al. 1972).

Hydration of leaves of helophytes was found to change also during the vegetative season, being — within the range of the experimental period — maximal in June. Water content in leaves decreased by the end of the vegetative season. Khashes, Bobro (1971) have reported in May to 57.8% in August. Thus, the pattern of changes during the that water content in leaves of *Phragmites communis* changed from 68.2% season resembled that observed in the present study. Likewise, Antipov (1961) has demonstrated a reduction of water content in leaves during the vegetative season in hygro- and mesophytes, and Nakhutsrishvili (1971) — in high-mountain grasses. Thus, it is evident that, irrespective of the environment, the hydration of leaves decreases during the vegetative season. Moreover, the behavior of water content in leaves during the season is independent of the site where representatives of a given plant species grow; this is confirmed by the fact that changes similar to those at present reported have been found in *Typha latifolia* also by Boyd (1970) and Antipov (1964).

On the other hand, seasonal changes in the hydration of plants were found to be related to their growth and different developmental stages, this having been observed also by Nakhutsrishvili (1971) for high-mountain grasses. Among the helophytes studied, *Phragmites communis* showed the lowest water content in leaves. At the same time, transpiration is lower in this species than in, for example, *Typha latifolia* (Bejdeman 1956). In studies of hygro-, meso- and xerophytes, Antipov (1961) has found that, compared with other plants, Phragmites, Typha and Schoenoplectus exhibit a low water content, least intense transpiration and high thermoresistance of cells. Leaves of these plants have a relatively well developed stomatal apparatus. On the grounds of these properties (Antipov (1971) has classified the above plants into the group of xeromorphic hygrophytes.

Studies of water content in leaves in accordance to their location on the stem showed that upper leaves contain less water than the lower ones. It has previously been demonstrated (Królikowska 1971) that transpiration is more intense in upper than in lower leaves. There are differences in the immediate environmental conditions between leaves growing in the upper and lower part of the stem. In case of the upper leaves ambient temperature is higher, whereas the relative humidity of the surrounding air is lower, owing to greater exposure to wind (Anti-

pov 1961). Lower leaves are less well illuminated, and show lower productivity than respiration (Szczepański 1973). Differences in the immediate environmental conditions between upper and lower leaves, as well as a dependence of water content in leaves upon these differences have been observed in cereals by Strebeyko, Karwowska (1958). In studies of leaves of *Mangifera indica*, Taylor (1970) has shown that, irrespective of the age of plant, water content per unit of leaf surface depends on the location on the stem, lower leaves being more hydrated than the upper ones.

Smaller hydration of lowest situated leaves, observed in this study by the end of the experimental period, was probably due to the outset of leaf wilting, this having been demonstrated in reed by Willer, Wodden (1943).

The effect of the immediate environmental conditions on the hydration of successive leaves was even more evident when comparison was made of leaf water content between reed occurring in a dense reed-belt and growing singly. Singly growing reed is more exposed to the action of climatic factors than that occurring in dense communities. It is more exposed to sunlight, and ambient air shows higher temperature and lower relative humidity. In a dense reed-belt, just above water level, the exchange of air saturated with water vapor is greatly reduced. In the case of plants growing singly, even just above the water level there are conditions inducing xeromorphism of leaves.

The results obtained in the present and previous (Królikowska 1971) studies prove that the physiological processes of helophytes are related to environmental conditions and internal water balance. The balance of water uptake and utilization is associated with climatic conditions and structure of helophytes. Changes in hydration observed in this investigation indicate that different parts of the plant show dissimilarities in water balance, which have a bearing on the processes of biomass production; these processes are, in fact, a matter of great interest. Thus, it would be advisable to observe the interrelation between helophyte hydration and their productivity.

5. SUMMARY

During the vegetative season of 1972, water content in leaves of several species of helophytes was studied.

Hydration of leaves was found to be highest during morning hours and lowest at noon. Fluctuations in water content at different times of day depended on the plant species and period of tests. *Typha latifolia* leaves showed a highest water deficit both in July and August. Three species of helophytes exhibited greater daily fluctuations in water content in July, and three — in August. Average water content during the day was higher for *Phragmites communis, Glyceria aquatica* and *Schoenoplectus lacustris* in July, and for *Typha latifolia, T. angustifolia* and *Acorus calamus* — in August.

Changes in water content in helophyte leaves were observed also during 3 months of the vegetative season. Leaves were most hydrated in June, and least

in August. The drop in water content in leaves by the end of the season was probably related to the beginning of leaf fading. Fluctuations in the hydration of leaves during the season varied in different helophyte species. The drop in water content in leaves was greatest in *Acorus calamus* and *Typha angustifolia*, and smallest in *Glyceria aquatica*.

Hydration of helophyte leaves depended on their location on the stem. Upper leaves contained less water than the lower ones, exposed to higher humidity. There were considerable differences in water content between the most and least hydrated leaf of an individual plant; this was probably related to differences in the age of leaves collected from one plant, as well as to the age of the plant as a whole.

In the case of *Phragmites communis* it was found that in younger plants an increase in the biomass of leaves was paralleled by a rise of their water content; this relationship did not apply to older leaves by the end of the vegetative season.

6. STRESZCZENIE

W sezonie wegetacyjnym 1972 r. przeprowadzono badania nad zawartością wody w liściach kilku gatunków helofitów.

Stwierdzono, że liście są najbardziej uwodnione w godzinach rannych, najmniej w południe. Wahania zawartości wody w ciągu dnia zależne były od gatunku rośliny i okresu, w którym prowadzono pomiary. Największy deficyt wodny liści w lipcu i sierpniu stwierdzono u *Typha latifolia*. Trzy gatunki helofitów wykazywały większe dzienne wahania uwodnienia w lipcu, trzy zaś w sierpniu. Średnio w ciągu dnia u *Phragmites communis*, *Glyceria aquatica* i *Schoenoplectus lacustris* zaobserwowano większą zawartość wody w lipcu, natomiast u *Typha latifolia*, *T. angustifolia* i *Acorus calamus* — w sierpniu.

Zmiany zawartości wody w liściach helofitów stwierdzono również w ciągu 3 miesięcy sezonu wegetacyjnego. Liście najwięcej uwodnione były w czerwcu, najmniej w sierpniu. Spadek zawartości wody w liściach pod koniec sezonu związany był prawdopodobnie z początkiem obumierania liści. Wahania wody w liściach w sezonie były różne u poszczególnych gatunków helofitów. Największy spadek zawartości wody stwierdzono w liściach *Acorus calamus* i *Typha angustifolia*, najmniejszy u *Glyceria aquatica*.

Zawartość wody w liściach helofitów zależała od ich położenia na łodydze. Stwierdzono, że liście wyżej położone zawierają mniej wody niż dolne liście, znajdujące się w bardziej wilgotnych warunkach, przy czym wystąpiły duże różnice w zawartości wody między najbardziej a najmniej uwodnionym liściem jednego osobnika. Związane to było prawdopodobnie z różnym wiekiem liści na jednej roślinie, oraz wiekiem całej rośliny.

U *Phragmites communis* stwierdzono, że u roślin młodszych wraz z przyrostem biomasy liści zwiększała się w nich zawartość wody, zależność ta nie realizowała się u liści starszych pod koniec sezonu wegetacyjnego.

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DISTRIBUTION OF LARVAE OF CHIRONOMIDAE (DIPTERA) IN CROSS-SECTIONS OF NAREW RIVER

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ABSTRACT

Species composition and distribution of the benthic Chironomidae fauna in two cross-sections of the Narew River were investigated in yearly cycle. The occurrence of larvae of 39 species was demonstrated. In the paracentral, sandy-muddy and sandy-peaty sampling stations, as well as in the sandy deposits of the main current of the river, the numbers of larvae found in fall exceeded those recorded in spring and summer. In summer larval density in both investigated cross-sections of the Narew River was closely similar.

1. INTRODUCTION

The Chironomidae fauna of rivers is not so far well known, though having been the subject of many studies.

On account of the domination of Chironomidae among the benthic fauna and, consequently, of its substantial share in the food supply of fish, as well as on the grounds of the abundance of rivers in Poland, the knowledge of the distribution, species composition and dynamics of the total numbers of this group is of considerable importance.

The benthic fauna of rivers is of special interest as experimental material, because its qualitative and quantitative composition undergoes marked fluctuations as a result of washing out during violent river floods and owing to other ecological factors (Tarwid et al. 1953, Markovsky, Olivari 1956, Kajak 1959 and others).

The purpose of the present study was to investigate the distribution of the benthic Chironomidae fauna in cross-sections of the Narew River in yearly cycle, and to gain insight into species composition and dynamics of its total numbers.

2. DESCRIPTION OF THE INVESTIGATED TERRAIN, MATERIAL AND METHOD

Tests were performed in two cross-sections of the river:

1. 1 km, downstream of the Narew River, from estuary of the tributary Turośnianka River — cross-section No. 1;
2. 6 km, upstream of the Narew River, from estuary of the tributary Supraśl River — cross-section No. 2.

During fall and spring rises the Narew River forms vast flood waters. In summer, in cross-section No. 1 the breadth of the river was 39 m and its depth 5 m, and in cross-section No. 2 — 36 m and 3.5 m, respectively.

To determine the species composition and the number of Chironomidae larvae, in 1968, once a month, samples were collected with the use of a loaded Ekman-

Birge bottom-sampler (catching area 225 cm²), along the whole breadth of the Narew River, at intervals of 2–3 m.

In both cross-sections three groups of sampling stations with similar bottom structure and numbers of larvae were distinguished:

stations 1A and 2A — 0–3 m from the riverside,
stations 1B and 2B — 3–13 m from the riverside,
stations 1C and 2C — central part of the river.

The bottom of stations nearest to the riverside was muddy and overgrown with vegetation. At a distance of 3–13 m from the riverside, the deposits were sandy-muddy (cross-section No. 1) and sandy-peaty (cross-section No. 2), with scattered tufts of macrophytes. In the central part of the river, in cross-section No. 1 the bottom was stony-sandy and in cross-section No. 2 it was covered with fine yellow sand.

The sediment collected was sifted through sieves (mesh surface 0.5 · 0.5 mm). The material was preserved in a 3% formalin solution, selected and examined in the laboratory. Species composition of larvae, pupae and the imagines bred was determined according to the keys of: Tshernovskij (1949), Romaniszyn (1958), Fittkau (1962), Pankratova (1970), Muragina-Koreneva (1957) and Goetghebuer (1936 a, b).

3. RESULTS

In the Narew River 39 species of Chironomidae were detected. Some species occurred unfrequently and in small proportion, e.g. *Microcricotopus bicolor* (Zett.), *Potthastia longimanus* (Kieff.), *Cryptochironomus ex gr. pararostratus* Harn. and others (Table I).

In yearly cycle, in stations of cross-section No. 1 of the Narew River, the mean number of Chironomidae larvae was 635 larvae/m². The numbers of larvae in muddy stations 1A situated next to the riverside amounted to 605 larvae/m², in paracentral, sandy-muddy stations 1B — to 1190 larvae/m² and in the central, stony part of the river (stations 1C) — to 110 larvae/m² (Table II). Changes in larval density in 1968, in cross-section No. 1, are recorded in Fig. 1. In muddy stations 1 A nearest to the riverside the numbers of larvae were maximal in May and September, and minimal in July and December. In paracentral, sandy-muddy stations 1 B the number of larvae averaged 1500 larvae/m² in the latter part of April. After a drop in spring, from July the number of larvae gradually increased to attain in December a maximum of 2250 larvae/m².

The numbers of larvae found in samples collected from the main current of the river (station 1 C at the stony river bottom) were lowest, as compared with all the samples taken in cross-section No. 1.

In stations of cross-section No. 2 of the Narew River, in 1968 the mean number of Chironomidae was 785 larvae/m² (Table II). In muddy stations 2 A located next to the riverside, from May the number of larvae gradually increased to attain in the early part of September a maximum of 1500 larvae/m² (Fig. 2). Subsequently, the number of larvae decreased to a minimum of several tens of larvae/m² in December. In paracentral, peaty-sandy stations 2 B and sandy stations of the main current 2 C, the numbers of larvae in fall exceeded those observed in spring and summer.

Table I. Mean numbers of larvae of various Chironomidae species in cross-sections Nos. 1 and 2 of the Narew River in 1968, expressed as percentages of the mean yearly total number

Species	Stations				
	1 A (%)	1 B (%)	2 A (%)	2 B (%)	2 C (%)
Ablabesymia ex. gr. monilis L.	—	2.20	0.70	1.53	0.61
Ablabesymia ex. gr. lentiginosa Fries	1.60	1.10	2.40	1.70	6.10
Clinotanypus nervosus (Meig)	5.60	0.30	4.20	0.50	0.67
Procladius choreus (Meig)	37.40	15.40	30.28	15.12	6.50
Cricotopus algarum (Kieff.)	1.20	0.40	0.70	—	0.61
Cricotopus inaequalis (Kieff.)	0.40	0.10	0.75	—	—
Cricotopus silvestris (Fabr.)	0.45	—	0.70	—	—
Microcricotopus bicolor (Zett.)	0.20	0.40	0.70	0.50	—
Potthastia longimanus Kieff.	0.10	1.45	—	1.00	0.61
Psectrocladius psilopterus Kieff.	—	3.50	—	2.50	—
Synorthocladius semivirens Kieff.	0.40	0.30	0.70	—	—
Thienemanniella flaviforceps Kieff.	0.50	1.45	0.70	1.57	—
Chironomus f.l. plumosus L.	2.50	0.50	2.10	0.95	0.64
Cricotopus inaequalis (Kieff.)	—	—	—	0.50	—
Chironomus f.l. thummi Kieff.	0.50	—	0.70	—	0.64
Cryptochironomus borysthenicus (Tshern.)	1.10	0.60	—	0.50	0.90
Cryptochironomus ex gr. defectus Kieff.	0.50	7.20	0.42	6.93	11.55
Cryptochironomus ex gr. vulneratus (Zett)	—	2.20	—	1.53	4.27
Cryptochironomus ex gr. pararostratus Harn.	—	0.20	—	—	0.30
Cryptochironomus sp.	—	—	—	—	0.88
Endochironomus ex gr. dispar (Meig)	0.80	—	1.41	—	—
Endochironomus tendes Fabr.	1.40	1.10	0.75	—	—
Endochironomus ex gr. signaticornis (Kieff.)	—	—	0.78	—	—
Glyptotendipes ex gr. gripekoveni Kieff.	1.10	2.10	—	1.70	0.90
Harnischia conjugens Kieff.	—	1.00	2.10	1.00	1.89
Harnischia fuscimana Kieff.	—	0.40	—	—	3.66
Limnochironomus nervosus (Staeg.)	0.30	0.10	0.70	—	—
Microtendipes ex gr. chloris (Meig.)	0.70	3.00	—	2.90	—
Paralauterborniella nigrohalteralis (Mall.)	—	1.50	—	1.53	4.27
Paratendipes ex gr. albimanus (Meig.)	3.60	3.20	3.45	—	—
Polypedilum ex gr. convictum (Walk.)	1.00	0.50	0.70	—	0.61
Polypedilum ex gr. nubeculosum (Meig.)	10.40	9.85	17.25	5.80	5.99
Polypedilum ex gr. pedestre (Meig.)	—	—	—	3.00	—
Polypedilum ex gr. scalaenum Schr.	16.35	10.05	12.78	2.72	11.40
Tanytarsus ex gr. exiquus (Ioch.)	—	—	—	—	0.61
Tanytarsus gregarius (K.) Edw.	5.0	16.40	4.85	15.88	14.12
Tanytarsus lobatifrons Kieff.	1.20	1.10	0.70	5.00	1.80
Tanytarsus ex gr. mancus (Walk).	5.50	12.40	7.00	23.73	19.88
Micropsectra praecox Meig.	1.20	—	2.40	1.90	0.62

Stations: 1A and 2A — muddy deposits (riverside), 1B — sand with mud (riverside), 2B — peat with sand (3-13 m from the riverside), 2C — sand (central part of the river).

Table II. Mean yearly numbers of Chironomidae larvae in the Narew River in 1968

Cross-section No.	Stations	Minimal depth (m)	Bottom	Mean number of larvae/m²	
1	1 A: 0–3 m from riverside	0.9	mud	605	635
	1 B: 3–13 m from riverside	1.5	sand with mud	1190	
	1 C: central part of river	3.5	stones and sand	110	
2	2 A: 0–3 m from riverside	1.4	mud	700	785
	2 B: 3–13 m from riverside	2.0	peat covered with sand	850	
	2 C: central part of river	5.0	sand	800	

The increases and drops in the numbers of larvae, presented in Fig. 1 and 2, were for the most part related to the dynamics of the dominant species.

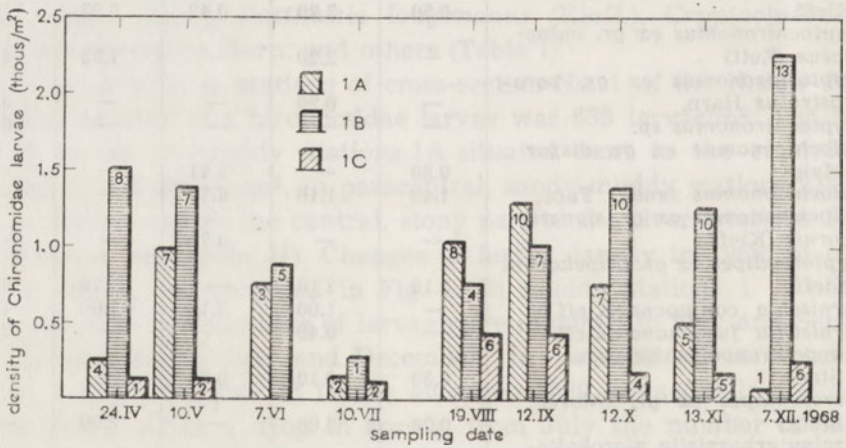

Fig. 1. Density (bars) and number of species (numbers within bars) of Chironomidae larvae in deposits of cross-section No. 1 of the Narew River in 1968. 1 A — muddy stations next to riverside, 1 B — paracentral, muddy-sandy stations, 1 C — sandy--stony central part of river

In muddy stations 1 A and 2 A situated next to the riverside larvae of *Procladius choreus* (Meig.), *Clinotanypus nervosus* (Meig.), *Plypedilum ex gr. nubeculosum* (Meig.) occurred most frequently. In paracentral, sandy-muddy (1 B) and peaty-sandy (2 B) stations, as well as in the sandy, central part of the river (stations 2 C), larvae of *Tanytarsus ex gr. mancus* (Walk.), *Tanytarsus gregarius* (K.) Edw. and *Polypedilum ex gr. scalaenum* Schr. were most common (Table I).

The numbers of larvae of the remaining Chironomidae species were lower (Table I).

The maximal numbers of pupae varied from 430/m² (on June 8th, in paracentral, muddy-sandy stations 1 B) to 600/m² (on Sept. 12th, in muddy stations 2 A nearest to the riverside).

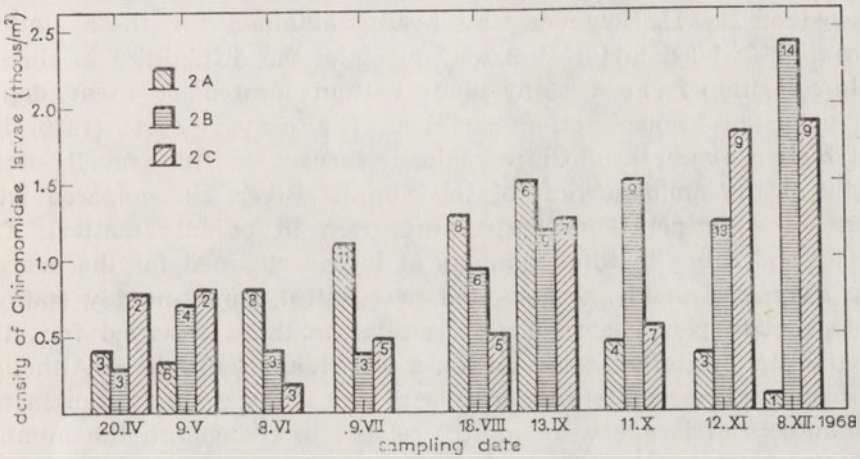

Fig. 2. Density (bars) and number of species (numbers within bars) of Chironomidae larvae in deposits of cross-section No. 2 of the Narew River in 1968. 2 A — muddy stations next to riverside, 2 B — paracentral, sandy-peaty stations, 2 C — sandy central part of river

4. DISCUSSION

The distribution of Chironomidae larvae in the investigated cross-sections was irregular throughout the year.

The fact that the number of larvae in the stations nearest to the riverside was lower in early spring and late autumn, as compared with the maximum observed in summer, is probably due to washing away of the benthos by the spring and fall river floods and, doubtless, also to migration to deeper sites. This interpretation is confirmed also by the high number of larvae in the central part of the river (cross-section No. 2) and in the paracentral stations (cross-sections No. 1 and 2) in late fall. Markovsky, Olivari (1956), as well as Lyakhov (1961) have reported maximal washing out of the benthic fauna in spring. The intensity of washing out of the benthic fauna is testified to by the results of Tarwid et al. (1953), who have observed larger amounts of benthic fauna carried away in the Vistula River, as compared with the population of the bottom of the river.

The total numbers of Chironomidae larvae found in late summer were closely similar along the whole cross-sections (except for the strony stations of the main current 1 C) and mostly exceeded those observed in

spring, whereas the numbers of larvae obtained in fall were higher than those recorded in summer (with the exception of the stations next to the riverside). Konstantinov (1944) and Kajak (1959) have found lowest numbers of Chironomidae in the main current of the river. In the investigated parts of the Narew River, lowest numbers of larvae were noted only at the stony-sandy bottom of the main current (cross-section No. 1). However, the results obtained for these stations are in all likelihood underestimated, owing to the difficulties in correct sample collection from a stony-sandy bottom located at great depth, when using an Ekman bottom-sampler. Niedźwiecki (1970) has found higher numbers of Chironomidae larvae in the gravelly-stony deposits of the main current of the Supraśl River, as compared with stations of a distinct deposit structure (rich in organic matter). The increases and drops in total numbers of larvae obtained for the nearest to the riverside, muddy stations and paracentral, sandy-muddy stations of cross-section No. 1 were closely similar to those observed for the respective stations of cross-section No. 2 (Fig. 1 and 2, Table I). Although a multispecies community was dealt with, the increases and drops in the total numbers of larvae were mostly related to changes in the number of the dominant species. The substantial numbers of pupae present in samples collected in spring and late summer indicate that also the flights of imagines exerted a marked effect on the decreases in the total numbers of larvae.

5. SUMMARY

To determine species composition and distribution of the benthic Chironomidae fauna in two cross-sections of the Narew River samples were collected with a loaded Ekman bottom-sampler (catching surface 225 cm²). Systematic studies were performed in 1968, demonstrating the occurrence of 39 Chironomidae species. The following three groups of sampling stations were distinguished:

1. 0–3 m from the riverside (stations 1 A and 2 A)
2. 3–13 m from the riverside (stations 1 B and 2 B)
3. central part of the river (stations 1 C and 2 C).

In paracentral muddy stations of cross-section No. 1 large increases in the number of larvae were observed twice (early part of May and September), and in the respective stations of cross-section No. 2 — only once (in mid September), with a maximal drop in late fall (Fig. 1 and 2). In the paracentral, sandy-muddy (1 B) and sandy-peaty (2 B) stations, as well as in the sandy deposits of the central current of the river (2 C), the numbers of larvae recorded in fall exceeded those observed in spring and summer.

In summer the larval population in various stations of both investigated cross-sections of the Narew River was closely similar (except for stony-sandy stations 1 C). The increases and drops in the numbers of larvae observed in spring and fall in the sandy-muddy stations of cross-section No. 1, as well as in the peaty-sandy and sandy stations of cross-section No. 2 were also nearly similar.

6. STRESZCZENIE

W celu określenia składu gatunkowego i rozmieszczenia bentonicznej fauny Chironomidae na przekroju poprzecznym rzeki Narew próby pobierano obciążonym czerpakiem dna typu Ekman (pow. 225 cm²) na dwóch odcinkach rzeki. Systematycz-

ne badania przeprowadzano w 1968 r. W okresie badań w rzece Narew stwierdzono występowanie 39 gatunków Chironomidae. Wyróżniono trzy grupy stanowisk:

1. od 0–3 m od brzegu (1 A i 2 A),
2. od 3–13 m od brzegu (1 B i 2 B),
3. środek rzeki (1 C i 2 C).

Na mulistych stanowiskach przybrzeżnych przekroju 1 stwierdzono dwukrotnie wysokie wzrosty liczebności larw (pierwsza połowa maja i września), zaś na przekroju 2 — tylko w połowie września, z maksymalnym spadkiem późną jesienią (Fig. 1 i 2). Na piaszczysto-mulistych (1 B) i piaszczysto-torfiastych (2 B) stanowiskach przyśrodkowych oraz w piaszczystych osadach głównego nurtu rzeki (2 C), liczebności jesienne dominują nad wiosennymi i letnimi.

W okresie letnim zasiedlenie larw na różnych stanowiskach przekrojów poprzecznych badanych odcinków rzeki Narew było zbliżone (z wyjątkiem kamienisto-piaszczystych stanowisk 1 C). Zbliżone są również wzrosty i spadki liczebności wiosną i jesienią na piaszczysto-mulistych stanowiskach przekroju 1 i torfiasto-piaszczystych oraz piaszczystych stanowiskach przekroju nr 2.

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A PRELIMINARY STUDY OF FEEDING RATES ON BACTERIAL FOOD BY ADULT FEMALES OF A BENTHIC NEMATODE, *PLECTUS PALUSTRIS* DE MAN 1880

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ABSTRACT

A technique is described for measuring ingestion rates of bacterial-feeding nematode, using radioactively labelled cells as food. Adult females of the freshwater species, *Plectus palustris* de Man 1880, were fed on *Acinetobacter sp.*, homogenously dispersed in a sloppy agar at a density of $5–10 \cdot 10^9$ cells/ml or $1.35–2.69 \cdot 10^{-3}$ g/ml dry weight. Ingestion rates at this food concentration were measured at 20°C and gave a mean value of 5000 cells/min. Assuming continuous feeding, the daily ingestion rate of a female worm was $1.94 \cdot 10^{-6}$ g dry weight and equivalent to 650% of the body weight (1.5 µg wet weight). Applying concurrently measured data on growth and reproduction (Schiemer et al., unpubl.) and on respiration (Klekowski et al., unpubl.), a preliminary daily energy budget for a reproducing female was calculated as follows: ingestion$=9.72 \cdot 10^{-3}$ calories; production$=$ $=0.983 \cdot 10^{-3}$ calories, which consisted mostly of egg production ($0.851 \cdot 10^{-3}$ calories); respiration$=0.219 \cdot 10^{-3}$ calories. Assimilation efficiency was 12%; production was 10% of consumed energy and 82% of assimilated energy.

1. INTRODUCTION

A considerable literature exists on feeding in nematodes, particularly in plant parasitic forms (Bird 1971, Croll 1973). Fewer observations have been made on non-parasitic species and even fewer for those occurring in freshwater benthic substrates. Doncaster (1962) describes the structure and mechanism of food intake for *Rhabditis oxycerca* and *Pelodera lambdiensis* and Mapes (1965) outlines the mode of pharyngeal pumping in three species, *Panagrellus silusiae*, *Aplectana brevicaudata* and *Rhabditis axei*. The only work attempting to measure feeding rates was by Soyza (1970, 1973) who studied *Aphelenchus avenae* which fed on fungal mycelia, using a piercing stylet and sucking out the cell contents by pulsations of the muscular pharynx and metacorpal bulb and valves.

This paper reports on the first attempts to determine ingestion rates of an adult benthic and parthenogenetic nematode, *Plectus palustris* de Man 1880, using radioactively labelled bacterial cells as food.

2. MATERIAL AND METHODS

Stock cultures of Plectus were set up on solid agar to which concentrated bacterial food was added at regular intervals and were kept at 20°C. The worms were in a good state of nutrition and grew into females with ovaries full of eggs. The body size of the adult females used in the feeding runs was not determined but they were young animals with a fresh weight of about 1.5 µg (Schiemer et al., unpubl.).

The bacterial species, *Acinetobacter sp.*, was grown on solid agar made up with organic Collins medium [1] and harvested when one week old. The food medium consisted of a sloppy agar, made up in inorganic Collins medium [1], to which a known number of Acinetobacter cells were added und uniformly dispersed with the aid of a manual glass homogeniser. The procedure of preparing the concentrated bacterial-agar food is illustrated in Fig. 1 A. Bacterial cells, washed

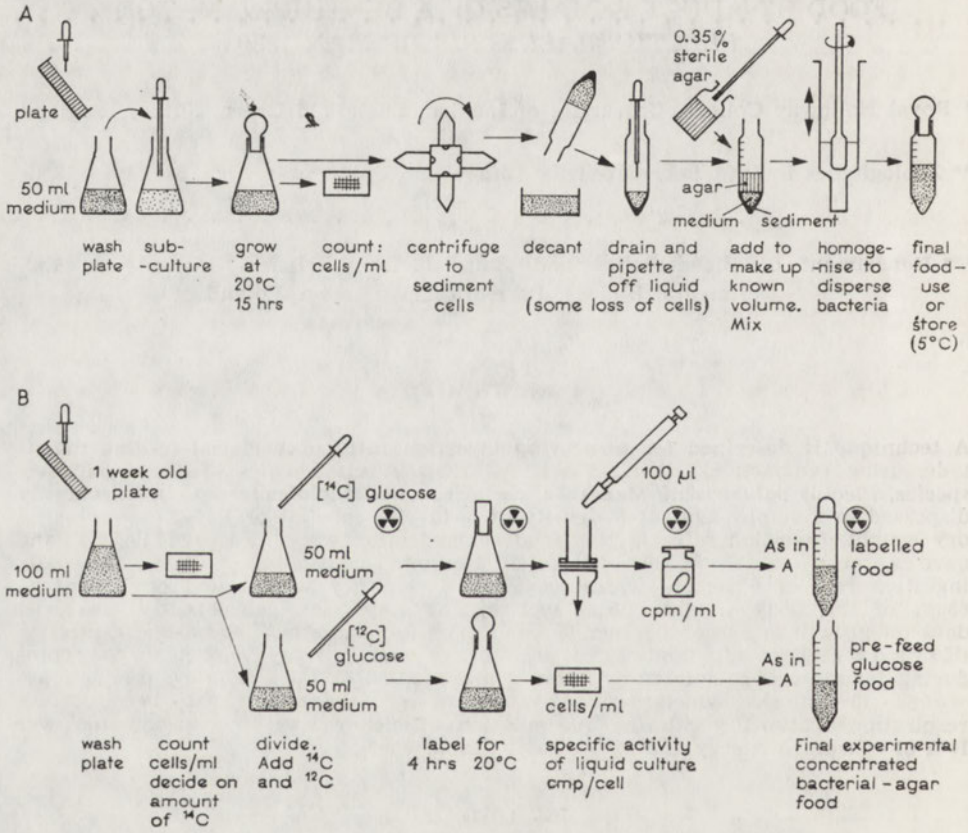

Fig. 1. Preparation of food. A — concentrated bacterial-agar food, B — experimental bacterial-agar food, labelled with [^{14}C] glucose or innoculated with [^{12}C] glucose

off the solid agar, were grown in liquid Collins medium at 20°C. While still in the log phase of growth, the bacterial density was estimated with a Helber chamber to a 95% confidence limit of ± 5.4%. A known volume of this counted liquid was centrifuged and the bacterial sediment re-suspended in 0.35% agar to produce

[1] Collins medium: 0.5 g soluble starch, 0.5 g bactopeptone, 0.5 g casein, 0.2 g K_2HPO_4, 4 drops of 0.01% $FeCl_3 \cdot 6H_2O$, 0.05 g $MgSO_4 \cdot 7H_2O$ made up to one litre with distilled water. Inorganic Collins medium was made up without starch, bactopeptone and casein.

a final density of between 5–10 · 10^9 cells/ml and an agar medium diluted by drainage water to 0.3%. Some loss of cells occurred during the processes of decantation and removal of excess drainage water. Since it proved impossible to check the final cell concentration of the agar food by direct counting, an estimate was obtained for the radioactively labelled food used in the feeding runs from the following equation:

$$\text{number of cells/ml} = \frac{\text{cpm/ml of labelled agar food}}{\text{specific activity as cpm/bacterium}}$$

where the specific activity of the bacterial cells was determined in the liquid medium, at the end of the labelling period and just prior to concentration into the agar, with 95% confidence limits of ± 6%.

Liquid cultures of known bacterial density were labelled with [^{14}C] glucose (specific activity 16.7 μCi per μg glucose). An investigation following the time course of [^{14}C] glucose uptake in various combinations of bacterial densities and glucose concentrations showed that in four hours at 20°C up to 40% of the available glucose was taken up by Acinetobacter cells and that the highest label achieved was 1000–1500 · 10^{-6} cpm/cell. Figure 2 demonstrates that the intensity of label per cell was

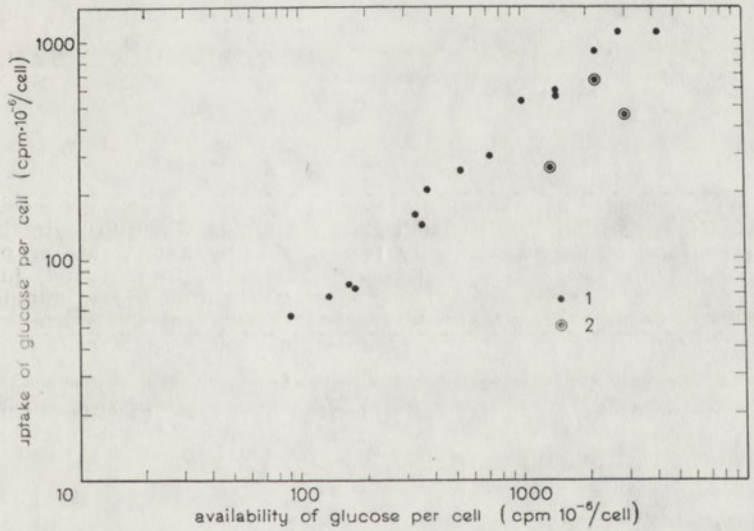

Fig. 2. The relationship between the uptake per cell and the availability per cell of [^{14}C] glucose at 20°C. 1 — 40% uptake, 2 — <40% uptake

directly related to the initial availability of [^{14}C] glucose per cell and that any desired intensity of label per cell could be readily obtained. The procedure of preparing the labelled experimental food is illustrated in Fig. 1 B. The specific radioactivity per cell for a particular run was measured initially in the liquid medium at the end of the labelling period and was monitored in the concentrated agar food throughout the course of the feeding run. The rate of change in cpm/cell of the agar food was relatively slow, being about 2.4% per hour; in runs with large numbers of worms, fresh food was made up every two hours from labelled liquid medium kept at 5°C in which the loss of label due to respiration was negligible, even over 48 hours.

During the first feeding experiments attempted, the worms exhibited a very characteristic "disturbed" movement when placed directly into highly labelled experimental food and started pumping only after some hours. Pre-feeding overnight in similar food to which some [^{12}C] glucose had been added reduced the period of disturbance, suggesting some kind of adaptation to the dissolved glucose levels present. By reducing the specific radioactivity of the cells to

$100 \cdot 10^{-6}$ cpm/cell ($3.4 \cdot 10^{-9}$ µg glucose/cell), the amount of dissolved glucose present in the food medium was kept down to between 2–4 µg/ml. In addition, worms were kept overnight in the same experimental food but with [^{12}C] glucose added in similar amounts as [^{14}C] glucose (see Fig. 1 B).

Worms destined as experimental animals were kept in concentrated food with $5–10 \cdot 10^9$ cells/ml for several days prior to the run. The condition of the food was checked by monitoring the daily egg production of females kept in individual cultures with a daily change of food. The sequence of events during a feeding run is illustrated in Fig. 3. Single nematodes were placed in about 100 µl of

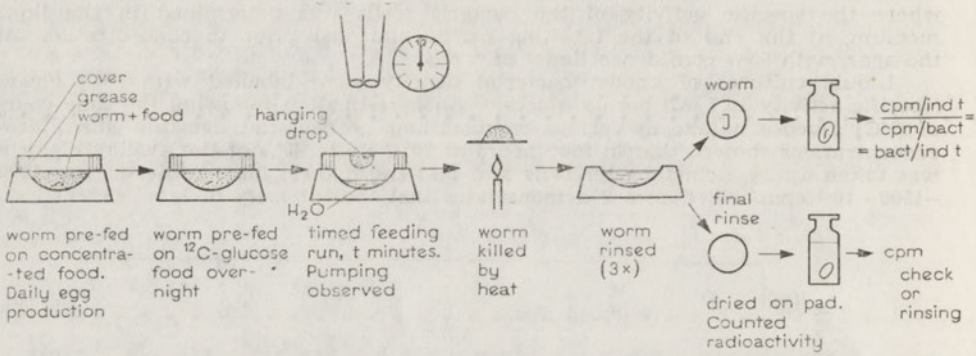

Fig. 3. The sequence of events during a feeding run

labelled food suspended as a hanging drop on the underside of a glass cover which was greased into position over a watch glass containing water to maintain a damp atmosphere. Good feeding was readily recognisable by a fast rate of pharyngeal pumping and bulb movements of between 150–250 pumps/min and, in the experiments reported here (Table I), pumping started within a few minutes of the transfer of the worm. After a timed period of pumping, the worm was killed quickly, by heating the glass cover, in order to avoid loss of cells by vomiting

Table I. Number of Acinetobacter cells ingested by female Plectus during feeding periods of various durations at 20°C (food concentration: $5–10 \cdot 10^9$ cells/ml)

Duration of feeding (min)	2	3	5	10	30
Minutes to start feeding	0–5	1–5	0–5	0.5–6	2–12
10^6 cells ingested per individual (Number of worms given in brackets)	0.011 (10) 0.004* (10)	0.044 (6) 0.007 (5) 0.014 (4)	0.023 (3) 0.036 (3) 0.011 (3) 0.009 (5) 0.011 (5) 0.005* (2)	0.0014* (3) 0.018 (3)	0.059 (3) 0.027 (3) 0.081 (1) 0.025 (1)
Mean per feeding period	0.011	0.022	0.018	0.018	0.048
Coefficient of variation (%)		±86	±66		±56

* worms not pumping continuously.

Mean ingestion rate as cells/minute and standard error = 5019±1354 (±27%) calculated from nine values for feeding periods 2, 3 and 5 minutes.

or by defaecation. The worm was rinsed in three washes of distilled water, placed on a millepore pad along with others, and dried at 60°C. Although worms were handled individually during feeding, radioactive counting had to be done on 2–10 individuals in order to obtain a good detectible count above background. Radioactive counts were carried out in a Panax liquid scientillation counter at 5°C in 10 ml of scintillant containing PPO and dimethyl POPOP in toluene. The efficiency of the counter varied between 77–82%. All counts, including the background, were taken to a standard deviation of ± 3% or less. Low counts, such as those for the nematodes, were repeated several times and those samples that varied more than ± 3% of the mean were rejected.

Worms were allowed to feed for various periods of pumping times ranging from 2–30 minutes, the pumping time being used to define the feeding time t. It was planned to determine the time taken to fill the gut so that, during the period of gut filling and before any appreciable assimilation had taken place, the radioactive content of the body could be used to calculate the number of cells ingested during t from which ingestion rates per minute could be obtained. Thus:

$$\text{number of cells ingested during } t = \frac{\text{mean cpm/nematode}}{\text{specific activity cpm/cell}}$$

This assumes no loss of radioactive material by vomiting or defaecating during killing, no leakage during washing and that no labelled bacteria adhered to the outside of the worms' bodies. Knowing the dry weight of an individual Acinetobacter, the ingestion rates in cell numbers could be converted to gravimetric units. After the initial filling of the gut with radioactive bacteria, the picture becomes more complicated since assimilated [^{14}C] begins to cumulate as body production and some to be respired as $^{14}CO_2$. No attempt was made to measure assimilation rates.

The dry weight of one Acinetobacter cell was calculated from the dry weight of $18.2 \cdot 10^{10}$ cells, obtained by drying to constant weight first in an oven at 60°C and then in a desiccator over silica gel at room temperature. The total dry weight was 0.049 g, giving an individual weight of $0.269 \cdot 10^{-12}$ g.

3. RESULTS

Table I gives the number of cells ingested during various feeding periods by well-fed female Plectus offered good food on which an average of 18 eggs/day could be produced and containing $5–10 \cdot 10^9$ Acinetobacter cells/ml. Conditions were good since the worms started feeding within a few minutes. These are mean values for the number of worms indicated in brackets after each rate. The variability of the results is demonstrated by the large coefficient of variation given as a percentage of the mean for feeding times 3, 5 and 30 minutes and contrasts with the combined error of ± 6.5% involved in estimating the specific activity of the bacterial cells and the radioactive content of the nematodes.

From Table I, it appears that the number of cells ingested during 3 to 10 minutes is similar and double the number ingested in 2 minutes. This is taken to mean that the time required to fill the gut lies somewhere between 3 and 10 min whereas the higher value obtained after a 30 min feed represents the cells contained in a full gut plus the cellular equivalent of the ^{14}C assimilated into the nematode body during the feeding period and not yet respired as $^{14}CO_2$. There is some supporting evidence for a short gut-filling time in Plectus. Observations on the interval of time between defaecations by a well-fed female feeding continuously

on the same food gave the following times: 3 min 4 sec, 2 min 45 sec, 3 min 18 sec, 2 min 41 sec, 2 min 53 sec, 3 min 7 sec — mean 2 min 5.8 sec. This suggests that the time to fill Plectus gut lies somewhere between 3 and 5 min.

On this basis, ingestion rates per minute feeding were calculated from the nine good values given for feeding periods of 2, 3 and 5 minutes in Table I and gave a mean value of 5000 cells/min. Observations of worms in good food at different periods of day or night suggests that Plectus pumps continuously under normal conditions. Thus assuming continuous feeding throughout 24 hours, the daily food intake is about $7.23 \cdot 10^6$ cells or $1.94 \cdot 10^{-6}$ g dry weight. This represents a daily ingestion of 650% of the body weight of a young female whose wet weight is 1.5 µg (Schiemer et al. unpubl.) and assuming the dry weight: wet weight ratio is 20% (Rainbow 1971). Further assuming that the calorific value of Acinetobacter cells is 5000 calories/g dry weight, the daily food energy intake is $9.72 \cdot 10^{-3}$ calories.

Whilst the ingestion of Plectus was being studied, work was also being done on its growth (Schiemer et al. unpubl.) and respiration (Klekowski et al. unpubl.) using animals from the same culture and fed on the same food. This provides an opportunity to calculate a preliminary daily energy budget for a young female Plectus producing 18 eggs/day. Table II gives values for the daily production and daily respiration in

Table II. Preliminary daily energy budget for a female Plectus, compared with daily budgets for adult *Aphelenchus avenae* (Nematoda) and *Brachionus plicatilis* (Rotifera)

	Plectus palustris	*Aphelenchus avenae*	*Brachionus plicatilis*
Temperature	20°C	26°C	20°C
Body size of female (µg)	1.5 (wet)	2.0 (wet)	0.158 (dry)
Food energy (cal/ml)	6.7–13.5	88	4.36
Volume grazed (µl/day)	0.72–1.44	$5.88 \cdot 10^{-3}$	24
Daily ingestion (% body wt.)	650	26	1000
Ingestion *C* (10^{-3} cal/day) (± SE of mean)	9.72 ±27%	0.52	7.99±28%
Assimilation *A* (10^{-3} cal/day)	(1.202)*	(1.185)*	1.539
Respiration *R* (10^{-3} cal/day)	0.219	0.394	0.634
Production *P* (10^{-3} cal/day)			
body growth	0.132	0.405	0
egg production	0.851	0.387	0.888
Efficiencies: *A/C* (%)	12	—	19
P/C (%)	10	—	11
P/A (%)	82	67	58
R/A (%)	18	33	41
Authors	present authors	Soyza (1973)	Doohan (1973)

( )* summed $P + R$.

calories for Plectus, which were derived as follows. Body growth is based upon a daily instantaneous growth rate of 0.08 (Schiemer personal communication) applied to a body wet weight of 1.5 µg and egg production upon the mean wet weight of a single egg being 0.043 µg. The dry weight: wet weight ratio for eggs is also taken to be 20% and the calorific value for both bodies and eggs as 5500 calories/g dry weight (Soyza 1973). The respiration of a young female at 20°C was measured as $1.9 \cdot 10^{-3}$ µl oxygen/hr and converted to energy liberated using Ivlev's oxycalorific coefficient of $4.8 \cdot 10^{-3}$ calories/µl oxygen. It is clear from Table II that the daily ingestion rate of $9.72 \cdot 10^{-3}$ calories is greatly in excess of the daily energy requirements to cover production of $0.983 \cdot 10^{-3}$ calories plus respiration of $0.219 \cdot 10^{-3}$ calories. Assimilated energy forms only 12% of the consumed energy and most of that goes into production (10%) and especially into egg production (8.8%). Thus, as much as 82% of the assimilated energy is channelled into production. Daily production is equivalent to as much as 60% of the body weight and daily respiration only 13%.

From the same basic data on production and respiration, it is possible to calculate how much energy is assimilated, minute by minute, during the time course of a feeding run. This may indicate after how many minutes of feeding that assimilated radiocarbon becomes an appreciable proportion of the measured body radiocarbon. Assuming instantaneous digestion and assimilation, which is not likely, the percentage of the consumed energy which is assimilated after feeding for 2, 3, 5, 10 and 30 minutes is 11.6, 8.7, 17.8, 35 and 39% respectively. This suggests that the calculated mean ingestion rate may be over-estimated by 10% or less but not more.

4. DISCUSSION

In *Aphelenchus avenae*, Soyza (1970, 1973) calculated the quantity of food ingested daily from the volume taken in per day and the calorific value per unit volume of food material. The volume taken in was estimated from the volume of the open valve, the number of pulsations of the valve per second and the time spent feeding during each day. As is shown in Table II, a nine-day old female ingested $5.88 \cdot 10^{-3}$ µl/day of food material containing $0.52 \cdot 10^{-3}$ calories/day (Soyza 1973). This is a daily ingestion that is only 26% of the body calories and is one order of magnitude lower than the value obtained for a female Plectus (Table II). Other small Aschleminthes, such as adult *Brachionus plicatilis* have daily ingestion rates (1000%) which are as high as those of Plectus when fed on an excess of a flagellate alga (Doohan 1973) and Pilarska (1971) records minimal and maximal feeding rates of 58 and 250% for *Brachionus rubens*, another rotifer of similar size. Much larger ani-

mals such as daphnids (*Daphnia magna* 120 µg dry weight) only manage to ingest daily 56% of the body weight under good food conditions (Duncan unpubl.). As can be seen in Table II, the volume grazed per day is also very low in Aphelenchus compared with Plectus and Brachionus so that, despite the higher food calories/ml available, the quantity of food ingested clearly does not cover the animal's daily energy requirements, as Soyza (1973) herself points out.

The assimilation efficiency of 12% for Plectus adults is very low but this may reflect surplus feeding in high food concentrations as well as the relative indigestibility of eubacterial cells with their somewhat thick, rigid cell walls. Moreover, the short gut-filling time and frequency of defaecation suggests a passage of particles through the gut which may be too rapid for effective digestion. Crofton (1966) notes that the frequency of defaecation is high in most of the nematodes investigated. Bastian (1966 in Bird 1971) records 4–5 minute intervals for Dorylaimus, Crofton (1966) records 3 minute intervals for Ascaris, accompanied by an almost complete emptying of the intestine but Mapes (1965) gives an interval of 26–33 minutes for *Panagrellus silusiae*. However, since the frequency of defaecation is directly related to the rate of pumping and the animal defaecates when the pumping pressure is unable to overcome the pressure within the intestine (Crofton 1966), intervals between defaecations are likely to vary with feeding rates. It is not known what proportion of Plectus intestinal contents are egested during defaecation but any digestive enzymes have only short time for action.

Only 10% of the food consumed was utilized as production which is clearly related to the low assimilation efficiency shown by Plectus because a very high 82% of assimilated energy is converted into production. Most of this goes into the production of eggs, since the adult body growth had slowed down to a daily instantaneous growth rate of only 0.08 compared with a larval growth rate of 0.42 (Schiemer et al. in prep.). Furthermore, provided with a daily change of concentrated food, female Plectus are capable of sustaining this level of reproductive production for up to six weeks (Schiemer et al. in prep.). Table II shows that the net production efficiencies are reasonably high in Aphelenchus and Brachionus and in the latter species this is entirely due to egg production.

It is interesting that, when provided with food of similar calorific content, the absolute values for daily ingestion, assimilation and egg production at 20°C are very similar for adult Plectus and Brachionus, both Aschelminthes of similar body size. The main differences are that Brachionus has a higher assimilation efficiency, no body growth and required more than double the respiratory energy to cover maintenance costs. It is tempting to relate this to the high cost of continuous ciliary

activity required for Brachionus feeding and locomotion (Doohan 1973 and in print) but Plectus adults also seem to pump continuously and this involves muscular work. However, the cause of higher respiration may be elsewhere. The ovigerous female Brachionus, used for the budget, had respiratory rates ($5.46 \cdot 10^{-3}$ µl/ind. · hr) double those for non-ovigerous adults ($2.66 \cdot 10^{-3}$ µl/ind. · hr) whose respiration approaches more nearly that of Plectus females ($1.9 \cdot 10^{-3}$ µl/ind. · hr). Of course, the non-ovigerous females will possess an ovary with developing oocytes but the "eggs", borne on the outside of the ovigerous female's body, represent separate developing individuals and are no longer dependent upon her for food. It would therefore seem erroneous to include their respiratory costs with those of the adult female. In this case, the daily respiration of a non-ovigerous Brachionus is $0.306 \cdot 10^{-3}$ calories, which is still slightly higher than for a similar Plectus female also containing active ovaries. The new daily assimilation is now $1.194 \cdot 10^{-3}$ calories, a value very similar to those of both Plectus and Aphelenchus. The new efficiencies are also more similar to those for Plectus: assimilation efficiency — 15%; net production efficiency (P/A) — 74% and R/A is 26%. These three aschelminth species are revealing very similar patterns of energy conversion.

It is interesting to speculate whether the ingested bacterial cells are concentrated during the pumping process, as is suggested by Doncaster (1962). The measured number of cells ingested in one pump is 25, calculated from an ingestion rate of 5000 cells/min and a pumping rate of 200 pumps/min. Without any concentration, the expected number of cells per pump is 67 (45—90), which is the number of cells contained in a volume of food ($5—10 \cdot 10^9$ cells/ml) equivalent to the volume of the open pharyngeal lumen. This is about $0.009 \cdot 10^{-3}$ µl, measured from a film still of one feeding female. This suggests that the food was not concentrated but that some loss of cells may occur during pumping.

The concentration of Acinetobacter used in this study appears to be a realistic one, judging by the densities recorded in the surface mm of bottom lake mud by Karsinkin, Kusnetzov (1931) and Kusnetzov (1935) (cited in Henrici, McCoy 1938). In both cases, direct counts gave bacterial numbers within 10^9 per ml.

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netobacter from Neusiedlersee bottom mud and of Mr. N. Hurley for preparation of media.

5. SUMMARY

Adult females of *Plectus palustris* were fed on bacterial food containing $5–10 \cdot 10^9$ cells/ml or $1.35–2.69 \cdot 10^{-3}$ g dry weight/ml. The mean ingestion rate was 5000 cells/min. The daily ingestion rate came to $1.94 \cdot 10^{-6}$ g dry weight and was equivalent to 650% of the body weight. A preliminary daily energy budget for a reproducing female was calculated and gave the following values: ingestion — $9.72 \cdot 10^{-3}$ cal, production — $0.983 \cdot 10^{-3}$ cal, respiration — $0.219 \cdot 10^{-3}$ cal. The assimilation efficiency was 12%. Production was 10% of the consumed energy and 82% of the assimilated energy.

6. ZUSAMMENFASSUNG

Mit Hilfe ^{14}C markierter Bakterienzellen wurde die Nahrungsaufnahmerate von adulten Weibchen von *Plectus palustris* bestimmt. Die Bakterienkonzentration des Nahrungsmediums betrug $5–10 \cdot 10^9$ Zellen/ml bzw. $1,35–2,69 \cdot 10^{-3}$ g Trockengewicht/ml.

Die durchschnittliche Nahrungsaufnahmerate betrug 5000 Zellen pro Minute. Dies entspricht $1,94 \cdot 10^{-6}$ g Trockengewicht/Tag bzw. 650% des Körpergewichtes.

Ein Energiebudget für reproduzierende Weibchen unter den gegebenen Nahrungsbedingungen ergab folgende Werte:

Nahrungsaufnahme pro Tag: $9,72 \cdot 10^{-3}$ cal,

Produktion: $0,983 \cdot 10^{-3}$ cal,

Respiration: $0,219 \cdot 10^{-3}$ cal.

Das bedeutet, dass unter den gegebenen experimentellen Bedingungen nur 12% der augenommenen Nahrungsenergie assimiliert werden. Die Produktion an Köppergewebe und Eiern beträgt 10% der aufgenommenen Energiemengen bzw. 82% der assimilierten Energiemenge.

7. STRESZCZENIE

Dorosłe samice nicienia *Plectus palustris* karmiono bakteriami znakowanymi za pomocą ^{14}C, przy koncentracji pokarmu $5–10 \cdot 10^9$ komórek/ml czyli $1,35–2,69 \cdot 10^{-3}$ g such.m./ml. Średnia szybkość pobierania pokarmu wynosiła 5000 komórek/min. Średnia dobowa racja pokarmowa wynosiła $1,94 \cdot 10^{-6}$ g such.m., albo 650% wagi ciała. Obliczono dobowy bilans energetyczny rozmnażającej się samicy: konsumpcja $-9,72 \cdot 10^{-3}$ cal, produkcja $-0,983 \cdot 10^{-3}$ cal, respiracja $-0,219 \cdot 10^{-3}$ cal, wydajność asymilacji pokarmu —12%; produkcja stanowiła 16% energii skonsumowanej w pokarmie i 82% energii zasymilowanej z pokarmu.

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CHEMICAL COMPOSITION OF THE ANTARCTIC AMPHIPODA *PARAMOERA WALKERI* STEBBING AND CHROMATOGRAPHIC ANALYSIS OF ITS LIPIDS

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ABSTRACT

Material for analysis was collected at the Soviet Antarctic Station Molodezhnaya in winter 1969 and summer 1972/1973. Lyophylized samples of *P. walkeri* have been analysed to determine the ash, chitin, lipids, and proteins content and also tested by burning in the microbomb calorimeter. Differences were found between the two generations of *P. walkeri* under examination. The observed variations in the components of each generation were slightly different depending on feeding conditions in the environment and the reproduction period. The composition of lipids in *P. walkeri* was the same regardless of differences in their age and the time of sample collection.

1. INTRODUCTION

Littlepage (1964) has observed seasonal changes in the lipids content in the Antarctic Copepoda and Euphausiacea species depending on the amount of food in the environment and the life cycle of the species. Pearse, Giese (1966) have analysed the content of water, lipids, proteins and carbohydrates in the body of the Antarctic benthic invertebrates. They have not found any differences as compared with the invertebrates inhabiting temperate zones, they suggest merely that the Crustacea living in cold waters may have a slightly greater amount of lipids than the other ones. The compiled results (Mauchline, Fisher (1969) from the chemical analyses of Euphausiacea found in cold and warm waters have not confirmed the above mentioned suggestion.

The object of the present study was the analysis of the chemical composition and calorific values of *Paramoera walkeri* Stebbing in various stages of the species life cycle which had been determined in the course of the studies on its biology and metabolism (Rakusa-Suszczewski 1872, Klekowski et al. 1973, Rakusa-Suszczewski, Klekowski 1973). In connection with the studies on the Antarctic fish feeding habits (Rakusa-Suszczewski, Piasek 1973) carried out simultaneously it has been necessary to determine the composition of their food, as well.

2. MATERIAL AND METHODS

The research was carried on at the Soviet Antarctic Station Molodezhnaya (67°49'S, 45°50'E). The Amphipoda *Paramoera walkeri* caught in the littoral zone

were divided into groups according to their size corresponding to various age of the examined population. Live crustaceans were homogenized and lyophylized either in a lyophylizator (10 samples collected between May and November 1969) or in a vacuum desiccator with P_2O_5 (3 samples collected in January 1972). Lyophylized samples prepared in 1969 were kept for about three years partly at the room temperature partly in dry ice. The remaining material was stored at about −20°C. Analyses were carried out according to Giese (1967) and Dowgiałło (in press) with some modifications. To determine the dry weight, samples of lyophylized material were dried first in a desiccator with $CaCl_2$, then in an oven at 60°C to constant weight. The ash content of a sample was established by igniting it to constant weight in an oven at about 500°C. For chemical analyses the material dried at 60°C was used. Total nitrogen was determined by the Kjeldahl procedure. For determination of chitin nitrogen another sample was used after the protein was removed from it by digesting it twice in 3n NaOH at 100°C for 20 minutes and contrifuging. Lipid nitrogen was determined in only one sample of the previously extracted lipids, by the Kjeldahl method. The level of lipids was established in samples of about 50 mg of dried material. These were extracted exhaustively with a mixture of 2 volumes of chloroform and 1 volume of methanol at 60°C and filtered through a degreased pad of cotton wool. The extracts were evaporated in a water bath, dried in a vacuum desiccator with KOH pellets. The residue was re-extracted with a mixture of equal volumes of chloroform and light petroleum ether, and filtered through cotton wool into tared thin wall flasks. The solvents were let to evaporate in to the air, the residue was dried to constant weight in a vacuum desiccator with solid KOH and weighed. The protein content was computed by multiplying the content of alkali soluble non-lipid nitrogen obtained from the difference between total nitrogen and lipid and chitin nitrogen by the 6.25 factor. The chitin was calculated by multiplying the chitin nitrogen level by the 14.5 factor. The hexose carbohydrates were determined only in one sample (Sept. 14, 1969). A sample of dry material was hydrolyzed with 1n H_2SO_4 at 100°C for 30 min, refilled with water up to 10 ml and centrifuged. The carbohydrates were determined in the supernatant using the anthrone colour reaction. Determination of the sum of hexose carbohydrates was carried out by suspending the sample in 2 ml of distilled water and 10 ml of anthrone solution in concentrated H_2SO_4 (d=1.78). From another sample a pigment blank was prepared by adding 2 ml of distilled water and 10 ml of H_2SO_4 of the same concentration. Both samples were heated at 100°C for 5 minutes and read at 610 nm against water. The reading for the sample was corrected for both pigment blank and reagent blank. Both methods of carbohydrate determination gave an identical result. The calorific value was determined by combustion of the material in the Phillipson type microbomb calorimeter. Mean values are given from of 3–5 analyses of each material.

A thin-layer chromatography of the extracts of lipids has been also performed. The 8.5 • 8.5 glass plates were covered with G-silica gel (Merck, Darmstadt, Germany), which immediately before the use was activated during 30 min at 100°C. Neutral lipids were separated by using solvent system — diethyl ether : light petroleum : glacial acetic acid (6.0 : 4.0 : 0.2 v/v) (Mangold, Malins 1960). Phospholipids were separated in solvent system — chloroform : methanol : water (6.5 : 2.5 : 0.4 v/v) (Wagner et al. 1961). Spots of lipids on the chromatogram were produced by the use of either the 10% phosphomolybdic acid in ethanol at 80°C or the ammonium sulphate solution (Zimiński, Borowski 1966) at about 170°C.

3. RESULTS AND DISCUSSION

Chemical analyses were performed on individuals of only two out of the three generations occurring in the *P. walkeri* population in the period between May and January. The chemical composition of individuals hatching in the spring was not analysed. The content of dry weight in the *P. walkeri* body was examined only once in the spring 1969 (Rakusa-Suszczewski 1972). As it has been observed, the relation between the length and dry weight of the body was expressed in the

double logarithmic plot by the formula $W = 0.00166 \cdot L^{2.80}$, and the relation between the length and the wet weight of the body was described by the formula $W = 0.0778 \cdot L^{3.13}$; where W — body weight (mg), L — body length (mm). In individuals of the younger generation dry weight amounted to 27% and in females with eggs to 23% of the wet body weight.

The content of chitin ranged from 5.0 to 9.7% and of ash from 22.2 to 31.3% of the dry body weight and showed differences between the

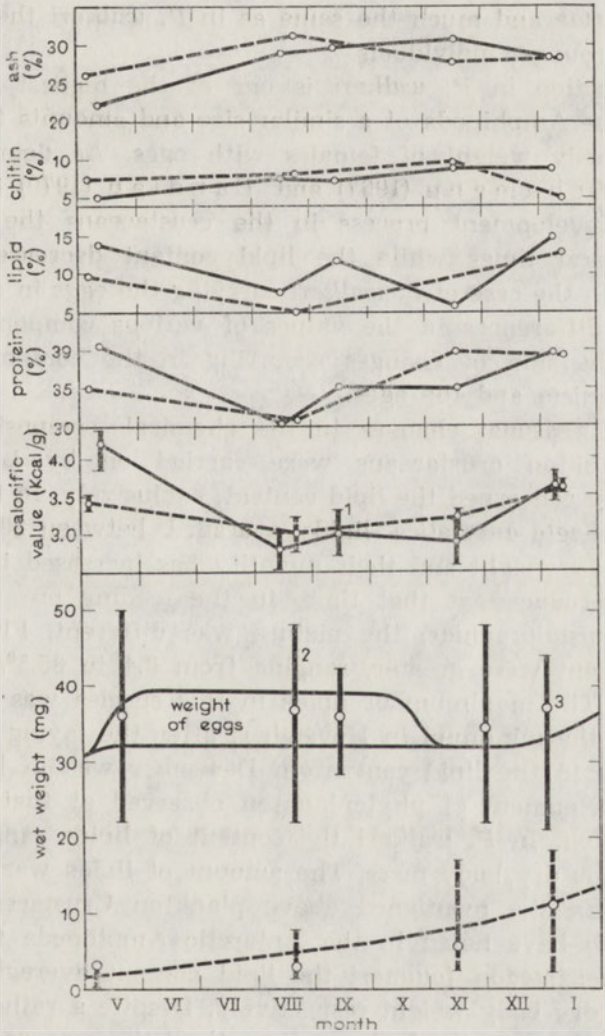

Fig. 1. Changes of chemical composition and calorific value of *Paramoera walkeri* Stebbing in the life cycle, in per cent of dry weight. Continuous line — older generation comprising only females, dotted line — younger generation. 1 — standard deviation, 2 — individual wet weight distribution, 3 — mean wet weight of individuals taken for analysis. Data for the individual wet weight distribution from Rakusa-Suszczewski (1972) and Rakusa-Suszczewski, Klekowski (1973)

two generations under examination Fig. 1). In the period of time from May to September the amount of ash and chitin was greater in the individuals of the younger generation. In the summer the situation changes, there is less of chitin in the younger individuals which corresponds with their quicker growth at that time and consequently a more frequent moulting. The ash content in *P. walkeri* as compared with the plankton crustaceans (Raymont et al. 1964) is considerably higher. Sushchenya, Abolmasova (1968) have obtained approximate values of the ash content (18.5—25.7%) in the Black Sea Amphipoda *Orchaestia bottae* and much the same as in *P. walkeri* there was more of ash in the younger individuals.

Egg production in *P. walkeri* is one of the highest among those observed in the Amphipoda of a similar size and amounts to about 18% of the wet body weight of females with eggs. As demonstrated by Pandian, Schumann (1967) and Pandian (1970) in the course of the egg development process in the crustaceans the ash content increases several times while the lipid content decreases nearly by a half. Thus, in the case of *P. walkeri* carrying the eggs in a marsupium, the seasonal differences in the values of various components are expressed by the sum of changes occurring in the composition of the maternal organism and the eggs.

Studies of seasonal changes in the chemical composition of the Antarctic plankton crustaceans were carried on by Littlepage (1964) but they concerned the lipid content, exclusively. In the predatory Copepoda *Euchaeta antarctica* lipids averaged between 28.1 and 46.1% of the dry body weight and their quantity has increased in winter due to the eggs produced at that time. In the feeding on phytoplankton (*Euphausia crystalorophias*) the picture was different. Fluctuations in the lipid content were greater, ranging from 9.4 to 35.5% of the dry body weight. The maximum of lipids in this species was noted in the early winter, the minimum in November, after the laying of eggs. The rapid increase in the lipid content in December was in line with the abundant development of phytoplankton observed at that time in the McMurdo region. In *P. walkeri* the content of lipids ranged from 4.8 to 14.8% of the dry body mass. The amount of lipids was considerably smaller than in the mentioned above plankton Crustacea. Pearse, Giese (1966) have noted in the Antarctic Amphipoda *Orchomonella plebs* and *Cheirimedon fougneri* the lipid content averaging 53% and 35% of their dry body weight respectively. Despite a rather small content of lipids found in the body of *P. walkeri* the seasonal fluctuations were relatively great and slightly variant in the two examined generations (Fig. 1). The amount of lipids in females is greater than in the younger generation. The highest values were noted in the summer to the late autumn. In winter the lipid content decreases. Simultaneously with the

development of the sub-ice microflora feeding conditions become considerably more favourable for the *P. walkeri* population, the content of lipids increases and the body weight of individuals increases, likewise (Rakusa-Suszczewski 1972). A repeated decrease in the amount of lipids and the lowest values in body of females were observed after the end of reproduction period and getting rid of the offsprings from the marsupium which occurs in November. Later on the lipid content in the body increases anew quicker in females, and in January it is again higher in them than in individuals of the younger generation.

The protein content in the body of *P. walkeri* was in the range of 30.6 to 39.5%. In the Antarctic benthic invertebrates protein constitutes, according to Pearse, Giese (1966), on the average 50% of the dry body mass. The values denoted in this study are distinctly lower. In the period between May and August the amount of protein decreases in both generations of the *P. walkeri* population. This decrease is greater in the females, which may come in result of their drawing energy from the protein in a higher degree than the younger individuals, due to the fact that their energetic reserves stored in the lipids had passed over into the earlier produced eggs. Food increase in the environment is followed by the protein content increase in the body of both generations. Release of the hatched offsprings from the mersupium has an unimportant effect on the decrease of protein content in the body of the females.

The content of carbohydrates in the examined sample containing females with eggs was rather low averaging 1.2% of the dry body weight.

The respective components in the body of the examined crustaceans do not sum up to 100%. In the subsequent analyses performed on a series of sample containing individuals from the younger generation only, 78.2, 74.7, 85.3 and 84.4% were obtained, for females — 80.5, 74.2, 83.9, 80.6 and 89.9%, respectively. This may be caused by the following reasons: 1. losses resulting from deamination of proteins; 2. losses resulting from oxidation of lipids and their incomplete solubility which probably is regained in the total calorific values after the burning of material in the microbomb calorimeter; 3. Hydration of mineral salts which in consequence leads to the removal of water when the ash content is determined at the temperature of 500°C. This water however is not lost by the samples when dried at the temperature of 60°C; 4. lack of the determination of carbohydrates and fragments of glycoproteins not-reacting with anthrone.

In a general way, the picture of changes in the chemical composition of the examined generation of *P. walkeri* is confirmed by the results obtained from the material burned in the microbomb calorimeter. Calorific value in both generations of *P. walkeri* ranges from 2.8 to 4.2 Kcal/g of the dry weight together with ash (Fig. 1). Greater seasonal changes occur in the females. The lowest calorific value in both ge-

nerations was noted in August. In the species with similar ash content to that in *P. walkeri*, such as Amphipoda *Orchestia bottae* and Isopoda *Asellus aquaticus*, calorific value was 3.8—4.4 Kcal/g (Sushchenya, Abolmasova 1968) and 2.9 Kcal/g (Prus 1972) respectively. Thus, by comparing its chemical composition and calorific value one can infer that *P. walkeri* makes a more valuable food for the benthic fish *Trematomus bernacchii*, which feeds on it during the summer season, than for *T. newnesi* and *T. borchgrevinki* living beneath the ice and feeding also on *P. walkeri* during the winter season (Rakusa-Suszczewski, Piasek 1973).

As results from the chromatographs presented in Fig. 2, the greater part of the lipids in *P. walkeri* consists of phospholipids which include mainly acidic phospholipids (Fig. 2 B). There occurs also a very small

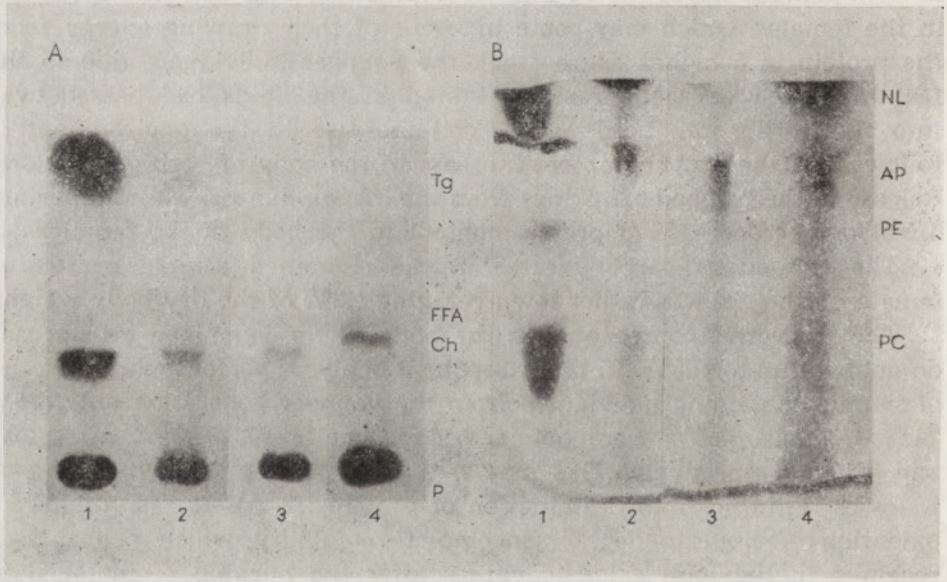

Fig. 2. Thin-layer chromatograms of *P. walkeri*. Glass plate (8.5×8.5 cm) covered with silica gel G. Solvent system: A — petroleum ether : diethyl ether : glacial acetic acid (6.0 : 4.0 : 0.2, by vol.), B — chloroform : methanol : H_2O (6.5 : 2.5 : 0.4, by vol.). NL — neutral lipids, AP — acidic phospholipids, PE — phosphatidyl ethanolamine, PC — phosphatidyl choline, Tg — triglycerides, FFA — free fatty acids, Ch — cholesterol, P — phospholipids. 1 — hen egg yolk, 2-4 — *P. walkeri*

amount of phosphatidylcholine and phosphatidylethanolamine. Neutral lipids contain mainly cholesterol and tryglicerides. There is only a trace of free fatty acids (Fig. 2 A). The fact that acidic phospholipids are the most important component of the phospholipids found in *P. walkeri* may indicate their essential role in the metabolic processes of the phospholipids (Murray, Magee 1972).

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We wish to express our deep gratitude to Mrs. H. Wątkowska for her valuable help. We are especially indebted to Dr. A. Dowgiałło for his advice and suggestions and to Mrs. H. Cybińska for the chromatographic analyses of lipids.

4. SUMMARY

The material was collected at the Soviet Antarctic station Molodezhnaya, in winter 1969 and summer 1972. Studies were carried on the Amphipoda *Paramoera walkeri* Stebbing. The samples of crustaceans were divided into two generations and after homogenization they were lyophylized. Chemical composition and calorific value were analysed in various periods of *P. walkeri* life cycle. Chromatography of lipids was also performed. The obtained results showed that the dry body weight content of ash ranged from 22.2 to 31.0%, chitin: 5.0–9.7%, lipids: 4.8–14.8%, protein: 30.6–39.5%. The carbohydrates content was examined only once, in females with eggs it was 1.2%. The calorific value ranged from 2.8 to 4.2 Kcal/g of dry body weight with ash. Seasonal changes in chemical composition depended on feeding conditions in the environment, age of generation and its reproduction time. The greater part of the lipids in *P. walkeri* consists of phospholipids which include mainly acidic phospholipids.

5. STRESZCZENIE

Materiały zebrano na radzieckiej stacji Mołodiożnaja w czasie zimy 1969 roku i lata 1972 roku. Obiektem badań były Amphipoda *Paramoera walkeri* Stebbing. Zebrane skorupiaki rozdzielano na dwie generacje, które badano oddzielnie. Próbki homogenizowano i poddawano liofilizacji. Analizowano skład chemiczny i kaloryczność *P. walkeri* w różnych okresach cyklu życiowego gatunku. Wykonano również analizę chromatograficzną lipidów. Rezultaty wykazały, że zawartość popiołu w suchej masie ciała wahała się od 22.2 do 31.0%, chityny od 5 do 9.7%, lipidów od 4.8 do 14.8%, białka od 30.6 do 39.5%. Zawartość węglowodanów zbadano w jednej próbie obejmującej samice noszące jaja; stanowiły one 1.2% suchej masy ciała. Kaloryczność w przeliczeniu na suchą masę ciała z popiołem wynosiła od 2.8 do 4.2 Kcal/g. Sezonowe różnice zawartości poszczególnych składników i kaloryczności ciała *P. walkeri* związane były z warunkami pokarmowymi w środowisku, wiekiem osobników i okresem reprodukcji gatunku. Główną część lipidów *P. walkeri* stanowią fosfolipidy, które zawierają głównie fosfolipidy kwaśne.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	2	269-273	1974

MARIA WIERZBICKA

HAEMOLYMPH CONCENTRATION IN CYCLOPOIDA COPEPODIDS DURING ACTIVE AND RESTING STAGE AND THE EFFECT OF 2,4-D SODIUM SALT

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ABSTRACT

The Δ°C value in Cyclopoida copepodids collected from plankton in their active stage was 0.62 whereas in copepodids awakened from their resting stage in the bottom sediments containing H_2S the Δ°C value was much lower, ranging from 0.24 to 0.28. The 2,4-D sodium salt herbicide caused an increase in Δ°C value in the latter up to 0.43–0.49, nevertheless, no death or locomotric disturbances cases were observed.

1. INTRODUCTION

Life cycle of Cyclopoida consists of two phase occurring in radically divergent environmental conditions. Copepodids IV and V (in a period characteristic for given species) give up their active life in plankton, dig themselves into bottom sediments of the water body, and go into resting stage lasting a few months (Birge, Juday 1908, Elgmork 1955, Wierzbicka 1962). This stage is characterized by torpidity, decreased metabolism, and accumulation of metabolic products in the alimentary tract, due to the plugging of intestine serving for excretion in the active life of Cyclopoida (Wierzbicka 1966, 1972 b).

The aim of this study was to ascertain whether in those extreme conditions of life and following changes in metabolism of organisms there are also occurring some osmotic changes in the body fluid of copepodids.

2. MATERIAL AND METHODS

The following species were under investigation: *Cyclops vicinus vicinus* Ulj., *Cyclops vicinus kikuchii* Smirn. and *Cyclops bohater* Kożm. The examined copepodids were sampled from plankton and bottom sediments of a clay-pit in Warsaw, containing sulphuretted hydrogen, between April and September (Wierzbicka, Kędzierski 1964). In the following years, in the samples from bottom sediments collected in the first half of October, copepodids were still found in the atmosphere containing sulphuretted hydrogen. Plankton material was sampled about a month before copepodids started to penetrate into the mud and again just before their resting stage (May 9 and 21). The mud samples were collected with an adequately loaded bolton-cloth net dragged along the bottom of the water body. In the laboratory copepodids awakened in the aerated water environment. The procedure of sampling bottom sediments and awakening of copepodids from their resting stage has been described in previous papers of the author (Wierzbicka 1966, 1972 b). Copepodids from bottom sediments of the clay-pit were awakened at the begin-

Table I. Osmotic changes (Δ°C) of the body fluid of the active copepodids Cyclopoida (from plankton) and at their resting stage (from mud)

Species	Environment	Date of sampling	Number of specimens	Δ°C (mean ± S. D.)
C. bohater V	Clay-pit plankton before resting stage	21.V.1971 9.V.1972	128	0.62 ± 0.033
C. vicinus kikuchii V	Clay-pit plankton before resting stage	21.V.1971	13	0.62 ± 0.023
C. vicinus kikuchii V	Clay-pit mud resting stage	6.VI.1972	41	0.28 ± 0.034
C. vicinus vicinus IV	Clay-pit mud resting stage	6.VI.1972	16	0.25 ± 0.029
C. vicinus vicinus IV	Clay-pit mud resting stage	28.VII.1972 3.X.1972	56	0.24 ± 0.032
C. vicinus vicinus IV	Clay-pit mud resting stage	28.VII.1972 3.X.1972	56	0.24 ± 0.032 *
C. vicinus vicinus IV	Powsińskie Lake plankton	17.V.1972	8	0.55 ± 0.033 *

* Differences highly significant, $p<0.001$.

Table II. Effect of 2,4-D sodium salt on osmotic values (Δ°C) of the body fluid in copepodids Cyclopoida taken at their resting stage from mud in a clay-pit in Warsaw

Species	Environment	Date of sampling	Number of specimens		Δ°C (mean±S. D.)		Significance p
			Treated	Control	Treated	Control	
C. vicinus kikuchii V	Clay-pit mud resting stage	6. VI. 1972	31	41	0.49±0.031	0.28±0.034	<0.001
C. vicinus vicinus IV	Clay-pit mud resting stage	6. VI. 1972	20	16	0.49±0.026	0.25±0.029	<0.001
C. vicinus vicinus IV	Clay-pit mud resting stage	3. X. 1972	17	6	0.43±0.053	0.26±0.022	<0.001
C. vicinus vicinus IV	Clay-pit mud resting stage	3. X. 1972	17	56	0.43±0.053	0.24±0.032	<0.001

ning of their resting stage (June 6), on July 28, and at the end of their resting stage (October 3). The period of dormancy of the examined species from the clay-pit in Warsaw lasts about 4 months i.e. from about June 1 to about October 15. Moreover, some specimens of *C. v. vicinus* IV sampled from plankton in the Powsińskie Lake, near Warsaw, has been examined, as well.

Osmotic concentration of the body fluid of copepodids was determined in a Ramsay type microcryoscope (Ramsay 1949, Ramsay, Brown 1955) modified by Klekowski (1963). The body fluid was taken into the capillary tubes immediately after the sampling of plankton or immediately after the awakening of copepodids from their resting stage. In experiments with the use of 2,4-D sodium salt the body fluid was taken after a few days of exposure. Copepodids were put into 35 ml of 2,4-D sodium salt solution at 15 mM/l concentration. The number of the examined individuals is given in Tables I and II. The specimens were superficially dried on filter paper, always in the same manner, and transferred into a drop of paraffin oil, where they are pierced, and their body fluid is drawn out into capillary tubes alternately with paraffin oil.

3. RESULTS

The Δ°C value of the body fluid of copepodids V — *C. bohater* and *C. v. kikuchii* — collected from plankton in clay-pit in May, before their resting stage, was 0.62 (Table I). Copepodids IV of *C. v. vicinus* from plankton sampled in the Powsińskie Lake had the Δ°C value in May 0.55. The Δ°C values in the specimens awakened from their resting stage in bottom sediments of clay-pit were much lower and ranged from 0.24, 0.25, for *C. v. vicinus* IV, to 0.28 for *C. v. kikuchii* V.

In the experiments using 2,4-D sodium salt (Table II) the Δ°C values for copepodids at the resting stage are higher and approach those observed in their plankton life i.e. for *C. v. kikuchii* V 0.49, and for *C. v. vicinus* IV 0.49, 0.43.

It is worth mentioning that in those experiments the examined copepodids awakened from their resting state have not shown any sensitivity to the effect of 2,4-D sodium salt. No death or locomotoric disturbance cases were found. The same lack of sensitivity was also observed in copepodids IV of *C. strenuus* Fisch. awakened from their resting stage in bottom sediments of the reservoir at Zaborów.

4. DISCUSSION

There is a lack of data on the Δ°C values of the body fluid of Cyclopoida, in the literature. Data concerning other freshwater crustaceans are also pretty scant. The Δ°C values of blood of some Malacostraca approach the values obtained in this study for copepodids sampled from plankton, as follows: *Astacus astacus* — 0.71 (Scholles 1933), *Asellus sp.* — 0.50 (Parry 1953), *Gammarus pulex* — 0.55 (Beadle, Cragg 1940). Whereas, a known Δ°C value for *Daphnia magna* given by Fritzsche (1917) (Robertson 1960) is 0.27. The adult *C. strenuus* specimens sampled from plankton and examined by Dr. E. Styczyńska-Jurewicz (personal communication) had the Δ°C value of about 0.4.

The obtained in this study considerable differences in osmotic properties of the body fluid occur in organisms with thoroughly different metabolism. The active organisms in plankton are characterized by intensive metabolism and excretion, whereas, metabolism in copepodids, neither moving nor feeding during their resting stage in mud, is suppressed and its products are accumulating in the plugged up and isolated from the environment alimentary tract (Wierzbicka 1966, 1972 b). The obtained results suggest the resting stage and 2,4-D sodium salt have an effect on physiological mechanisms regulating osmotic pressure in the body fluids of Copepoda Cyclopoida.

It seems to be worthy of notice that copepodids IV of *C. strenuus* awakened from their resting stage were not sensitive to the effect of 2,4-D sodium salt i.e. neither death nor paralysis cases has been observed. On the other hand, experiments with plankton organisms of the same species have shown a high rate of mortality in copepodids IV at the same concentration 15 mM/l of 2,4-D sodium salt (Wierzbicka 1974).

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5. SUMMARY

By the means of a microcryoscope the freezing point depression of the body fluid has been determined in copepodids IV and V active sampled from plankton (*C. bohater* V, *C. v. kikuchii* V, and *C. v. vicinus* IV) and those at their resting stage taken from bottom sediments of a clay-pit in Warsaw (*C. v. kikuchii* V, and *C. v. vicinus* IV). Moreover, the effect of 2,4-D sodium salt on osmotic properties of the body fluid in copepodids has been examined on the same material. The obtained results show that the Δ°C value of the body fluid of copepodids taken from plankton immediately and a month earlier before their resting stage is 0.62. Whereas, in those awakened from their resting stage in bottom sediments containing sulphuretted hydrogen it is lower — 0.24, 0.25, 0.28. Under the effect of 2,4-D-Na the Δ°C value increases in specimens awakened from their resting stage and comes up to 0.43, 0.49. Whatsoever, no death or locomotoric disturbance cases have been observed in copepodids.

6. STRESZCZENIE

Przy pomocy microcryoscopu określono punkt zamarzania cieczy ciała kopepoditów IV i V aktywnych z planktonu (*C. bohater* V, *C. v. kikuchii* V i *C. v. vicinus* IV) oraz wziętych ze stanu spoczynku w osadach dennych glinianki w Warszawie (*C. v. kikuchii* V i *C. v. vicinus* IV). Poza tym na tym samym materiale zbadano wpływ 2,4-D-Na na własności osmotyczne cieczy ciała kopepoditów. Okazało się, że Δ°C cieczy ciała kopepoditów z planktonu tuż przed stanem spoczynku i miesiąc przed nim jest wyższe i wynosi 0,62. Natomiast u okazów obudzonych ze stanu spoczynku w osadach, zawierających siarkowodór, wynosiło 0,24, 0,25, 0,28. Pod wpływem 2,4-D-Na u okazów obudzonych ze stanu spoczynku Δ°C podnosi się do 0,43. 0,49. Natomiast nie zaobserwowano żadnych porażeń ani śmiertelności kopepoditów.

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INFLUENCE OF 2,4-D SODIUM SALT ON THE SURVIVAL OF SOME COPEPODA SPECIES

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ABSTRACT

In experiments with 2,4-D sodium salt, used at 2.5, 5.0, 10.0, 15.0, and 20.0 mM/l concentrations, a considerable sensitivity and high mortality rate has been observed in the active plankton organisms: *Cyclops strenuus* Fischer and *Eudiaptomus graciloides* Lillj. The critical concentration was 10.0 mM/l. On the other hand, 2,4-D sodium salt used at the same concentrations had no effect on copepodids IV and V of the following species: *Cyclops strenuus* Fischer, *Cyclops bohater* Kozm., *Cyclops vicinus vicinus* Ulj., and *Cyclops vicinus kikuchii* Smirn., examined shortly before their resting stage or awakened from resting stage.

1. INTRODUCTION

In their life cycle Cyclopoida are passing through two thoroughly different stages i.e. an active one in plankton and a resting one in bottom sediments of water bodies. Depending on the particular species either copepodids IV and V go through the resting stage. It lasts a few months (in the reservoirs under examination from about four to nine months). The resting stage is characterized by immobility and decreased metabolism. Excretion occurring during the whole active life of Cyclopoida also through its alimentary tract is held up during the resting stage, due to the closing up of the alimentary tract with plugs. This, in consequence, causes accumulation of metabolic products in it (Wierzbicka 1966, 1972 a). It seemed to be a matter of interest to compare the effect of 2,4-D sodium salt on those two so different, as regards physiology, organisms of copepodids: the active ones from plankton and those at their resting stage in bottom sediments of reservoirs.

2. MATERIAL AND METHODS

The specimens used in experiments were collected from three water bodies: 1. from plankton in the artificial reservoir within the precincts of the Institute of Experimental Biology, Warsaw (*Cyclops strenuus* Fischer and *Eudiaptomus graciloides* Lillj.), 2) from a clay-pit in Warsaw (plankton and bottom sediments — *Cyclops bohater* Kozm., *Cyclops vicinus vicinus* Ulj., and *Cyclops vicinus kikuchii* Smirn.), and 3. from bottom sediments of an astatic reservoir at Zaborów (*Cyclops strenuus* Fischer).

Experimental conditions are given in Table I. In the first type of experiments carried out on plankton population of Copepoda, sampled in the time of their full development from the artificial reservoir, plankton was placed in twenty 1000 ml flasks, each filled with 500 ml solution of reservoir water and various

Table. I. Conditions of the experiments

Experiment No.	Environment	Date	Species	Number of specimens	Number of vessels	Capacity (ml)	Temp. (°C)	Concentration of 2,4-D sodium salt (mM/l)
1	Plankton artificial reservoir	3–6.XII.1971	*C. strenuus* *E. graciloides*	1651	20	500	0.5–5	0, 2.5, 5.0, 10.0, 15.0, 20.0
2	Plankton clay-pit before resting stage	11–17.VI.1971 12–17.VI.1971	*C. bohater* V *C. bohater* V	20 8	4 2	24 24	6 6	0, 2.5, 5.0, 7.5, 10.0 0, 5.0, 10.0
3	Mud clay-pit resting stage	10–20.VI.1972	*C. v. vicinus* IV *C. v. kikuchii* V	70	6	24	6	0, 15.0
4	Mud clay-pit resting stage	5–9.X.1972 7–12.X.1972	*C. v. vicinus* IV *C. v. vicinus* IV	15 12	3 3	35 35	6 6	0, 15.0 0, 15.0
5	Mud astatic dried-up water body resting stage	13–25.I.1973 15–25.I.1973 26–30.I.1973 27–30.I.1973	*C. strenuus* IV *C. strenuus* IV *C. strenuus* IV *C. strenuus* IV	6 5 11 9	2 1 1 1	35 35 35 35	6 6 6 6	0, 15.0 0, 15.0 0, 15.0 0, 15.0

concentrations of chemically clean 2,4-D sodium salt (December 3–6, 1971). Specimens from one flask of each concentration and the control one were examined every day. In each flask there were, on average, 79 *C. strenuus* and 15 *E. graciloides* individuals. All flasks were immersed in the reservoir, so as to get the same temperature in the experimental conditions as in the natural ones. During the whole experiment animals were feeding on phytoplankton provided in flasks (alimentary tracts of the specimens were examined several times). Thus, the created conditions were very similar to the natural ones.

In the second type of the experiments *C. bohater* copepodids V from plankton of the clay-pit were used. The specimens were already prepared to go into the resting stage in bottom sediments of the clay-pit. Solutions of 2,4-D sodium salt, in this experiment and the following ones, were prepared with left-over for some time tap water. In this type of experiments and all the following ones specimens were not fed. Copepodids awakened from their resting stage can survive without food up to two months (Wierzbicka 1972 a).

In the third and fourth type of experiments copepodids IV — *C. v. vicinus* and copepodids V — *C. v. kikuchii* were used while awakened from their resting stage in bottom sediments of the clay-pit.

In the fifth type of the experiments 2,4-D sodium salt was applied to copepodids IV — *C. strenuus* awakened from their resting stage in bottom sediments of the astatic, devoid of water, reservoir at Zaborów.

Together with the five types of experiments parallel control experiments were carried on, just the same as the others but without use of herbicide.

The procedure of collecting samples from bottom sediments and awakening of copepodids from their resting stage has been described in the previous papers (Wierzbicka 1966, 1972 a). In the clay-pit samples were collected by means of a properly loaded plankton net. Specimens were awakened by sifting mud under the running tap water. In the astatic reservoir at Zaborów a 5 cm thick blocks of mud, with surfaces corresponding to the surfaces of the aquaria, were cut out from the bottom and put into aquaria filled up with, left-over for some time, tap water.

3. RESULTS

The first type of experiments in shown in Fig. 1, 2, 3.

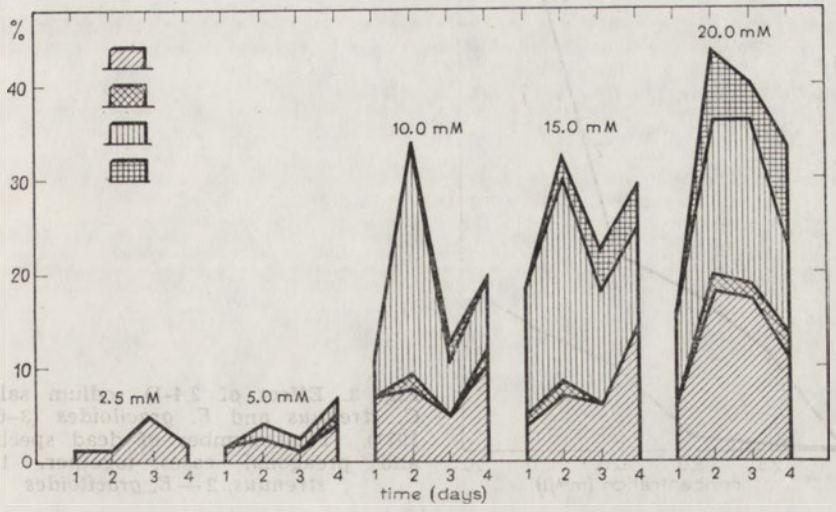

Fig 1. Effect of 2,4-D sodium salt on *Cyclops strenuus* Fisch. (3–6.XII.1971). 1 — nauplii and copepodids I–IV, dead specimens, 2 — copepodids V and adults, dead specimens, 3 — nauplii and copepodids I–IV, specimens in preagonal state, 4 — copepodids V and adults, specimens in preagonal state

Together with the increase in strength of 2,4-D sodium salt concentration the mortality rate of *C. strenuus* specimens was increasing simultaneously. Moreover, a higher mortality rate was observed in

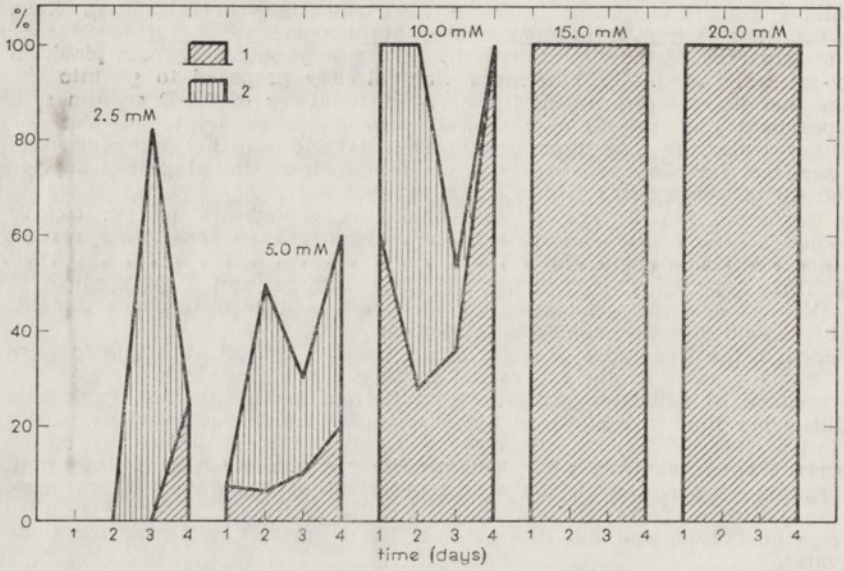

Fig. 2. Effect of 2,4-D sodium salt on *Eudiaptomus graciloides* Lillj. (3–6.XII.1971). 1 — all developmental stages, dead specimens, 2 — all developmental stages, specimens in preagonal state

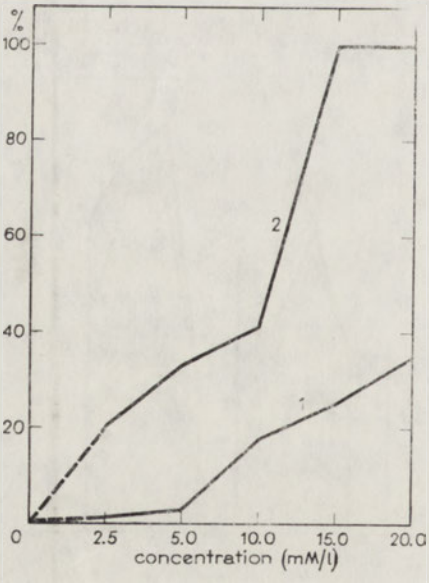

Fig. 3. Effect of 2,4-D sodium salt on *C. strenuus* and *E. graciloides* (3–6.XII. 1971). Total number of dead specimens and preagonal cases, together. 1 — *C. strenuus*, 2 — *E. graciloides*

nauplii and copepodids I–IV. There were also observed cases of locomotoric disturbance in some degree similar to the cases differentiated

in copepodids awakened from their resting stage and subjected to the effect of hydrogen sulphide water (Wierzbicka, Kędzierski 1964). At that time "state 4" has been differentiated, characteristic for resting stage, when antennules are turned backwards and legs forward and the animals does not react to the touch and the following "state 5", when antennules are wide apart, legs are turned backwards, and the animal does not react of the touch. This is the state of agony. The affected specimens when transferred into aerated water were getting active again. In the present experiment besides the described "state 4" in many cases some animals with the same arrangement of antennules and legs were, nonetheless, sensitive to the touch. Those two conditions found in the present experiment has been called preagonal states.

It has been observed that the reaction to 2,4-D sodium salt (mortality and preagonal states) was more intensive in the earlier development stages ranging from nauplius to copepodids IV, inclusively. Copepodids V and adult specimens react to a much lesser degree to the effect of 2,4-D sodium salt.

Data obtained for *E. graciloides* are shown in Fig. 2. Here, just as in the instance of *C. strenuus*, there were observed cases of locomotoric disturbance, noticeably: the observed specimens stopped to move by leaps, changing the course of their motion by a characteristic movement, instead of that they moved onwards along a straight line. Their antennae were also visibly affected, gradually the speed of the whirling motion became slower, the speed of the animal movements decreased and finally they became completely motionless. The observed cases of locomotric disturbance, typical for the "preagonal" state occurring already at 2.5 mM/l concentration of 2,4-D sodium salt, on the second day of the experiment. As can be seen from Fig. 1 and 2 the critical concentration for *C. strenuus* and *E. graciloides* is 10.0 mM/l. At that concentration the number of dead nauplii and copepodids I–IV has increased significantly. On the fourth day of the experiment the mortality rate of *E. graciloides* was already a 100 per cent. At the 15.0 and 20.0 mM concentrations during the whole experiment nothing but dead specimens of that species were found. Figure 3 shows jointly all the development stages and all the preagonal and deed cases, during the four days of the experiment. It can be seen distinctly that *C. strenuus* is much less sensitive than *E. graciloides*.

In the second type of experiments (Table I) copepodids V of *C. bohater* species from plankton, immediately before going into their resting stage, were not sensitive to the effect of 2,4-D sodium salt. They behaved just the same as the control specimens and after the end of the experiment they buried themselves, instantaneously, into the mud at the bottom of the laboratory vessels where they had been transferred.

Also, in the third and fourth type of experiments (Table I) the awa-

kened copepodids IV of *C. v. vicinus* and *C. v. kikuchii* were not sensitive to 2,4-D sodium salt and did not show any symptoms of locomotoric disturbance.

In the fifth type of experiments (Table I) the awakened copepodids of *C. strenuus*, likewise, were not sensitive to the effect of that herbicide. Plugs closing up their intestines during their resting stage were thrown out at the same time as in the control specimens (during the time of two days). In general, in the experiments with copepodids, awakened from their resting stage, no differences at all were observed between the behaviour of the specimens under the effect of 2,4-D sodium salt and the control animals.

4. DISCUSSION

A comparison of the experiments on active organisms and the awakened from their resting stage, or taken from plankton but shortly before the resting stage, shows completely different reactions of those organisms namely, a high sensitivity of the former and lack of sensitivity of the latter. They, not only, endure the effects of 2,4-D sodium salt in perfect state and are not subject to any symptoms of locomotoric disturbances but, moreover, immediately after being transferred into a vessel with some mud at its bottom, they bury themselves in it, at once. This proves that physiological mechanisms in active organisms differ from those in the animals at their resting stage. Equally insensitive to the effects of 2,4-D sodium salt are the awakened copepodids of the species living in the clay-pit *(C. bohater, C. vicinus vicinus, C. vicinus kikuchii)* and spending the time of their resting stage in sulphuretted hydrogen environment in condition of anoxia (Wierzbicka, Kędzierski 1964) and also the awakened copepodids of *C. strenuus* from bottom sediments of a dried up astatic water body.

The results from the comparison of sensitivity to the effects of 2,4-D sodium salt, at the same 15.0 mM concentration, of the same stages in the development of copepodids IV of *C. strenuus*, give cause to reflection. Copepodids IV, leading an active life in plankton, have shown the highest sensitivity (alongside with nauplii and copepodids I, II, and III). Copepodids IV, awakened from their resting stage, were completely insensitive. This concerns *C. strenuus* as well as other examined species belonging to the same O. F. Müller genus.

In the literature there is a lack of data as regards the effects of 2,4-D sodium salt on Cyclopoida and Calanoida, therefore, the mentioned-above observations seem to be the first attempt at determination of the reactions of those organisms to that agent. There are some data concerning Cladocera (Klekowski, Zvirgzds 1971, and Kaniewska (unpublished) have investigated the survival of *Simocephalus vetulus*.

Klekowski, Zvirgzds (1971) suggest that concentrations higher than 7.5 mM are critical for the survival of *S. vetulus*. In the present experiment the critical concentration is 10 mM/1, there is a distinct increase in dead specimens and preagonal cases of *C. strenuus* (nauplii and copepodids I–IV). At that concentration, on the fourth day of the experiment there was a 100 per cent mortality of *E. graciloides* population, at the concentrations of 15.0 mM/l and 20.0 mM/l. *E. graciloides* mortality rate was 100 per cent from the very first to the last day of experiment. In the experiments described by Kaniewska, at the 6.98 mM/l concentration there was after 96 hours 50 per cent of mortality in *S. vetulus* population, whereas, higher concentrations (9 and 10 mM/l) caused a 100 per cent mortality rate already after 24 hours. As results from the above comparisons Copepoda species examined in the present study are less sensitive to the effects of the 2,4-D sodium salt herbicide than *Simocephalus vetulus*, a representant of Cladocera.

From the above presented results one could draw some suggestions for fishing industry, as follows: the use of a herbicide containing 2,4-D sodium salt is indicated at the time when, the most numerous and most useful for fish in the ponds and lakes, organisms living in plankton during the cold seasons of the year, such as various species of Cyclops genus, are at their resting stage in the mud (from about June 1 to about October 15). Thus, the herbicide "PIELIK" which is more toxic than 2,4-D sodium salt should be applied only at the time when in the bottom sediments there is a reserve of organisms in the form of copepodids at their resting stage.

Acknowledgements

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5. SUMMARY

Experiments have been carried out on the effects of 2,4-D sodium salt on the survival of some species of Copepoda. For experiments active specimens were taken from plankton (*C. strenuus* and *E. graciloides*), others were either awakened from their resting stage in the mud of bottom sediments or sampled shortly before the resting period (*C. strenuus, C. bohater, C. v. vicinus, C. v. kikuchii*). Concentrations of the 2,4-D sodium salt were, as follows: 2.5, 5.0, 10.0, 15.0, and 20.0 mM/l.

The results of experiments have shown a high sensitivity of *C. strenuus* active specimens from plankton and especially of nauplii and copepodids I–IV, inclusively. Cases of locomotoric disturbances, characteristic for preagonal state, have been differentiated. *E. graciloides* specimens have also shown symptoms of locomotoric disturbances and their mortality rate was much higher. The critical concentration for both species was 10.0 mM/l.

On the other hand, copepodids IV of *C. strenuus* and copepodids IV and V of other, mentioned above, species of Cyclops O. F. Müller genus, awakened from their resting stage, has been completely insensitive to the effects of 2,4-D sodium

salt. No cases of locomotoric disturbances have been observed, the specimens behaved just the same as the control ones and after the end of experiment they have buried themselves anew in the mud.

6. STRESZCZENIE

Przeprowadzono eksperymenty nad wpływem soli sodowej 2,4-D na przeżywalność kilku gatunków Copepoda. Do eksperymentów wzięto okazy aktywne z planktonu (*C. strenuus* i *E. graciloides*) i budzone ze stanu spoczynku w mule osadów dennych względnie będące tuż przed okresem spoczynku (*C. strenuus, C. bohater, C. v. vicinus, C. v. kikuchii*). Stężenia wynosiły: 2,5, 5,0, 10,0, 15,0 i 20,0 mM.

Stwierdzono znaczną wrażliwość okazów aktywnych *C. strenuus* z planktonu zwłaszcza nauplii i kopepoditów od I do IV włącznie. Wyróżniono porażenia o charakterze lokomotorycznym, charakteryzujące stany przedagonalne. Również *E. graciloides* wykazał porażenia lokomotoryczne, przy czym śmiertelność jego była znacznie większa. Krytycznym stężeniem dla obu gatunków było 10,0 mM/l.

Przeciwnie, kopepodity IV C. strenuus jak i kopepodity IV i V innych wymienionych gatunków rodzaju Cyclops O. F. Müller, budzonych ze stanu spoczynku, okazały się zupełnie niewrażliwe na działanie soli sodowej 2,4-D: nie zauważono żadnych porażeń, okazy zachowywały się jak w kontrolnych, po eksperymencie zagrzebywały się ponownie w mule.

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TOXIC EFFECTS OF 0,0,DIETHYL-0-PARANITROPHENYL PHOSPHOROTHIOATE OR FOLIDOL ON THE MORPHOLOGY AND NUCLEAR APPARATUS OF A FEW FRESHWATER CILIATES

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ABSTRACT

The effect of 0,0,Diethyl-0-Paranitrophenyl Phosphorothioate on a few fresh water ciliates was studied. Changes in the morphology and nuclear apparatus of the ciliates were observed. The population density of the normal and treated was compared. The results obtained show that a concentration of 50 µl/100 ml Folidol was lethal, 40 µl/100 ml sublethal and 1 µl/100 ml did not cause death immediately.

1. INTRODUCTION

Protozoan distribution in fresh water is affected by changes in temperature, salinity, pH, light, dissolved oxygen content, carbon dioxide and the purity of the water. A change in any one of these factors bring about a change in the morphology and the normal functioning of the organisms (Noland, Gojdics 1967). It has been reported that chlorinated organic pesticides can be biologically concentrated and are a potential threat to life in the aquatic ecosystem. It has been established that DDT remains in the soil for a long time after discontinuation of its application and will be in the surface drainage for many years (Hindrin, Bennett 1971). In the present investigation, the effect of different concentrations of 0.0.Diethyl-0-Paranitrophenyl Phosphorothioate or Folidol (an organophosphate insecticide that is a major water pollutant) on the morphology and nuclear apparatus of five ciliates has been studied.

2. MATERIAL AND METHODS

Cultures of *Spirostomum ambiguum major*, *Spirostomum ambiguum minor*, *Blepharisma intermedium*, *Blepharisma seshachari* and *Frontonia leucas* were grown in a medium of hay infusion. The stock culture of hay infusion was prepared by boiling 10 gms of Ragi hay (*Eleusine coracana*), 200 g of garden soil in a litre of distilled water for 6 hours and filtered. This was diluted 1 : 100 ml distilled water before use and fortified with horlicks malt (made by Hindustan milk food manufacturers), 10 mg/100 ml before use at room temperature 26±1°C.

For the experiments, 50 organisms of each of the five species were inoculated into 100 ml of culture medium with 10 mg of horlicks. Three bottles served as controls and 1 µl/100 ml Folidol was added to the other three bottles. The capacity of the bottles was 125 ml and were of hard glass. The number of organisms in three alliquots of 1 ml of the medium was counted daily for a week and the average taken.

Normal and organisms treated with 1 µl/100 ml Folidol were fixed daily over a period of seven days in Carnoy's fixative, stained with Feulgen

using light green as counter stain. Camera lucida drawings were made and the length and width of the organisms was measured. The experiments were run in triplicate, the standard deviation for the population density of both normal and treated was calculated.

40 µl/100 ml and 50 µl/100 ml Folidol were also used to study the lethal and sublethal concentrations of the pesticide. The immediate response of the ciliate were recorded.

3. RESULTS

The stress of the pesticide on the ciliates increased as its concentration increased from 1 to 50 µl/100 ml Folidol. 50 µl/100 ml Folidol proved lethal, immediately after inoculation, *S. ambiguum major* wriggled, became very much elongated, repeatedly contracted and relaxed. *Spirostomum ambiguum minor* became thin and long. *Frontonia leucas* burst within five minutes. *Blepharisma intermedium* and *Blepharisma seshachari* were normal for two hours. All the species burst after three hours.

A concentration of 40 µl/100 ml Folidol was sublethal. *Frontonia leucas* was inactive and burst after 30 minutes. *Spirostomum ambiguum major* struggled and immediately in size (595 µ in length and 280 µ in width) later it contracted, measured 411 µ in length and 140 µ in breadth. 24 hours later both species of Spirostomum had burst. Blepharisma species elongated, their activity remained normal however they were eliminated after five days.

The ciliates in the present study were eliminated in the following order in 1 µl/100 ml Folidol: *Blepharisma seshachari* after 3 days, *Spirostomum ambiguum major* after 6 days, *Spirostomum ambiguum minor* after 7 days. *Blepharisma intermedium* and *Frontonia leucas* were present even after 8 days. Changes in the morphology and nuclear apparatus were also seen with a concentration of 1 µl/100 ml Folidol. The pesticide affects the macronucleus in two ways. It causes elongation and breakage of the cylindrical macronucleus of *Blepharisma intermedium* and beaded macronucleus of *Blepharisma seshachari* and Spirostomum species. There was no change in the oval condensed macronucleus of *Frontonia leucas* (Fig. 4 B).

Morphological changes took place with Folidol. On the 4th day the cytoplasm of *Blepharisma intermedium* was full of vacuoles and on the 5th day *Blepharisma intermedium* and *Spirostomum ambiguum minor* showed abnormal morphology (Fig. 1 B, 3 D).

The nuclear apparatus was also affected. In a few ciliates two macronuclei were found (Fig. 1 C). Abnormal binary fission occurred, daughter individuals receiving unequal number of macronuclei (Fig. 1 E). Fusion of the ciliates at the anterior end as in conjugation was observed (Fig. 1 C), but the ciliates failed to separate. From the 5th day onwards giant individuals that were twice the body size of the normal ciliates appeared (Fig. 1 F).

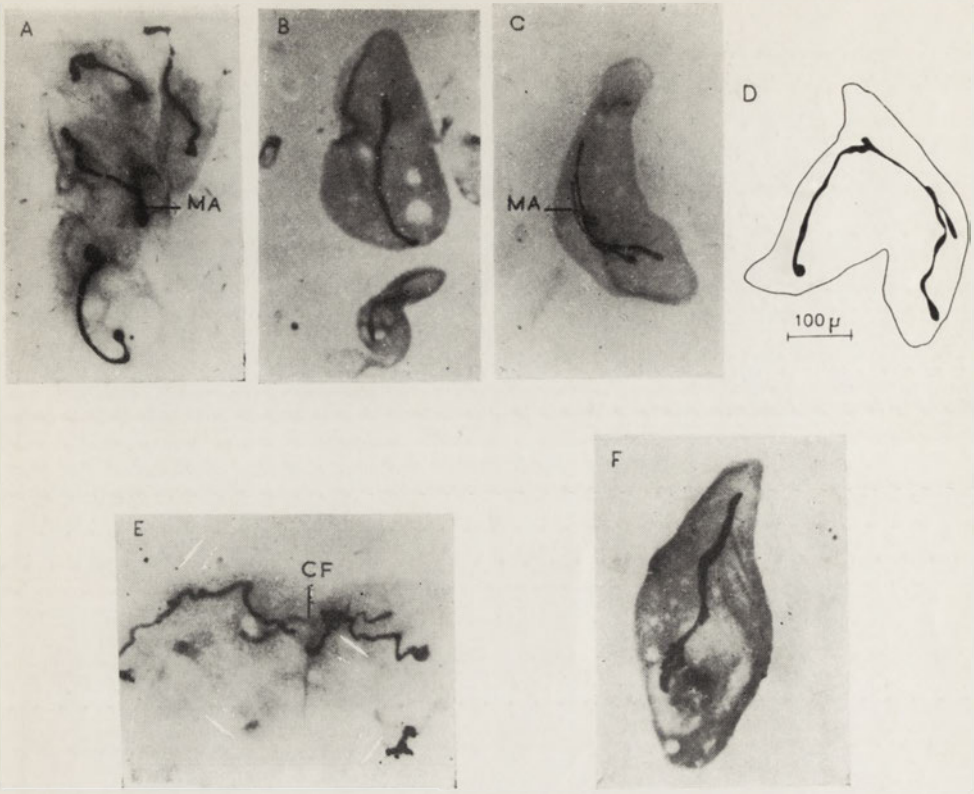

Fig. 1. *Blepharisma intermedium* in 1 μl/100 ml Folidol. A — after 2 days: macronucleus (MA) elongated and broken, B — after 5 days; abnormal morphology, C — after 6 days; double macronucleus (MA), D — after 6 days; fusion of two ciliates, E — after 6 days; irregular number of macronuclei in the daughter individuals during binary fission (CF — cleavage furrow), F — after 7 days; giant

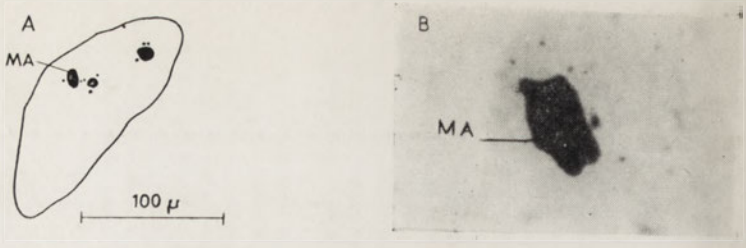

Fig. 2. *Blepharisma seshachari* in 1 μl/100 ml Folidol after 1 day. A — macronucleus (MA) distorded, B — beads nature not clear, clumping of the beads into an irregular mass

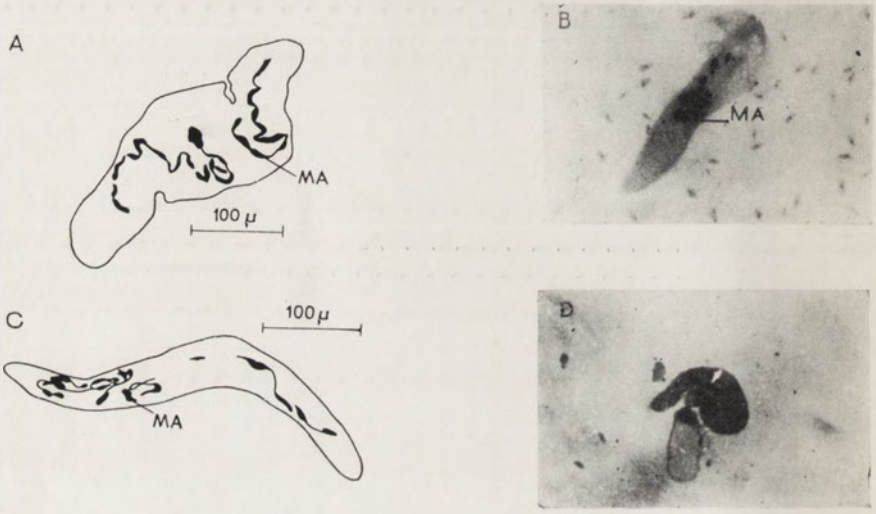

Fig. 3. A — *Spirostomum ambiguum major* after 6 days in 1 μl/100 ml Folidol; macronucleus (MA) broken.
Spirostomum ambiguum minor in 1 μl/100 ml Folidol. B — after 2 days; macronucleus (MA) distorded, C — after 2 days; macronucleus (MA) distorded, D — after 5 days; abnormal morphology

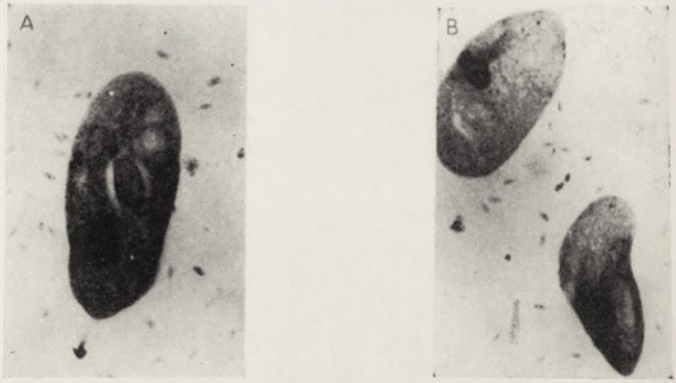

Fig. 4. *Frontonia leucas* in 1 μl/100 ml Folidol. A — after 1 day; no change, B — after 6 days; no change

Changes in the morphology, behaviour, nuclear apparatus and size of the organisms treated with 1 µl/100 ml Folidol has been observed and the results have been summarized in the Table I and II. This concentration is not highly toxic.

The population density increased in the normal over seven days when compared to the organisms treated with 1 µl/100 ml Folidol (Fig. 5).

Table I. Changes in the morphology and behaviour when treated with 1 µl/100 ml Folidol

Day	Change in	*Blepharisma intermedium*	*Blepharisma seshachari*	*Spirostomum ambiguum major*	*Spirostomum ambiguum minor*	*Frontonia leucas*
1st	Morphology	Normal	Becomes bigger in size and paler	Normal	Elongated	Becomes smaller
	Behaviour	Normal	Inactive	Normal	Normal	Active
2nd	Morphology	Normal	Size reduction	Elongated	Elongated, thin	Small, dark
	Behaviour	Active	Inactive	Active	Not very active	Active
3rd	Morphology	Thinner, pale with vacuoles	Burst	Reduction in size	Reduction in size	Small, dark
	Behaviour	Active	—	Lethargic	Inactive	Active
5th	Morphology	Changes in shape, anterior end pointed, large number of vacuoles, pale	—	Elongated, thin	Elongated	Small
	Behaviour	Inactive	—	Inactive	Inactive	Active
6th	Morphology	Abnormalities, organisms were fused, abnormal binary fission (Fig. 1 DE)	—	Reduction in size	Reduction in size	Change in shape, anterior end pointed
	Behaviour	Inactive	—	Inactive	Inactive	Active
7th	Morphology	Giants (Fig. 1 F)	—	Reduction in size, thin	Reduction in size, thin	Small, dark
	Behaviour	Inactive	—	Inactive	Inactive	Active

Table II. Changes in the nuclear apparatus and size when treated with 1 µl/100 ml Folidol

Day	Item	*Blepharisma intermedium*	*Blepharisma seshachari*	*Spirostomum ambiguum major*	*Spirostomum ambiguum minor*	*Frontonia leucas*
0	Length (µ) Width (µ)	295 ± 1 8 172 ± 3.09	100 ± 1.4 54 ± 2.8	590 ± 1.8 277 ± 1.4	309 ± 1.8 45 ± 3.09	411 ± 1.6 140 ± 1.4
1st	Length (µ) Width (µ) Nuclear apparatus	326.6 ± 1.1 174 ± 7.1 Distorted, macronucleus is broken (Fig. 1A)	148 ± 18.8 76.5 ± 1.7 Beaded nature broken, in some single irregullar nucleus (Fig. 2)	472.6 ± 22.9 152.6 ± 5.7 Normal (Fig. 3A)	355 ± 33.6 52 ± 5.7 Broken	272 ± 10.5 124 ± 9.9 Normal (Fig. 4)
2nd	Length (µ) Width (µ) Nuclear apparatus	431 ± 6.8 161 ± 4.7 Distorted long	— Broken	654 ± 13 109 ± 2 Normal	— — Broken (Fig. 3BC)	244 ± 2 166 ± 5.5 Normal
3rd	Length (µ) Width (µ) Nuclear apparatus	414 ± 22.8 133 ± 6.8 Elongated and distorted	— — —	380 ± 21 106 ± 8 Normal	290 ± 3.6 85 ± 1.8. Normal	378 ± 12 98.6 ± 7 Normal
5th	Length (µ) Width (µ) Nuclear apparatus	410 ± 23 117 ± 8 Abnormal	— — —	556 ± 11.6 132.6 ± 5.5 Normal	447 ± 48.5 80.6 ± 0.008 Broken	308.6 ± 15.5 175 ± 17 Normal
6th	Length (µ) Width (µ) Giants: Length (µ) Width (µ) Nuclear apparatus	270.6 ± 12.7 80.6 ± 8.8 449 ± 57 201 ± 1.8 Double macronucleu (Fig 1C)	= — —	355 ± 12 142 ± 6.4 Broken (Fig. 2B)	160.6 ± 1.8 60 ± 1.4 Broken	184 ± 7.6 82 ± 10.5 Normal
7th	Length (µ) Width (µ) Giants: Length (µ) Width (µ) Nuclear apparatus	291 ± 12.8 80 ± 2.3 464 ± 7.3 203 ± 12 Broken	— — —	260 ± 16 102 ± 1.14 Broken	252.6 ± 7.7 41 ± 0.007 Broken	210 ± 18.3 81.5 ± 8.8 Normal

4. DISCUSSION

The effects of dissipation of the pesticide DDT from the environment on animals specially fish and fish eating birds is well known (Chesters, Konrad 1971). Very little information is available on the effects of Folidol an organophosphate pesticide that is widely used in India. The susceptibility of organisms to pesticides differs, their

effects become manifest in species at various levels of the food chain. The Protozoan community occupy the second level in the food chain, they serve as food mostly for juvenile tertiary consumers. Elimination

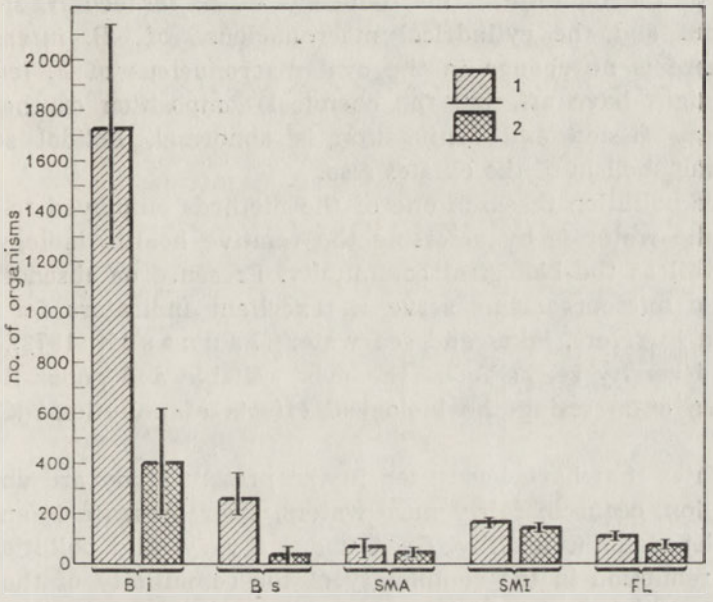

Fig. 5. Density of normal (1) and treated with 1 µl/100 ml Folidol (2) Protozoan populations. Bi — *Blepharisma intermedium* (8 days), Bs — *Blepharisma seshachari* (3 days), SMA — *Spirostomum ambiguum major* (6 days), SMI — *Spirostomum ambiguum minor* (7 days), FL — *Frontonia leucas* (8 days). Vertical lines — standard deviations

of Protozoans would break the food chain and bring about changes in the ecosystem. Results of this investigation show that 50 µl/100 ml Folidol is lethal, 40 µl/100 ml sublethal and 1 µl/100 ml will not destroy the ciliates immediately. However morphology and behaviour is affected. Normal activities like growth and reproduction are abnormal or retarded.

Earlier observations have shown that differences in the kind and amount of food material bring about conspicuous differences in form and structure of Protozoa (K u d o 1966). K i d d e r et al. (1940) observed that in *Tetrahymena vorax*, bacteria feeders were tailed, saprozoic forms were fusiform to ovoid, carnivores and cannibals were irregularly ovoid. With 1 µl/100 ml Folidol the shape of all the five ciliates studied changes. There is reduction in the size of the two species of Spirostomum, *B. seshachari* and *F. leucas* while giant formation occurs in *B. intermedium*. Binary fission and conjugation are abnormal. Giant formation and abnormal conjugation have been observed with changes in temperature and hydrogen ion concentration of the culture

medium (Kasturi Bai et al. 1971). Giant formation is an unbalanced metabolic response (Giese 1938).

The nuclear apparatus of the ciliates are also affected. Folidol causes breakages of the moniliform macronucleus of *B. seshachari*, species of Spirostomum and the cylindrical macronucleus of *B. intermedium*. Though there is no change in the oval macronucleus of *F. leucas* the pesticide might have affected the chemical composition of the nucleic acids. Binary fission and conjugation is abnormal, Folidol seems to affect the metabolism of the ciliates also.

In water pollution research one of the methods employed to test the purity of the water is by assessing the relative health, biology of the water as well as the biological community. Presence or absence of certain aquatic microorganisms serve as excellent indicators for degrees of pollution in rivers, lakes and sea water (Jannasch 1972). Change in species diversity seems to be the most reliable and generally applicable means of assessing the biological effects of pollution (Cairns 1972).

The ciliates that have been used in the present study are ubiquitous in distribution, occur in fairly pure waters, their presence is an indication that the water is healthy. Even under a very mild pollution stress there is a reduction in the complexity of the community of the ciliates studied. The number of individuals per species is reduced when treated with 1 µl/100 ml Folidol. With the increase in the use of pesticides in agriculture, toxicity towards the nontarget organisms have always been a concern of responsible users of biological toxicants (Kaufman Plimmer 1972). Knowledge of the concentrations of the pesticide that will destroy the zooplankton is essential in water pollution control. The results of this investigation might be useful in limiting the quantity of Folidol that could be released into fresh waters to protect the biota and retain the purity of the water.

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5. SUMMARY

In this investigation the tolerance of five fresh water ciliates *Spirostomum ambiguum major*, *Spirostomum ambiguum minor*, *Blepharisma intermedium*, *Blepharisma seshachari* and *Frontonia leucas* that are normally found occurring together in stagnant pools, to different concentrations of Folidol has been studied. 50 µl/100 ml and 40 µl/100 ml proved lethal while 1 µl/100 ml was not highly toxic. However this concentration brought about changes in the morphology and nuclear apparatus of the ciliates. The response of the ciliates chosen for the present study to the pesticide differed. *Frontonia leucas* showed the least change both in morphology and nuclear apparatus, while it proved lethal to *B. sesha-*

chari after three days. There was a reduction in its size, the macronucleus lost the moniliform appearence. *S. ambiguum major* remained normal on the first day, but was greatly elongated by the second day and continued to be for two to three days. Response of *S. ambiguum minor* was similar to that of *S. ambiguum major*, but the nuclear apparatus appeared to be broken and distorted after 24 hours. Morphological changes were seen after 5 days in *B. intermedium* when some transformed into giants while in others the posterior end became rounded. Abnormal binary fission resembling budding was seen. Fusion of the anterior end as in conjugation was observed. The macronucleus showed significant changes. These ranged from distortions to double macronuclei. In general division rate in all the ciliates was slower than the normal rate. Even under a mild pollution stress, the ciliates studied are eliminated from the aquatic biota. The Protozoan community occupies the second level in the food chain, they serve as food mostly for juvenile tertiary consumers. Elimination of even a few Protozoans would break the food chain and bring about changes in the ecosystem.

6. ZUSAMMENFASSUNG

Untersucht wird die Widerstandsfähigkeit von fünf Frischwasserziliaten *Spirostomum ambiguum major, Spirostomum ambiguum minor, Blepharisma intermedium, Blepharisma seshachari* and *Frontonia leucas,* die normalerweise zusammen in stehenden Gewässern leben, gegen verschiedene Konzentrationen von 50 l/ml und 40 l/100 ml waren tödlich, während 1 l/100 ml nicht sehr giftig war. Diese Konzentration jedoch verursachte Veränderungen in der Morphologie und in Aufban des Zellkernes der Ziliaten. Hede der zur Untersuchung ausgewählten Ziliaten reagierte verschieden auf diese Pestizid (Folidol). *Frontonia leucas* wies die geringsten Veränderungen in Morphologie und Aufbau des Zellkernes, während Folidol auf *Blepharisma seshachari* nach drei Tagen eine tödliche Wirkung hatte. Es war eine Schrumpfund in der gröpe za beobachten und der Makrokern verlor sein moniliformiges Aussehnen. *Spirostomum ambiguum major* blieb unverändert am ersten Tag aber wurde länger und blieb so, bis es nach 6 Tagen abstarb. Der Aufban des Zellkernes von *Spirostomum ambiguum minor* wurde nach 24 Stunden deformiert. Morphologische Veränderungen traten deformiert. Morphologische Veränderungen traten nach 5 Tagen bei Blepharisma intermedium ein. Einige entwickelten Riesenwuchs während das Hinterteil bei den anderen sich abrundete. Bei allen Ziliaten waren Wachstum und Vermehrung entweder abnormal oder verzögert. Abnormale binäre Spaltung, einer Knospenbildung ähnlich, war bei *Blepharisma intermedium* zu beobachten. Die Ziliaten fügten sich aneinander am Vorderteil wie bei einer Konjugation. Der Makrokern zeigte auffällige Veränderungen. Diese Veränderungen reichten von deformationen bis zur Verdoppelur von Makrokern. Im allgemeinen war die Spaltungsgeschwindigkeit langsamer als normalerweise bei Ziliaten. Unter dem Einflup einer leichten Pollution wurden die Ziliaten von der Fauna des Wassers ausgeschieden. Die Protozoen stellen die zweite Stufe in der Nahrungskette dar. Sie dienen als Nahrung, hauptsächlich für junge tertiäre Verbraucher. Die Eliminierung von auch nur wenigen Protozoen wird die Nahrungskette unterbrechen und Veränderungen im ökologischen System bewirken.

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BIOLOGICAL STUDIES OF THE SEWAGE-TREATMENT PROCESSES IN THE CITY OF TORUŃ

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ABSTRACT

Sewage, containing 66% of industrial waste and 34% of municipal sewage, was purified in the laboratory-type models, using the activated sludge method. During the whole period of the experiment, Ciliata were occurring in abundance, either sessile, or crawling among the flocs. The other species of microfauna, except *Flagellata n. det.* and Rotatoria, were present only sporadically. It serves to show that the sewage treatment process followed a normal course. The shortening of the sewage aeration time from 6 to 3 hr did not have any significant effects on the species composition, on the other hand, it has brought about considerable fluctuations in the number of individuals of each particular species. The results of bacteriological tests have shown a high degree of sewage purification in respect of all the experiments that had been carried out. The degree of destruction of psychrophilic and mesophilic bacteria averaged 83.1%. The *B. coli* titre of the influent sewage was $1 \cdot 10^{-6}$ and of the purified sewage flowing out from the settling tanks — $1 \cdot 10^{-5}$.

1. INTRODUCTION

To raise the standard of efficiency of the process of sewage purification by means of activated sludge a biological control is indispensable. Observation of the microorganisms entering into the composition of sludge and knowledge of technological parameters allow to determine conditions conducive to optimal efficiency of a sewage treatment plant. Each degree of sewage purification has its own characteristic set of organisms in the activated sludge. Good knowledge of those various species of organisms makes possible to draw conclusions about the actual run of the purification process. The occurrence of this or that kind of species is conditioned by the following factors: concentration of some substances in the sewage that are either nutritious or harmful to the organisms, the amount of dissolved oxygen, duration of sewage aeration in the tank, the loading of activated sludge, the temperature, the pH value, type of the sewage treatment plant, etc. Regularity of the course of purification processes depends on ecologic balance between microorganisms forming the activated sludge. The knowledge of microfauna composition, total number of bacteria population, and titre of Bacterium coli allow to infer whether the determined conditions are promoting or inhibiting sewage purification processes.

Therefore, while searching for the most economical techniques to be used in the town of Toruń municipal sewage treatment plant thorough biological observations have been carried on, simultaneously.

2. MATERIAL AND METHODS

The treated sewage was a condensed mixture (Żołnowski et al. 1973). The point in stake was to obtain the experimental sewage consistency similar to that of municipal sewage in Toruń, as foreseen for 1985. For that reason, sewage was sampled from collectors situated nearby factories and residential quarters in quantities agreeing in proportion with the composition of sewage, as anticipated in 1985. In accordance with prospective plans of town-development, Toruń will supply to the municipal sewage treatment plant 111,718 m^3 of sewage per day, derived in 66% from industry and 34% from households. 78% of the industrial wastes will come from "ELANA" — The Artificial Fiber Plants, 6% from "MERINOTEX" — The Combing and Spinning Mills, 4% from The Bone-Glue Works, 2% from Slaughterhouses and Meat Canning Plants, and 10% from smaller factories.

The BOD_5 values of the prepared sewage mixture fluctuated within the range of 186–416 mg O_2/l, the mean value averaged 245 mg O_2/l. The Chemical Oxygen Demand values ranged from 315 to 784 mg O_2/l, mean value — 525 mg O_2/l. Oxidation values were constantly within the range of 53–131 mg O_2/l. The pH value of the sewage in the settling tank ranged from 7.5–8.2, mean value of the reaction was 7.9. In accordance with the results reported by Żołnowski et al. (1973) the treated sewage contained 37% of substances not easily decomposed by means of activated sludge. Particularly resistant to biochemical treatment are: fats (20.2–38.4 mg/l), detergents (9.6–14.4 mg/l), iron (5.0–29.0 mg Fe^{+3}/l), copper (0.7–7.3 mg Cu/l), and heavy metals (Cu, Zn, Cr, Ni) averaging about 3.19 mg/l.

The normal development of microorganisms in activated sludge must have been, doubtlessly, influenced by biogenous substances found in the artificially condensed sewage used in experiments in quantities, as follows: organic nitrogen (6.3–16.2 mg/l), ammonium nitrogen (1.4–15.8 mg/l), inorganic nitrogen averaging 12.8 mg/l, and phosphorus (1.31 mg/l).

Under laboratory conditions, experiments were carried out in two aquarium-type aeration tanks with build in dividing walls separating the settling tanks. Capacity of the former — 6.5 l, of the latter — 1.8 l. Air obtained from a compressor was spread out through the aquarium-type diffusers. Altogether, four experiments were performed, differing from one another, mainly, by duration of the sewage aeration time. In Experiment I, the time of sewage passage through the tank was 6 hrs, in II — 5 hr, in III — 4 hr, in IV — 3 hr. Experiments I and III were carried on from May 15 to June 30, and the II and IV from November 13 to December 23, 1972. The very thick sewage has been diluted with tap water in Experiments II and IV, only. The period of collecting materials for the present study was conditioned by the technological observations simultaneously carried on under the direction of Henryk Żołnowski, M. Sc.

Samples for qualitative and quantitative analyses were collected twice a week. To obtain the exact mean value of the number of the microorganisms actually present in the activated sludge small samples were collected from various places in the tank and poured all together into one bottle to form one large sample. On the whole, 52 samples were analyzed.

On the basis of live samples a detailed list of species was established, relying on classification systems of Kahl (1930/1935, 1934), Calaway, Lackey (1962), Voigt (1956/1957), Bartoš (1959); next living individuals were counted. All the data in the Table I are to be considered as approximate results of quantitative analysis since they included, sometimes, floating individuals and this makes the exact calculation rather difficult. Microfauna was always analyzed alive only a few minutes after the sampling. A Sedgwick-Rafter type, flat, plexiglass, 1 ml cell with bottom divided into 1000 grids was used for calculations. Since the activated sludge was of great density the content of the sample in the calculating cell was always diluted 1 : 10 with tap water.

Sewage for bacteriological analyses was sampled next day after it had been fetched from Toruń. The degree of destruction of the total number of psychro- and mesophilic bacteria and the titre of Bacterium coli were examined. Bacteriological inoculations were performed twice in each of the four Experiments (I, II, III, IV), mentioned above.

The total number of bacteria was determined with the method of subsequent dilutions of 1 ml of sewage. The number of psychrophilic bacteria was determined after 72-hour incubation on agar-agar, at 20°C, and of mesophilic ones

Table I. List of microfauna species found in the investigations

Species	Time of sewage aeration (mean hr)			
	6 (Exp. I)	5 (Exp. II)	4 (Exp. III)	3 (Exp. IV)
	Mean number of ind. per 1 ml			
Amoeba limax Duj.	1191	1352	1943	2386
Amoeba proteus (Leidy)	788	912	1761	0
Amoeba radiosa Ehrb.	750	937	1155	6218
Cochliopodium granulatum Pen.	215	164	144	136
Euglypha alveolata Duj.	5	3	1	0
Actinosphaerium eichhorni Ehrb.	0.05	0	0	0
Peranema trichophorum (Ehrb.)	0.3	0.1	0.01	0
Euglena viridis Ehrb.	0	0	0	1
Flagellata n. det.	1250	3410	5181	12.280
Trachelophyllum pusillum Per., Clap.	1297	1533	2510	3237
Hemiophrys fusidens Kahl	0.1	0.1	0.1	0
Litonotus carinatus Stokes	0.3	11	32	92
Litonotus crinitus Grandori	5	3	0.3	6
Chilodonella cucullulus (Müll.)	130	92	15	2
Chilodonella uncinata Ehrb.	667	158	55	8
Paramecium caudatum Ehrb.	4	2	0.4	0.1
Colpidium campylum (Stok.)	0.1	0	0.01	0
Drepanomonas revoluta Pen.	6	1	5	0
Oxytricha fallax Stein	0.1	3	0	2
Oxytricha ludibunda Stokes	0.3	2	1	1
Euplotes aediculatus Piers.	0.5	2	0.2	1
Euplotes moebiusi Kahl	191	210	129	81
Euplotes affinis Duj.	519	320	253	105
Aspidisca lynceus Ehrb.	0.2	0.1	0	0
Aspidisca costata (Duj.), Clap.	1438	1597	147	162
Epistylis plicatilis Ehrb.	1533	910	931	241
Opercularia curvicula Pen.	3	4	15	0
Opercularia phryganeae Kahl.	12	21	0	15
Opercularia minima Kahl	29	198	250	317
Opercularia elongata (Kellicott)	0	0	11	0
Opercularia microdiscum Faure	37	62	46	82
Opercularia coarctata Clap.,, L.	1403	1612	1951	2180
Vorticella putrina Müll., Kent.	0	48	0	87
Vorticella convallaria L.	2100	1540	1907	842
Vorticella octava Stokes	360	1110	661	571
Vorticella microstoma Ehrb.	0	1242	0	1606
Acineta grandis Kent	3	0.8	0.6	0 3
Acineta foetida Maupas	1	0.2	0.1	0.1
Tokophrya quadripartita Clap.	0.7	1	1	0
Podophrya fixa Müll.	0.02	4	0.02	4
Nematodes n. det.	10	15	8	5
Habrotrocha bidens (Gosse)	2	1	4	0.1
Philodina roseola Ehrb.	16	22	26	25
Rotaria rotatoria (Pallas)	5	2	4	1
Epiphanes senta (Müll.)	0.2	0.1	0.01	0.01
Colurella colurus (Ehrb.)	1	205	12	2
Lepadella patella (Müll.)	0.2	1	1	0.1
Lecane stichaea Harring	2	0	2	1
Lecane inermis (Bryce)	3	2	1	1

Species	Time of sewage aeration (mean hr)			
	6 (Exp. I)	5 (Exp. II)	4 (Exp. III)	3 (Exp. IV)
	Mean namber of ind. per 1 ml			
Monostyla lunaris (Ehrb.)	0.1	1	0.2	0.1
Monostyla closterocerca (Schmarda)	0.2	0	2	1
Cephalodella gibba (Ehrb.)	0.1	1	2	1
Cephalodella gracilis (Ehrb.)	0.2	3	0.2	0.1
Dicranophorus grandis (Ehrb.)	1	0.1	0.01	0.01
Encentrum lupus Wulfert	3	2	0.3	1
Gastrotricha n. det.	0.3	1	0.2	0.1
Oligochaeta n. det.	0.1	0.1	0.1	0.1
Copepoda n. det.	0.01	0.1	0.01	0.01
Number of samples	13	13	13	13
Total number of found species	54	52	51	47
Mean number of ind. per 1 ml	13,984	17,722	19,170	30,702

after 24-hour incubation in the same agar medium, at 37°C. Each determination was repeated three times.

The Bacterium coli titre was determined with the fermentation method using Ejkman's liquid substratum. Results were read after 24 and 48-hour incubation, at 37°C. The presence of Bacterium coli was detected on the basis of acidification of the medium and the presence of gas in the Durham tubes.

3. RESULTS

Results from qualitative and quantitative microscopy studies are shown in Table I. Altogether 53 species were recorded, 6 of them belonged to Rhizopoda, 2 — to Flagellata, 31 — to Ciliata, 14 — Rotatoria. Species of very small Flagellata, Nematoda, Gastrotricha, Oligochaeta, and Copepoda, were not identified. In further discussion individuals belonging to the mentioned systematic units ranking above a species will be regarded as single species. The sewage under treatment contained about 66% of industrial waste causing a mass-production of Amoeba individuals which, as described by McKinney, Gram (1956), appear in the activated sludge regenerating after previous contamination. The density of *Amoeba limax* and *A. radiosa* population was increasing simultaneously with the shortening of the activated sludge aeration time. The occurrence of *Amoeba proteus* followed a similar course, however, their presence was not detected during the shortest aeration time (Experiment IV). The number of *Cochliopodium granulatum* individuals was decreasing slightly together with the shortening of aeration time. Scarce shells of dead at all times, *Euglypha alveolata*, were found, particularly, at the longer periods of aeration

time. Few specimens of *Actinosphaerium eichhorni* were encountered in the first days of the experiment i.e. at the beginning of addition of the examined sewage to the earlier cultivated activated sludge.

Some few individuals of *Peranema trichophorum* were observed at the longer-lasting, and some of *Euglena viridis* at the shortest aeration of the activated sludge. The number of very small specimens determined as *Flagellata n. det.* decreased, just as observed in many former studies and confirmed by Klimowicz (1970), simultaneously with the shortening of aeration time, which increases the activated sludge loading.

Together with the progressing shortening of the activated sludge aeration time there was observed an increase in the numbers of individuals of the following Ciliata species: *Opercularia minima, O. microdiscum,* and *O. coarctata,* and a decrease in such species as: *Chilodonella cucullulus, Ch. uncinata, Euplotes affinis, Aspidisca costata, Epistylis plicatilis,* and *Vorticella convallaria.* It is worth mentioning that individuals of *Vorticella putrina* and *V. microstoma* appeared in great numbers only in the period between November 13 and December 23, at 5-hr and 3-hr aeration times (Experiments II and IV) which must have been produced by some unaccounted for specificity of the sewage and not by the duration of aeration. As concerns other species of Ciliata it was difficult to perceive any regularity in their occurrence since those specimens were observed only sporadically and quantitative changes occurred most frequently in unpredictable periods of time.

A considerable admixture of industrial wastes prevented the development of all the multicellular organisms (Metazoa). As results from Table I the occurrence of Nematodes, Rotatoria, Gastrotricha, Oligochaeta, and Copepoda was sporadic. It should be emphasized that out of 14 Rotatoria species only *Colurella colurus* occurred in greater numbers and just in one experiment.

Results from bacteriological analyses of the sewage are shown in Table II. In Experiment I the average degree of the psychrophilic bacteria destruction amounted to 88.0%, of mesophylic ones — 75.8%. In Experiment II it averaged 92.2% for the former and 95.0% for the latter. In Experiment III it averaged 86.7% and 51.7%, respectively. In Experiment IV — 87.0 and 83.3%, respectively. The titre of Bacterium coli was $1 \cdot 10^{-6}$ in the sewage before treatment and $1 \cdot 10^{-5}$ in the purified sewage; it was the same in all the four experiments.

After a comparative estimation of the results obtained in four experiments one can say that the degree of destruction of psychrophilic and mesophilic bacteria was fairly high, averaging 83.1%. The Bacterium coli titre was also considerably reduced from $1 \cdot 10^{-6}$ in the inflowing sewage to $1 \cdot 10^{-5}$ in the purified one. It has been noticed that the duration of the sewage aeration time did not have any significant

Table II. Total number and reduction per cent of psychrophilic and mesophilic bacteria and Bacterium coli titre

Date of experiment	Bacteria	Sewege before treatment		Tank No. 1			Tank No. 2		
				Time of sewage aeration (hr)					
				6 (Exp. I)			4 (Exp. III)		
		Total number/ml	Coli titre	Total number/ml	Reduction (%)	C li titre	Total number/ml	Reduction (%)	Coli titre
15.V—30.VI	Psychrophilic	8000,000 7900,000	$1 \cdot 10^{-6}$	900,000 1000,000	88.8 87.3	$1 \cdot 10^{-5}$	1300,000 900,000	84.8 88.6	$1 \cdot 10^{-5}$
	Mesophilic	2000,000 1800,000		300,000 600,000	85.0 66.7		600,000 1:00,000	70.0 33.4	
				5 (Exp. II)			3 (Exp. IV)		
13.XI—23.XII	Psychrophilic	6200,000 7000,000	$1 \cdot 10^{-6}$	170,000 900,000	97.3 87.2	$1 \cdot 10^{-5}$	1000,000 700,000	84.0 90.0	$1 \cdot 10^{-5}$
	Mesophilic	3400,000 3000,000		122,000 200,000	96.7 93.4		234,000 500,000	93.2 83.4	

effect on the degree of the bacteria destruction. Only once in Experiment III it was strikingly low averaging 33.4%, but this can be, surely, explained by some measuring errors.

4. DISCUSSION

The four experiments with various aeration times showed similar results of the quantitative and qualitative composition of microfauna (Table I). Changes in aeration time did not produce violent destruction of some species or created optimal growth conditions for others. Changes in sewage loading effected by the shortening of the time of sewage aeration considerably greater fluctuations in the number of individuals than in the species composition (cf. Klimowicz 1970).

In all the experiments Ciliata species, sessile or crawling on the floccules, were predominant. In the opinion of Ardern, Lockett (1936), Baines et al. (1953), McKinney, Gram (1956), Curds, Cockburn (1970 a, b), and Klimowicz (1970), they are characteristic for a high degree of sewage purification. The mass occurrence of the Ciliata species crawling or attached to the flocs, characteristic for an effective sewage purification by the activated sludge, happened for the greater part simultaneously. There was not observed a mass destruction of the sessile and crawling Ciliata and their replacement by mass occurrence of Flagellata and loosely floating among the flocs — Ciliata.

Table II shows also a stable, remaining at a similar level, destruction of the total number of bacteria population and a constant titre of Bacterium coli. The identical titre of Bacterium coli obtained in all the experiments may be explained by the fact of keeping the sewage in the barrels during the transport and in consequence bacteriological processes had already begun 1—2 days earlier before bacteriological inoculations. Results from bacteriological examinations suggest that the shortening of the time of sewage aeration can be carried on in further experiments. The growth of filiform bacteria *Sphaerotilus natans f. dichotomus* was observed only in Experiments I and III. The development of Spaerotilus was insignificant and did not effect the bulking of activated sludge. This should be considered as a favourable factor in the further development of the sewage treatment plants for the town of Toruń in the nearest future. On the basis of some studies (Klimowicz 1969) a mass development of filiform bacteria was dreaded.

5. SUMMARY

Bacteriological and hydrobiological studies were carried out in order to get precise information about the course of the sewage purification processes. Bacteriological analyses had in view determination of the degree of sewage purification on

the basis of destruction of the total number of the bacteria population and the Bacterium coli titre. The scope of hydrobiological observations covered the determination of species composition and of total number of individuals of the microfauna species. The purpose of all the experiments was to determine technological parameters for the most economical means of sewage treatment in the town of Toruń.

The majority of the organisms identified in the activated sludge have preserved continuity of occurrence and that can be a guarantee of a uniform degree of sewage purification. Considerable changes in the duration of sewage aeration did not bring about the foreseen substantial effects on the composition of organisms. The shortening of the sewage aeration time caused much greater fluctuations in the number of individuals of particular species than in the composition of species. Mass occurrence of Ciliata crawling on flocs or sessile, characteristic for the activated sludge — ripe and effectual in purifying sewage, happened for greater part simultaneously. Reciprocal elimination was not observed in the time of the occurrence of those two groups. Rotatoria appeared in the course of all the experiments, sporadically. Their limited development can be explained by the harmful effects of the admixture of industrial wastes in the sewage.

Results from bacteriological analyses indicated a high degree of sewage purification in all the experiments. The degree of psychrophilic and mesophylic bacteria destruction averaged 83.1%. The titre of Bacterium coli in flowing in sewage was $1 \cdot 10^{-6}$ and in the purified sewage flowing out of settling tank — $1 \cdot 10^{-5}$. The composition of the detected organisms indicated that the biological sewage purification process was normal in all the four experiments. So, one can say that the determined aeration time of 3, 4, 5, and 6-hr duration produced favourable conditions for the normal course of the sewage treatment processes.

6. STRESZCZENIE

Przeprowadzono badania bakteriologiczne i hydrobiologiczne, które informowały o przebiegu procesu oczyszczania ścieków. Badania bakteriologiczne miały na celu określenie stopnia oczyszczania ścieków na podstawie redukcji ogólnej ilości bakterii i miana Bacterium coli. Zakres badań hydrobiologicznych obejmował określenie składu gatunkowego i liczebności mikrofauny. Celem przeprowadzonych doświadczeń było ustalenie parametrów technologicznych stwarzających najekonomiczniejszy sposób oczyszczania ścieków miasta Torunia.

Większość organizmów, zidentyfikowanych w osadzie czynnym, zachowała ciągłość występowania, co winno gwarantować równomierny stopień oczyszczania ścieków. Znaczne zmiany czasu napowietrzania ścieków nie wywierały przewidzianych zasadniczych wpływów na zestaw organizmów. Skracanie okresów napowietrzania powodowało znacznie większe wahania w liczebności osobników poszczególnych gatunków niż w ich zestawie gatunkowym. Masowe występowanie orzęsków pełzających po kłaczkach i osiadłych, charakterystyczne dla dojrzałego, dobrze oczyszczającego ścieki osadu czynnego, następowało przeważnie równocześnie. Nie stwierdzono wzajemnego wykluczania się w czasie występowania tych dwóch grup. Wrotki występowały sporadycznie we wszystkich doświadczeniach; brak ich masowego rozwoju należy tłumaczyć szkodliwym dla nich wpływem domieszki ścieków przemysłowych.

Wyniki badań bakteriologicznych wykazują wysoki stopień oczyszczania ścieków we wszystkich doświadczeniach. Stopień redukcji bakterii psychrofilnych i mezofilnych wyniósł średnio 83,1%. Miano Bacterium coli dopływających ścieków wynosiło $1 \cdot 10^{-6}$, a w oczyszczonych, odpływających z osadników wtórnych — $1 \cdot 10^{-5}$. Skład wykrytych organizmów wykazał, że proces biologicznego oczyszczania ścieków we wszystkich 4 doświadczeniach przebiegał prawidłowo. Okazało się więc, że stosowane czasy napowietrzania 3, 4, 5 i 6 godzin stwarzały pomyślne warunki dla normalnego przebiegu procesu oczyszczania ścieków.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	21	2	301-307	1974

IRENA CABEJSZEK

Associate professor Irena Cabejszek, Ph. D. in Natural Sciences, retired scientific worker of the State Institute of Hygiene, former secretary of the Hydrobiological Committee of Department II of Biological Sciences, Polish Academy of Sciences, died in Warsaw on Sept. 12, 1972.

Irena Cabejszek was born on Oct. 1, 1910, in Cracow. After completion of secondary education, in 1929 she took up biological studies at the Faculty of Mathematics and Natural sciences of the Jagiellonian University. In 1934 she was awarded the degree of M. S. in botany. While yet a student, she took up research work under the direction of Prof. Jadwiga Wołoszyńska, Ph. D., and Prof. Alfred Lityński, Ph. D. She investigated the plankton of the Biała Przemsza River, while working during 1934–1935 as deputy-assitant at the Chair of Pharmaceutical Botany, Jagiellonian University. Immediately after having taken her M. S. degree, for one year she taught biology at the girls' high-school in Miechów. Subsequently, till 1938 she prepared her doctor's dissertation as holder of a scholarship of the Warsaw Scientific Society, collaborating with the Biological Station in Pińsk and investigating the Diatoma of Polesia waters. Among other findings, she described a new species — *Fragilaria zasumiensis n. sp.* Cab. In 1938 she was awarded the doctor's degree in hydrobiology at the Jagiellonian University. In March 1939 she accepted employment as senior assistant at the former Waters Division of the State Institute of Hygiene in Warsaw, where under the direction of Prof. Marian Stangenberg, Ph. D., she investigated the plankton of polluted water bodies.

For the duration of World War II Prof. Cabejszek did not leave her Department. Forced to perform routine services imposed by the Nazi administration, she secretely engaged in describing the plankton material collected prior to the war. Although no research work was possible during Poland's occupation, she prepared a paper on the plankton of the Niemen River, which was published only after restoration of peace.

After Poland's liberation Prof. Cabejszek moved to the city of Łódź to continue research on sanitary protection of waters at the local branch of the State Institute of Hygiene. Under trying post-war conditions she very actively participated in the reactivation of the State Institute of Hygiene. In 1946 she returned to Warsaw where she was appointed adjunct and head of the Laboratory of Hygiene and Sanitary Protection of Surface Waters, State Institute of Hygiene. There she initiated investigations on the pollution of main Polish rivers. Moreover, she was employed as scientific worker at the Chair of Communal Hygiene, Medical Academy in Warsaw. In co-operation with Prof. Jan Just, Ph. D., she was engaged in teaching at the Sanitary and Epidemiological Studium, Medical Academy in Warsaw, as well as in performing research and organizational activities at the State Institute of Hygiene. Subsequently, Irena Cabejszek was awarded the degree of Associate Professor, and continued studies of the sanitary conditions in Poland. She greatly appreciated the participation of hydrochemists

and bacteriologists in research on environmental pollution; by bringing together various specialists, she promoted co-operative studies. Her ample professional record mainly comprises collaborative publications, with participation of biologists of various specialities and chemists.

In 1956 the Institute of Water Management was set up and gradually took over studies of water pollution. Prof. Cabejszek greatly assisted in the organization of this new unit, helped in research planning, instructed young scientific workers who could always rely on her for advice, and acted as consultant of some projects (e.g. construction of the Zegrzyński dam reservoir).

Prof. Cabejszek made an enormous contribution of teaching and organizational work to the co-operation with Sanitation and Epidemiology Stations. By way of professional patronage, consisting of supervision of many courses, collective and individual instruction as well as consultations, she raised the standard of performance of these regional institutions.

By the end of the fifties Prof. Cabejszek took up a series of new broad investigations on the biocenosis of rivers polluted with various industrial wastes. Study was made of the role played by wastes of the sugar, paper and petrochemical industries in the development of complexes of organisms under the physico-chemical conditions of river water, changing as a result of pollution and self-purification. One of these investigations dealing with the pollution of Vistula, in the region of Płock, by wastes of the developing petrochemical works was continued over several years and found practical application at the time when a waste purification station was designed for this plant.

At the beginning of the sixties Prof. Cabejszek started with her coworkers a series of studies using biotests. Investigation was made of the effect of phenols, cyanides and some metals on water biocenosis, with daphniae used as test organism.

In 1965 Irena Cabejszek was awarded the degree of professor. At this time she focused attention on the problem of water pollution from the standpoint of human health protection. She undertook pioneer studies using warm-blooded animals as test organisms for determination of noxious levels of detergents, and subsequently of pesticides, in drinking water.

Investigations on health hazard from pesticides were conceived on a wide scale, including studies of river seston, biotests, physico-chemical properties of pesticides and physiological studies. Investigations of this series, still being continued at the Department of Communal Hygiene, State Institute of Hygiene, of the grounds of their wide scale and broad perspectives are an important contribution to the development of sanitary hydrobiology in Poland. Prof. Cabejszek has initiated studies of pesticides, in the aspect of environmental protection, at a time when the effects of chemicalization of agriculture still evoked no concern in this country. The last paper of this series, with Prof. Cabejszek as co-author, was published already after her death (1973).

In addition to strenuous research activities at the State Institute of Hygiene and at Department II of the Polish Academy of Sciences, Prof. Cabejszek effectively performed social work. She was charter member of the Warsaw Branch of the Polish Hydrobiological Society, performing a number of organizational functions. Together with her coworkers, she put much energy into organization, in August 1965, of the XV Limnological Congress in Warsaw. At her suggestion, a Conference on "Pesticides in surface waters" was organized in May 1970. The postulates put forward at this conference relative to restriction of the use of insecticides were carried into effect, in the form of practical measures aimed at environmental protection.

Grave illness did not prevent Prof. Cabejszek from taking part in many scientific and social undertakings. In the last years of her life she effectively worked as President of the Employees' Committee of the State Institute of Hygiene. She prepared publication of methods used in sanitary hydrobiology. However, grave illness made progress. Whoever knew Prof. Cabejszek, was aware that she was severely overworked; nevertheless, she seemed to be capable of resisting over-exertion, always being young in spirit and full of optimism.

On Jan. 1, 1971, she untimely retired, without — however — losing touch with her coworkers, whom she continually helped in research work and management of the Laboratory.

The life-work of Prof. Irena Cabejszek is an important contribution to the development of sanitary hydrobiology as an applied science in the domain of medicine. She proved the usefulness of hydrobiological investigations for man's

health protection, she promoted their standing and rendered their continuation possible.

In appreciation of her merits, Prof. Irena Cabejszek was awarded the Decoration for Exemplary Work in the Health Service, Gold Cross of Merit and Companion's Cross of Poland's Revival.

Peace to her memory.

Janina Stanisławska

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1935

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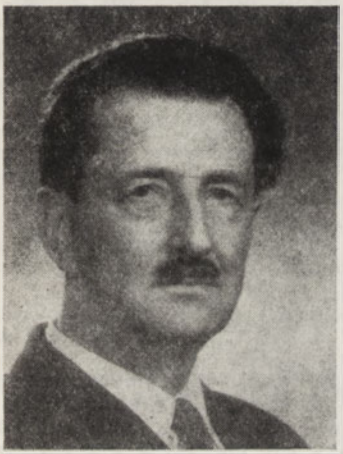
PRZEMYSŁAW OLSZEWSKI



On Dec. 16, 1972, science lost a prominent hydrobiologist of great merit. After grave illness Prof. Przemysław Olszewski, Ph. D., untimely passed away.

Prof. Olszewski was born on June 23, 1913 Cracow. His father was a painter and his mother — a teacher. In Cracov he attended grammar school and Nowodworski High School of humanistic type, which he completed in 1931 and then took up studies at the Agricultural Faculty of the Jagiellonian University. From the beginning, his interests gravitated towards fishery and hydrobiology. While yet being a student, he attended a course in limnology at the Hydrobiological Station, at Lake Wigry, and a course in oceanography at the Naval Station, at the Hel Peninsula.

By the end of his third academic year he took up research work within the scope of his M. S. thesis dealing, at his own request, with the biology of Pond Czerwony Stawek Pańszczycki in the Tatra Mountains. While becoming acquainted with the literature of the subject, he became aware of the scarcity of knowledge of the aquatic environment, especially that of the Tatra Mountains. In addition to his M. S. thesis, he performed some hydrochemical studies reported in the paper "Some data on the chemistry of waters in the region of Hala Gąsienicowa". Already at this time, his main scientific interests assumed their final shape.

He presented the most important results of this series of investigations in the paper "Stratification of mountain lakes in summer" sent to Paris to be delivered at the Limnological Congress. In collaboration with Meisels, he studied gas saturation and gas equilibrium in a water column, and independently investigated the indices of aerobic transformations in water.

In 1936, he accepted employment as assistant at the Department of Ichthyobiology and Fishery of the Jagiellonian University in Cracow. He participated in the organization of the Experimental Fishing Station of the Jagiellonian University in Mydlniki.

During World War II, after reestablishment, by the invader's authorities, of the former Department of Fishery of the Jagiellonian University as a service unit for the fishing industry, together with other fellow-workers he returned to employment at this Department. At this time he carried out investigations on the dam reservoir Rożnowskie, described his results obtained before the outbreak of war in the Tatra Mountains and prepared his Doctor's thesis. In 1945, soon after Poland's liberation he was conferred the Doctor's degree and appointed to the post of adjunct at the Department of Fishery of the Jagiellonian University. At the same time, between 1945–1949 he taught hydrobiology at the Fishery High School in Cracow.

In 1946 he was charged with the duties of lecturer and examiner in hydrobiology and ichthyology at the Agriculture and Forestry Department of the Jagiellonian University. Between 1949–1951 he directed a number of investigations carried out for a M.S. thesis, was reviewer of the respective theses and took part in

examinations for a M.S. degree. At the invitation of the Agricultural University in Warsaw, during 1950–1951 he commuted to Warsaw, to lecture on hydrochemistry and water pollution.

New inclinations and former interests drew Prof. Olszewski's attention to the largest Polish lake complex, i.e. the Mazurian Lakes. In 1951 he was appointed Head of the Hydrochemical Laboratory at the Fishing Station in Giżycko. Soon afterwards he was transferred to the newly set up Faculty of Fishery of the Agricultural Academy in Olsztyn (at present: Agricultural and Technical Academy in Olsztyn), to become Head of the Department of Hydrochemistry and to lecture on this subject. After some years he was appointed Head of the Chair of Limnology. There he was conferred in 1956 the degree of professor, and in 1965 — of full professor.

At the first stage of his activities in the Mazurian Lake land, Prof. Olszewski and his group were engaged upon gaining general information on these lakes. Together with J. Paschalski, he published the main results concerning the hydrochemistry of 170 lakes in the paper: "Preliminary limnological characterization of some lakes in the Mazurian Lake District". Against the background of these results, he presented his fundamental thermo-typological concept in papers: "Graduation in the intensity of the wind effects on lakes", "The influence of seiches on lake life", "Wirbelströmungen in dem Hypolimnion der Seen" and "Upper limit of the hydrogen sulphide zone in the thermocline of lakes".

At the same time, on the basis of his profound knowledge of thermology and dynamics of water masses, as well as of nutrient transformations, Prof. Olszewski took up the problem of lake recultivation. The first experiment, preceded by broad complex studies of the whole ecosystem and of its catchment area was set up at Lake Kortowskie in 1956. The first results were presented in communications: "The removal of lake hypolimnion", "Versuch einer Ableitung des hypolimnisches Wassers aus einem See" and "Die Ableitung des hypolimnischen Wassers aus einem See".

Prof. Olszewski's untimely death prevented him from carrying into realization the program of recultivation of several other lakes, intended and prepared by preliminary studies.

The experiment on Lake Kortowskie was an application of a pioneer method which permitted successful solution of the important problem of preventing excessive entrophication of this lake. This investigation was one of the first extensive projects carried out by a group of various specialists using a multidirectional approach to solve, under the direction of Prof. Olszewski, complex scientific problems. In addition to the development of methods for proper fish management of lakes and their protection from entrophication, Prof. Olszewski and his group pursued collection of fundamental data on lakes. Unfortunately, there was no time enough for him to personally describe the results obtained for over 400 lakes.

Moreover, Prof. Olszewski greatly contributed to studies and to a theoretical interpretation of the water pollution processes. He presented a picture of the migration of impurities in lakes.

On the grounds of comprehensive studies treated on a broad basis, in the last publications Prof. Olszewski summarized his views on the problem of trophy and saprobity of lakes in papers: "Trophy and saprobity", "Search for means of effective prevention of lake degradation" and "Establishment of principles of appropriate lake protection".

Prof. Olszewski's research work was paralleled by teaching and educational activities. His lectures achieving a very high standard both in contents and form, delivered in fine Polish and standing out by an unconventional literary form, always attracted a multitude of students. He created an authentic school of modern limnology. Under his direction a number of students were conferred the degrees of M.S., Ph. D. and associate professor. Bringing together, in his complex investigations, a team of coworkers, he conveyed his knowledge and traced scientific goals. As a highly cultivated man, he moulded their personality. His perseverance in solving difficult scientific problems, his honesty and conscientiousness set an example for his pupils.

Prof. Olszewski was a fervent propagator and advocate of the cause of environmental protection. His contribution to water protection was specially important. He organized the Faculty of Water and Inland Fishery Protection, laying the foundations of teaching programs in the field of biological protection of waters.

Prof. Olszewski performed a number of responsible functions and held many posts of eminence (cf. enclosed list).

As a tribute to his scientific and organizational attainments, Prof. Olszewski

was awarded the Medal of the Tenth Anniversary of Polish People's Republic, Gold Cross of Merit, Gold Decoration "Meritorious for Varmia and Mazuria", Companion's Cross "Polonia Restituta" and others, as well as the scientific award of the Minister of Technique, Science and Academic Education, and the scientific prize of the Polish Academy of Sciences.

Aleksandra Sikorowa

LIST OF FUNCTIONS PERFORMED BY PROF. P. OLSZEWSKI Ph. D.

1. Head of Chair of Limnology, Agricultural University in Olsztyn.
2. Dean of former Faculty of Fishery, Agricultural University in Olsztyn.
3. Deputy Dean of former Division of Inland Fishery, Agricultural University in Olsztyn.
4. Director of the Institute of Hydrobiology and Water Protection.
5. Chairman of the Editorial Committee of *Zeszyty Naukowe WSR* (Scientific Papers of the Agricultural University in Olsztyn).
6. Member of Scientific Council of the Institute of Applied Biology, Agricultural University in Olsztyn.
7. Member of the Committee of Experts on academic programs, attached to the Main Council of Academic Education.
8. Member of the Advisory Group for Development of Young Professionals, attached to the Main Council of Academic Education.
9. Member of the Scientific and Technical Council of the Ministry of Agriculture — Fishery Commission.
10. Member of the Scientific Council of the Institute of Inland Fishery.
11. Member of the presidium of the Hydrobiological Committee, Polish Academy of Sciences.
12. Chairman of the Main Board and of the Division in Olsztyn, of the Polish Hydrobiological Society.
13. Member of the Editorial Committee of the *Polish Archives of Hydrobiology.*
14. Member of the Scientific Council of the Department of Water Biology, Polish Academy of Sciences.
15. Member of the Main Board of the Society of Agricultural Engineers and Technicians (SITR).
16. Chairman of the Fishery Section of the Society of Agricultural Engineers and and Technicians.
17. Member of the Olsztyn Voivodeship Committee for the Preservation of Nature.
18. Member of the M. Kopernik Society of Natural Scientists.
19. Member of the Polish Geophysical Society.
20. Member of the Society for Development of the Regained Territories.
21. Member of the Social and Cultural Association "Pojezierze" ("Lakeland").
22. Member of the Linguistic Culture Society.
23. Member of Societas Internationalis Limnologiae.
24. Member of the Freshwater Biology Association.
25. Member of CS Limnologicka Společnost.
26. Member of the Polish Teachers' Association.
27. Member of the Association of Jagiellonian University Graduates.
28. Member of the Association of Agricultural Faculty Graduates of the Jagiellonian University.
29. Councillor of the People's Town Council in Olsztyn.
30. Member of the Committee for Protection of Water Resources.
31. FAO Expert.

LIST OF MORE IMPORTANT PAPERS BY PROFESSOR PRZEMYSŁAW OLSZEWSKI

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