INSTYTUT BIOLOGII DOŚWIADCZALNEJ
IM. M. NENCKIEGO POLSKIEJ AKADEMII NAUK

THE NENCKI INSTITUTE OF EXPERIMENTAL
BIOLOGY POLISH ACADEMY OF SCIENCES

POLSKIE ARCHIWUM HYDROBIOLOGII

POLISH ARCHIVES OF HYDROBIOLOGY

VOL. XIX

No. 4

Founded by
A. LITYNSKI, M. BOGUCKI

Editor
R. Z. KLEKOWSKI

PWN • WARSZAWA 1972

POLSKIE ARCHIWUM HYDROBIOLOGII
jest kontynuacją ARCHIWUM HYDROBIOLOGII I RYBACTWA

POLISH ARCHIVES OF HYDROBIOLOGY
formerly ARCHIVES D'HYDROBIOLOGIE ET D'ICHTHYOLOGIE

Polskie Archiwum Hydrobiologii existing as such since 1953, is a continuation of *Archiwum Hydrobiologii i Rybactwa* founded in 1926 under the editorship of A. Lityński; during the period 1926 to 1939 and in 1947, thirteen volumes of *Archiwum Hydrobiologii i Rybactwa* have appeared, volume XII, 3, 4, published in September 1939, being almost entirely destroyed due to war action.

Nakład: 870 (759+111) ark. wydawn. 15,5 ark. druk. 10,25. Oddano do składania we wrześniu 1972 r. Podpisano do druku w styczniu 1973 r. Druk ukończono w styczniu 1973 r. Papier druk. sat. III kl. 80 g.

Druk. Białostockie Zakłady Graficzne. Zam. 3652/72. A-103

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	343–359	1972

E. FISCHER

A YEARLY CYCLE OF CHANGES IN DYNAMICS OF PRODUCTION OF THE CHEMOAUTOTROPHIC BACTERIA IN BOTTOM SEDIMENTS OF A WATER BODY

Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

ABSTRACT

This study was to investigate the yearly cycle of production of the chemoautotrophic bacteria in bottom sediments of small water bodies. Relationship existing between the level of the chemoautotrophic bacterial biomass production, the number of bacteria and the production dynamics of bacterial population, expressed by a coefficient, was determined. It was stated that seasonal changes in the 24 hr production value of the bacterial biomass do not correspond always with the seasonal changes in number of the bacterial cells. Whereas, there is a relationship between the biomass production and the coefficient of production dynamics of bacteria. Hence, the bacterial biomass production is controlled by other factors than those influencing the effective number of bacteria existing in the bottom sediments.

1. INTRODUCTION

The problem of the productivity of inland water bodies has come lately to the fore of limnological studies. In this problem the matter of the production of bacteria takes an important place, since they make a highly valuable food for all the water animals feeding upon microorganisms. Furthermore, bacteria on way of metabolic transformations supply the environment with chemical compounds indispensable for plant nutrition.

One of the most important of the functions performed by bacteria in the water bodies is mineralization of the organic matter and transformation of the dissolved in water organic and inorganic substances into the living substance of their own cells (ZoBell 1946, Kriss 1959, Kuznetzov 1952, Gambarian 1962). The mineralization process mobilises organic compounds comprised in the decaying plants and animals abiding in a water body and in that way the cycle of circulation of the basic chemical elements, such as: carbon, nitrogen, phosphorus, and sulphur, is closed. In part, the assimilated organic and inorganic compounds serve, among other uses, for production of the bacterial biomass, which in its turn provides a base of food supply for water animals feeding on bacteria.

The composition of bacterial cells and their caloricity approximate fish and man tissues (Kazakov, Pronina 1941, Klejmenov 1952, Rodina 1958). Moreover, the bacterial capacity to produce vitamin substances (Anderson 1948, Goodwin 1952, Beran 1966, Hospodka 1966) qualifies them as a valuable food material for lots of animal organisms. Therefore, production of bacteria in the inland water bodies is of a particular consequence in limnological studies.

The hitherto published, relatively numerous papers concerning limnological microbiology contain estimates on either the number of bacteria (Kuznetzov 1956, 1958, 1959 a, b, Rodina 1963, Jannasch 1954, Beljackaja 1958, Potter 1964 and others) or else on the 24 hr production of the bacterial biomass in the collected samples (Kuznetzov 1955, Sorokin 1955, 1957, 1958 a, b, Kuznetzov et al. 1966, and others). Both of those two lines of investigations, no matter how important for limnological research, do not give a sufficient material for determination of the basic feature of the bacterial biomass production process, namely, the dynamics of development of the bacterial population. In one case the amount of the bacterial biomass, actually stored up at the moment of collecting the sample, is determined, in the other case — the amount of bacterial biomass produced in a unit of time. Although, those values characterize the actual state of a water body, as considered from the specific viewpoint of microbiology, nevertheless, neither of the two values, when analysed separately, characterizes the dynamics of development of bacterial population.

The feature characterizing the production activity of the bacterial cells, in pure cultures and under conditions of unrestrained development, i.e. under conditions imposed upon the culture — is the generation time of cells. The development of a bacterial culture proceeds, as it is well known, in five phases (Monod 1942, 1949, 1950, Precht et al. 1955). From the point of view of increase of the number of cells in the culture the most essential phase is this, in which bacterial cells are dividing. This stage is called the exponential or logarithmic phase and is expressed by the following formula:

$$N_t = N_0 2^{\frac{t}{T}} \tag{1}$$

where N_t — the number of cells after the time of the experiment, N_0 — the initial number of cells, t — the time of duration of experiment, while T — the time needed for the cell division, which characterizes the productive activity. Formula (1), however, may be applied solely to pure cultures and by no means to mixed populations (Fischer 1966 a). Methods used at present do not allow to determine the generation time in a mixed bacterial population living in its native environment. Obviously, it comes as result of the diversity of kinds of bacteria existing in a water body and of the division of their cells occurring in different periods of time. Furthermore it results from the fact that in their native environment the growth of bacteria takes place, for one thing, under the conditions of restrained development, for the other, in the conditions of a variable supply of substrata. Restraint of bacteria development in biocenosis of a water body is caused by consumption of bacteria by zooplankton, bacteriostatic or bactericidal action of some plants, etc. The generation time of bacteria is also affected by the varying physicochemical state of the environment.

Correlations between bacterial population and the prevailing conditions in a water body are presented in Fig. 1, in a simplified manner. As can be seen, the process of bacterial biomass production is influenced by chemical properties of the environment and by climatic factors. On the other side, the metabolic products of bacteria bear effect on the chemical properties of environment. Those, in turn, influence biocenosis of plants and animals, which has an effect on the number of the living cells in bacterial population.

Climatic factors affect alike both the production activity of bacteria and of all the rest of biocenosis. Simultaneously, they bear effect on the chemical composition of the environment, since by acting on production activity they regulate thereby the supply of metabolites. Moreover, rainfalls contain many a time considerable quantity of nitro-compounds, which quite often may be of a great consequence for biocenosis of a water body (Nowotny-Mieczyńska, Gołębiowska 1960).

Thus, climatic factors can accelerate the process of bacterial biomass production and also activate the organisms reducing bacterial population. For instance, the rise of water temperature of a few degrees may, by increasing the productive activity of bacteria, at the same time activate some bactericidal agents that will destroy that population.

In the shaded water bodies and in the periods of a reduced inflow of luminous energy into the water, a particular role is played by chemoautotrophic bacteria in the generation of the living organic substance. So, in our investigations of water (Fischer 1970) for instance, in the autumn and winter seasons the quantities of

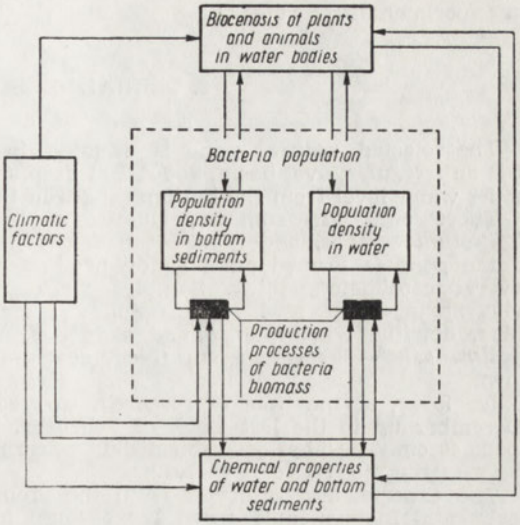


Fig. 1. Interdependence of bacterial population, the remaining biocenosis, abiotic environment, and climate conditions

assimilated carbon, in the 24 hr process of the chemoautotrophic bacteria production, were values of a similar order, and they were many times greater than quantities of carbon assimilated in the process of photosynthesis (Fischer 1966 b, Table I).

Table I. Minimum and maximum values from determinations of photosyntesis in water of the experimental reservoir in comparison with simultaneously determined production of chemoautotrophic bacteria in the bottom sediments

Season	Date	Photosynthesis (μg C/m²)	Chemosynthesis in bottom sediments * (μg C/m²)
Spring	10.V.1966	3000	37,860
	23.III.1967	311,400	112,800
Summer	30.VI.1966	50,880	424,500
	16.VI.1966	2240,000	406,500
Autumn	27.X.1966	912	28,140
	30.IX.1966	13,650	77,700
Winter	27.II.1967	0	23,760
	5.I.1967	960	36,300

* Calculations included a 3 cm thick layer of bottom sediment.

Worth of particular notice is the production of chemoautotrophic bacteria at the bottom layers of the water body. The bottom zone is enriched all the time with simple mineral compounds owing to the decomposition of the sinking dead remains of plants and animals. Thus, the heterotrophic microorganisms, producing in the bottom sediments on the way of their own metabolic processes abundant sources of food and energy for the chemoautotrophic bacteria, are creating for them thereby very favourable conditions of the development (Sorokin 1955).

The object of this work was characterization of the dynamics of bacterial population in the bottom sediments in a yearly cycle of the biomass production process of chemoautotrophic bacteria.

For investigation two small water bodies were selected — a natural pond and an experimental reservoir.

2. TERRAIN DESCRIPTION

The selected natural pond is situated in the area of the Kampinos Forest. It is an irregular, oval basin, about 2 m deep, 8 m wide, and 14 m long. The distance of the water level from the bottom ranges in the course of a year from 70 to 180 cm.

There occur, in summer, clumps of *Sparganium simplex, Typha latifolia, Equisetum arrense,* and *Elodea canadensis* prevailing at the bottom. The bottom of the pond is formed on a sandy bedding and has a layer of bottom sediment several centimeters thick. Samples were collected in places moderately silty with arrangement of layers as follows: semifluid detritus — layer about 3 cm thick, detritus with fine-grained sand — layer about 5 cm thick, grained, pale--yellow sand — layer 4—5 cm thick, coarse-grained gravel — layer reaching deep down.

Ice layer, at the time of research, covered the area in the period from mid--December up to the last week of February. The maximal thickness amounted to about 40 cm. The physicochemical data referring to the pond and bottom sediments are presented in Fischer (1972).

The experimental reservoir is in the premises of the Nencki Institute of Experimental Biology in Warsaw. It was built in 1962. It is a ditch, of a truncated--pyramid shape, 120 cm deep, and 4 m² area at the top surface. The hollow has been lined first with boards then with closely fused igelite insulation foil. The bottom of reservoir was formed with the surface layer of detritus from the bottom sediments of the investigated natural water body. Water was also taken from there to fill up the experimental reservoir. Vascular plants were not introduced. Thickness of the formed bottom layer, during the period of research, amounted to 22 cm, depths of water ranged from 36 to 6 cm.

In the year of our investigations (1966) there was abundant emergent vegetation. The water surface was covered with *Lemna minor.* In the water, the presence of Cladocera, Copepoda, Protozoa, Rotatoria, and also of insect larvae (Ephemeroptera, Diptera and others) was recorded.

The thickness of the ice and the time of its occurrence is presented in Fig. 2. Temperature changes and some results of chemical analyses are shown in Table II.

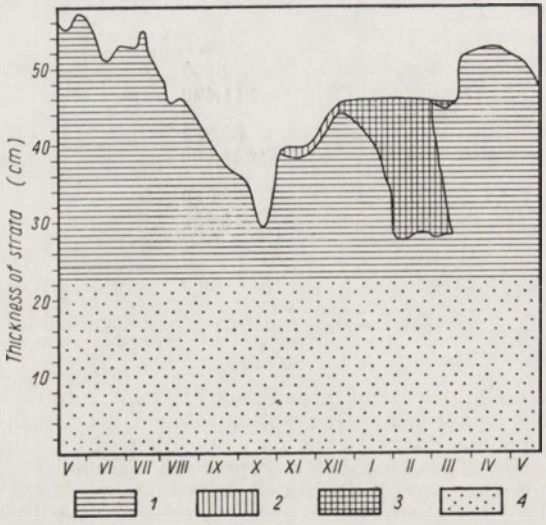


Fig. 2. Forms of water in the experimental reservoir during the time of investigations. 1 — water, 2 — initial phase of congelation, 3 — ice cover, 4 — bottom sediments

3. METHODS

Aiming at the characterization of dynamics of the bacterial population in the examined bottom sediments in the production process of the chemoautotrophic bacteria biomass, the assessment of the number of bacterial cells in bottom sediments and the 24 hr assimilation of carbon by the chemoautotrophic sediment bacteria were carried out. In result, it was possible to determine the coefficient of the production dynamics.

In order to be able to obtain as complete characteristics as possible, of the conditions under which the examined processes were taking place, the following had to be assessed:

The number of bacterial cells in water of the experimental reservoir,

The 24 hr assimilation of carbon in water of the experimental reservoir, with access of luminous energy,

24 hr assimilation of carbon in the reservoir water, without of the luminous energy access,

Inflow of luminous energy below the surface level of the reservoir water at the following wave-length ranges: 400—500, 500—600, and 600—700 mμ,

Continuous registration of temperature, during the period of investigations, at two levels of the experimental pond, and temperature measurements in the bottom sediments and in water of the natural pond.

Furthermore, the pH and contents of ammonium salts, nitrites, nitrates, phosphates, sulphates, carbon dioxide, and oxygen, in water were determined in both water bodies under investigation.

Sampling. Samples were collected from the natural pond at a 3 m distance from the edge of a landing stage built up for permanent use, with that purpose in view. In the experimental reservoir, sampling of the material from the central part of the reservoir bottom was done likewise from a stationary platform. Samples from reservoir were collected once a week, whereas from the natural water body at monthly intervals. Bottom sediments from both of them were dipped out with a sterilized Karzinkin bottom sampler (Rodina 1965). The top layer of the mud column was skimed and 30 ml of mud was collected from each dip. To collect one sample the sampler was dipped up, three times, every 3—5 minutes, from spots distant from one another not more than 20 cm. The collected mud was taken over to the laboratory in a thermos flask, previously sterilized. The time of transport from the natural water body did not exceed 2 hr, and from the experimental reservoir — 30 minutes.

Bacteria count in the bottom sediments. The number of cells of the bacterial population abiding in the bottom sediments was determined by the method of direct counting of cells on the membrane filters (Erlich 1955, Jannasch 1958, Jannasch, Jones 1959). To this purpose Coli-5 filters, made in Gottingen, were used.

Measurement of 24 hr chemoautotrophic bacteria production. Production of chemoautotrophic bacteria were denoted in units of carbon assimilated from CO_2. For determination of carbon assimilated by bacteria there was used a method with application of the labelled carbon ^{14}C (Sorokin 1955). For measurements of radioactivity the gas flow detector was used, from the Firm Nuclear Chicago mod. 47, with 45% performance. At the assessment of value of carbon assimilated by microorganisms allowances were made for correction of the quenching effect, on the sample.

The assimilated carbon in the process of chemoautotrophic production of bacteria was calculated after the formula:

$$C_{CH} = C \frac{a}{R} \tag{2}$$

where: C_{CH} — quantity of assimilated carbon, during the time of the experiment, by bacteria contained in a volume unit of the examined sediment (μgC/l · 24 hr), C — carbon in carbon dioxide and carbonates contained in the examined sediments determined according to the method applied by Sorokin (1955), (μgC/l), a — radioactivity of bacteria residue obtained from filtration of the known volume of experimental fluid (imp/min · ml), R — radioactivity of the experimental material after addition of the $NA_2{}^{14}CO_3$ solution (imp/min · ml).

Table II. Some results of physicochemical analyses of water and bottom sediments for the experimental reservoir (11.V.1966—11.V.1967)

Date	Water									Sediment	
	Po_4−−− (mg/l)	SO_4−− (mg/l)	NH_4+ (mg/l)	NO_2− (mg/l)	NO_3− (mg/l)	O_2 (mg/l)	CO_2 (mg/l)	pH	Temp. (°C)	Temp. (°C)	CO_2 (mg/l)
11.V	0.130	30.44	0.288	0	0.06	—	156.9	6.9	11.0	10.5	366.0
19.V	0.085	23.86	0.10	0.001	0.10	1.2	107.0	6.6	13.5	12.0	—
26.V	0.095	27.43	0.10	0.001	0.10	2.7	106.3	6.8	16.0	12.5	—
2.VI	0.140	26.33	0.27	0	0.10	3.6	160.6	6.8	10.5	9.5	294.0
10.VI	0.145	28.52	0.10	0	0.02	1.6	108.9	7.0	10.5	11.0	146.0
16.VI	0.280	30.16	0	0	0	2.7	108.5	7.0	17.0	18.0	585.0
30.VI	0.113	—	0.05	0	0.06	0.9	31.5	6.7	14.5	14.5	513.0
7.VII	0.116	23.45	0.19	0	0.04	T	160.6	6.9	17.5	17.5	585.0
15.VII	0.115	20.98	0.08	T	0.04	0.6	101.2	6.6	15.0	16.0	294.0
20.VII	0.121	22.23	0.14	0.001	0.06	0.2	157.3	6.6	21.0	21.0	475.0
28.VII	0.092	17.69	0.04	0	0.02	0	158.4	6.8	18.0	17.0	257.0
4.VIII	0.090	15.13	0.04	T	0.08	0.3	143.7	6.6	17.0	16.0	513.0
11.VIII	0.093	19.33	0.04	0	0.04	0.2	121.7	6.7	17.5	17.0	548.0
8.IX	0.069	45.66	0.05	0	0.06	1.8	101.9	6.9	14.5	14.5	330.0
15.IX	0.062	49.37	0.04	0	0.06	2.9	86.9	6.8	11.5	11.5	438.0
23.IX	0.042	56.77	0.04	0	0.08	5.6	91.3	6.8	10.0	10.0	110.0
30.IX	0.045	66.21	0.04	0	0.08	6.4	156.2	7.2	10.5	10.0	330.0
6.X	0.065	73.64	0.04	0	0.08	3.1	122.1	7.0	15.0	15.0	147.0
13.X	0.034	53.89	0.04	0	0.08	4.8	94.6	7.4	12.0	12.0	330.0
20.X	0.093	85.98	0.12	0	0.04	4.0	94.6	7.1	13.0	13.0	147.0
27.X	0.074	86.39	0.08	T	0.08	5.8	69.3	7.0	10.5	10.5	475.0
3.XI	0.103	92.97	0.04	0	0.08	8.5	84.3	7.0	4.0	7.0	73.0
10.XI	0.144	96.68	0.04	T	0.08	6.6	42.5	7.0	5.0	8.0	115.0
17.XI	0.129	99.15	0.03	T	0.08	3.7	110.0	7.1	3.0	4.0	136.0
24.XI	0.124	88.86	0.08	T	0.08	6.0	36.3	6.8	2.5	4.5	183.0
2.XII	0.143	74.05	0.23	0.001	0.08	5.3	101.2	6.6	2.5	2.2	—

8.XII	0.150	66.29	0.58	0.001	0.16	1.1	79.2	6.9	1.0	2.5	15.0
15.XII	0.157	69.94	0.58	0.001	0.16	1.6	145.9	6.6	0.5	2.0	293.0
22.XII	0.125	73.23	0.40	0.001	0.08	0	50.6	7.0	0.5	2.0	257.0
29.XII	0.205	76.11	0.58	0	0.08	0	85.8	6.6	0.5	1.0	438.0
5.I	0.165	69.53	0.78	0	0.06	0.5	85.8	6.6	0.5	1.0	330.0
12.I	0.220	77.75	1.36	0	0.08	0	222.1	7.1	0.5	0.5	403.0
19.I	0.385	58.42	1.55	T	0.12	0	233.2	7.5	0.5	0.5	438.0
27.I	0.385	23.63	2.33	0.003	0.12	0	160.6	7.4	0.5	0.5	400.0
3.II	0.284	34.97	2.33	0.004	0.12	0.16	223.3	6.8	0.5	0.5	257.0
9.II	0.270	34.15	1.94	0.017	0.12	0	115.9	6.8	0.5	0.5	110.0
16.II	0.720	31.26	2.72	T	0.12	0	158.4	6.7	0.5	0.5	330.0
27.II	—	—	—	—	—	—	165.7	6.7	0.5	0.5	110.0
3.III	0.299	27.98	1.55	0.004	0.04	0.5	119.5	6.7	1.0	0.8	220.0
9.III	0.106	23.86	0.78	0.014	0.04	4.4	117.7	6.7	4.5	4.0	330.0
16.III	0.077	28.39	0.04	0.004	0.04	11.9	19.1	7.2	3.0	4.5	403.0
24.III	0.020	32.91	0.04	0	0.12	12.4	61.6	7.0	4.5	5.0	550.0
31.III	0.072	—	0.04	0	0.12	8.2	12.5	7.1	4.0	6.0	585.0
6.IV	0.033	41.33	0.12	0	0.12	8.4	67.5	7.1	5.0	5.0	73.0
13.IV	0.051	46.08	0.08	T	0.04	4.2	80.7	7.1	9.0	9.0	660.0
20.IV	0.060	56.36	0.08	T	0.04	6.1	146.7	7.2	7.0	7.0	438.0
27.IV	0.057	46.49	0.06	T	0.04	5.3	24.2	7.2	6.5	6.0	36.0
4.V	0.063	46.90	0.04	T	0.04	5.3	52.8	7.2	8.0	7.5	36.6
11.V	0.063	49.37	0.50	T	—	4.0	61.4	7.2	11.0	11.0	147.0

0 — not found, T — traces, — — no measurements was made.

Estimation of the coefficient of the production dynamics of the chemoautotrophic bacterial biomass. The characteristic feature of production activity of the bacterial population in the water bodies is expressed by the amount of biomass produced, during a unit of time, e.g. 24 hr, by any taken at choice, but always the same, initial number of bacterial cells. This coefficient can be expressed by the following formula:

$$\gamma = \frac{\Delta B}{N} \tag{3}$$

where: γ — coefficient of production dynamics of the bacterial population, ΔB — 24 hr increment of the bacterial population biomass in a volume unit of the examined material, N — initial number of bacterial cells in a volume unit of the examined material.

In the adopted method, the 24 hr increment of the chemoautotrophic bacteria was expressed by the quantity of carbon, derived from CO_2 and carbonates from the environment (C_{CH}), built up into the produced cells. This value is presented in μg C. The quantity unit for the number of cells is one million of bacterial cells. Using the mentioned above units the formula for the coefficient of the production dynamics of the examined population will be as follows:

$$\gamma = \mu g\ C/10^6 \text{ cells} \cdot 24 \text{ hr} \tag{4}$$

Saying it in another way, that coefficient designates the yield efficiency of one milion of bacterial cells in producing, on the way of chemoautotrophic synthesis, a new biomass of bacteria in 24 hr.

Studies on photosynthesis in phytoplankton. Examination of photosynthesis was carried out in like manner as investigations on bacterial production i.e. by the method with application of labelled carbon ^{14}C.

Measurements of temperature. Temperature of water and bottom sediments in the natural body of water was measured by the means of an insulated thermometer (Paschalski 1961). In the experimental reservoir, all during the time of investigations, a continuous recording of temperature was carried on, which made possible the observation of temperature changes both in the course of 24 hr time and during the seasons of the year, as well. Examinations were performed at two levels, 3 cm below the surface of detritus and 15 cm above the bottom of the reservoir, by means of a recorder equipped with platinum thermometers.

Measurements of luminous energy, inflowing under the water level. Measurement was performed by means of irradiance integrators fitted out with detectors with properly corrected spectrum.

Chemical analyses of water environment were carried out according to Just, Hermanowicz (1955).

4. RESULTS

Results from calculating the number of bacteria in bottom sediments show that sediments in the pond are many times more abounding with bacterial flora than sediments in the experimental reservoir (Fig. 3). In both water bodies considerable seasonal changes were observed in the number of bacterial cells abiding in the bottom sediments. The minimum of the number of bacteria occurs in the summer season. In the course of a year the changes in the number of bacteria in sediments of the pond are within the range from $1700 \cdot 10^9$ to $15{,}850 \cdot 10^9$ per one litre of sediment. In the experimental reservoir — from $4.3 \cdot 10^9$ to $115.4 \cdot 10^9$ cells per one litre of sediment.

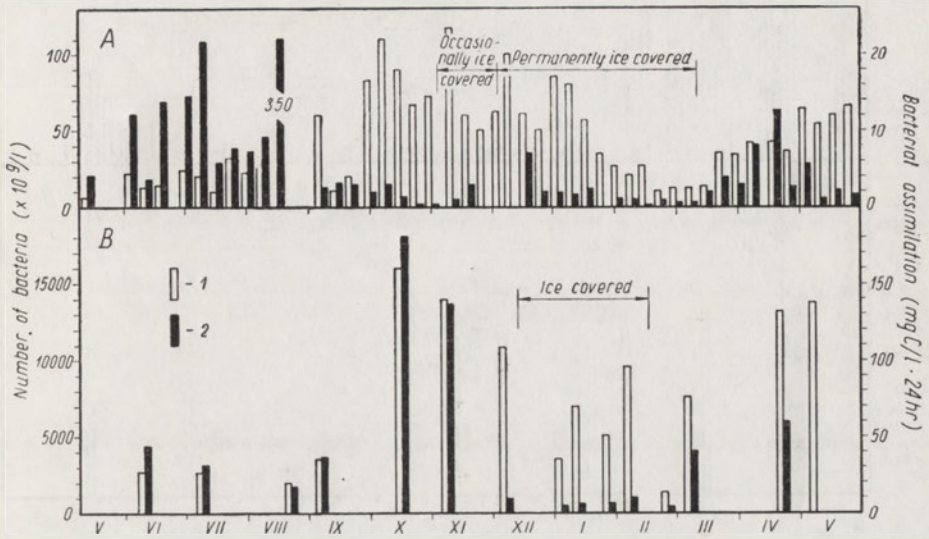


Fig. 3. Comparison of the number of bacteria and the 24 hr bacterial assimilation of carbon in bottom sediments of the two water bodies. 1 — number of bacteria, 2 — bacterial assimilation of carbon. A — experimental reservoir, B — natural pond

The 24 hr assimilation of carbon by microorganisms in the process of chemoautotrophic production of bacteria is likewise subject to considerable seasonal changes (Fig. 3). The quantities found for the natural water body were ranging, in the course of a year, from 5.6 up to 181.0 mg of carbon assimilated during 24 hr by bacteria comprised in one litre of sediment, for experimental reservoir from 0.1 up to 35 mg.

On the basis of the obtained results there were calculated the values illustrating production dynamics of the population in producing the chemoautotrophic bacterial biomass. As can be seen from the data presented in Fig. 4, the calculated coefficient is many times lower for the bacterial population in sediments of the pond than in sediments of the experimental reservoir.

It was stated that the variability of the coefficient γ in each of the two investigated water bodies follows a different seasonal course. In the experimental reservoir, the values of the coefficient found in the spring time are considerably higher than the values obtained in the autumn season. In the natural pond the situation is reversed. Chemical and temperature determinations for the experimental reservoir are presented in Table II. Assimilation values in the process of photosynthesis and chemosynthesis in water are shown in Fig. 5. The flow of the radiant energy into the water layer in the depth of 15 cm below the water surface in the spring period (from the vanishing of ice till the end of May) amounted to: in

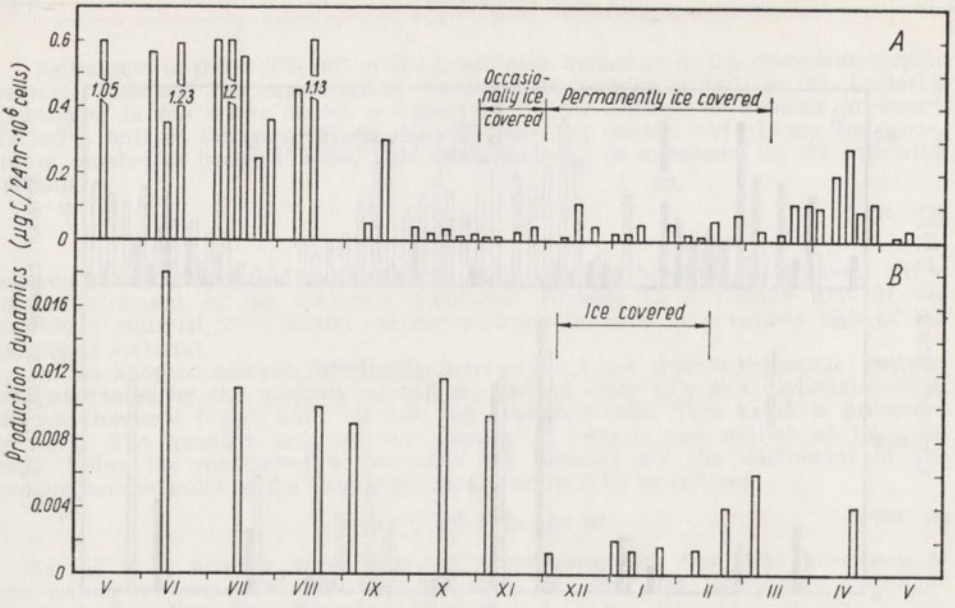


Fig. 4. Comparison of production dynamics coefficients of chemoautotrophic bacteria in the bottom sediments of the two water bodies. A — experimental reservoir, B — natural pond

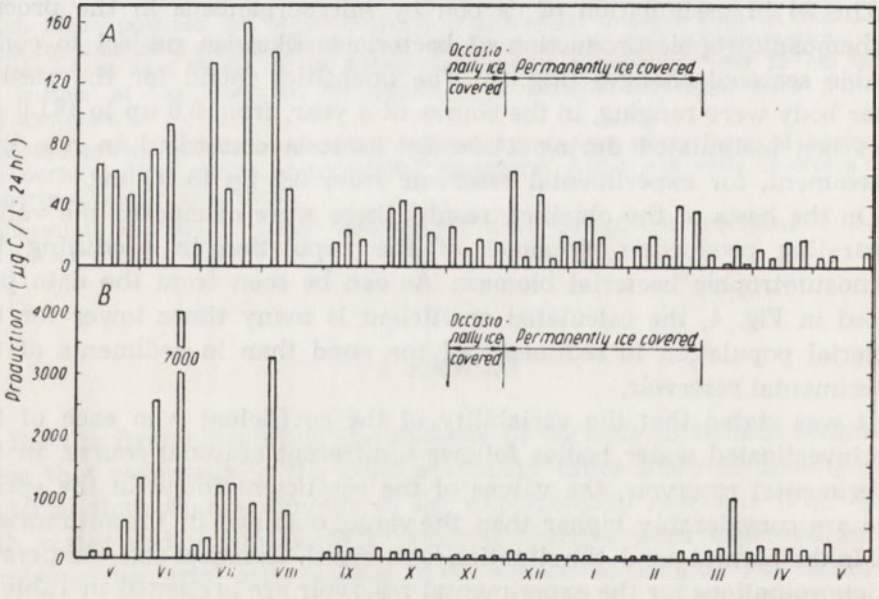


Fig. 5. Comparison of the chemoautotrophic bacterial production in the bottom sediments with photosynthesis in water of experimental reservoir. A — chemoautotrophic bacterial production in bottom sediments, B — photosynthesis

the 400—500 mμ band — 1.4 kcal/cm², in the 500—600 mμ band — 21 kcal/cm², in the 600—700 mμ — 2.3 kcal/cm². In the summer period (June—August): 3.6, 4.8, and 5.5 kcal/cm², respectively.

5. DISCUSSION

It follows from the obtained results that the problem of biomass formation of autotrophic bacteria calls for a careful consideration from three points of view, namely:

1. the amount of chemoautotrophic bacterial biomass produced in the process of synthesis in a unit of time,

2. the total number of bacterial cells in the population producing the autotrophic biomass,

3. the efficiency of the bacterial population in producing the autotrophic biomass.

The two first points of view tend to characterization of the water body as regards the 24 hr value of the autotrophic bacterial production and the actual number of bacteria, the third one aims at characterization of the bacterial population in the water body in respect of its efficiency in the production of the autotrophic biomass.

Results obtained from the estimation of the coefficient γ of the population in the two water bodies albeit they differ from each other in their absolute values, nevertheless, they show a conformable tendency to the changes occurring in the summer and winter seasons. In the summer time the coefficients are of a maximum value, whereas in winter their value is the lowest. However, while comparing the spring and autumn season we have observed some differences in the changes of the coefficient values for the two water bodies under investigation. As it seems, those differences may be explained by the different quantitative composition of the bacterial population and also by different surroundings of the two water bodies. The experimental reservoir lacking the natural ground and drainage area could not be supplied in the autumn with biogenic substances at a high degree as the pond situated nearby a plough-land and a meadow.

The coefficient γ values are under an apparent influence of the astaticism of the investigated water bodies. Here this influence shows up much more strongly than it could have been observed at the examination of the number of bacteria and the autotrophic 24 hr production of the bacterial biomass. The ratio of the coefficient highest value to the lowest one for the natural pond, with greater static equilibrium, amounted to 18.6, whereas for the experimental reservoir to 1230. This seems to be quite comprehensible, since the production activity of the bacterial

population is in the first place dependent on the influences of the climate. On the other hand, the influence of climate on the abundance of bacteria in a water body and the 24 hr production of the biomass manifests itself through the changes in their production activity. The high degree of astaticism in the experimental reservoir resulted from a rather small quantity of water markedly subject to the influence of the atmospheric conditions (Fig. 2) (Fischer 1972). In our work, it was assumed that the activity of the autotrophic production of bacterial population is expressed by the adopted coefficient γ. In order to check up whether that coefficient is in fact related to the activity of autotrophic production of the bacterial population, it has been examined in the yearly cycle as a function of temperature and the contents of carbon dioxide in the investigated material, as the basic component of the nutrient substrata (Fig. 6 and 7). It became evident that it is subject, in some measure, to the commonly known regularities.

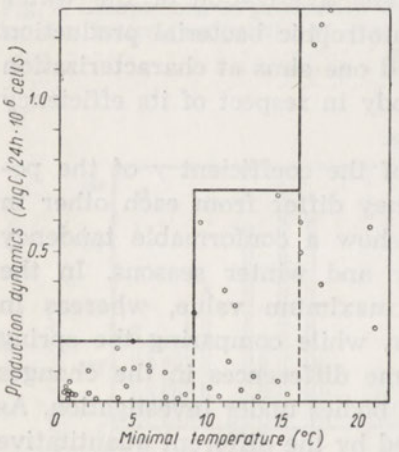


Fig. 6. Dependence of the coefficient γ of chemoautotrophic bacterial production dynamics upon the 24 hr minimum temperature in bottom sediments of the experimental reservoir

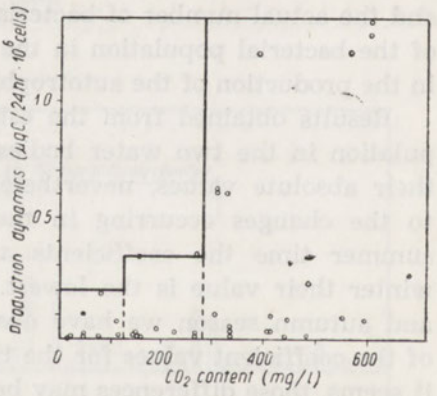


Fig. 7. Dependence of the coefficient γ of chemoautotrophic bacterial production dynamics upon the carbon dioxide contents in bottom sediments of the experimental reservoir

At the 24 hr minimum temperatures, not beyond 9°C (Fig. 6) the coefficient of production activity γ does not exceed the value of 0.2 µgC/10^6 · 24 hr. At the 24 hr minimum temperatures not above 16°C, the coefficient does not exceed the 0.7 µgC/10^6 · 24 hr value, while all higher values of the coefficient are to be found in the range of the minimum temperatures higher than 16°C.

Experimental values of the coefficient do not trace a regular curve since in natural conditions, i. e. where many factors bear on the development of bacteria, one cannot expect such regular dependences as those occurring in pure cultures under settled conditions. About regularity of arrangement, of the results obtained from the coefficient determinations, one can draw conclusions merely on the basis of the course of the curve delimiting the top ranges of the area, where those results are grouped (Fig. 6). The top limit of the area runs, as can be seen, at the higher level for higher temperatures and at a lower one for lower temperatures. Since, it is a matter of course that, even in the most favourable conditions of the bacterial population development the temperature changes are limiting its activity and that the lower is the temperature the lower is the production activity value, so it seems that Fig. 6. allows the assumption that the coefficient γ is in fact a coefficient of the autotrophic production activity of the bacterial population.

A similar situation is found in Fig. 7, where are presented the coefficient γ values and their dependence on the amount of the carbon dioxide contents in one litre of bottom sediments. Here we have also three sectors singled-out. The coefficient γ value does not exceed 0.2 μgC/$10^6 \cdot 24$ hr at the CO_2 contents below 120 mg/l. Within the range up to 280 mg CO_2/1 the coefficient γ values do not exceed the 0.38 μg C/$10^6 \cdot 24$ hr. Higher values of the coefficient γ occur at the CO_2 contents in the sediments greater than 280 mg/l.

By analysing the results obtained from our work it was stated that the bacterial population, abiding in the bottom sediments of the investigated water bodies, shows seasonal changes in the 24 hr bacterial biomass production values, the number of bacteria, and the production activity of the bacterial biomass, as well. However, the course of the seasonal variation runs in a different way in each of the three groups of results. This ensues from the fact that each of the three examined values forms under the influence of different factors.

Thus, in summer, for instance, in the time of the occurrence of the smallest number of bacteria we have stated a high production of their biomass, which can be explained solely by the high production activity of the population. The simultaneous presence in the waters of a great number of organisms feeding on bacteria allows to presume that the phenomenon of the summer minimum of the bacteria number is due, above all, to the destruction of their cells. Hence, one can say that in a water body the summer season is the period of the most intensive turnover in the bacterial biomass.

An inverse situation has been stated in the winter time. The great number of bacteria population does not result from its high producti-

vity since the production activity, being dependent in such a great measure on the physicochemical conditions, is in that period of time quite inconsiderable, probably due to the low temperatures. Therefore, the great density of bacteria population is doubtlessly conditioned by the diminished consumption of their cells.

Thus, in the winter season the bacterial biomass turnover is minimal.

Albeit, the carried out investigations refer only to the chemoautotrophic production of bacteria, nevertheless, they constitute an integral part of the research on the problems of the bacterial productivity in the waters. The extensiveness and complexity of this problem require further methodical studies, as the methods applied at present make it necessary to use numerous approximations.

Acknowledgements

My grateful thanks are due to Dr. A. Dowgiałło for his clear-sighted consultation and his care devoted to the chemical analyses used in the present paper.

6. SUMMARY

The research concerned the dynamics in a yearly cycle of the chemoautotrophic bacterial production in the bottom sediments of two small water bodies: a natural pond situated in the area of Kampinos Forests and an experimental reservoir situated in the premises of the Institute of Experimental Biology, Polish Academy of Sciences.

The collected samples served for determination of the 24 hr assimilation of carbon absorbed in the process of the chemoautotrophic bacterial production, using the method of labelled carbon ^{14}C and the density of bacterial population determined by direct microscopic counting. Basing on those two determinations, production dynamics of bacterial population in the autotrophic bacterial biomass production were estimated. This value was expressed in microgrammes of the assimilated carbon per one million bacterial cells.

It has been stated that all three of the investigated values are subject in the course of the year to considerable fluctuations, however, they occur in a different way in each of them.

The production dynamics of the chemoautotrophic bacteria shows the highest values in the summer time, when the number of bacteria is at its minimum. A reversed situation is found in the winter season — at the highest number of bacteria, the production dynamics are low, which is concurrent with a small amount of the produced biomass. It becomes quite evident that factors determining the number of bacteria in small water bodies are different from those influencing production dynamics.

The low number of bacteria at the high production activity in the summer time allows to infer that the bacterial biomass turnover in the water biocenosis is high in this period of time contrary to the winter season where the situation is inverted.

The variability of the 24 hr chemoautotrophic bacterial production level is related to the variability of the production activity in such an essential manner that the seasonal variability of both those values is, in general, of a very similar character. Hence, one can come to conclusion that the level of the chemoautotrophic bacterial biomass production is dependent above all, on those factors which bear effect on the efficiency of that production, while the effect of the abundance of bacterial cells becomes apparent only in sporadic cases.

The computed coefficient of production dynamics in the chemoautotrophic bacterial biomass production shows some characteristic regularities in relationship to temperature of the environment and the CO_2 contents, which allows to infer that in fact this coefficient really determines the activity of the chemoautotrophic production of bacterial population in the bottom sediments.

7. STRESZCZENIE

Badano dynamikę chemoautotroficznej produkcji bakterii w cyklu rocznym w osadach dennych dwóch małych zbiorników wodnych: zbiornika naturalnego, położonego na terenie Puszczy Kampinoskiej, oraz zbiornika doświadczalnego, położonego na terenie doświadczalnym Instytutu Biologii Doświadczalnej PAN.

Z pobranych próbek oznaczano: dobową asymilację węgla pobieranego w procesie chemoautotroficznej produkcji bakterii, stosując metodę znaczonego węgla ^{14}C, oraz liczebność populacji bakteryjnej, oznaczaną metodą bezpośredniego liczenia mikroskopowego. Na podstawie tych dwóch oznaczeń określano dla poszczególnych prób dynamikę produkcyjną populacji bakterii w produkcji autotroficznej biomasy bakterii. Wielkość tę wyrażano w mikrogramach przyswajanego węgla przypadającego na 1 milion komórek populacji.

Stwierdzono, że wszystkie trzy badane wartości ulegają w ciągu roku znacznym wahaniom, jednakże wahania te kształtują się różnie.

Dynamika produkcji chemoautotroficznych bakterii wykazuje najwyższe wartości w okresie lata, kiedy liczba bakterii przechodzi przez swoje minimum. Odwrotnie sytuacja przedstawia się w okresie zimowym — przy najwyższych liczbach bakterii dynamika jest niska, czemu towarzyszy w tym czasie mała ilość wyprodukowanej biomasy. Staje się oczywiste, że czynniki kształtujące liczbę bakterii w małych zbiornikach są odmienne od czynników sterujących ich dynamiką produkcyjną.

Niska liczba bakterii przy dużej aktywności produkcyjnej w okresie lata pozwala wnioskować, że obrót biomasą bakteryjną w biocenozie zbiornika jest w tym okresie duży, w przeciwieństwie do okresu zimowego, kiedy obserwujemy sytuację odwrotną.

Zmienność poziomu dobowej chemoautotroficznej produkcji bakteryjnej powiązana jest ze zmiennością aktywności tej produkcji w sposób tak istotny, że zmienność sezonowa obydwu tych wielkości ma charakter na ogół bardzo zbliżony. Stąd można wnioskować, że poziom produkcji chemoautotroficznej biomasy bakteryjnej uzależniony jest przede wszystkim od tych czynników, które mają wpływ na aktywność tej produkcji, podczas gdy wpływ liczebności komórek populacji uwidacznia się tylko w sporadycznych przypadkach.

Przyjęty w pracy wskaźnik dynamiki produkcji biomasy bakterii chemoautotroficznych wykazuje pewne charakterystyczne prawidłowości powiązań z temperaturą środowiska oraz zawartością CO_2, co pozwala na wnioskowanie, że w osadach dennych określa istotnie aktywność chemoautotroficznej produkcji populacji bakteryjnej.

8. REFERENCES

Anderson, C. C. 1948. *An introduction to bacteriological chemistry.* Edinburgh, E. and S. Livingstone.

[Beljackaja, Ju. S.] Беляцкая, Ю. С. 1958. Сезонные изменения общего числа и биомассы бактерий в воде трех озер разных типов [Seasonal changes of the total number and biomass of bacteria in the water of three lakes of different types]. *Mikrobiologija,* 27, 113–119 [Engl. summ.].

Beran, K. 1966. Application of continuous cultivation in research. In: I. Málek, Z. Fencl [Eds] *Theoretical and methodological basis of continuous culture of microorganisms.* 317—406, Prague, Publ. House Čechoslov. Akad. Sci.

Erlich, R. 1955. Technique for microscopic count of microorganisms directly on membrane filters. *J. Bact.,* 70, 265—268.

Fischer, E. 1966 a. Metodyka badań bakteriologicznych związanych z zagadnieniem produktywności [Methods of bacteriological investigations dealing the problem of productivity]. *Kosmos, Zesz. probl.,* 13, 117—126.

Fischer, E. 1966 b. Dynamics of autotrophic bacterial production in the bottom sediment of astatic pools. *Verh. int. Ver. Limnol.*, 16, 539—542.
Fischer, E. 1970. Production of chemosynthetizing bacteria as a chain of energy recuperation by aquatic biocenosis. *Pol. Arch. Hydrobiol.*, 17, 109—115.
Fischer, E. 1972. Seasonal changes of the number of nitrogen cycle bacteria in bottom sediments of a pool. *Pol. Arch. Hydrobiol.*, 19, 37—52.
[Gambarian, M. E.]Гамбарян, М. Е. 1962. К методике определения интенсивности деструкции органических веществ в денных отложениях глубоковых водоемов [A contribution to the technic of assaying the rate of destruction of organic matter in benthic sediments of deep reservoirs]. *Mikrobiologija*, 31, 895–898 [Engl. summ.].
Goodwin, T. W. 1952. *The comparative biochemistry of the carotenoids.* London, Chapman and Hall.
Hospodka, J. 1966. Industrial application of continuous fermentation. In: I. Málek, Z. Fencl [Eds] *Theoretical and methodological basis of continuous culture of microorganisms.* 493—645. Prague, Publ. House Čechoslov. Akad. Sci.
Jannasch, H. W. 1954. Ökologische Untersuchungen der planktischen Bakterienflora im Golf von Neapel. *Naturwissenschaften*, 41, 42.
Jannasch, H. W. 1958. Studies on planktonic bacteria by means of a direct membrane filter method. *J. gen. Microbiol.*, 18, 609—620.
Jannasch, H. W., Jones, C. E. 1959. Bacterial populations in sea water as determined by different methods of enumeration. *Limnol. Oceanogr.*, 4, 128—139.
Just, J., Hermanowicz, W. 1955. *Fizyczne i chemiczne badanie wody do picia i potrzeb gospodarczych* [Physical and chemical studies of the tap water and for the economical purpose]. Warszawa, PZWL.
[Kazakov, E. I., Pronina, M. V.] Казаков, Е. И., Пронина, М. В. 1941. Химический состав различных форм планктона и бентоса [Chemical composition of various planktonic and benthic forms]. *Trudy Lab. Genez. Saprop.*, 2, 49–52.
[Klejmenov, I. Ja.] Клейменов, И. Я. 1952. Химический и весовой состав основных промысловых рыб [Chemical and weight compositions of economically important fish species]. Moskva, Piščepromizdat.
[Kriss, A. E.] Крисс, А. Е. 1959. Морская микробиология (глубоководная) [Marine microbiology (deep-sea). Moskva. Izdat. Akad. Nauk SSSR.
[Kuznetzov, S. I.] Кузнецов, С. И. 1952. Роль микроорганизмов в круговороте веществ в озерах [Role of microorganisms in turnover of matter in lakes]. Moskva, Izdat. Akad. Nauk SSSR.
[Kuznetzov, S. I.] Кузнецов, С. И. 1955. Использование радиоактивной углекислоты C^{14} для определения сравительной величины фотосинтеза и хемосинтеза в ряде озер различных типов [Application of radioactive carbon dioxide C^{14} to the estimation of relative values of photosynthesis and chemosynthesis in lakes of various types]. In: A. A. Imšeneckij [Red.] *Izotopy v mikrobiologii*, 126–135. Moskva, Izdat. Akad. Nauk SSSR.
[Kuznetzov, S. I.] Кузнецов, С. И. 1956. Микробиологическая характеристика вод и грунтов Байкала [Microbiological characteristics of Baikal water and soils]. *Trudy Bajkal. limnol. Sta.*, 15, 388–396.
[Kuznetzov, S. I.] Кузнецов, С. И. 1958. Численность бактерий в Рибинском водохранилище [Bacteria density in Rybinsk dam reservoir]. *Bjull. Inst. Biol. Vodohram.*, 1, 7–10.
[Kuznetzov, S. I.] Кузнецов, С. И. 1959 a. Микробиологическая характеристика волжских водохранилищ [Microbiological characteristics of Volga dam reservoirs]. *Bjull. Inst. Biol. Vodohran.*, 1 (4), 69–81.
[Kuznetzov, S. I.] Кузнецов, С. И. 1959 b. Микробиологическое исследованние Горьковского водохранилища [Microbiological investigations of Gorki dam reservoir]. *Bjull. Inst. Biol. Vodohran.*, 3, 2–4.
[Kuznetzov, S. I., Romanenko, V. I., Karpova, N. S.] Кузнецов, С. И., Романенко, В. И., Карпова, Н. С. 1966. Численность бактерий и продукция органического вещества в водной массе Рыбинского водохранилища в 1963 и 1964 гг. [Number of bacteria and production of matter in water mass in Rybinsk reservoir in 1963 and 1964]. *Trudy Inst. Biol. vnutr. Vod*, 13, 123–132.
Monod, J. 1942. *Recherches sur la croissance des cultures bactériennes.* Paris.
Monod, J. 1949. The growth of bacterial cultures. *A. Rev. Microbiol.*, 3, 371—394.
Monod, J. 1950. La technique de culture continue. Théorie et applications. *Annls Inst. Pasteur, Paris*, 79, 390.
Nowotny-Mieczyńska, A., Gołębiowska, J. 1960. *Krążenie azotu w przyrodzie* [Nitrogen circulation system in the nature]. Warszawa, PWRiL.

Paschalski, J. 1961. Termometr naczyńkowy napełniany na określonej głębokości [Sounding thermometer filled at a chosen depth]. V Zjazd Hydrobiol. Pol., Gdańsk 1961, Streszcz. ref., 148–149.

Potter, L. F. 1964. Planktonic and benthic bacteria of lakes and ponds. In: H. Heukelekian, N. C. Dondero [Eds] *Principles and applications in aquatic microbiology*. 148–166, New York, J. Wiley.

Precht, H., Christophersen, J., Hensel, H. 1955. *Temperatur und Leben*. Berlin, Springer Verl.

[Rodina, A. G.] Родина, А. Г. 1958. Микроорганизмы и повышение рыбопродуктивности прудов [Microorganisms and the increase of fish production in ponds]. Moskva, Izdat. Akad. Nauk SSSR.

[Rodina, A. G.] Родина, А. Г. 1963. Содержание бактерий в детрите озер Приладожья [The bacterial content of detritus in the lakes of the Ladoga area]. *Microbiologija*, 32, 1031–1037 [Engl. summ.].

[Rodina, A. G.] Родина, А. Г. 1965. Методы водной микробиологии. Практическое руководство [Methods of water microbiology. Practical guide]. Moskva, Izdat. „Nauka".

[Sorokin, Ju. I.] Сорокин, Ю. И. 1955. О бактериальном хемосинтезе в иловых отложениях [Bacterial chemosynthesis in mud deposits]. *Mikrobiologija*, 24, 393–399.

[Sorokin, Ju. I.] Сорокин, Ю. И. 1957. Роль хемосинтеза в продукции органического вещества в водоемах. I. Подледный хемосинтез в водной толще Рыбинского водохранилища [The rôle of chemosynthesis in the production of organic substances in water receptacles. 1. The chemosynthesis within the water layer of the Ribinskoe water receptacle in winter]. *Mikrobiologija*, 26, 736–744 [Engl. summ.].

[Sorokin, Ju. I.] Сорокин, Ю. И. 1958 a. Роль хемосинтеза в продукции органического вещества в водохранилищах. II. Изучение хемосинтеза в иловых отложениях с помощью C^{14} [The rôle of chemosynthesis in the production of organic substance in reservoirs. II. A study of chemosynthesis in silt depositions by means of C^{14}]. *Mikrobiologija*, 27, 206–213 [Engl. summ.].

[Sorokin, Ju. I.] Сорокин, Ю. И. 1958 b. Роль хемосинтеза в продукции органического вещества в водохранилищах. III. Продуктивность хемосинтеза в водной толще в летний период [The rôle of chemosynthesis in the production of organic substance in water reservoir. III. Summer productivity of chemosynthesis in water]. *Mikrobiologija*, 27, 357–365 [Engl. summ.].

ZoBell, C. E. 1946. *Marine microbiology. A monograph on hydrobacteriology*. Waltham, Mass., Publ. the Chronica Botanica.

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	361–368	1972

A. DEUAR

TRIALS FOR MAKING USE OF *SACCHAROMYCES CEREVISIAE* (HANSON) AS FOOD FOR *SPIROSTOMUM AMBIGUUM* (EHRBG)

Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

ABSTRACT

Experimental trials were made to use *Saccharomyces cerevisiae* as food for *Spirostomum ambiguum*. Observations of the survival of *S. ambiguum* in various concentrations of *S. cerevisiae* suspensions have shown a decrease in the number of protozoa in the course of experiments. The observed dynamics of formation of food vacuoles dependent on the time of exposition is characteristic for the indigestible food. The *Saccharomyces cerevisiae* cells obtained from excreted food vacuoles have shown a high rate of capability for reproduction. The obtained results suggest that *Saccharomyces cerevisiae* is not an adequate food for *Spirostomum ambiguum*.

1. INTRODUCTION

In view of some particular purposes, e.g. bioenergetic studies, there is a need to find some kind of food which would be easily available in pure state, i.e. free from all other mingled organisms, easy to handle in all sorts of manipulation, e.g. counting, and would not multiply in the course of the experiment. As concerns the food for Ciliata, yeast meets all the required conditions.

In the protozoological literature one can find data referring to the use of yeast as food for protozoa. Loeffer (1936), for instance, made use of *Saccharomyces cerevisiae* to feed *Paramecium caudatum* on, Glaser, Coria (1930) fed the same protozoan species with *Saccharomyces elipsoideus*, Miśkiewicz (unpubl.) grew *Chilodonella cucullulus* culture on *Saccharomyces cerevisiae*. Brutkowska (unpubl.) used *Saccharomyces cerevisiae* as food for *Paramecium caudatum*. Yet, Brutkowska (unpubl.) and Loefer (1936) used as food substance yeast, which did not derive from pure cultures. Brutkowska, for instance, made use of yeast got from grocer's shops. It has been demonstrated in investigation by Ilnicka-Olejniczak (1968) that such kind of yeast contains merely about 60% of *Saccharomyces cerevisiae* Breed I, Okocim strain, the rest of it consists of bacteria.

Since it had been observed that *Spirostomum ambiguum* cultured on edible yeast are multiplying it was decided to check up whether a pure strain of yeast would be an appropriate food for this species of Ciliata.

2. MATERIAL AND METHODS

Spirostomum ambiguum (Ehrbg) used in experiments was taken from cultures maintained by the Department of Biology, at the Nencki Institute of Experimental Biology, in Warsaw.

Reserve *S. ambiguum* cultures were grown in sterile conditioned tap water, taken from municipal water supply, and fed on bacteria. Water used for culturing after having been sterilized in an autoclave was kept for about two months in glass vessels before being actually used for experiments.

Saccharomyces cerevisiae, Breed I, Okocim strain, were obtained from the Institute of Fermentation Industry in Warsaw, which supplies the country food industry with pure strains of yeast. Yeast before being taken for experiments was cultured on barley malt infusion with 2% agar, at 26°C, during 48 hr. In the experiments, yeast suspension was prepared as food for *S. ambiguum*, by shaking up a clod of yeast taken from some other 48 hr old culture in conditioned sterilized water. The *S. cerevisiae* cell concentration in suspension was determined by means of Bürcker hematocytometer.

There have been carried out: 1. observations of *S. ambiguum* reproduction in various *S. cerevisiae* concentrations, 2. observations of food vacuoles formed by that protozoan.

1. The reproduction of *S. ambiguum* was investigated in nine 10 ml cultures in Petri dishes. Three different concentrations of *S. cerevisiae* suspension were used. In each of the three concentrations, tests were repeated three times (Table I). The

Table I. Changes in the number of *Spirostomum ambiguum* individuals in the cultures, in the course of experiments

Age of culture (days)		Number of *S. ambiguum* individuals			
		Yeast concentration (ind./ml)			Control
		$3.5 \cdot 10^4$	$35.0 \cdot 10^4$	$350.0 \cdot 10^4$	
Replicate I	0	100	100	100	100
	1	192	100	95	101
	2	50	100	90	105
	3	50	100	93	107
	4	50	100	95	107
	5	45	93	80	103
	6	45	80	70	111
Replicate II	0	100	100	100	100
	1	98	93	100	100
	2	98	90	101	100
	3	60	82	80	108
	4	60	85	73	115
	5	60	80	70	115
	6	53	65	72	118
Replicate III	0	100	100	100	100
	1	95	95	100	90
	2	97	96	98	91
	3	97	90	95	94
	4	98	80	94	94
	5	90	71	94	98
	6	85	65	92	102

duration of exposure in all cultures was the same, i.e. 6 days. During that time, the experimental environment has not been refreshed. The *S. cerevisiae* cell concentration was checked twice a day and supplemented to the initial level by addition of few drops of thick, freshly prepared suspension. The course of the factual changes of the concentration of *S. cerevisiae* cells in cultures is presented in Fig. 1.

Protozoa for experiments were taken out from reserve cultures. Before being used in the tests they were rinsed from the superfluous bacteria by washing them three times in conditioned sterilized water. The duration of a single bath was 20 min and there was 20 ml of liquid per every protozoan. In consequence of the washing of protozoa, only very few single bacterial cells were transferred into experimental cultures, so they probably did not play any worth mentioning role as

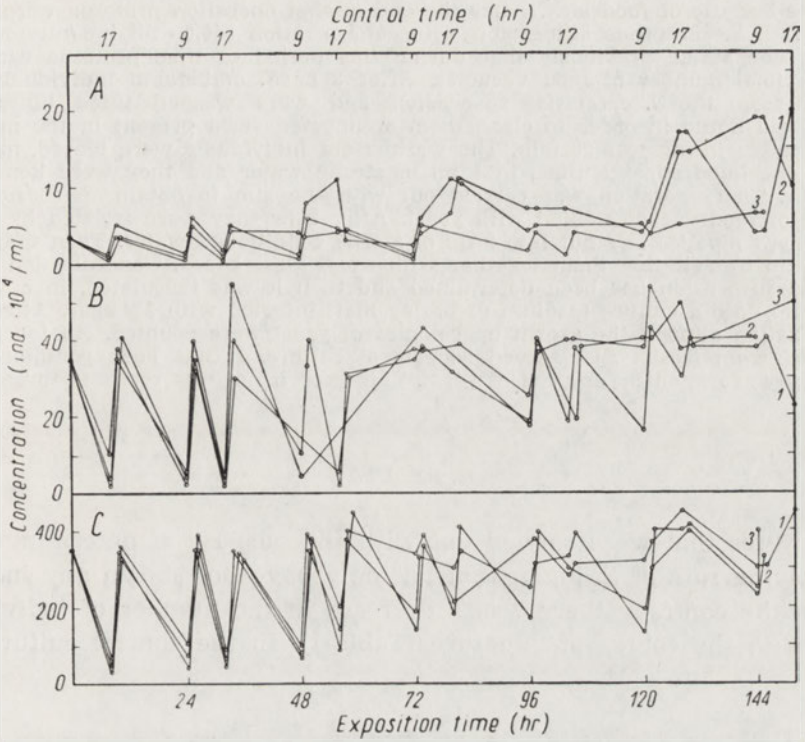


Fig. 1. Changes in concentrations of *S. cerevisiae* in the cultures in course of the experiment. Initial concentration: A — 3.5 · 10^4 ind./ml, B — 35.0 · 10^4 ind./ml, C — 350.0 · 10^4 ind./ml. 1 — 3 — successive repetitions

a supplementary source of food. The initial number of protozoa in each of the cultures amounted to one hundred individuals. The number of protozoa under culture was checked every day by counting them with the naked eye directly in the Petri dishes.

2. The examination of food vacuoles consisted in counting them within the *S. ambiguum* body and also in observations of the vacuoles excreted into the surrounding environment.

The food vacuoles were counted in the bodies of protozoa derived from four other cultures especially set up with that purpose in view. Those cultures were inoculated and reared in the analogical manner with the cultures described above. In the Variant I, one culture was started for each of the following *S. cerevisiae* concentrations: 3.5, 35.0, 350.0, · 10^4 ind./ml. They have been cultivated for 6 days without any changes in the medium. In the Variant II, one culture was started in concentration of 35.0 · 10^4 ind./ml. It has been reared for 10 days. On the 5th day of the experiment, i.e. at the time when protozoa did not form food vacuoles any more, the medium was exchanged completely. In both variants of the culture protozoa were taken out and the food vacuoles were counted under the microscope. Simultaneously with the cultures under experiment there were cultivated analogous ones, from which protozoa were collected and served to supplement the missing number of protozoa that were killed in the process of counting the food vacuoles.

Investigations on excreted vacuoles were carried out in the following experiment: protozoa rinsed off from the superfluous bacteria in analogical manner as mentioned above, were transferred and put for 2 hr into sterile conditioned water.

During this time, *S. ambiguum* excreted food vacuoles previously formed in a bacterial environment and simultaneously the protozoa could not produce any more vacuoles because of food lack. After the end of that operation protozoa were transferred into *S. cerevisiae* suspension with concentration $350.0 \cdot 10^4$ ind./ml for 3 hr. It bas been stated previously that during this period of time protozoa can form the maximal number of food vacuoles. After 3 hr *S. ambiguum* individuals were isolated from the *S. cerevisiae* suspension and were washed three times with a sterilized liquid in order to clean them of uneaten yeast present in the medium. The time of rinsing was 20 min. The washed out individuals were placed, in number of one hundred at a time, in 1 ml of sterile water and they were kept there for 2 hr. That operation was carried out with the aim to obtain a considerable density of food vacuoles filled with yeast. After 2 hr they were isolated by means of capillary pipettes and put into 2 ml of sterile conditioned water. Then they were broken up by agitating them together with small glass beads. Concentration of the obtained suspension has been determined and then it was inoculated, in a volume of 0.01 ml, into a culture medium of barley malt infusion with 2% agar. After 48 hr of incubation at 26°C the grown up colonies of yeast were counted. An inoculation prepared from fresh yeast served as control culture. It has been presumed that one colony grows out of one cell of yeast. This experiment was repeated three times.

3. RESULTS

Cultures that were carried out with the purpose of determining the multiplying rate of *S. ambiguum* not only have not shown any increase but on the contrary there was a decrease in the number of individuals evident in the course of exposure (Table I). In the control culture, i.e.

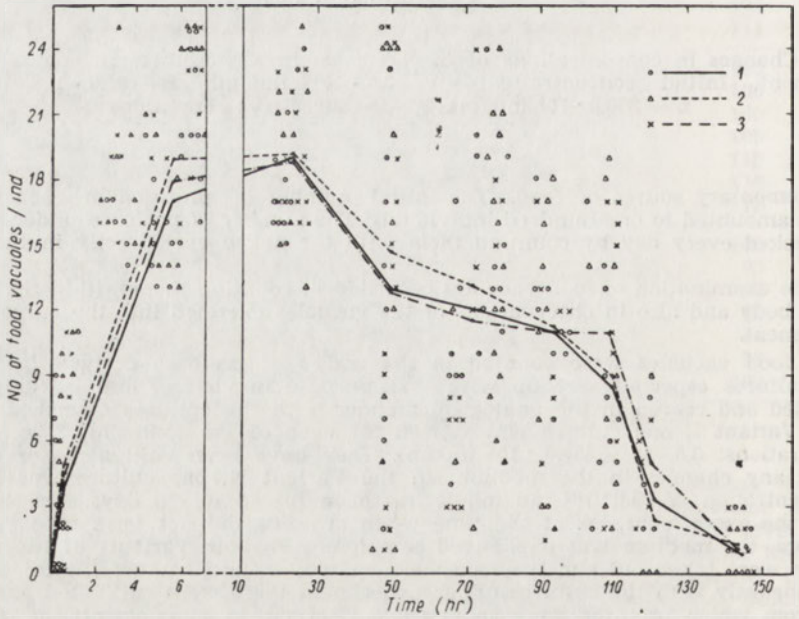


Fig. 2. Relation between the average number of food vacuoles formed by *S. ambiguum* and the time of exposure in various concentrations of *S. cerevisiae*: 1 — $3.5 \cdot 10^4$ ind./ml, 2 — $35.0 \cdot 10^4$ ind./ml, 3 — $350.0 \cdot 10^4$ ind./ml

with bacterial food, there was noticed, nevertheless, a small increase of the protozoan population. It is worth noting that in measure with the time passing by there remained in the culture more and more of uneaten yeast (Fig. 1). Thus, it can be assumed that the amount of consumed yeast has decreased.

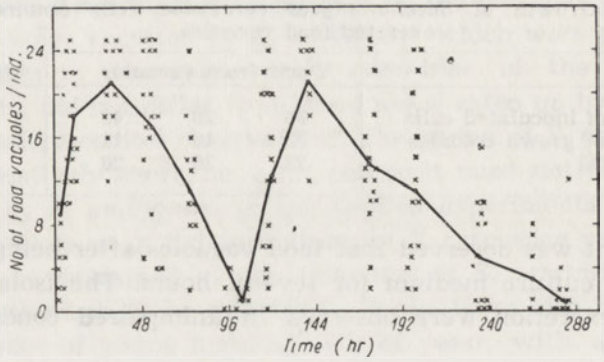


Fig. 3. Relation between the average number of food vacuoles formed by *S. ambiguum* and the time of exposure in the culture, where the medium was exchanged, with *S. cerevisiae* concentration of $35.0 \cdot 10^4$ ind/ml

Results from the counting of food vacuoles found in one individual of *S. ambiguum* are presented in Fig. 2 and 3. So, as it can be seen the number of food vacuoles was not dependent, upon the food concentration (Fig. 2). Their number was increasing rapidly right at the beginning of the experiment, about 25 vacuoles could be found already after 2—4 min from the start. The smallest differentiation in the number of vacuoles found in one individual was observed in the time from 3 to 30 hr from the beginning of experiment. After 48 hr differentiation in their number becomes greater, more and more often there are found protozoa with a very small number of vacuoles. In the Variant I of the experiment (Fig. 2) a complete lack of food vacuoles was observed after 145 hr from its start. This moment coincided with a rapid dying out of protozoa, which before their death had gone through a phase of deformation of their shape. In the culture of the Variant II (Fig. 3) the complete lack of vacuoles occurred after about 110 hr. Here, the violent dying out of protozoa was not observed. At the time when the protozoa were not producing food vacuoles any more, the whole cultured environment was exchanged. The process of formation of vacuoles has been repeated. This time, the utter disappearance of vacuoles occurred again but after 280 hr from the start of the experiment, i.e. after 170 hr from the complete exchange of the environment.

Results from the research on the vitality of yeast consumed previously by *S. ambiguum* are presented in Table II. Yeast cells remained in the food vacuoles 3—5 hr and after the breaking up of vacuoles were inoculated in barley infusion medium fixed with agar. The growth was observed in 73, 33, 25%. The control culture showed the 86% growth of inoculated yeast.

Table II. Growth of *Saccharomyces cerevisiae* cells obtained from excreted food vacuoles

	Yeast from vacuoles			Control
Number of inoculated cells	30	28	42	50
Number of grown colonies	22	10	11	43
Growth (%)	73	36	26	86

Moreover, it was observed that food vacuoles after being ejected can subsist in the culture medium for several hours. The isolated food vacuoles after excretion were observed in unimpaired condition after a time of 15 hr.

4. DISCUSSION

The increase in number of protozoa in various concentrations of *S. cerevisiae* was not stated in the carried out investigations. Even at the greatest food concentration, i.e. $350.0 \cdot 10^4$ ind./ml, the number of protozoa was on the decrease (Table I).

The picture of changes in the numbers of the food vacuoles formed in the body of a protozoan in relation to the time of exposure was as follows: at first, the protozoa formed a great many of food vacuoles, then the number of vacuoles dwindled to the nought. After the renewing of culture medium the formation of vacuoles started anew. Dembowski (1922) obtained a similar picture in his investigations as result of feeding *Paramecium caudatum* on alum carmin, that is an indigestible substance. So, one can assume that this kind of dynamics in the formation of food vacuoles is quite characteristic for Ciliata while provided with indigestible food.

S. cerevisiae derived from excreted food vacuoles retained partially its capacity for growth. The percentage of growing cells of *S. cerevisiae* derived from the excreted food vacuoles is lower than the one obtained from the control culture, viz., 73, 33, 25% as compared with 86% in the control test. It is not possible to deduce from the experiments, presented above, whether the decrease of yeast vitality was produced by the environment of food vacuoles, or by their deterioration in the process of isolation.

To be able to make sure of indigestibliness of *S. cerevisiae* yeast for *S. ambiguum* it would be necessary to carry on the process of isolation of the food vacuoles excreted by protozoa remaining much more longer than 3 hr in the yeast suspension and to use a lower food concentration that the one applied presently. Such circumstances would exclude the conjecture of indigestibliness of yeast resulting from eventual overeating of protozoa. It seems, however, that overeating did not take place in the present research. This might be supported by the fact that yeast obtained from food vacuoles excreted by *S. ambiguum* which were previously fed for several days in suspension of *S. cerevisiae* of the concentration $3.5 \cdot 10^4$ ind./ml, did not differ from yeast never eaten up by the protozoa, according to microscopical observations. The period of *S. ambiguum* starvation was relatively short, i.e. 2 hr, and so it must not cause excessive consumption by *S. ambiguum* in the applied experiments.

The probable cause of indigestibliness of *S. cerevisiae* yeast by *S. ambiguum* may be explained by the thickness of *S. cerevisiae* cell membrane. There is much to be said for it, if one takes also into consideration the absence of young budding cells of yeast, with a thinner cell membrane, in the yeast obtained from the excreted vacuoles.

Further observations, unreported in this paper, concerning the *S. ambiguum* behaviour in the suspension of yeast from grocer's shop showed that *S. ambiguum* has multiplied in the medium containing yeast and bacteria. At the same time, presence of yeast in the food vacuoles of that protozoan was stated not only after a few hours, but also after several days of its stay in the culture, which indicates a continual consuming of yeast. It is possible that in this kind of environment the cell membrane of yeast was damaged by bacteria and in this way it became more suitable to be used as food by *S. ambiguum*. That could be the reason why Loefer (1936) and Brutkowska (unpubl.) have obtained positive results while feeding *Paramecium caudatum* upon *S. cerevisiae* suspension.

On the basis of results presented herewith, one can assume that *Saccharomyces cerevisiae* are as food either entirely indigestible for *Spirostomum ambiguum*, or else only partially digestible, viz. in its young forms.

5. SUMMARY

Trials were made to test the use of *Saccharomyces cerevisiae* Breed I, Okocim strain, as food for *Spirostomum ambiguum* (Ehrbg).

Growth of *S. ambiguum* in pure suspension of *S. cerevisiae* was carefully observed. Three cultures were set up with *S. cerevisiae* concentrations as follows: 3.0, 35.0, 350.0, ind. $\cdot 10^4$/ml. Sterile conditioned water was used as culture medium, all the time of the duration of the experiment it was not exchanged. In none of the three cultures any growth of protozoa has been stated, on the contrary there was a marked decrease in their number (Table I).

The number of food vacuoles formed by *S. ambiguum* was regularly checked up in all three concentrations, above mentioned (Fig. 1 and 2). Dynamics of formation of the food vacuoles was typical for indigestible food.

The isolation of the food vacuoles excreted into environment was carried out. After breaking them up, the obtained yeast suspension was inoculated in barley malt infusion with 2% agar. In result, the growth in 75, 33, and 25% was obtained of the inoculated yeast cells; the growth in the control culture amounted to 86%.

The obtained results suggest that *Saccharomyces cerevisiae* used as food are indigestible for *S. ambiguum.*

6. STRESZCZENIE

Wykonano próby zastosowania *Saccharomyces cerevisiae* Rasa I, szczep Okocim, jako pokarmu dla *Spirostomum ambiguum* (Ehrbg).

Obserwowano rozmnażania się *S. ambiguum* w czystej zawiesinie *S. cerevisiae.* Założono po 3 hodowle w następujących koncentracjach *S. cerevisiae:* 3.0, 35.0, 350.0 • 10^4 os./ml. Środowiskiem hodowlanym była sterylna woda odstała; przez cały czas doświadczenia nie wymieniano środowiska. W żadnej hodowli nie stwierdzono rozmnażania się pierwotniaków, a przeciwnie, spadek ich liczby (Tab. I).

Przeprowadzono kontrolę liczby wodniczek pokarmowych tworzonych przez *S. ambiguum* w 3 podanych wyżej koncentracjach (Fig. 1 i 2). Dynamika tworzenia się wodniczek pokarmowych była charakterystyczna dla pokarmu niestrawialnego.

Dokonano izolacji wodniczek pokarmowych wydalonych do środowiska. Po rozbiciu ich uzyskaną zawiesinę drożdży wysiano na brzeczkę jęczmienną z 2% agarem. Uzyskano wzrost w 75, 33 i 25% posianych komórek drożdży; wzrost w kontroli wynosił 86%.

Wyniki sugerują, że *Saccharomyces cerevisiae* jest pokarmem niestrawialnym dla *S. ambiguum.*

7. REFERENCES

Dembowski, J. 1922. O wyborze pokarmu i tzw. zjawiskach pamięciowych u *Paramaecium caudatum* [Über die Nahrungswahl und die sog. Gedächtniserscheinungen bei *Paramaecium caudatum*]. *Pr. Inst. M. Nenckiego,* 1(1), 1—37 [German summ].

Glaser, R. W., Coria, N. A. 1930. Methods for pure culture of certain protozoa. *J. exp. Med.,* 51, 787—806.

Ilnicka-Olejniczak, M. 1968. Zagadnienie czystości mikrobiologicznej w produkcji drożdży piekarnianych [Problem of production of bacteria-free baker-yeast]. Mat. Symp. "Zag. techn. prod. drożdży piek." 52—58, Warszawa, Inst. Przem. Farm.

Loefer, J. B. 1936. A simple method for maintaining pure-line mass cultures of *Paramaecium caudatum* on a single species of yeast. *Trans. Am. Microscop. Soc.,* 55, 255—256.

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	369–376	1972

M. WIERZBICKA

DISTRIBUTION OF CYCLOPOIDA COPEPODITES IN THE RESTING STAGE IN BOTTOM SEDIMENTS OF ASTATIC RESERVOIRS

Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

ABSTRACT

Quantitative analysis of vertical and horizontal distributions of copepodites IV and V of four Cyclopoida species (*Cyclops strenuus* Fischer, *Cyclops furcifer* Claus, *Acanthocyclops gigas* (Claus), and *Acanthocyclops bicuspidatus* (Claus)), being in resting stage in bottom sediments of two astatic reservoirs was made. Significant differences were found in horizontal distribution of copepodites in one of reservoirs during July and August. Vertical copepodite distributions are different in both reservoirs; in both ones, copepodites did not penetrate deeper than 5 cm, and maximal amounts were in 1—2 cm layer.

1. INTRODUCTION

In an astatic reservoir, as it dries up, the Cyclopoida copepodites prepare themselves for their resting stage. When a reservoir is dry the copepodites IV and V remain in its bottom sediment.

The aim of the paper was to analyse quantitatively the vertical and horizontal distributions of resting copepodites in bottom sediments of two astatic reservoirs. Horizontal distribution in one of them (Field Larger Reservoir) was discussed in Wierzbicka (1966). In the present paper, informations about vertical distribution in bottom sediment, as well as the comparison of both reservoirs are supplemented.

2. TERRAIN DESCRIPTION

The investigated astatic reservoirs are situated in the same depression among fields under cultivation of the State Farms in Zaborów, near Kampinos Forests, about 30 km from Warsaw. They were named Field Larger and Field Smaller Reservoirs. During the spring high level of thawy water, both reservoirs are sometimes confluent (Fig. 1). The area of Field Larger is 940 m², maximal depth — 2.3 m, and of Field Smaller — 115 m² and up to 1 m, respectively. The reservoirs are filled with thawy water in March and dry up in the end of June (Larger) or even in May (Smaller). So the resting stage of copepodites lasts about 9 months. Substratum of Field Larger Reservoir is covered with thick layer of mouldering leaves, its humus is black. Substratum of the Smaller one is rather sandy-loamy, and there is fewer of mouldering leaves. Both are surrounded with trees and bushes: willows, poplars and sloes. In summer, the bottom of the Larger one is covered with groundling bramble sprouts which manure the soil. In 1964, humidities of bottom sediment in the deepest place of Field Larger were 30% (October 20),

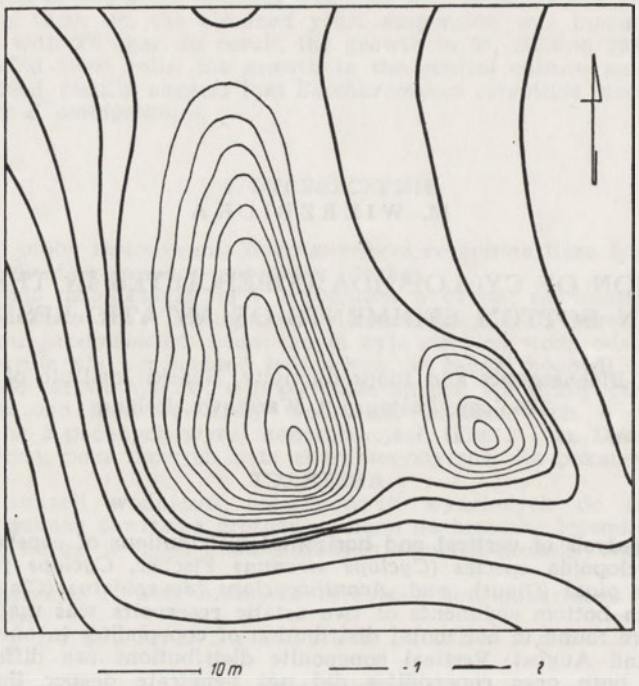


Fig. 1. The map of the astatic reservoirs in Zaborów. Lines — contour lines drawn every 25 cm. 1 — reservoir, 2 — surrounding ground

30.51 and 29.43% (July 4). But it was only 13.42% in the Smaller one immediately after its early drying up (May 18, 1966). (The samples of bottom sediments for humidity determinations were dried at 105°C up to constant weight).

3. MATERIAL AND METHODS

The same species of Cyclopoida live in both reservoirs: *Cyclops strenuus* Fischer, *Cyclops furcifer* Claus, *Acanthocyclops gigas* (Claus), and *Acanthocyclops bicuspidatus* (Claus).

For analyses of distribution of copepodites in their resting stage, quantitative samples of bottom sediments were drawn on July 9 and August 5, 1964 in Field Smaller Reservoir and on October 4, 1964 in Larger one.

In Smaller one the samples were drawn along the longer axis of the reservoir, forming an angle of 25° with the marked direction N—S in Fig. 2 AB. The area of the "macrosample" was 510 cm^2 and its capacity was 2550 cm^3. The samples were drawn with a shovel of appropriate dimensions. The area of the "macrosample" was similar to that of aquaria where the samples were submerged in water to awake the copepodites. Before the sampling, some aquaria (24×30 cm) were exposed, with samples of bottom sediment drawn on June 26, 1964, with different layers: 0—5, 5—10, 10—20 and 20—30 cm. It was found out that there were no specimens below 5 cm. Therefore only 5 cm high samples were drawn later on.

Besides, 25 "microsamples" were drawn from both reservoirs in order to analyse the penetration of copepodites in their resting stage into the depth of bottom sediments, and to determine their vertical distribution ("microanalysis").

In July samples for "microanalysis" were drawn from Field Smaller by cutting 4 blocks of sediment with the surfaces of 8×8, 8×8.5, 9×4, and 3.5×4 cm. In the first block the following layers were tested: 0.0—0.5, 0.5—1.5, and so on, up to 4.5

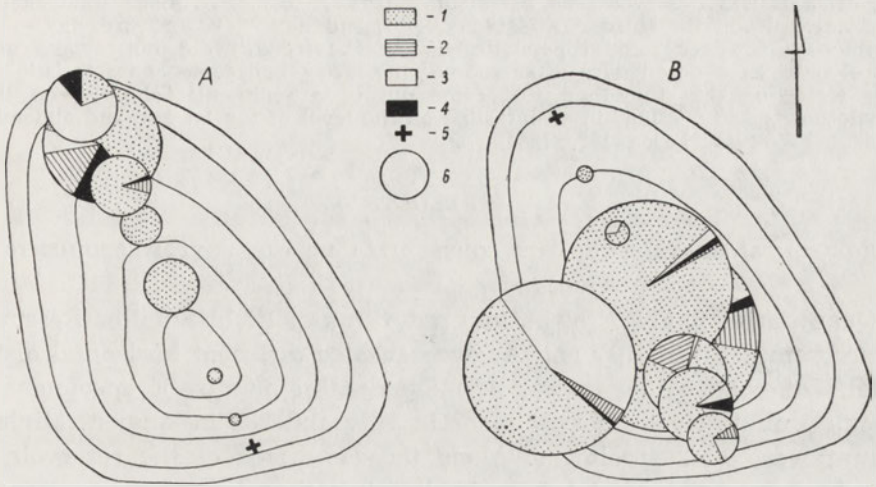


Fig. 2. Horizontal distribution of copepodites of Cyclopoida in their resting stage in bottom sediments of Field Smaller Reservoir. 1 — *C. strenuus*, 2 — *C. furcifer*, 3 — *A. gigas*, 4 — *A. bicuspidatus*, 5 — no specimens, 6 — 20 specimens. A — July, B — August

cm, then 4.5—8.5 and 8.5—18.5 cm. It was shown that there were no specimens below 2.5 cm. In the other blocks, the whole sediment contents were tested together.

In August (Smaller) and in October (Larger), 21 "microsamples" were drawn with a special sampler constructed according to an idea of mgr S. Kędzierski. It was a cylinder, 5 cm in diameter and 19.6 cm^2 in section. A suitable sucker pushed a drawn sample out of the cylinder into a polyetilen sack. The samples were 5 cm high. In the laboratory, successive disks (0—1, 1—2, 2—3 and 3—5 cm high) were cut, placed in beakers, and submerged in water to awake the copepodites. 5 "microsamples" were drawn by means of this sampler from Field Smaller, and 16 — from Field Larger.

In Table I the results of only those samples are presented in which each centimeter of sediments was examined.

Table I. Vertical distribution of copepodites IV and V of four Cyclopoida species being in resting stage in bottom sediments (one specimen in cm^3 of mud) (1964)

Field Smaller				Field Larger	
1 sample of 9.VII		3 samples of 5.VIII		8 samples of 4.X	
Layer (cm)	cm^3 of mud	Layer (cm)	cm^3 of mud	Layer (cm)	cm^3 of mud
0.0—0.5	5.1	0—1	3.4	0—1	51.6
0.5—1.5	9.1	1—2	1.3	1—2	9.8
1.5—2.5	31.0	2—3	4.6	2—3	14.1
		3—5	29.5	3—5	24.1

Since it was found that copepodites of different species do not awake simultaneously, the experimental vessels were investigated several times. It was stated that copepodites of *C. strenuus* awaked most early: from dozens of minutes to

several hours after submerging, and copepodites IV of *A. bicuspidatus* — most late, often it was observed only after some days. Also e.g. *A. gigas* V awaked in great amounts on the third day (1st day — 1, 2nd day — 9, and 3rd day — 104 specimens). However some copepodites IV of *C. strenuus* did not awake untill several or even a dozen days after submerging. They had some characteristic features testifying that they had just come out of a sediment: "shields" on their cephalothorax and a "plug" in an intestine on the level of the 1st and 2nd abdominal segments (Wierzbicka 1966, 1967).

4. RESULTS

HORIZONTAL DISTRIBUTION OF COPEPODITES IN THE BOTTOM SEDIMENTS

Quantitative analyses of bottom sediments of Field Smaller Reservoir ("macrosamples") in July and August revealed different horizontal distributions of copepodites (Fig. 2 AB). Maximal amounts of specimens in a single sample were 125 specimens in July and 332 in August. Minimal amount were 1—2 specimens. Along the same axis of the reservoir, in July the largest amounts were on its northern shore where it was 1 specimen per 20.4 cm^3 of mud. In August they were in the deepest place and where both reservoirs are sometimes confluent (Fig. 1); in these places it was 1 specimen per 7.7 cm^3.

In August there were nearly 3 times more specimens per sample in the most abundant samples than in July; in the whole of the investigated area there were about 3 times more specimens in August, too.

Figure 2 AB presents quantitative species composition in Field Smaller: the predominating species is *C. strenuus,* and *A. gigas,* inhabiting mostly the shallower places, occurs in great amounts. *C. furcifer* and *A. bicuspidatus* are in lesser amounts.

It was found out as early as in 1954—1956 that copepodites of Cyclopoida came to their resting stage when the level of water was rather high (about 1 m in Larger and 30 cm in Smaller one) and at the temperature between 10 and 20°C.

VERTICAL DISTRIBUTION OF COPEPODITES IN THE BOTTOM SEDIMENTS

In all samples drawn from Field Smaller Reservoir in July, in the layer up to 2.5 cm there was only *C. strenuus* IV. It was even more numerous in the layer of 0.0—0.5 cm than of 0.5—1.5 cm (Table I). In July *C. strenuus* was the only species in the "microsamples" drawn from the centre of the reservoir as well as in the "macrosamples" drawn from the deeper places.

In August it was *C. strenuus* IV which was still the most numerous, occurring mostly in 1—2 cm layer. In 0—1 cm layer it was two times less numerous, and below 2 cm its amounts were low. *A. gigas* V was numerous in 1—2 cm layer, and three times less numerous in 0—1 cm layer. *C. furcifer* IV was found in small amounts in 1—2 cm layer, but

A. bicuspidatus was not found at all. Quantitative species composition in this reservoir was also characterized by the "microsamples"; it can be defined as one where *C. strenuus* is predominating, *A. gigas* V lives in its periphery, *C. furcifer* IV occur in small amounts and *A. bicuspidatus* IV — in very small amounts (Fig. 2 B).

In the most settled layer (1—2 cm) there was 1 specimen in 1.3 cm^3 of mud (Table I). In 0—1 cm layer there were remarkably many specimens (one in 3.4 cm^3). Below 2 cm there was only one specimen per 4.6 and 29.5 cm^3 of mud. The value of "microsamples" was confirmed by recounting the specimens in whole "microsamples" drawn in July and August. In July, there was one specimen in 20.0 cm^3 of sediment, which corresponds with the "macrosample" with the greatest amount of specimens, i.e. one specimen in 20.4 cm^3. In August, there was 1 specimen per 3.4 cm^3 ("microsample") and 7.7 cm^3 ("macrosample"). This difference can be explained by specificity of sampling, however, the increase tendency of the number of specimens in August is markedly visible.

In Field Larger the greatest number of specimens was found in the deepest place, in south-eastern part of the reservoir (Fig. 1 and Wierzbicka 1966). It turned out that in 0—1 cm layer only single individuals could be rarely found (1 specimen in 51.6 cm^3 of sediment). It should be added that in the samples from this layer, submerged in water, numerous nauplii of *Diaptomus castor* (Jurine) were found. Copepodites occurred mostly in the layer 1—2 cm (1 specimen in 9.8 cm^3 of sediment). Below 2 cm they were less numerous. The smallest species, *A. bicuspidatus* IV, occurred in all layers, even in the deepest investigated one. *C. strenuus* IV was evenly distributed in all layers, but only one specimen of *A. gigas* V was found in 0—1 cm layer and none of *C. furcifer* IV. It corresponds with quantitative composition of species in "macrosamples" where *A. gigas* V and *C. furcifer* IV were considerably less numerous. Also the "microsample" with the greatest number of *A. bicuspidatus* IV corresponds with the "macrosample" where this species was most numerous. Thus it can be stated that "microsamples" were representative for copepodite distribution in bottom sediments of the reservoir.

COMPARISON OF COPEPODITE DISTRIBUTION IN BOTTOM SEDIMENTS OF BOTH RESERVOIRS

Both investigated reservoires are characterized by different species predomination: in Field Larger Reservoir, *A. bicuspidatus* is prevailing and *C. strenuus* is numerous, whereas in Smaller one *C. strenuus* is predominating and *A. bicuspidatus* is the least numerous (Fig. 2 AB and Wierzbicka 1966). *C. furcifer* is the least numerous in Larger and quite numerous in Smaller one. *A. gigas* is similarly distributed.

Such contrasting quantitative relationships in both reservoirs which

have even been confluent at high water level, can be explained by different characters of their substrata. Chemical analyses could perhaps be instructive.

There were also differences in vertical distributions: in Field Smaller, the 0—1 cm layer was settled more densely than in Larger one. The reasons could be seen in characters of substrata (more dry and dense in Smaller one) as well as in the span of time from the beginning of the resting stage (June): the samples were drawn from Smaller in July and August, and from Larger in October. The latter hypothesis seems to gain more ground in that in July copepodites penetrated only up to 2.5 cm layer, and in August already up to 5 cm. In July they were even more numerous in 0.0—0.5 than in 0.5—1.5 cm layer.

The features common for both reservoirs are following: maximal densities of copepodites were in 1 — 2 cm layer (in August and October); *A. gigas* prefers the shores of reservoirs and thus shallower places, whereas *A. bicuspidatus* tends to choose deeper ones which is visible especially in Larger.

5. DISCUSSION

The investigations of astatic reservoirs, presented above, allow to state that the drawn samples ("micro-" and "macrosamples") represent qualitative and quantitative distributions of copepodites being in resting stage (and thus they provide a characteristics of the settlement of a given reservoir without the need to investigate plankton), if the following requirement is met: sufficiently long period of observation of samples submerged in water (or their particular layers when vertical distribution is examined). This condition results from a non-simultaneous awaking of copepodites of various species, as well as of particular individuals of a given species.

If all species of a given reservoir are expected to be found in bottom sediments, samples should be drawn along its several axes (it was mentioned that some species such as *A. bicuspidatus* and *C. strenuus* prefer the deepest place of a reservoir, the other, e.g. *A. gigas*, prefer shallower places in the shores).

Since the distribution of copepodites depends on the time of their resting in mud (vertical distribution), as well as on weather conditions, multiple sampling seems to be required.

In the Field Smaller from which the samples were drawn in July and August, copepodites were more numerous in the layers 0.0—1.5 and 1—2 cm and in July they did not penetrate below 2.5 cm in some samples. However in Larger one there were nearly no copepodites in 0—1 cm layer and they penetrated fairly deeper. The reason could be more dry and dense substratum of Field Smaller, as well as that the copepodites did

not have time to penetrate deeper. Some observations testify that after their entering into the sediment, copepodites could move and change their resting place (Wierzbicka 1962). Champeau (1966) observed that *D. odessanus* remained active in strongly humidified soil of an astatic reservoir. This species was most numerous in 0–10 cm layer and it penetrated up to 20 cm. *C. strenuus* examined by Elgmork (1959) in non-astatic reservoirs, penetrated up to 10, 15, and even 20 or 30 cm. In the present paper, *C. strenuus* penetrated only to 5 cm. The reason of the differences is probably the substratum, more dense and grown with roots of the surrounding trees and bushes in Zaborów.

Figure 2 AB presents horizontal distributions of copepodites from Field Smaller, quite different in July and August. It could be supposed that in July the specimens were diffused in sediment out of the deepest place, and in more rainy August they gathered in wetter places.

So, according to Mikulski (personal information and 1965), in July 1964 there was 32.5 mm of rainfalls and in August — 62.4 mm, which is 38.69 and 86.67% of the mean of many years' rainfalls (the data concern Dziekanów Leśny situated also on the fringe of Kampinos Forests, 15 km to the north-west from the investigated reservoirs). In July the heaviest rainfall (8 mm) was on 10th, one day after the samples were drawn, whereas in August small rainfalls were constant.

Since during the summer the investigated reservoirs are never filled with water, the probable reason of the described differences was that the copepodites awaked when the substrata were more wet, they drifted with water along the slope and digged themselves in the sediment again. The observations of copepodites which awake quite soon after the sediment is submerged in water, testify to it (e.g. *C. strenuus* IV awakes sometimes after about an hour), especially that the maximum of their vertical distribution was in 1–2 cm layer, and even in 0.0–0.5 cm layer in July.

Acknowledgements

I wish to thank sincerely to mgr S. Kędzierski and Mr. Cz. Farfura for the help in sampling the material.

6. SUMMARY

Horizontal and vertical distributions of copepodites IV and V in resting stage of four Cyclopoida species (*Cyclops strenuus* Fischer, *Cyclops furcifer* Claus, *Acanthocyclops gigas* (Claus) and *Acanthocyclops bicuspidatus* (Claus)) were investigated in bottom sediments of two astatic reservoirs, deprived of water from the end of June until March of a next year, situated closely to each other among fields under cultivation near the Kampinos Forests, about 30 km from Warsaw.

There were significant differences in quantitative species composition in both reservoirs (Field Larger and Smaller Reservoir). Moreover, there were significant differences in horizontal distribution of copepodites in Field Smaller between July and August. There were also differences in vertical distribution: in Field Smaller there were more copepodites in the top layer of sediment (0–1 cm) than in Larger one, and they penetrated only to 2.5 cm.

The following similarities were found: maximal numbers of copepodites were in 1–2 cm layer in both reservoirs in August and October; by then specimens did not penetrate deeper than 5 cm; the same species from both reservoirs prefered shallower or deeper places.

The reasons of differences were probably different substrata, chemism of reservoirs (not dealt with in the present paper), the span of time from the beginning of the resting stage (influencing the vertical distribution), and of climatic conditions (influencing the horizontal distribution of copepodites).

7. STRESZCZENIE

Zbadano rozmieszczenie poziome i pionowe kopepoditów IV i V, będących w stanie spoczynku, 4 gatunków Cyclopoida (*Cyclops strenuus* Fischer, *Cyclops furcifer* Claus, *Acanthocyclops gigas* (Claus) i *Acanthocyclops bicuspidatus* (Claus)) w osadach dennych dwóch zbiorników astatycznych, pozbawionych wody od końca czerwca do marca następnego roku, leżących obok siebie wśród pól uprawnych w odległości około 30 km od Warszawy, na skraju Puszczy Kampinoskiej.

Stwierdzono, że zbiorniki te (Śródpolny Większy i Śródpolny Mniejszy) wykazują istotne różnice w ilościowym układzie gatunków. Zwracają uwagę zasadnicze różnice w rozmieszczeniu poziomym kopepoditów w zbiorniku Mniejszym w dwóch kolejnych miesiącach, w lipcu i w sierpniu. Różnice występują również w rozmieszczeniu pionowym w tych dwóch zbiornikach: w Mniejszym więcej kopepoditów znajduje się tuż pod powierzchnią osadów (0–1 cm) niż w drugim zbiorniku i w lipcu kopepodity przeniknęły w głąb osadów tylko do 2.5 cm.

Stwierdzono następujące podobieństwa: maximum w pionowym rozmieszczeniu przypada w obu zbiornikach na warstwę 1–2 cm (w sierpniu i w październiku). W tych dwóch miesiącach okazy nie przenikają w głąb osadów dennych poniżej 5 cm. Jednakowe gatunki w obu zbiornikach wykazują predylekcję do płytszych lub głębszych miejsc.

Przyczyny różnic zapewne leżą w zróżnicowanym podłożu zbiorników, chemizmie zbiorników, nie uwzględnionym w tym opracowaniu, w odległości czasowej od początku okresu spoczynku (wpływającej na rozmieszczenie pionowe kopepoditów) oraz od warunków atmosferycznych, które mogą wpłynąć na zmiany w rozmieszczeniu poziomym kopepoditów.

8. REFERENCES

Champeau, A. 1966. Contribution a l'étude écologique de la faune des eaux temporaires de la Haute Camargue. *Arch. Oceanogr. Limnol.*, 14, 309–357.

Elgmork, K. 1959. Seasonal occurrence of *Cyclops strenuus strenuus* in relation to environment in small water bodies in southern Norway. *Folia limnol. scand.*, 11, 1–196.

Mikulski, Z. 1965. *Zarys hydrografii Polski* [The outline of hydrography of Poland]. Warszawa, PWN.

Wierzbicka, M. 1962. On the resting stage and mode of life of some species of Cyclopoida. *Pol. Arch. Hydrobiol.*, 10, 215–229.

Wierzbicka, M. 1966. Les résultats des recherches concernant l'etat de repos (resting stage) des Cyclopoida. *Verh. int. Ver. Limnol.*, 16, 592–599.

Wierzbicka, M. 1967. Obserwacje nad stanem spoczynku niektórych gatunków Cyclopoida [Observations on the resting stage of some species of Cyclopoida]. Streszcz. ref. 133–135 VII Zjazd Hydrobiol. Pol. Świnoujście, Warszawa, Pol. T-wo Hydrobiol.

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	377–381	1972

K. W. OPALIŃSKI

FRESHWATER FAUNA AND FLORA IN HASWELL ISLAND (QUEEN MARY LAND, EASTERN ANTARCTICA)

Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

ABSTRACT

Flora and fauna of a small freshwater lake in Haswell Island (Queen Mary Land, Eastern Antarctica) is described. The island has a character of an Antarctic oasis. In the investigated lake, Rotatoria, Tartigrada, Cyanophyta, Diatomae and Chlorophyta were found. Such composition of freshwater flora and fauna is typical for Eastern Antarctica.

1. INTRODUCTION

Flora and fauna of Antarctic oases, especially of lakes and small freshwater reservoirs frequently occurring in all Antarctic oasis, are not sufficiently known yet.

Biocenoses of Antarctic lakes are characterized by small number of species, and consequently, by simple ecological relationships. In these lakes, filamentous Cyanophyta and Diatomeae are markedly predominating. Among not numerous Chlorophyta, coccoidal forms are prevailing (cf. Bryant 1945, Hirano 1965, Goldman 1970).

The aim of the present paper is to determine the species composition of flora and fauna in one lake in Haswell Island (Eastern Antarctica). Similar investigations have not been carried out in this island until now.

2. TERRAIN DESCRIPTION AND METHODS

Haswell Island (66°31′S, 93°00′E) is the largest one of the archipelago of the same name, being situated near the shores of Queen Mary Land. Haswell Island lies about 2.5 km from an ice-barrier of the continent; its area is 1 km² and maximal height 91 m a.s.l.

There are some freshwater lakes in the island, lasting or periodical ones. The lakes are supplied with organic substances and biogenic salts by guano of birds, mainly Adelie penguins (*Pygoscelis adeliae* Homb et Jac.) which live in the island in numerous colonies.

Gollerbach, Syroyechkovsky (1960) estimated that birds carry about 100 t of organic substances per year from sea to Haswell Island.

The samples were drawn from organic layer of the bottom of a small lake in north-western part of the island on December 31, 1969 (Fig. 1). The lake is about 50 m in diameter and about 2 m in depth. It was covered with ice 1–2 cm thick.

The samples were drawn with a plankton net (Hensen type), pulled along the bottom. The obtained material was conserved with 4% formaline. The samples are qualitative only on account of the sampling method.

Fig. 1. Haswell Island; the sampling lake (December 31, 1969)

3. RESULTS

Fauna in the investigated reservoir is rather poor: Rotatoria and Tartigrada are its only representants (Table I). Cyanophyta, Diatomeae and Chlorophyta compose the flora there. Cyanophyta are most numerous; they are 53% of the total of both fauna and flora in the lake. Diatomeae take the second place (23%) and Chlorophyta account for 17%. All the animal organisms account for somewhat more than 2%. The remaining 5% are hardly identificable cysts and spores (probably of Cyanophyta) as well as remnants of plants and animals (mainly Rotatoria) damaged during conservation.

The numbers of species of both Cyanophyta and Diatomeae are similar (cf. Table I).

4. DISCUSSION

It is interesting that only two animal taxonomic groups (Tartigrada and Rotatoria) occurred in the investigated lake. Donner (1972) found five Rotatoria species in living unpreserved samples from this lake. In other oases of Eastern Antarctica, e.g. Bunger Hills, Vestfold Hills, Thala Hills, Langhovde, lake fauna is represented by a greater number of taxonomic groups: Protozoa (Testaceae), Nematoda, Rotatoria, Tarti-

Table I. Freshwater fauna and flora composition in Haswell Island
(% of the total density)

Species	%
Rotatoria	
Philodina gregaria Murray	+
Adienta grandis Murray	+
Genus undetermined	1
Tartigrada	
Hypsibius sp.	+
Cyanophyta	
Tetrachloris merismopedioides Skuja	14
Gloeocapsa cohaerens var. antarctica (Bréb) Wille	14
Phormidium frigidum F. E. Fritsch	13
Chloroglea sp.	8
Lyngybya antarctica Gain.	2
Oscillatoria irrigua Kütz.	1
Calotrix sp.	+
Binuclearia tatrana Wittr.	+
Diatomeae	
Navicula mutica var. ventricosa (Kütz) Cl.	11
Achnanthes marginulata Grun.	7
Amphora holsatica Hust.	1
Pinnularia borealis Ehr.	1
Nitzschia sp.	+
Fragilariopsis rhombica Hust.	+
Fragilariopsis antarctica (Castr.) Hust.	+
Charcotia actinochilus Hust.	+
Bidulphia sp.	+
Chlorophyta	
Prasiola crispa (Ligthf.) Menegh.	11
Chlorella antarctica (Fritsch) Wille	6
Undetermined	5

+ — less than 1%

grada and Cyclopoida, Harpacticoida, Cladocera (cf. Korotkevich 1958, Sudzuki, Shimoizumi 1967, Opaliński 1972).

Among flora of the lake, two groups, endemic for the Antarctica, were found: *Gloeocapsa cohaerens var. antarctica* (Bréb) Wille and *Chlorella antarctica* (Fritsch) Wille. Both the species constitute 10% of all plant species occuring there and about 20% of the total density of plants. These values are similar to the respective data for the Thala Hills oasis (cf. Opaliński 1972).

Among Diatomeae, beside cosmopolitic forms commonly occurring in Antarctic oases, such as *Pinnularia borealis* Ehr. or *Navicula mutica var. ventricosa* (Kütz) Cl., some typically sea species were found: *Fragilariopsis rhombica* Hust., *Fragilariopsis antarctica* (Castr.) Hust., *Charcotia actinochilus* Hust., and *Bidulphia sp.*, which are common in Antarctic seas (cf. Kozlova 1964). They were probably brought to the lake by the birds inhabiting the island; low densities of those species testify to it.

Occurrence of nitrofil forms in the lake, such as Prasila and Oscillatoriales, is the proof that the lake is supplied with allochthonic matter originating from bird faeces (cf. Matsuda 1968).

Flora and fauna composition in Haswell Island is similar to the general pattern of living organisms distribution in the lakes of Eastern Antarctic oases.

5. SUMMARY

In December 1969, qualitative samples of organic sediments were drawn from the bottom of the freshwater lake in Haswell Island (Queen Mary Land, Eastern Antarctica). Numerous colonies of birds live in the island. The lakes in the island are supplied with several organic and mineral substances originating from guano of gulls and penguins.

Among flora and fauna of the investigated lake, the following groups were found: Tartigrada and Rotatoria (2% of the total density of flora and fauna), Cyanophyta (53%), Diatomeae (23%) and Chlorophyta (17%).

Only two among 19 plant species — *Gloeocapsa cohaerens var. antarctica* (Bréb) Wille (Cyanophyta) and *Chlorella antarctica* (Fritsch) Wille (Chlorophyta) — are endemic plants in Antarctica.

Among Diatomeae, four brackish species were found, characteristic for Antarctic seas (*Fragilariopsis rhombica* Hust., *F. antarctica* (Castr.) Hust., *Charcotia actinochilus* Hust. and Bidulphia sp.). These species were probably brought to the lake by birds.

The mentioned inflow of allochthonic matter into lakes makes the development of nitrofil forms, such as Prasila and Oscillatoriales, possible.

The investigated lake is similar, as to its flora and fauna composition, to other lakes in the shore oases of Eastern Antarctica.

6. STRESZCZENIE

W grudniu 1969 r. pobrano jakościowe próbki organicznych osadów z dna słodkowodnego jeziora na wyspie Haswell (Queen Mary Land, Antarktyda Wschodnia). Na wyspie tej znajdują się liczne kolonie ptaków. Jeziora znajdujące się na wyspie zasilane są w szereg związków organicznych i mineralnych pochodzących z guana mew i pingwinów.

We florze i faunie badanego jeziora stwierdzono występowanie Tartigrada i Rotatoria (2% ogólnej liczebności flory i fauny), Cyanophyta (53%), Diatomeae (23%) i Chlorophyta (17%).

Tylko dwa spośród 19 gatunków roślin — *Gloeocapsa cohaerens var. antarctica* (Bréb) Wille (Cyanophyta) i *Chlorella antarctica* (Fritsch) Wille (Chlorophyta) — są endemitami kontynentu antarktycznego.

Wśród Diatomeae stwierdzono występowanie 4 gatunków słonowodnych, charakterystycznych dla mórz antarktycznych (*Fragilariopsis rhombica* Hust., *F. antarctica* (Castr.) Hust., *Charcotia actinochilus* Hust. i *Bidulphia sp.* Gatunki te zapewne zawleczone zostały do jezior przez ptaki.

Wspomniany wyżej dopływ materii allochtonicznej do jezior umożliwia rozwój form nitrofilnych, jak Prasiola i Oscillatoriales.

Pod względem składu flory i fauny badane jezioro podobne jest do jezior występujących w przybrzeżnych oazach Antarktydy Wschodniej.

7. REFERENCES

Bryant, H. M. 1945. Biology at East Base, Palmer Peninsula, Antarctica. *Proc. Am. phil. Soc.*, 89, 256–269.

Donner, J. 1972. Bericht über Funde von Rädertieren (Rotatoria) aus der Antarktis. *Pol. Arch. Hydrobiol.*, 19, 251–252.

Goldman, C. R. 1970. Antarctic freshwater ecosystems. In: M. W. Holdgate [Ed] *Antarctic ecology*. II, 609–627, London, Academic Press.

[Gollerbach, M. M., Syroyechkovsky, E. E.] Голлербах, М. М., Сыроечковский, Е. Е. 1960. Биогеографические исследования в восточной Антарктиде в летний сезон 1957 г. [Biogeographical investigations in East Antarctica in summer season 1957]. *Trudy sov. antarkt. Eksped.*, 9, 197–207.

Hirano, M. 1965. Freshwater algae in the antarctic regions. In: J. van Mieghem, P. van Oye [Eds] *Biogeography and ecology in Antarctica*. 127–193, The Hague. W. Junk, (Monographiae Biologicae, 15).

[Korotkevich, V. S.] Короткевич, В. С. 1958. Население водоемов оазисов в восточной Антарктиде [Animal population of „Oasis" lakes in the East Antarctica]. *Inf. Byull. Sov. antarkt. Eksped.*, 3, 91–98.

[Kozlova, O. G.] Козлова, О. Г. 1964. Диатомовые водоросли индийского и тихоокеанского секторов Антарктиды [Diatoms of the Indian and Pacific sectors of the Antarctic]. Moskva, Izdat. „Nauka".

Matsuda, T. 1968. Ecological study of moss community and microorganisms in the vicinity of Syowa Station, Antarctica. *JARE sci. Rep., Ser. E*, 19, 1–58.

Opaliński, K. W. 1972. Flora and fauna of freshwater bodies of the Thala Hills oasis (Enderby Land, Eastern Antarctica). *Pol. Arch. Hydrobiol.*, 19, 383–398.

Sudzuki, M., Shimoizumi J. 1967. On the freshwater microfauna of the Antarctic region. II. Stability of faunistic composition of antarctic microorganisms. *JARE sci. Rep., special issue*, 1.

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	383–398	1972

K. W. OPALIŃSKI

FLORA AND FAUNA IN FRESHWATER BODIES OF THE THALA HILLS OASIS (ENDERBY LAND, EASTERN ANTARCTICA)

Department of Bioenergetic and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

ABSTRACT

The present paper deals with the quantitative and qualitative composition of fauna and flora of some freshwater bodies in the Antarctic oasis Thala Hills (67°40′S, 45°50′E) in the Enderby Land region, Eastern Antarctica. Resemblance of flora and fauna abounding in particular water bodies was studied by means of the dendrite method. Causes of the sparsity of living organisms in water bodies in the Antarctic oases, as well as problems of plant and animal life in Antarctica are discussed.

1. INTRODUCTION

Environmental conditions of Antarctic oases such as: presence of ground not covered with ice, positive heat balance, warming of rocks in the summer season up to the temperature above 0°C, occurrence of water in liquid state, chemical and physical disintegration of rock (cf. Korotkevich 1969, Bardin 1968) are favourable for the settling of living organisms.

Flora and fauna of the Antarctic oases is very scarce. Land fauna consists of a few species of Acarina (cf. Dalenius 1965), Colembola and Diptera (cf. Gressit 1965). In the moss communities and water bodies there are Actinopoda, Rhizopoda, Ciliata, Rotatoria, Gastrotricha, Tartigrada, Nematoda (Sudzuki, Shimoizumi 1967), Turbellaria (Armitage, House 1962); in some lakes there also occur Cyclopoida, Harpacticoida, and Cladocera (Korotkevich 1958, Akatova 1964, Heywood 1970).

Flora is represented by mosses (cf. Savich-Lyubitskaya, Smirnova 1969) and lichens (cf. Dodge 1965, Golubkova 1969). In water bodies Diatomeae, Cyanophyta, Chlorophyta (Hirano 1959, 1965), Xanthophyceae (Lavrenko 1966), Glaucophyta (Wille 1924) and mosses (Savich-Lyubitskaya, Smirnova 1964) can be found.

The rate of biological processes in the Antarctic oases is very slow and this consequently slows down the process of regeneration of the destroyed biocenoses. The main source of devastation of the natural environment are scientific exploration bases established within the area of oases. Use of caterpillar tractors, geological surveys, littering up with refuse, garbage, and leavings of fuel and lubricants, cause the wasting away of mossy turf, pollution in the lakes, changes in the natural drainage area, and introduction of new kinds of plants and animals trailed into Antarctica along with food and equipment for polar explorers and alien to the living organic world of oases.

The object of the present work is an assessment of quantitative and qualitative composition of flora and fauna of one of the Antarctic oases (the Thala Hills

Oasis [1], Enderby Land) at the moment when human intervention has not yet upset the natural order in biological environment of the oasis.

Flora and fauna of the Thala Hills Oasis has not been investigated thus far. Bardin et al. (1969) makes mention of some species of algae collected in various spots in the oasis, Fukushima (1970) reports five Diatomeae species found in two lakes of this oasis.

2. TERRAIN DESCRIPTION AND METHODS

Thala Hills Oasis (67°40'S, 45°50'E) lies in Enderby Land, in Eastern Antarctica. It is a typical littoral oasis (cf. Korotkevich 1969) situated in the fork of Campbell Glacier (eastward) and Hays Glacier (westward). The northern border of the oasis are waters of the Alasheyev Bight.

The oasis area is ca. 9 km², there are three ranges of hills running towards the north-west. The height of the hills is up to 100 m above sea level. They are formed of crystalline schist and gneiss, i.e. rocks characteristic for the archaeozoic East Antarctic Plateau.

Within the area of the oasis there are about 40 water bodies with their surfaces ranging from several hundred up to several hundred thousand m² area, and their depths is from 0.3 up to 27 m. They are supplied with water from the melting masses of ice and snow, in the summer season there occurs the overflowing of some lakes and then water flows out into some other lake lying beneath, further down (cf. Simonov 1970) or else down into the sea (cf. Klokov 1970).

They are all freshwater lakes. Their bottom is covered with orogenic-type sediments, i.e. coarse rocky material or, rather rarely, loam. Bottom sediments are overlaid with a bacterio-algal layer, ranging from 1 cm up to 1 m of thickness, depending on the size of the water body. Presence of that bacterio-algal substratum is very characteristic for Antarctic lakes (Korotkevich 1969). A greater part of the living organisms living in the waters of the oasis are closely connected with that layer.

Taking into consideration the thermic and ice conditions one can divide the water bodies of the oasis into three groups: 1 — large lakes, not thawing in summer, with all year round inverse thermic stratification and the temperature at the bottom of 4°C; 2 — lakes 3–5 m deep, thawing in summer for a period of about 50 days (cf. Fukushima 1961) with temperature at the bottom, in winter, from 0° to 4°C; 3 — small and shallow lakes, in winter freezing down to the bottom, in summer warming up even to temperatures in the order of +12°C (Simonov 1968).

For the purpose of hydrobiological studies, ten water bodies were selected outside the territory of Molodezhnaya Station (Fig. 1, Table I). Among the selected lakes one belongs to the first group (Glubokoye), four to the second group (lakes No. 2, 5, 11 — Fig. 2 and No. 12) and five to the third group (No. 1, 4, 6, 8, 9). During the period from March 19 to April 15, 1969, eighteen samples of the bacterio-algal substrata were collected from those lakes. Samples were drawn up from the bottom with Ekman's dipper, of water-tight construction and with 225 cm² surface. Plankton samples were collected with Apstein-type plankton net (qualitative samples) from lakes No. 1, 2, 11 and 12.

The collected materials were preserved with 4% solution of formalin. Determination of species and of density of organisms population was carried out after our return home.

The quantity of animals and plants was determined in preparative samplings (20–25) extracted from each of the collected samples and afterwards the obtained values were converted to the number of individuals per volume unit (1 ml) of the bacterio-algal substratum.

---

[1] Since 1962, Russians have extended the name "Molodezhnaya", given to their Research Station built in Thala Hills oasis, upon the whole territory of that oasis. In Soviet papers Thala Hills oasis is mentioned under the name of "Molodezhnaya Oasis" (cf. Simonov 1968).

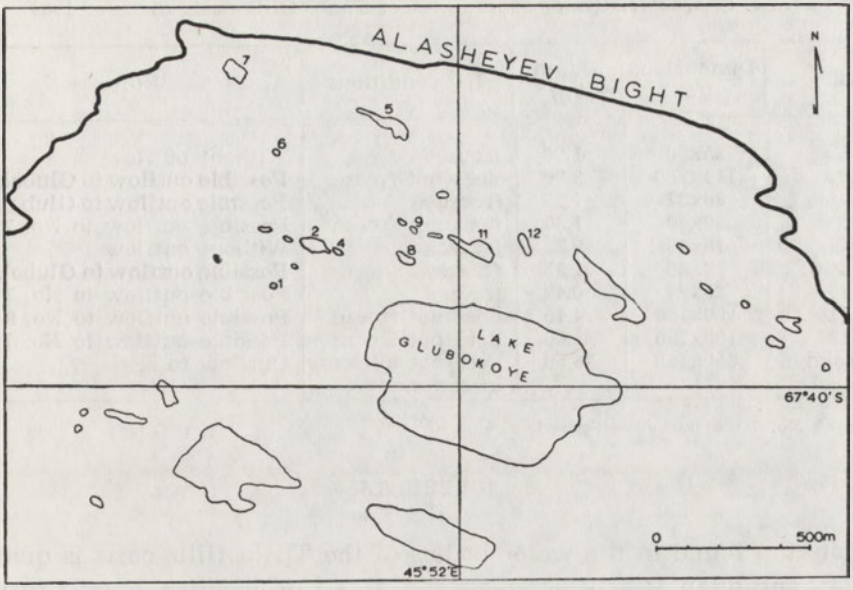


Fig. 1. The central part of Thala Hills oasis. Numerals denote the lakes under survey

Fig. 2. Thala Hills oasis. Lake No. 11 covered with ice (March 25, 1969)

Table I. Characteristics of some lakes of Thala Hills oasis (data of 1969)

Lake	Dimensions (m)	Depth (m)	Ice conditions	Notes
No. 1	35x50	1.20	freezes	Without outflow
No. 2	114x200	2.30	does not freeze	Possible outflow to Glubokoye
No. 4	36x37	1.25	freezes	Possible outflow to Glubokoye
No. 5	30x60	1.85	does not freeze	Possible outflow to No. 7*
No. 6	16x28	0.32	freezes	Without outflow
No. 8	8x20	1.25	freezes	Possible outflow to Glubokoye
No. 9	20x24	0.48	freezes	Possible outflow to No. 7*
No. 11	100x150	4.15	does not freeze	Possible outflow to No. 9
No. 12	100x150	2.00	does not freeze	Possible outflow to No. 7*
Glubokoye	850x450	26.50	does not unfreeze in summer	Outflow to sea

* Lake No. 7 was not investigated.

3. RESULTS

Plankton found in the water bodies of the Thala Hills oasis is quantitatively and qualitatively deficient and, in all probability, it fails to play any greater role in the life cycle of the water bodies (cf. Heywood 1967). In the plankton samplings only small quantities of Diatomeae were found, belonging to the following species, presented in the sequence of occurrence order, namely: *Pinnularia gibba var. parva* (Ehr.) Grun, *P. borealis* Ehr., *Navicula gibbula* Cl., *Achnanthes marginulata* Grun., *Stauroneis anceps* Ehr., *Melosira sp.*, *Navicula hungarica var. capitata* (Ehr.) Cl., and *Rophalodia sp.*

No animal organisms were found in the water column.

A prevalent majority of living organisms cluster in the bacterio-algal layer at the bottom of the lakes. Frequencies of particular species in the population of the water bodies under survey is presented in Table II.

Lake fauna is very deficient; it consists of the following organisms: Testacea (48.4% of the total density of population), Tartigrada (26.3%), Rotatoria (24.0%), and Nematoda (1.3%). In neither of the lakes in the oasis the presence of Arthripoda was recorded.

The average density of fauna in all the lakes amounts to 38.8 individuals per 1 ml, but the frequency and the distribution of various species varies from one lake to another, e.g. the greatest number of Testacea species and individuals occurs in Glubokoye Lake; in the other ones they either occur sporadically or are not present at all. Nematoda, similarly, exist only in three lakes (No. 5, 8, and 9). On the contrary, *Lepadella patella* O. F. Müller (Rotatoria) and *Hypsibius chilensis* Plate (Tartigrada) count among animals occurring regularly and in fairly large numbers. The average density of *L. patella* amounts to 5.3 ind. per 1 ml, and *H. chilensis* — 9.8. Frequency distribution of both those species amounted to 38.9% of the total fauna.

Flora in the lakes of the oasis is also rather poor as concerns quality, but, on the contrary, fairly rich with respect to quantity. The most numerous are Diatomeae and Cyanophyta, their numbers amount to several hundred thousand of cells per 1 ml. The most frequently occurring Diatomeae species are the following: *Navicula seminulum* Grun., *Pinnularia gibba var. parva* (Ehr.) Grun., *Pinnularia sp.*, *Achnanthes marginulata* Grun. Those four species constitute 90% of the total numbers of Diatomeae and 63.3% of the total number of flora specimens. Among Cyanophyta, the most commonly occurring is *Phormidium glaciale form. longiarticulata* (W. and G. S. West) Wille constituting 94.0% of the total number of Cyanophyta and 22.4% of the total numbers of flora. Another species occurring in all the lakes, though in small numbers, is *Oscilatoria irrigua* Kütz.

Chlorophyta, except the fairly regularly occurring *Chlorosphaera antarctica* Fritsch, are found rather rarely and not in all the lakes and the density of their population reaches the value in the order of scores of thousand individuals per 1 ml.

In lakes No. 1, 2, 5, 8, and Glubokoye, small numbers of *Oedogonium sp.* were recorded.

In the investigated lakes of the oasis, the frequency distribution among the various groups of flora is as follows: Diatomeae — 70.2%, Cyanophyta — 28.1%, Chlorophyta — 1.6%, Oedogoniales — 0.1%. In three lakes under survey, No. 8, 9 and 11, with respect to the density of population Cyanophyta are prevalent, in the others — Diatomeae.

On the basis of investigation of quantitative and qualitative composition of flora and fauna, "average differences" were computed (cf. Perkal 1963) (Table III). A dendrite of those differences was build up (cf. Florek et al. 1951) and it is presented in Fig. 3. Taking away of the longest sides of the dendrite, one after another successively, does not

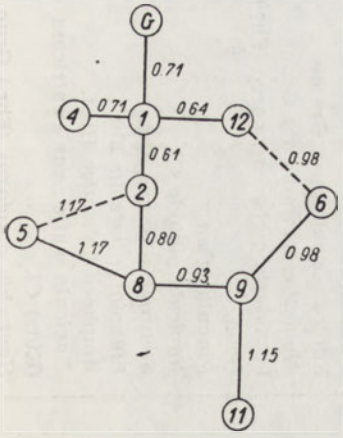


Fig. 3. Dendric classification of the investigated lakes in the Thala Hills oasis based on quantitative and qualitative composition of flora and fauna. Numerals in circles denote the Lake No., G — Glubokoye Lake. Segments of lines and numerals between the circles denote "distances" from one lake to another

Table II. Density of population of flora and fauna in the selected lakes of Thala Hills oasis (No. of ind./ml of bacterio-algal layer)

Species	Lake									
	No. 1	No. 2	No. 4	No. 5	No. 6	No. 8	No. 9	No. 11	No. 12	Glubokoye
Rhizopoda										
Difflugia manicata Penard	—	—	—	+	18	—	+	+	—	50
Centropyxis constricta Ehr.	—	—	—	+	—	+	—	—	—	10
Chryptodifflugia sp.	—	—	—	—	—	—	—	—	—	20
Waiesella sp.	—	—	—	—	—	—	—	—	—	90
Arcella sp.	—	—	—	—	+	—	—	+	—	—
Nematoda										
Nematoda sp.	—	—	—	5	—	+	+	—	—	—
Rotatoria										
Lepadella patella O. F. Müller	3	11	—	19	+	7	3	+	+	10
Macrotrachela sp.	—	+	+	10	+	4	5	+	+	—
Genus undetermined	—	3	—	—	—	7	10	+	—	—
Tartigrada										
Hypsibius (Diphascon) chilensis Plate	6	8	10	5	+	11	5	33	10	10
Hypsibius mertoni	—	—	—	5	+	+	+	+	—	—
Diatomeae										
Navicula seminulum Grun.	338,512	481,734	11,219	375,285	225,522	142,164	13,949	1188	201,372	682,870
Ahnanthes marginulata Grun.	3408	14,598	1726	15,240	75,596	2154	2262	297	6984	44,535
Linnularia gibba var. parva (Ehr.) Grun.	10,224	131,382	3445	228,600	—	49,542	1508	77,517	12,804	2969
Pinnularia sp.	81,792	—	56,095	38,100	66,330	28,002	8671	891	25,608	29,630
Navicula gibbula Cl.	10,224	14,598	6904	3810	—	36,618	—	—	—	2969
Stauroneis anceps Ehr.	6816	7299	2589	+	—	2154	—	—	—	53,442
Pinnularia borealis Ehr.	+	—	+	—	26,532	—	377	297	—	26,721
Amphora holstatica Hust.	—	51,093	863	—	—	12,924	—	297	—	—
Navicula mutica var. ventricosa (Kütz) Cl.	—	—	4315	1905	+	—	377	—	—	—
Hantzchia ampioxys (Ehr.) Grun.	3408	—	—	—	30,954	—	—	—	—	23,752

Coconeis costata Greg.	—	—	—	1905	—	—	—	—	1164	—
Cosinodiscus furcatus Karsten	—	—	—	1905	—	—	—	594	—	—
Triceraticum sp.	—	—	—	+	—	—	—	—	—	—
Epithemia sorex Kütz.	—	—	+	—	—	—	—	—	—	—
Genus undetermined	—	—	—	—	—	—	377	—	—	—
Cyanophyta										
Phormidium glaciale f. longiarticula (W. and G. S. West) Wille	19,863	58,294	65,660	286,938	46,636	271,274	321,944	352,840	25,160	very numerous
Oscilatoria irrigua Kütz.	186	305	—	4586	+	+	+	+	+	260
Synechococcus maior Schröter	—	—	—	4200	14	33	22	—	+	750
Gleocepsa magna (Brebisson) Hollerbach*	—	+	—	19,486	+	25,216	27,510	+	—	150
Schizotrix antarctica F. E. Fritsch	—	+	—	+	+	+	—	113	514	+
Nostoc sphaericum Vauch.	—	—	—	45,850	—	9834	+	+	+	—
Oscilatoria simplicissima Gom.	—	—	—	+	46	—	—	—	—	—
Synechococcus sp.	—	—	—	+	+	—	2	—	—	—
Calotrix sp.	—	—	—	+	—	—	+	—	—	+
Genus undetermined	—	—	—	—	—	4586	4586	—	—	—
Chlorophyta										
Chlorosphaera antarctica Fritsch	60	44	19	1000	236	5922	+	+	40	+
Cosmarium subtumidum Norst.	3	3	—	+	6	+	—	70,568	3068	+
Cosmarium cucurbita G. S. West*	—	—	—	4202	614	—	—	1842	—	—
Cosmarium regnelli Wille*	—	—	—	—	—	—	—	1228	—	—
Binuclearia tatrana Wittr.	—	—	—	+	—	—	—	—	—	—
Oedogoniaes										
Oedogonium sp.	13	1226	—	6104	6104	2	—	—	—	70

* — uncertain determination of species, — — not found, + — less than 1 ind./ml.

Table III. "Average differences" between freshwater lakes of Thala Hills oasis with respect to qualitative and quantitative composition of their flora and fauna

Lake	No. 2	No. 4	No. 5	No. 6	No. 8	No. 9	No. 11	No. 12	Glubokoye
No. 1	0.61	0.64	1.29	1.00	0.93	1.20	1.29	0.71	0.71
No. 2		0.76	1.17	1.41	0.80	1.41	1.29	0.98	1.17
No. 4			1.59	1.20	0.93	0.98	1.22	0.80	1.20
No. 5				1.49	1.17	1.39	1.66	1.34	1.56
No. 6					1.44	0.98	1.24	0.98	1.05
No. 8						0.93	1.41	1.05	1.29
No. 9							1.15	0.98	1.37
No. 11								0.88	1.71
No. 12									1.34

cause its disintegration into smaller parts, but only just the falling apart of single points of the dendrite. This facts evidences the homogenous character of the studied lakes in the oasis with respect to the quantitative and qualitative accumulation of flora and fauna. The greatest "average differences" are revealed by lakes No. 5 and 11.

4. DISCUSSION

Animals occurring in the water bodies of the Thala Hills oasis are for the greater part cosmopolites common to other oases in Antarctica and found in other parts of the world. Genera and species of Protozoa listed in Table II were also recorded at Lang Hovde (69°12′S, 39°44′E), Prince Olaf Coast and McMurdo (77°32′S, 166°12′E), Ross Island (Sudzuki 1964, Sudzuki, Shimoizumi 1967). Rotatoria, found in the lakes under survey, are widespread all over the area of Antarctica, they were noticed at Vestfold Hills (68°35′S, 78°10′E), Ingrid Christensen Coast, Bunger Hills (66°10′S, 101°00′E), Queen Mary Land (Kutikova 1958), Obrutshev Hills (66°33′S, 99°51′E), Queen Mary Land (Korotkevich 1958) and Lang Hovde (Sudzuki 1964). Lepadella and Macrotrachela are considered to be typical of the Antarctic microfauna (Sudzuki, Shimoizumi 1967). Tartigrada, viz. *Hypsibius chilensis* and *H. mertoni* were recorded at Lang Hovde (Sudzuki 1964). Nematoda occurring in the lakes of the oasis were not determined. Information on the subject of Nematoda can be found in Kirjanova (1958).

All animal organisms found in the Thala Hills oasis lakes were described from observations of moss community in other Antarctic oases (cf. Sudzuki, Shimoizumi 1967).

Diatomeae existing in the lakes of the oasis, are likewise cosmopolitan and ubiquitous forms, they are pioneering organisms in the settling process in new youthful water bodies. The greater part of the Diatomeae species enumerated in Table II is well known from the Northern Hemis-

phere and Europe (Hirano 1965, Siemińska 1964) and a whole range of species, as for instance, *Stauroneis anceps* Ehr., *Pinnularia borealis* Ehr., *Hantzschia amphioxys* (Ehr.) Grun., and *Navicula seminulum* Grun. are present in the Spitsbergen (Foged 1964). The same species that are found in the Thala Hills oasis are also present in the other Antarctic oases, e.g. Schirmacher Ponds (70°45'S, 11°40'E), Princess Astrid Land (Lavrenko 1966, Aleshinskaya, Bardin 1965), Lang Hovde (Hirano 1959), and Bunger Hills (Korotkevich 1958). The endemic forms of Diatomeae were not found.

From among nine species of Cyanophyta found in Thala Hills oasis two — *Phormidium glaciale form longiarticulata* (W. and G. S. West) Wille and *Schizotrix antarctica* F. E. Fritsch — are endemic to Antarctica (cf. Wille 1924, Hirano 1965). All the other Cyanophyta found there are cosmopolitan organisms. The phenomenon of formation of the bacterio-algal layer at the bottom of the Antarctic lakes by the various species of Phormidium and of bacteria was described by Heywood (1967). Liano (1959) and Goldman (1970) consider Phormidium as being the most extensively widespread Antarctic Cyanophyta genus.

From among Chlorophyta, only *Chlorosphaera antarctica* Fritsch is endemic to Antarctica (Hirano 1959). All the Chlorophyta, mentioned in Table II, had been already recorded in some other Antarctic oases (cf. Hirano 1959, 1965, Lavrenko 1966). Oedogoniales in the Antarctic region were found only in the South Orkney Islands (60°40'S, 45°30'W) by Hirano (1965).

The endemic species (*Phormidium glaciale form longiarticulata, Schizotrix antarctica*, and *Chlorosphaera antarctica*) make up 9.7% of various plant species in the Thala Hills oasis and 26.3% of the density of flora population (out of this *P. glaciale form longiarticulata* — 22.4%). In comparison with other oases in Antarctica, the quantity of endemic organisms in the Thala Hills oasis is very small (cf. Bardin et al. 1969).

According to Fukushima (1970), the endemic species in the diatomaceous flora of Enderby Land oases constitute from 9 to 20% of the total number of species. In those oases, according with Fukushima (1970), cosmopolites are preponderant. This author believes that the boundary line, between zones of preponderance of the cosmopolitan and the endemic forms, runs within the 68°S and 77°S.

Despite the differences between the lakes under investigation, with respect to their sizes, the thermic and ice conditions, etc., the composition of their flora and fauna is fairly homogenous (Fig. 3). It is a phenomenon quite easy to understand, if one takes into consideration the eurytopical character of the bulk part of the occurring species, the restricted inflow of new species (geographic isolation) and a small number of the specific forms — endemites.

Uniformation of the flora and fauna of water bodies in the oasis is also influenced by the facility of penetration of the whole area of the oasis by the living organisms or their sporophyte forms, etc., due to such agents as wind (cf. Rudolph 1970), seasonal temporary confluence of some water bodies, as well as the presence of moss turf on the lakes shores with settled groups of organisms of a similar composition of species to those abiding in the waters (cf. Sudzuki 1964).

The qualitative sparsity of organisms living in the lakes of oasis comes as result of a series of causes. One of them is a very limited inflow of biogenetic elements from the drainage area. The wash out of mineral salts from the bedrock is impeded by the permanently frozen ground (thawing in summer reaches down to 30—100 cm deep — Simonov 1968) and by the blowing away of the degraded rock waste. Many authors are of the opinion that life in the Antarctic lakes is possible only owing to the inflow of allochtonic substances such as bird faeces. Coming slowly down the slope with the melting snow, bird excrements supply lakes with biogenetic substances, above all with phosphoric and nitro-compounds (cf. Dalenius 1965, Holdgate 1967, Longton 1967, Matsuda 1968, Bardin et al. 1969). Lakes situated in proximity of bird colonies are well provided with nitro-compounds and there might even develop some nitrofile forms, e.g. Prasiola and Oscilatoriace (Bardin et al. 1969). In the Thala Hills oasis bird colonies do not exist but the lakes are enriched by birds appearing in the oasis sporadically. The nearest bird colonies are on the isles McMahon (67°39′S, 45°59′E) and Myall (67°40′S, 45°45′E), about 5 km away from the oasis.

The second cause of the scarcity of living organisms in the water bodies of Antarctica is the high instability of environmental conditions prevailing in the lakes. In summer time, water temperature changes are of the 17°C order in the course of 48 hours (MacNamara 1969), in winter small lakes freeze all over down to the bottom. In a whole year cycle, there are considerable changes in the concentration of substances dissolved in water in result of the freezing process, since ice may constitute over 50% of lake capacity (cf. MacNamara 1969). In summer time they unfreeze and are ice-free for a period of about two months, in winter the accumulated snow on the surface of ice brings in effect a considerable increase of pressure inside the water bodies (jets of water piercing the ice and spurting forth). In the period of polar nights, the sunlight does not reach the water in the lakes.

The third cause, an important one, of the scantiness of life in the lakes of Antarctica is the fact that Antarctic oases and water bodies, too, are geologically very youthful. Geological age of oases is estimated to be but several thousand years (cf. Mellor 1959), e.g. Schirmacher Ponds — 6000 years (Simonov 1967), Bunger Hills — 4500 years (Różycki 1960), Hut Point (77°51′S, 166°37′E), Roos Island — 500 years (Llano

1959). It is estimated that the coasts of Antarctica became accessible to the settling in of living organisms only about 10 thousand years ago (Holdgate 1967). Nevertheless, even at the time when it becames possible to settle in, the process itself was strongly limited by the geographic isolation of Antarctica.

Thus, geographic isolation is another significant cause of the deficiency of living organisms in Antarctic oases. All the oases are set apart from one another by vast areas of ice desert and separated from the nearest land by boundless spaces of the sea. Air currents (cf. Dubentsov 1969) and sea currents (cf. Knox 1970) counterbalance the isolation of Antarctica. Wace (1965), on the basis of his observations and experiments, rules out the possibility of a transfer of plants and animals or their sporophyte forms etc. into Antarctica by sea currents, winds, or in alimentary canals of birds. The greater part of the authors, however, is of a contrary opinion. Gressitt (1965) and Tilbrook (1967) describe the transferring of insects into Antarctica by air currents, Matsuda (1968) — the transfer of plants, Sieburth (1965) — the transferring of bacteria.

The phenomenon of the transmission of living organisms by the winds in the Northern Hemisphere has been a well known fact, since a long time (cf. Gislen 1948, Polunin 1958). It seems that this phenomenon plays a much smaller role in the Arctic Zone than in Antarctica, as a consequence of the distance separating Antarctica from the nearest land.

Another important agent, besides the winds, facilitating the settling in of living organisms in Antarctic oases, are birds. Matsuda (1968) describes the presence of live Cyanophyta in faeces of a McCormick's skua (*Catharacta maccormicki* (Saund)). This confirms Bardin's thesis (Bardin et al. 1969) that birds eating algae without digesting them transfer them alive to other territories. Likewise, living organisms may be carried along on feathers and legs of birds, this is probable, so much more, in view of the fact that, e.g. on the sub-antarctic islands the beginning of the migration period coincides in time with the season of seed, fruit, egg, sporophyte forms, etc. production by the local flora and fauna (Falla 1960). Taylor (1954) basing on the information data, concludes that birds can be the main agent for spreading living organisms in the Southern Hemisphere and thereby in Antarctica, as well; his arguments are as follows: 1. a great number of sea birds in the Southern Hemisphere, 2. the capacity of those birds for covering long distances in a relatively short time (strong winds, West Wind Drifts), 3. fruits, seed, etc. provided with winged or sticky structures, 4. lubrication of the organisms carried on the feathers with the rump gland secretion to give protection against the effect of sea water.

It stands to reason that only very small numbers from among the

organisms brought into Antarctica through air currents and birds happen to find their way into oases, as the total oases area amounts up to hardly 0.06% of the total area of Antarctica, and much less of them, still, would prove to be able to vegetate in the ecological conditions prevailing in Antarctic oases.

Acknowledgements

I wish to thank sincerely Mrs. H. Bożdyńska, M. Sc., for help in determination of Diatomeae.

5. SUMMARY

This paper deals with the composition of flora and fauna in several freshwater lakes of the Antarctic oasis Thala Hills (67°40′S, 45°50′E), Enderby Land, Eastern Antarctica. For research, water bodies were selected with their natural environment not yet disturbed by man (pollution, changes in water intake, etc.).

In the Antarctic freshwater lakes, the greater part of living organisms concentrates in the bottom layer where the so-called bacterio-algal substratum is formed. The main elements of that layer consists of various species of Phormidium genus (Cyanophyta), as well as bacteria and Diatomeae. Fauna is also connected with that layer. Plankton in the Antarctic lakes is very poor, it consists mainly in Diatomeae.

In the investigated lakes of the Thala Hills oasis there has been stated the presence of five species of Protozoa, three species of Rotatoria, two species of Tartigrada, and Nematoda (species not determined). Flora of the lakes is represented by fifteen Diatomeae species, nine Cyanophyta species, five Chlorophyta species and Oedogoniales. The most frequently occurring representants of fauna are: *Lepadella patella* O. F. Müller and *Hypsibius chilensis* Plate. The prevalent representants of flora are: Diatomeae (*Navicula seminulum* Grun., *Pinnularia gibba var. parva* (Ehr.) Grun.) and Cyanophyta (*Phormidium glaciale forma longiarticulata* (W. and G. S. West) Wille).

Cosmopolitan forms are prevalent in the fauna and flora of the oasis. Occurrence of endemic forms was stated only among Chlorophyta (*Chlorosphaera antarctica* Fritsch) and Cyanophyta (*P. glaciale forma longiarticulata, Schizotrix antarctica* F. E. Fritsch).

On the whole, endemites constitute 9.7% of species and 26.3% of density of total flora population in the lakes of the oasis. The number of endemic species in other oases of the Enderby Land is of a similar order.

Despite some differences in the density of population in each particular lake of the oasis (Table II), nevertheless, analysis carried out by means of dendrite classification method indicates homogeneity of the lakes under survey with respect to quantitative and qualitative composition of flora and fauna. This homogeneity may come as result of the influence of a range of factors, as, for instance, temporary joining together of waters from the lakes, giving in consequence the intermingling of flora and fauna of the water bodies.

Mechanism of the settlement of the species living at present in Antarctic oases is not definitely explained yet. In all probability, the greatest role in the process of settling is played by the transferring of living organisms (or their sporophyte forms) into Antarctic oases by birds or through air currents.

6. STRESZCZENIE

W pracy przedstawiono skład flory i fauny kilku zbiorników słodkowodnych antarktycznej oazy Thala Hills (67°40′S, 45°50′E), Enderby Land, Wschodnia Antarktyda. Do badań wybrano zbiorniki, których środowisko naturalne nie zostało jeszcze zakłócone przez człowieka (zanieczyszczenia, ujęcia wody, itp.).

W słodkowodnych zbiornikach antarktycznych większość organizmów żywych koncentruje się w warstwie przydennej, gdzie tworzy się tzw. warstwa bakteryjno-glonowa. Głównymi elementami tej warstwy są różne gatunki rodzaju Phormidium (Cyanophyta) oraz bakterie i Diatomeae. Z warstwą tą związana jest również fauna. Plankton jezior antarktycznych jest bardzo ubogi, składa się głównie z Diatomeae.

W badanych zbiornikach oazy Thala Hills stwierdzono występowanie pięciu gatunków Protozoa, trzech gatunków Rotatoria, dwu gatunków Tartigrada i Nematoda (gatunków nie oznaczano). Flora zbiorników reprezentowana jest przez 15 gatunków Diatomeae, 9 — Cyanophyta, 5 — Chlorophyta i Oedogoniales. Najczęściej spotykanymi przedstawicielami fauny są *Lepadella patella* O. F. Müller i *Hypsibius* (*Diaphascon*) *chilensis* Plate, we florze dominują Diatomeae (*Navicula seminulum* Grun., *Pinnularia gibba var. parva* (Ehr.) Grun.) i Cyanophyta (*Phormidium glaciale forma longiarticulata* (W. et G. S. West) Wille).

We florze i faunie oazy dominują formy kosmopolityczne. Występowanie form endemicznych stwierdzono tylko wśród Chlorophyta (*Chlorosphaera antarctica* Fritsch) i Cyanophyta (*P. glaciale forma longiarticulata, Schizotrix antarctica* F. E. Fritsch).

Ogółem endemity stanowią 9,7% gatunków i 26,3% liczebności flory zbiorników wodnych oazy Thala Hills. Na podobnym poziomie kształtuje się ilość gatunków endemicznych w innych oazach Enderby Land.

Pomimo pewnych różnic w zasiedleniu poszczególnych zbiorników oazy (Tab. II) analiza przeprowadzona metodą uporządkowania dendrytowego wykazuje jednorodność wszystkich badanych zbiorników pod względem ilościowego i jakościowego składu flory i fauny. Jednorodność ta może być wynikiem działania szeregu czynników, jak na przykład okresowe łączenie się zbiorników, co prowadzi do przemieszczania się flory i fauny.

Mechanizmy zasiedlania oaz antarktycznych przez zamieszkujące je obecnie gatunki nie są jeszcze ostatecznie wyjaśnione. Prawdopodobnie w procesie zasiedlania oaz największą rolę odgrywa przenoszenie organizmów żywych (lub ich form przetrwalnikowych) przez ptaki i prądy powietrzne.

7. REFERENCES

[Akatova, N. A.] Акатова, Н. А. 1964. Нахождение *Daphniopsis studeri* Rühe (Cladocera) в озере „оазиса" Вестфоль (Восточная Антарктида) [The occurence of *Daphniopsis studeri* Rühe (Cladocera) in lake of the Vestfold Oasis (East Antarctica)]. *Issledovanija Fauny Morej*, 2 (10), 185–188.

[Aleshinskaya, Z. V., Bardin, V. I.] Алешинская, З. В., Бардин, И. В. 1965. Диатомовая флора оазиса Ширмахера [Diatom flora in the Shirmacher Oasis]. *Inf. Byull. sov. antarkt. Eksped.*, 54, 47–49.

Armitage, K. B., House, H. B. 1962. A limnological reconnaissance in the area of McMurdo Sound, Antarctica. *Limnol. Oceanogr.*, 7, 36–41.

[Bardin, V. I.] Бардин, В. И. 1968. Перигляциял [The pariglacial]. In: *Geografija Antarktidy*. 306–375, Moskva, Izdat. „Mysl'".

[Bardin, V. I., Gerasimenko, L. M., Pusheva, M. A.] Бардин, В. И., Герасименко, Л. М., Пушева, М. А. 1969. Некоторые данные о распространении водорослей в западной части Восточной Антарктиды [Some data on algae distribution in western part of East Antarctica]. *Inf. Byull sov. antarkt. Eksped.*, 73, 41–49.

Dalenius, P. 1965. The acarology of the Antarctic regions. In: J. van Mieghem, P. van Oye [Eds] *Biogeography and ecology in Antarctica*. 414–430, The Hague, W. Junk (*Monographiae Biologicae*, 15).

Dodge, C. W. 1965. Lichens. In: J. van Mieghem, P. van Oye [Eds] *Biogeography and ecology in Antarctica*. 194–200, The Hague, W. Junk (*Monographiae Biologicae*, 15).

[Dubentsov, V. R.] Дубенцов, В. Р. 1969. Аэроклиматическая характеристика южного полушаря [Aeroclimatic characteristics of the southern hemisphere]. In: E. J. Tolstikov [Red.] *Atlas Antarktiki*, II, 342–346, Leningrad, Gidrometeorologičeskoe Izdat.

Falla, R. A. 1960. Oceanic birds as dispersal agents. *Proc. R. Soc. Lond., Sect. B*, 152. 655–659.

Florek, K., Łukaszewicz, J., Perkal, J., Steinhaus, H., Zubrzycki, S. 1951. Taksonomia wrocławska [On Wrocław taxonomy]. *Przegl. antrop.*, 17, 191–211 [Engl. summ.].

Foged, N. 1964. *Freshwater diatoms from Spitzbergen.* Tromso-Oslo, Universitetsforlaget.

Fukushima, H. 1961. Algae vegetation in the Ongul Islands, Antarctica. *Antarctic Rec.*, 11, 149–151.

Fukushima, H. 1970. Notes on the diatom flora of Antarctic inland waters. In: M. W. Holdgate [Ed] *Antarctic ecology.* I, 232–238, London, Academic Press.

Gislen, T. 1948. Aerial plankton and its conditions of life. *Biol. Rev.*, 23, 109–126.

Goldman, C. R. 1970. Antarctic freshwater ecosystems. In: M. W. Holdgate [Ed] *Antarctic ecology,* II, 609–627, London, Academic Press.

[Golubkova, N. S.] Голубкова, Н. С. 1969. Лишайники [Lichens]. In: E. J. Tolstnikov [Red.] *Atlas Antarktiki,* II, 388–492, Leningrad, Gidrometeorologičeskoe Izdat.

Gressitt, J. L. 1965. Biogeography and ecology of land arthropods of Antarctica. In: J. van Mieghem, P. van Oye [Eds] *Biogeography and ecology in Antarctica.* 431–490. The Hague, W. Junk (*Monographiae Biologicae,* 15).

Heywood, R. B. 1967. Antarctic ecosystems. The freshwater lakes of Signy Island and their fauna. *Phil. Trans. R. Soc. Lond., Ser. B,* 252, 347–362.

Heywood, R. B. 1970. The mouthpart and feeding habits of *Parabrotes sarsi* (Daday) and *Pseudoboeckella silvestri* Daday (Copepoda, Calanoida) In: M. W. Holdgate [Ed.] *Antarctic ecology.* I, 239–650, London, Academic Press.

Hirano, M. 1959. Notes on some algae from Antarctic collected by the Japanese Antarctic Research Expedition. *Spec. Publ. Seto mar. biol. Lab.*, 3, 1–13.

Hirano, M. 1965. Freshwater algae in the antarctic regions. In: J. van Mieghem, P. van Oye [Eds] *Biogeography and ecology in Antarctica.* 127–193, The Hague, W. Junk (*Monographiae Biologicae,* 15).

Holdgate, M. W. 1967. The antarctic ecosystems. *Phil. Trans. R. Soc. Lond., Ser. B,* 252, 363–383.

[Kirjanova, E. S.] Кирьянова, Е. С. 1958. Антарктические представители пресноводных нематод рода Plectus Bastian (Nematodes, Plectidae) [Antarctic freshwater nematodes of the genus Plectus Bastian (Nematodes, Plactidae)]. *Inf. Byull. sov. antarkt. Eksped.*, 3, 101–103.

[Klokov, V. D.] Клоков, В. Д. 1970 Прорыв вод из озера Глубокого на станции Молодежной [Water flush from the Lake Glubokoye at Molodezhnaya Station]. *Inf. Byull. sov. antarkt. Eksped.*, 77, 96–99.

Knox, G. A. 1970. Antarctic marine ecosystems. In: M. W. Holdgate [Ed.] *Antarctic ecology.* I, 68–79, London, Academic Press.

[Korotkevich, V. S.] Коротевич, В. С. 1958. Население водоемов оазисов в Восточной Антарктиде [Animal population of "Oasis" lakes in the East Antarctic]. *Inf. Byull. sov. antarkt. Eksped.*, 3, 91–98.

[Korotkevich, Ye. S.] Короткевич, Е. С. 1969. Оазисы [Oases]. In: E. J. Tolstikov [Red.] *Atlas Antarktiki,* II, 563–574, Leningrad, Gidrometeorologičeskoe Izdat.

[Kutikova, L. A.] Кутикова, Л. А. 1958. К фауне коловраток с побережья Восточной Антарктиды [Contributions to the rotifer fauna from the shores of the East Antractic]. *Inf. Byull. sov. antarkt. Eksped.*, 3, 99.

[Lavrenko, G. Ye.] Лавренко, Г. Е. 1966.О водорослях одного из озер в районе станции Новолазаревской [On the algae from the lakes in the Novolazarevskaya Station area]. *Inf. Byull. sov. antarkt. Eksped.*, 56, 57–61.

Llano, G. A. 1959. Antarctic plant life. *Trans. Am. geophys. Un.* 40, 200–203.

Longton, R. E. 1967. Plant and invertebrate ecology. Vegetation in the maritime Antarctic. *Phil. Trans. R. Soc. Lond., Ser. B,* 252, 213–235.

Matsuda, T. 1968. Ecological study of moss community and microorganisms in the vicinity of Syowa Station, Antarctica. *JARE Sci. Rep., Ser. E,* 19, 1–58.

[MacNamara, E. E.] Макнамара, Э. Э. 1969. Некоторые лимнологические данные и наблюдения на Земле Эндерби [Some limnologic data and observations from Enderby Land Antarctica]. *Inf. Byull. sov. antarkt. Eksped.*, 72, 25–33.

Mellor, M. 1959. Variations of the ice margins in East Antarctica. *Geogr. J.*, 125, 230–235.

Perkal, J. 1963. *Matematyka dla przyrodników i rolników* [Mathematics for scientists and agronomists.]. Warszawa, PWN.

Polunin, N. 1958. The botany of ice-island T-3. *J. Ecol.*, 46, 323–347.

[Różycki, S. Z.] Ружицкий, С. З. 1960. О времени образования оазиса Бангер-Хилс [On the dating of the Bunger Hills oasis]. *Inf. Byull. sov. antarkt. Eksped.*, 20, 10–14.

Rudolph, E. D. 1970. Local dissemination of plant propagules in Antarctica. In: M. W. Holdgate [Ed.] *Antarctic ecology*. II, 812–817, London, Academic Press.

[Savich-Lyubitskaya, L. I., Smirnova, Z. N.] Савич-Любицкая, Л. И., Смирнова, З. Н. 1964. Глубоководный представитель рода Plagiothecium Br. et Sch. в Антарктиде [Deep-water representative of Plagiothecium Br. et Sch. in the Antarctic]. *Inf. Byull. sov. antarkt. Eksped.*, 49, 33–39.

[Savich-Lyubitskaya, L. I., Smirnova, Z. N.] Савич-Любицкая, Л И., Смирнова, З. Н. 1969. Мхи Восточной Антарктиды [Bryoflora of East Antarctica]. In: E. I. Tolstikov [Red.] *Atlas Antarktiki*. II, 484–492, Leningrad, Gidrometeorologičeskoe Izdat.

Sieburth, J. McN. 1965. Microbiology of Antarctica. In: J. van Mieghem, P. van Oye [Eds] *Biogeography and ecology of Antarctica*. 267–295, The Hague, W. Junk (*Monographiae Biologicae*, 15).

Siemińska, J. 1964. Chrysophyta II, Bacillariophyceae. *Flora słodkowodna Polski*, 6, Warszawa, PWN.

[Simonov, I. M.] Симонов, И. М. 1967. Эоловое вывертивание в оазисах Восточной Антарктиды [Eolation in East Antarctica oases]. *Inf. Byull. sov. antarkt. Eksped.*, 62, 37–43.

[Simonov, I. M.] Симонов, И. М. 1968. Физико-географическая характеристика оазиса Молодёжного [Physico-geographical characteristic of Molodezhnaya oasis]. *Trudy sov. antarkt. Eksped.*, 38, 6–11.

[Simonov, I. M.] Симонов, И. М. 1970. Озера антарктических оазисов [Lakes of the Antarcitc oases]. *Trudy arkt. antarkt. naučno-issled. Inst.*, 294, 142–171.

Sudzuki, M. 1964. On the fresh water microfauna of the Antarctic region. I. Moss-water community at Lang Hovde. *JARE sci. Rep., Ser. E*, 19, 1–14.

Sudzuki, M., Shimoizumi, J. 1967. On the fresh water microfauna of the Antarctic region. II. Stability of faunistic composition of antarctic microorganisms. *JARE sci. Rep., special issue*, 1.

Taylor, B. W. 1954. An example of long distance dispersal. *Ecology*, 35, 569–572.

Tilbrook, P. J. 1967. The terrestrial invertebrate fauna of the maritime Antarctic. *Phil. Trans. R. Soc. Lond., Ser. B*, 252, 261–278.

Wace, N. M. 1965. Vascular plants. In: J. van Mieghem, P. van Oye [Eds] *Biogeography and ecology in Antarctica*. 201–266, The Hague, W. Junk (*Monographiae Biologicae*, 15).

Wille, N. 1924. Süsswasseralgen von der Deutschen Südpolar-Expedition auf dem Schiff „Gauss". *Dtsch. Südpolar-Exped.*, 7, Bot.

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	399–401	1972

S. RAKUSA-SUSZCZEWSKI

RESPIRATION OF THE ANTARCTIC FISH EGGS (*TREMATOMUS BORCHGREVINKI* BOUL.)

Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

Fish of the Trematomus genus live at the coast of the Antarctic at temperatures of water invariably below 0°C with fluctuations never exceeding 2°C. They are extremely stenothermic (Wohlschlag 1960), and showing a decrease of motion activity at the rise of temperature from —1.8 to +1.8°C, which was confirmed experimentally (Wohlschlag 1962). At the rise of temperature from +2.0 to 3.0°C the metabolic processes are slowing down (Wohlschlag 1963). The lethal temperature for species of Trematomus genus is about +6.0°C (Somero, DeVries 1967). The effect of temperature changes on metabolism of the brain and gill tissues has been also investigated (Somero, DeVries 1967). In order to find out how the unterfilized eggs of fish of the species *Trematomus borchgrevinki* are reacting under a rise of temperature, several experimental tests have been carried out and their results are presented in this notice.

Investigations were performed at the Soviet Antarctic station Molodezhnaya, in February 1972. Eggs were taken out from the ovary of live fish catched under ice at the shores of an oasis. At that time the diameter of an egg was up to 1.5 mm. The average wet mass of eggs that were used for four experiments weighed about 840 mg. Eggs were placed first into plastic-net containers and then they were put into Erlenmayer flasks of about 200 ml capacity filled with sea water of about 29‰ salinity. The Erlenmayer flasks were immersed into a thermostat with temperature of —1.0°C. After a period of 6 hr the same eggs were transferred to temperature of +6.0°C and later on into thermostats with temperatures of + 11.0°C. The amount of oxygen used by eggs has been determined by marking its contents in the Erlenmayer flasks by the Winkler's method. Results of measurements are shown in Fig. 1. One can see but incon-

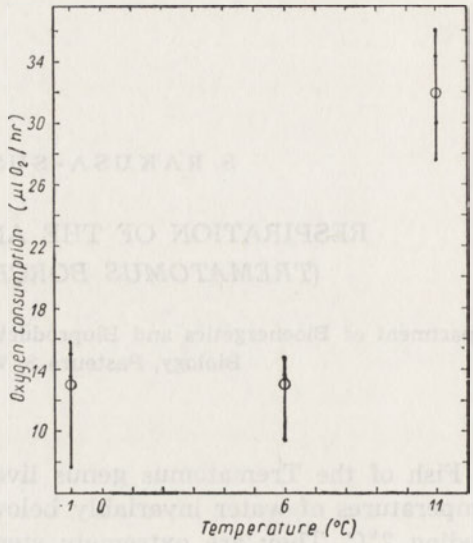


Fig. 1. Respiration of *T. borchgrevinki* eggs in various temperatures. o — mean values

siderable differences of oxygen consumption at temperatures —1.0 and +6.0°C and an increase in oxygen consumption at the range of +6.0 and +11.0°C. At the range of the temperature of survival of mature fish, eggs do not react at its rise, above those temperatures metabolism increases simultaneously with the temperature rise. According to Somero, DeVries (1967) the gill tissues of *T. bernaschii* also do not show contrary to its brain any sensitivity to temperature changes. Examination in vitro of the brain of *T. bernacchii* has shown an increase of oxygen consumption in the range of temperatures from about —3.0°C to the temperature determined as lethal or about +6.0°C. In that experiment however fishes were not acclimated. While investigating the metabolism of tissues of *Salmo gairdneri*, at temperatures +8.0 and +16.0°C, Evans et al. (1962) have obtained contrary results. The brain did not show any essential increase of metabolism after acclimation of fishes during 43 days at temperatures mentioned above, metabolism of liver was more active at +8.0 than at +16.0°C and metabolism of gill tissues increased with the rise of temperature.

The differing results of temperature tolerance observed in some Antarctic fishes, in general carried out in acute experiments, may come in consequence of the lack of sufficiently long period of acclimation and the substantial significance of that fact has been recently pointed out by Ivleva (1972).

REFERENCES

Evans, R. M., Purie, F. C., Hickman, C. P. 1962. The effect of temperature and photoperiod on the respiratory metabolism of rainbow trout (*Salmo gairdneri*). *Can. J. Zool.*, 40, 107–118.

[Ivleva, I. V.] Ивлева, И. В. 1972. Влияние температуры па скорость метаболизма пойкилотермных животных [Influence of temperature on metabolism rate in poikilothermic animals]. *Usp. sovrem. Biol.*, 73, 134–155.

Somero, G. N., DeVries, A. L. 1967. Temperature tolerance of some antarctic fishes. *Science*, 156, 257–258.

Wohlschlag, D. E. 1960. Metabolism of an antarctic fish and the phenomenon of cold adaptation. *Ecology*, 41, 287–292.

Wohlschlag, D. E. 1962. Metabolic requirements for the swimming activity of three antarctic fishes. *Science*, 137, 1050–1051.

Wohlschlag, D. E. 1963. An antarctic fish with unusually low metabolism. *Ecology*, 44, 557–564.

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	403–419	1972

A. WITKOWSKI

CHARACTERISTIC OF *COTTUS GOBIO* L. FROM STREAMS DZIKA ORLICA AND KAMIENNY POTOK IN LOWER SILESIA

Zoological Museum of Wrocław University, 21 Sienkiewicza, Wrocław, Poland

ABSTRACT

Morphological and taxonomic analyses were made of two populations of *Cottus gobio* L. from Dzika Orlica (catchment area of River Elba), and from Kamienny Potok (catchment area of Oder). Significant differences were found between the populations examined in the respect of their meristic and plastic features. It was found that *Cottus gobio* feeds mainly on larvae of aquatic insects.

1. INTRODUCTION

The object of this paper was bullhead, *Cottus gobio* Linnaeus 1758 (synonims: *Cottus microstomus* Heckel, *Cottus ferrugineus* Heckel and Kner, *Cottus gobio* Kessler). According to Koli (1969), *Cottus petiti* Bácescu and Bácescu-Mester corresponds to *Cottus gobio* L., *Cottus gobio var. microcephalus* Kessler corresponds to *Cottus poecilopus* Heckel, and *Cottus gobio var. roseus* Odenwall corresponds to *Myoxocephalus quadricornis* L.

The literature on morphology and biology of *Cottus gobio* L. in Poland is restricted to one paper by Starmach (1965) including a description of this species from the Raba River.

In Poland, *Cottus gobio* occurs mainly in montane streams and rivers. It is rarely found in lowlands (Kaj 1954, Jaśkowski 1962, Penczak 1968 a, b).

In the northern region of its occurrence, this species is often found in lowland rivers and lakes (Nybelin 1969), and also in marine bays (Koli 1969).

Vladykov (1931), Berg (1949), Müller (1960), Starmach (1965), and Koli (1969) reported on the distribution of this species. It occurs in rivers and streams throughout almost entire Europe (except for South Spain, Italy, Greece and also Denmark, Scotland, Norway, and Ireland). According to the authors mentioned, the eastern boundary of occurrence of this species runs through Ural. However Chaban, Bogdanov (1960), Fyodorov (1962), and Gundrizer (1966) found *Cottus gobio* also in the Irtysh River and the Katunia River.

The typical form of *Cottus gobio* L. occurs in Europe and numerous sub-species evolved on peripheries of its region. So *Cottus gobio koshewnikowi* Gratzianow occurs in the Kama and Oka rivers, in Finland, Sweden and in Ural, and *Cottus gobio milvensis* Soldatov — in the River Pechora. *Cottus gobio jaxartensis* Berg occurs in the catchment area of Syr Darya, *Cottus gobio natio* Pellegrini Vladykov in the River Cisa, and *Cottus gobio hispaniolensis* Bácescu and Bácescu-Mester — in the upper course of Garonne River.

The aim of this paper was to describe morphological and biological characteristics as well as to define taxonomic position of two populations of *Cottus gobio*

L. from streams situated in two different catchment areas: the Elba and Oder rivers.

2. DESCRIPTION OF THE STUDY AREA

The material was gathered from two streams: Dzika Orlica (catchment area of Elba) and Kamienny Potok (catchment area of Oder) (Fig. 1).

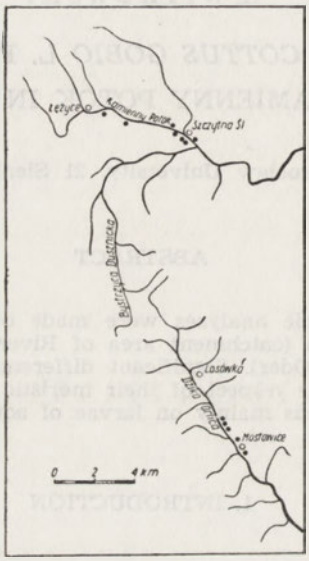


Fig. 1. The map of investigated streams. Black circles — stations of sampling

Dzika Orlica starts at the altitude of 760 m a.s.l. in the Bystrzyckie mountains. It is left bank affluent of the Elba River. Lohnisky (1963) reported that the upper course of the stream is inhabited by *Salmo trutta m. fario* L., *Cottus gobio* L., *Thymallus thymallus* (L.), and also by *Lampetra planeri* (Bloch) of Cyclostomi. When gathering material for this work, *Phoxinus phoxinus* (L.) and *Anguilla anguilla* (L.) were also encountered.

Kamienny Potok runs through the catchment area of Dusznicka Bystrzyca, a left bank affluent of Nysa Kłodzka (river basin of Oder). It starts at the altitude of 650 m a.s.l. on the slopes of hills called Lewińskie Wzgórza.

When gathering the material it was found that apart from *Cottus gobio* L. other species, namely, *Salmo trutta m. fario* L., *Nemacheilus barbatulus* (L.) and *Lampetra planeri* (Bloch) of Cyclostomi also occur in this stream. *Leucaspius delineatus* (Heckel) which is also present there, is not a typical representative of Salmon region (Starmach 1956). It came from a fish pond where it is abundant, and the pond has an outflow to Kamienny Potok.

3. MATERIAL AND METHODS

The material from Kamienny Potok was mainly gathered in the town Szczytna Śląska, some specimens were also caught in the region near to the stream source. A total of 166 individuals was caught from May to September 1969, and 15 specimens were caught during the spawning in March 1970 and 1971.

The material from Dzika Orlica, 34 individuals, was gathered in the vicinity of Mostowice in July 1970.

The fish were preserved in 4% formalin and transferred to 75% alcohol.

Thirty one measurements of plastic and meristic features were taken according to the Taliev's scheme (Starmach 1965) supplemented by Pravdin's (1966) scheme (Fig. 2).

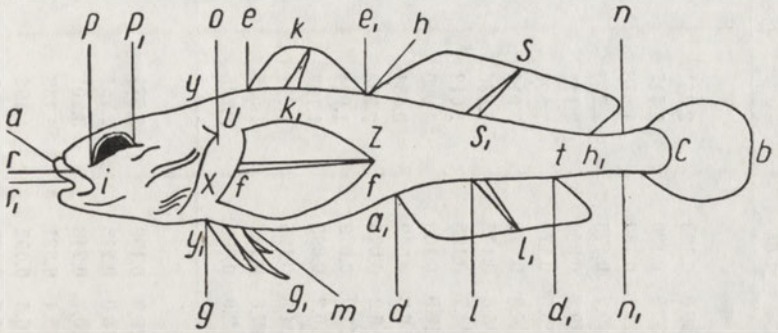


Fig. 2. Scheme of measurements: a–b longitudo totalis, a–c longitudo corporis, a–o longitudo capitis, a–e distantia praedorsalis, e–e_1 longitudo D I, h–h_1 longitudo D II, z–c distantia postdorsalis, a–d distantia praeanalis, a_1–d distantia inter anus et A, d–d_1 longitudo pinnae analis, t–c longitudo caudae, m–m_1 summa altitudo corporis, n–n_1 minima altitudo corporis, k–k_1 altitudo pinnae dorsalis I, s–s_1 altitudo pinnae dorsalis II, v–x longitudo pinnae pectoralis, f–f_1 altitudo pinnae pectoralis, g–g_1 altitudo pinnae ventralis, l–l_1 altitudo pinnae analis, a–p spatium praeorbitales, p–p_1 diameter oculi horizontalis, p_1–o spatium postorbitales, a–i_1 longitudo maxillae, r–r_1 latitudo maxillae, y–y_1 summa altitudo capitis, w–w_1 spatium inter oculos

The fish were measured with compasses to the accuracy of 0.5 mm. Statistical elaboration of the material included calculation of mean (*M*), standard error (*m*), standard deviation (σ) and coefficient of variation (*V*). The significance of differences between the populations examined was tested by Student test (*t*).

In order to make these data comparable with those of other authors, the analysis of populations of bullheads from Dzika Orlica and Kamienny Potok was carried out without distinguishing age and sex classes at first. Later on whole material from Dzika Orlica and 30% of material from Kamienny Potok were divided into age and sex classes. Thus arranged material was analysed by using the scheme of plastic features.

After weighing and measuring the fish, their alimentary canals were removed in order to analyse their contents. Thirty four alimentary canals of the fish from Dzika Orlica and 47 of those from Kamienny Potok were dissected. In each group two fish had empty alimentary canals.

The role of taxonomic groups in the food of *Cottus gobio* was expressed by: 1. percentage of the fish whose alimentary canals contained a given item in relation to the total number of fish dissected (this way had been used by Hartley (1948), Pliszka et al. (1951), Paschalski (1959)); 2. number and weight of the total of individuals of a given taxonomic group in the food, expressed as percentage of all the organisms found in the alimentary canals. Average weights of more numerous organisms were accepted after Pliszka et al. (1951).

Fecundity of females was obtained as a total number of roe in an ovary. Eggs were counted in the preserved state.

4. RESULTS

PLASTIC FEATURES

Analysis of plastic features in the two populations studied is presented in Tables I, II, and III.

Table I. Plastic features of *Cottus gobio* L. from Dzika Orlica ($n = 34$) and Kamienny Potok ($n = 166$). M — mean, m — standard error, σ — standard deviation, V — coefficient of variation, t — significance of difference

Feature	Dzika Orlica			Kamienny Potok			t
	$M \pm m$	σ	V	$M \pm m$	σ	V	
Percentage of longitudo corporis							
Longitudo capitis	28.8 0.165	0.963	3.346	26.8 0.183	2.351	8.772	8.09
Distantia praedorsalis	32.8 0.219	1.278	3.894	33.9 0.120	1.547	4.566	4.40
Longitudo D I	18.6 0.171	1.000	5.358	18.1 0.130	1.678	9.250	2.32
Longitudo D II	40.1 0.452	2.635	6.578	39.7 0.175	2.266	5.703	0.82
Distantia postdorsalis	48.4 0.153	1.982	4.092	49.8 0.369	2.142	4.301	3.50
Distantia praeanalis	53.5 0.279	1.734	3.241	56.5 0.171	2.208	3.903	9.15
Distantia inter anum et A	4.3 0.135	0.794	18.385	4.9 0.070	0.914	18.657	3.94
Longitudo pinnae analis	27.3 0.229	1.338	4.897	28.8 0.142	1.836	6.365	5.68
Longitudo caudae	17.9 0.251	1.468	8.108	14.7 0.125	1.612	10.964	11.41
Summa altitudo corporis	18.4 0.194	1.131	6.141	18.9 0.160	2.069	10.921	1.98
Minima altitudo corporis	7.3 0.092	0.540	7.387	6.3 0.049	0.634	6.368	6.95
Altitudo pinnae dorsalis I	7.8 0.120	0.705	8.949	6.2 0.076	0.985	15.768	11.60
Altitudo pinnae dorsalis II	12.0 0.184	1.077	8.926	12.2 0.079	1.024	8.363	0.99
Longitudo pinnae pectoralis	10.1 0.160	0.933	9.235	10.2 0.090	1.167	11.389	0.54
Altitudo pinnae pectoralis	24.1 0.247	1.457	6.035	24.8 0.125	1.611	6.487	1.87
Altitudo pinnae ventralis	19.0 0.143	0.853	4.479	17.8 0.122	1.575	8.834	6.30
Altitudo pinnae analis	12.7 0.125	0.729	5.735	12.0 0.096	1.245	10.320	2.35
Percentage of longitudo capitis							
Spatium praeorbitales	31.3 0.538	3.131	10.017	28.9 0.199	2.571	8.882	4.18
Diameter oculi horizontalis	17.4 0.289	1.688	9.661	18.0 0.213	2.751	15.242	1.68
Spatium postorbitales	53.5 0.462	2.693	5.457	50.7 0.248	3.201	6.311	4.95
Longitudo maxillae	35.7 0.691	4.028	11.278	35.4 0.321	4.138	11.674	0.39
Latitudo maxillae	7.1 0.173	1.010	14.145	6.5 0.092	1.192	18.124	3.01
Summa altitudo capitis	53.6 0.663	3.868	7.223	61.9 0.437	5.635	9.104	10.57
Spatium inter oculos	17.2 0.289	1.651	9.550	24.4 0.325	4.196	17.157	12.03

Table II. Dimensions of body in age classes of *Cottus gobio* L. from Dzika Orlica (ranges and means)

Age	Males						Females					
	2 ($n = 2$)		3 ($n = 8$)		4 ($n = 8$)		2 ($n = 3$)		3 ($n = 7$)		4 ($n = 6$)	
Weight (g)	4.5—5.4	4.9	7.2—9.9	8.6	10.5—15.6	13.9	3.6—8.1	5.2	5.2—8.2	7.0	7.9—14.5	12.0
Longitudo totalis (mm)	73—78	75	85—93	89.3	95—108	102.8	71—88	77	78—91	84.7	95—104	99.9
Longitudo capitis (mm)	16—20	18	21—23	21.8	23—27	25.5	17—21	18.3	19—22	20.7	22—26	24.6
Percentage of long. corporis												
Longitudo capitis	26.6—30.3	28.5	27.7—30.9	28.7	28.5—29.8	29.4	28.2—28.8	28.5	27.8—28.7	28.2	27.5—30.2	29.0
Distantia praedorsalis	31.4—33.3	32.2	31.9—33.8	33.0	31.9—34.8	33.5	32.7—35.5	34.0	30.1—33.8	31.7	30.4—34.8	32.6
Longitudo D I	16.4—18.1	17.3	17.9—19.2	18.4	17.2—20.9	19.4	18.6—18.9	18.7	16.1—19.8	18.4	17.5—19.6	18.6
Longitudo D II	39.3—39.4	39.3	37.6—42.1	40.3	37.7—41.9	39.6	39.6—42.3	40.8	36.6—44.1	39.9	38.3—43.7	40.6
Distantia postdorsalis	48.4—49.1	48.8	48.6—51.3	50.3	47.2—50.2	48.6	49.3—50.8	50.0	47.9—51.4	49.8	47.6—50.0	49.2
Distantia praeanalis	55.7—56.0	55.8	50.0—54.9	52.8	51.1—57.4	53.9	50.0—57.6	53.3	52.1—55.8	53.3	52.3—55.0	53.7
Dist. inter anum et A	3.2—6.0	4.6	2.7—5.2	3.9	3,2—5.5	4.3	3.3—6.7	4.8	3.8—5.1	4.3	3.1—5.2	4.2
Long. pinnae analis	26.2—27.2	26.7	25.3—30.1	28.0	25.2—28.2	26.8	25.8—28.8	27.2	25.0—29.6	26.8	26.7—29.5	27.8
Longitudo caudae	16.6—19.6	18.1	16.6—19.7	17.6	15.3—22.5	18.4	15.5—20.3	18.1	16.8—19.7	18.1	15.9—19.7	17.7
Summa altitudo corporis	18.1—18.8	18.5	16.6—20.5	18.7	17.5—22.2	19.0	16.9—18.6	17.6	16.1—19.1	18.1	14.6—20.0	18.3
Minima altitudo corp.	6.5—6.8	6.7	6.9—7.8	7.4	6.5—8.0	7.6	6.7—6.8	6.8	6.4—8.1	7.2	6.0—8.1	7.2
Altitudo D I	7.3—7.5	7.4	7.0—9.7	7.8	6.8—8.6	7.7	6.8—8.4	7.9	6.4—9.4	7.8	7.5—8.5	7.9
Altitudo D II	12.1—13.1	12.6	11.5—13.8	12.2	10.1—12.6	11.5	10.3—13.5	11.9	10.9—14.0	12.3	10.4—15.1	12.1
Long. pinnae pectoralis	8.1—10.6	9.3	8.9—11.1	10.1	9.7—11.7	10.8	7.7—10.1	8.7	9.4—10.8	10.1	8.7—10.4	10.0
Altitudo pinnae pector.	25.7—26.2	25.9	23.0—26.7	24.2	20.6—25.9	23.2	23.7—27.1	24.9	22.0—26.0	23.6	23.1—26.1	24.6
Altitudo pinnae ventr.	18.0—19.6	18.8	16.6—19.4	18.7	17.2—20.9	18.6	18.9—20.3	19.8	18.9—20.0	19.5	18.1—20.9	19.2
Altitudo pinnae analis	11.4—13.6	12.5	11.5—13.8	12.2	10.1—12.6	11.5	11.8—12.0	11.9	10.9—14.0	12.3	10.4—15.1	12.1
Percentage of long. capitis												
Spatium praeorbitales	30.0—37.4	33.7	28.5—36.3	31.9	26.9—34.6	32.0	29.4—35.2	33.3	21.0—31.8	28.8	28.1—33.3	30.6
Diameter oculi horiz.	17.5—18.7	18.1	15.9—19.0	18.0	13.8—19.2	16.0	17.6—20.5	19.6	15.7—19.0	18.8	14.8—20.0	17.0
Spatium postorbitales	50.0—55.5	52.5	45.4—56.5	53.1	52.1—57.6	54.0	52.9—52.9	52.9	47.6—57.8	52.8	50.0—56.5	53.6
Longitudo maxillae	30.0—31.2	30.6	31.8—41.8	36.3	38.4—42.3	38.9	29.4—35.2	31.3	28.5—36.6	33.0	33.3—44.0	38.0
Latitudo maxillae	5.5—6.2	5.8	5.7—9.0	7.2	6.5—8.6	7.5	5.8—6.4	5.7	5.7—9.4	7.2	6.2—8.4	7.2
Summa altitudo capitis	55.0—56.2	55.6	48.5—59.0	54.9	47.8—57.6	53.5	47.0—58.8	52.9	47.3—58.8	53.0	48.0—60.0	54.2
Spatium inter oculos	17.5—18.7	18.1	14.2—20.4	17.9	15.2—19.2	17.3	14.7—17.6	16.6	15.0—19.0	16.8	14.7—18.1	16.1

Table III. Dimensions of body in age classes of *Cottus gobio*

Age	Males					
	2 (n=3)		3 (n=10)		4 (n=8)	
Weight (g)	3.0—6.6	4.2	3.1—9.1	6.0	8.1—15.8	12.2
Longitudo totalis (mm)	64—74	69	66—91	81.5	90—106	98.2
Longitudo capitis (mm)	16—19	17.6	17—21	19.2	20—26	23.3
Percentage of long. corp.						
Longitudo capitis	29.0—30.1	29.5	26.7—31.4	28.3	25.9—30.5	28.5
Distantia praedorsalis	33.9—37.0	35.4	32.3—35.8	34.2	32.2—35.2	33.6
Longitudo D I	13.7—17.7	16.1	16.4—20.2	18.2	17.8—21.9	19.1
Longitudo D II	41.1—41.9	41.5	38.5—43.4	40.7	37.6—41.8	39.8
Distantia postdorsalis	47.1—50.0	48.7	47.0—51.3	48.4	47.6—49.4	48.3
Distantia praeanalis	52.8—59.6	53.1	54.2—60.0	57.7	51.8—59.7	56.3
Distantia inter anum et A	3.7—4.8	4.1	4.2—7.0	5.3	3.3—5.8	4.4
Longitudo pinnae analis	26.4—29.4	27.1	26.8—33.8	29.2	28.1—35.4	30.1
Longitudo caudae	16.9—17.7	17.4	13.2—17.4	15.1	11.3—15.4	13.7
Summa altitudo corporis	16.9—19.6	18.1	16.4—22.3	19.2	19.7—22.8	21.5
Minima altitudo corporis	6.4—7.8	6.9	5.8—7.1	6.3	5.4—7.8	6.5
Altitudo D I	5.8—7.5	6.6	5.6—9.9	6.7	5.0—7.0	6.4
Altitudo D II	12.9—15.6	13.9	10.8—13.4	12.1	10.1—14.2	12.0
Longitudo pinnae pector.	9.4—9.8	9.6	8.4—11.8	10.2	9.3—12.3	10.8
Altitudo pinnae pector.	24.1—27.4	26.0	23.0—26.7	24.4	20.9—25.8	23.1
Altitudo pinnae ventr.	17.6—18.8	17.4	17.1—19.7	18.2	15.7—19.0	17.4
Altitudo pinnae analis	11.3—12.9	11.9	10.7—12.8	11.8	10.3—13.0	12.0
Percentage of long. cap.						
Spatium praeorbitales	25.0—33.3	28.7	22.2—33.3	29.6	25.0—32.0	29.9
Diameter oculi horiz.	16.6—20.0	18.4	14.2—20.0	16.9	14.2—20.0	17.9
Spatium postorbitales	46.6—50.0	48.8	47.3—55.5	50.2	50.0—57.1	51.9
Longitudo maxillae	31.1—38.8	34.4	31.5—40.0	37.3	29.1—42.3	35.1
Latitudo maxillae	5.6—6.6	6.3	5.7—8.0	6.3	5.7—8.6	6.4
Summa altitudo capitis	56.2—61.1	59.1	57.8—66.6	61.3	57.6—68.1	62.6
Spatium inter oculos	22.2—26.6	24.6	21.0—35.0	26.0	20.0—30.7	25.9

It permitted: 1. to ascertain the significant differences of 15 features between the two populations of two catchment areas and to define the significance of differences, 2. to draw attention to the fact that some features used in T a l i e v's and P r a v d i n's schemes have a high coefficient of variation and thus are of a little diagnostic value (Table I), 3. to find out which of the features examined change with age of fish, 4. to define differences of some features characteristic to males and females of *Cottus gobio* L. (Tables II and III).

Average values of such features as: altitudo pinnae dorsalis I, longitudo caudae, longitudo capitis, altitudo pinnae ventralis, spatium postorbitale and praeorbitale, minima altitudo corporis, and latitudo maxillae, are larger in bullheads from Dzika Orlica than from Kamienny Potok.

The population from Kamienny Potok differs in larger means of such features as: spatium inter oculos, summa altitudo capitis, distantia praeanalis and postdorsalis, and distantia inter anum et A. The remaining average values show no higher differences (Table I).

Application of Student test permitted to find that 15 plastic features,

L. from Kamienny Potok (ranges and means)

	Females			
5 (n=2)	2 (n=5)	3 (n=6)	4 (n=4)	5 (n=1)
19.4—19.8 19.6	3.3—4.2 4.2	4.5—7.6 5.8	8.4—13.0 10.9	13.3
119—132 125	67—74 69	75—87 80	93—103 99	123
29—34 31	15—17 16	17—21 18.3	22—25 23.2	31
29.0—30.6 29.8	26.1—29.3 27.6	24.6—28.4 27.0	26.8—29.4 28.2	30.3
33.3—33.3 33.3	32.2—36.2 34.1	32.8—35.6 33.8	28.2—32.9 31.5	34.3
18.1—20.0 19.0	15.2—19.3 16.8	16.4—19.4 18.3	16.2—18.7 18.6	18.6
38.6—44.1 41.3	35.5—40.0 37.9	38.3—41.2 40.0	37.2—40.2 38.6	41.7
49.0—49.5 49.2	45.7—50.0 48.3	46.5—50.7 48.9	47.5—50.0 48.5	47.0
57.0—61.2 59.0	54.2—58.1 55.8	53.9—60.2 56.2	55.1—56.9 56.3	57.8
5.0—5.4 5.2	3.4—5.9 4.6	4.6—6.8 5.8	3.8—6.0 4.9	4.4
28.0—30.6 29.3	25.8—30.9 28.3	26.1—28.7 27.3	25.5—32.5 28.9	30.3
15.6—17.1 16.3	11.8—18.9 14.8	12.3—17.1 14.8	11.5—15.1 13.1	13.7
20.0—20.7 20.3	15.5—18.1 16.9	15.0—18.7 17.3	17.0—20.5 19.1	20.5
6.5—7.2 6.8	5.6—7.2 6.5	4.5—6.8 5.7	5.8—6.9 6.5	6.8
4.0—7.2 5.6	6.4—7.2 6.9	5.4—7.8 6.4	4.6—6.4 5.7	6.8
11.7—12.0 11.8	11.8—14.2 12.7	10.4—12.3 11.8	11.6—12.8 12.0	11.7
10.5—13.5 12.0	8.0—9.0 8.6	8.2—10.7 9.6	9.7—11.5 10.5	10.7
22.0—24.3 23.1	22.5—27.2 25.2	23.8—27.3 25.1	23.8—25.5 24.5	26.4
16.0—17.1 16.5	14.5—20.0 17.2	16.4—20.5 18.3	17.3—19.8 18.5	17.6
11.0—13.5 12.2	11.2—12.7 12.0	11.1—12.5 12.0	10.9—14.1 12.5	12.7
27.5—29.4 28.4	23.5—33.3 28.8	23.5—31.5 28.0	26.0—30.0 28.1	29.0
17.2—20.5 18.9	17.6—20.0 18.8	16.6—22.2 18.6	17.3—20.4 18.6	16.1
53.4—55.8 54.6	40.0—53.3 48.6	50.0—58.8 53.7	50.0—52.2 51.6	54.8
34.4—38.2 35.8	29.4—35.2 32.5	29.4—38.8 32.6	36.0—39.1 37.2	37.0
8.6—8.8 8.7	5.0—6.6 6.1	5.2—6.6 5.8	5.4—8.6 7.4	6.4
58.6—68.1 62.6	47.0—62.5 53.8	57.1—61.1 59.1	56.0—62.5 59.6	58.0
23.5—24.1 23.8	17.6—26.6 21.3	16.6—27.7 23.6	25.0—30.4 27.7	29.0

mentioned above, showed significant differences in these two populations ($t>3$) (Table I).

Variation of plastic features in the populations studied was rather high; the coefficient of variation (V) reached up to 18%. Features with high variation (above 10%) and those with low variation (below 10%) were distinguished in bullheads from the two streams. In bullheads from Kamienny Potok, the first group is represented by distantia inter anum et A, longitudo maxillae, latitudo maxillae, diameter oculi horizontalis and spatium inter oculos in the two populations as well as altitudo pinnae dorsalis I, longitudo pinnae pectoralis, longitudo caudae and summa altitudo corporis.

The first group of features was highly variable in the two populations and therefore should be considered as less useful for diagnosis.

The majority of plastic features in bullheads from Kamienny Potok showed also a higher coefficient of variation.

The analysis of plastic features revealed also that some of them such as: summa and minima altitudo corporis, longitudo pinnae dorsalis I, altitudo pinnae analis, spatium praeorbitale, latitudo and longitudo

maxillae increase with age in both sexes. However altitudo pinnae dorsalis I and II, altitudo pinnae pectoralis and ventralis decrease with age (Tables II and III).

When comparing size of bullheads in the two streams, it was found that the fish from Dzika Orlica were larger and heavier than those from Kamienny Potok.

Sexual dimorphism was expressed in that males of the two populations were heavier and had larger dimensions (almost all) than females. Such dimensions as diameter oculi horizontalis and altitudo pinnae ventralis were larger in females.

MERISTIC FEATURES

The comparative analysis of meristic features of *Cottus gobio* is presented in Tables IV—VI.

The analysis permitted to conclude: 1. Meristic features of *Cottus gobio* of the two populations examined from Silesia are within the limits of characteristic of this species. 2. Number of vertebrae and rays in dorsal fins and in anal fin differ in the two populations from Silesia, both in the distribution of values and in means, and the differences are significant (Student test). The population from Dzika Orlica shows higher number of vertebrae and rays in the both dorsal fins than the population from Kamienny Potok, but the latter had higher number of rays in the anal fin. 3. Number of rays in the first dorsal fin is the least variable feature (D I). 4. The most variable feature is the number of rays in the second dorsal fin (D II). Almost each station differs one from another and the difference is statistically significant. 5. The number of vertebrae and rays in the anal fin (A) can be considered as characteristic for the two populations of different catchment areas.

FOOD

Analysis of food in the two populations of *Cottus gobio* showed that the larvae of aquatic insects constitute over 98% of the food ingested (Table VII).

In the bullheads of the two localities, Tendipedidae larvae were most abundant, rather abundant were also Plecoptera larvae and in the fish from Kamienny Potok larvae of Ephemeroptera.

Differences in the food composition of *Cottus gobio* from Dzika Orlica and Kamienny Potok are expressed in the number of organisms of other taxonomic groups. The fish from Dzika Orlica ate more often larvae of Megaloptera (*Sialis sp.*) and Diptera (larvae and pupae) than

Table IV. Number of vertebrae in *Cottus gobio L.*

Catchment area or region	Stand (Author)		n	Number of vertebrae 30	31	32	33	34	35	$M \pm m$		σ	t
Volga (Kama)	a	Sylva (Zinoviev 1963)	—	—	—	—	+	+	—	33.50			
Finland	b	Vihtijoki (Koli 1969)	103	—	—	9	58	35	1	33.27	0.066	0.63	b/i=3.9, b/h=5.6 b/g=2.0, b/f=0.09, b/e=16.6, b/c=12.5
	c	Taivassolo (Koli 1969)	42	1	13	24	4	—	—	31.74	0.103	0.67	c/i=13.0, c/h=5.5, c/g=7.5, c/f=10.2, c/e=1.7
Danube	d	Cisa (Vladykov 1931)	64	—	+	+	—	—	—	31.50			
Oder	e	Kamienny Potok (present paper)	56	1	1	53	1	—	—	31.96	0.043	0.32	e/i=17.9, e/h=6.0, e/g=9.0, e/f=3.1
Elba	f	Elbe (Koli 1969)	65	—	—	8	33	23	1	33.26	0.084	0.68	f/i=3.6, f/h=5.0, f/g=2.5
	g	Vltava (Koli 1969)	44	—	—	11	25	8	—	32.93	0.099	0.66	g/i=14.8, g/h=2.3
	h	Dzika Orlica (present paper)	34	—	—	11	21	2	—	32.61	0.098	0.57	h/i=8.3
Weser	i	Fulda (Koli 1969)	46	—	—	—	17	23	3	33.70	0.087	0.59	
France	j	(Dorier 1942)	—	—	—	+	+	—	—	32.50			
	k	(Spilmann 1961)		+	+	+	+	—	—	31.50			

Table V. Number of rays in *Cottus gobio* L.

	Stand (Author)	n	Dorsal fin (D I)					Dorsal fin (D II)							Anal fin (A)						
			6	7	8	$M \pm m$	σ	15	16	17	18	19	$M \pm m$	σ	10	11	12	13	14	$M \pm m$	σ
	Pechora (Koli 1969)	7	—	7	—	7.0 0.000	0.000	—	—	6	1	—	17.1 0.133	0.358	—	1	4	2	—	12.1 0.242	0.640
	Kama (Volga) (Koli 1969)	26	—	14	12	7.5 0.094	0.500	—	4	17	5	—	17.0 0.115	0.588	—	5	12	8	1	12.2 0.153	0.783
	Ladoga (Koli 1969)	15	4	9	2	6.9 0.164	0.619	2	8	5	—	—	16.2 0.162	0.630	—	6	3	6	—	12.0 0.230	0.894
Finland	Vihtijoki (Koli 1969)	89	12[a]	65	11	7.0 0.058	0.551	—	16	57	16	—	17.0 0.063	0.599	—	13	50	21	1[f]	12.2 0.075	0.713
Finland	Taivassolo (Koli 1969)	66	1	49	16	7.2 0.055	0.451	12	38	16	—	—	16.1 0.079	0.648	2	30	31	3	—	11.5 0.077	0.633
Sweden	Köngamä (Koli 1969)	34	6	24	3	6.9 0.092	0.540	6	24	4	—	—	15.9 0.092	0.540	2	15	16	1	—	11.5 0.111	0.650
Sweden	Lule älv (Koli 1969)	64	—	35	29	7.5 0.062	0.500	—	9	42	13	—	17.0 0.073	0.586	—	11	37	16	—	12.1 0.083	0.669
Danube	Cisa (Vladykov 1931)	64	3	40	21	7.2 0.062	0.500	2[c]	38	21	2	—	16.3 0.082	0.663	2	35	25	2	—	11.4 0.076	0.608
Danube	Orava (Balon 1956)	3	—	1	2	7.2 0.383	0.668	1	—	2	—	—	16.3 0.312	0.541	—	1	2	—	—	11.6 0.086	0.149
Danube	Hron (Kux, Weisz 1960)	20	—	18	2	7.1 0.066	0.300	—	5	14	1	—	16.8 0.110	0.496	—	6	11	2	1	11.6 0.184	0.825
Vistula	Poprad (Kux, Weisz 1960)	20	—	19	1	7.1 0.049	0.223	1	5	12	2	—	16.7 0.156	0.700	—	9	8	3	—	11.7 0.156	0.700
Vistula	Raba (Starmach 1965)	100	—	100	—	7.0 0.000	0.000	—	—	25	67	8	17.8 0.054	0.541	—	—	—	21	79	13.7 0.041	0.417
Oder	Stenava (Kux, Weisz 1960)	20	1	15	4	7.1 0.106	0.479	1	—	19	—	—	16.6 0.188	0.529	—	1	11	8	—	12.3 0.128	0.564
Oder	Kamienny Potok (present paper)	166	10	150	6	6.9 0.023	0.375	3[g]	21	137	4	—	16.8 0.039	0.502	—	—	22	136	8	12.8 0.031	0.417
Elba	Elba (Oliva 1956)	42	2	32	8	7.1 0.089	0.579	7	17	15	3	—	16.3 0.131	0.850	4	19	15	4	—	11.4 0.122	0.794
Elba	Elba (Koli 1969)	71	4	50	17	7.2 0.061	0.518	1	13	46	19	1	17.0 0.087	0.740	1	21	42	7	—	11.8 0.074	0.631
Elba	Vltava (Koli 1969)	44	—	21	23	7.5 0.075	0.500	16	22	5	1	—	16.8 0.131	0.890	1	16	25	2	—	11.8 0.092	0.690
Elba	Dzika Orlica (present paper)	34	2	19	13	7.3 0.099	0.580	—	—	18	14	2	17.5 0.106	0.618	2	7	21	4	—	11.7 0.123	0.720
Elba	Otava (Skořepa 1967)	167	5	113	48[b]	7.1 0.042	0.545	3	28	85	47	3[d]	17.1 0.058	0.761	23[e]	76	52	6	9	11.3 0.075	0.976
	Fulda (Weser) (Koli 1969)	45	7	22	16	7.2 0.099	0.669	—	3	20	19	3	17.5 0.106	0.719	—	1	15	23	6	12.8 0.105	0.715

a 1 — 5 rays, b 1 — 9 rays, c 1 — 14 rays, d 1 — 20 rays, e 1 — 10 rays, f 1 — 15 rays, g 1 — 14 rays.

Table VI. Student test comparison of numbers of rays in different populations of *Cottus gobio* L.

Stand	Fulda	Otava	Dzika Orlica	Vltava	Elba (Oliva 1956)	Elba (Koli 1969)	Kamienny Potok	Raba	Cisa	Lule älv	Köngamä	Taivassolo
First dorsal fin (D I)												
Vihtijoki	1.8	1.3	2.6	5.3	2.3	0.9	1.6	0.0	2.2	1.1	0.9	2.5
Taivassolo	0.0	1.4	0.8	3.2	0.0	0.9	5.0	3.6	0.0	3.5	2.9	
Köngamä	2.3	2.1	3.0	5.3	2.8	1.6	0.0	1.1	2.7	7.5		
Lule älv	2.6	5.3	1.7	0.0	3.4	3.7	9.0	8.0	3.2			
Cisa	0.0	1.1	0.8	2.9	0.0	0.8	4.1	2.9				
Raba	2.0	2.3	3.0	6.6	3.2	1.1	4.3					
Kamienny Potok	2.9	4.1	3.9	7.6	4.6	2.1						
Elba (Oliva 1956)	0.7	0.0	1.5	3.4	0.9							
Elba (Koli 1969)	0.0	1.3	0.8	3.1								
Vltava	2.4	2.2	1.6									
Dzika Orlica	0.7	1.8										
Otava	0.9											
Second dorsal fin (D II)												
Vihtijoki	4.0	1.3	4.0	1.4	0.0	5.0	3.2	11.1	6.1	0.0	10.7	9.8
Taivassolo	10.6	10.3	10.6	4.5	7.6	1.3	8.0	17.8	1.7	8.4	1.6	
Köngamä	11.4	11.0	11.4	5.6	8.6	2.5	9.0	15.7	3.2	9.4		
Lule älv	3.9	1.0	3.9	1.3	0.0	4.6	1.2	8.8	6.3			
Cisa	8.9	8.0	8.9	3.2	5.7	0.0	5.5	15.3				
Raba	2.5	10.1	2.5	7.0	7.8	10.5	15.1					
Kamienny Potok	6.1	4.3	6.1	0.0	2.1	3.6						
Elba (Oliva 1956)	7.1	5.5	7.1	2.7	4.4							
Elba (Koli 1969)	3.6	0.9	3.6	1.2								
Vltava	4.1	2.0	4.1									
Dzika Orlica	0.0	3.3										
Otava	3.3											
Anal fin (A)												
Vihtijoki	4.6	8.4	3.4	3.3	3.8	5.5	7.4	17.6	7.4	0.8	5.2	6.8
Taivassolo	9.9	1.8	1.3	2.5	2.8	0.6	15.6	25.2	0.9	5.3	0.0	
Köngamä	8.5	1.4	1.2	2.0	2.2	0.6	11.3	18.6	0.7	4.3		
Lule älv	5.1	7.1	2.7	2.4	2.7	4.9	7.9	17.3	6.1			
Cisa	10.7	0.9	2.0	3.3	3.7	0.0	17.0	26.7				
Raba	16.6	28.2	15.3	18.8	22.6	17.7	17.5					
Kamienny Potok	0.0	18.5	8,6	10.3	12.5	11.1						
Elba (Oliva 1956)	8.6	0.6	1.7	2.6	2.8							
Elba (Koli 1969)	7.7	4.7	0.6	0.2								
Vltava	7.1	4.2	0.6									
Dzika Orlica	6.7	2.7										
Otava	11.5											

the fish from Kamienny Potok. They also feed on larvae of Trichoptera, which were not found in the stomachs of fish from Kamienny Potok.

Other invertebrate groups such as Hydrocarina, Copepoda, Oligochaeta, Nematoda, and Coleoptera constitute insignificant percentage of the food

Table VII. Incidence of different taxonomy groups in alimentary canals of *Cottus gobio* L. and frequency of feeding in them (l — larvae, p — pupae)

Food group	Dzika Orlica						Kamienny Potok					
	Food				Feeder		Food				Feeder	
	No.	%	Weight (g)	%	No.	%	No.	%	Weight (g)	%	No.	%
Tendipedidae l	742	75.96	10,425.6	65.53	30	93.75	1233	80.69	17,755.2	67.83	42	93.33
Diptera varia l	36	3.68			16	50.00	16	1.04			10	22.22
Diptera varia p	25	2.55	366.0	2.18	6	18.75	4	0.26	120.0	0.44	3	6.66
Megaloptera l *(Sialis sp.)*	34	3.48	1047.2	6.43	12	37.50	14	0.92	441.0	1.68	12	26.66
Ephemoroptera l	17	1.74	489.7	3.04	8	25.00	120	7.86	4092.0	15.63	19	42.22
Plecoptera l	73	7.47	2248.4	14.03	22	68.75	99	6.47	3049.2	11.65	30	66.66
Simulium l	12	1.22	6.0	0.03	7	21.87	21	1.31	10.5	0.05	5	11.11
Trichoptera l	23	2.26	1439.8	8.76			—	—	—	—	—	—
Coleoptera	4	0.41	—	—	2	12.50	1	0.07	—	—	1	2.22
Hydrocarina	2	0.21	—	—	2	12.50	—	—	—	—	—	—
Copepoda	3	0.31	—	—	3	9.37	—	—	—	—	—	—
Ostracoda	—	—	—	—	—	—	9	0.59	—	—	7	15.55
Oligochaeta *(Lumbricus sp.)*	—	—	—	—	—	—	4	0.27	—	—	4	8.88
Nematoda	6	0.62	—	—	3	9.37	7	0.46	—	—	6	13.33
Total	977	100.00	16,022.7	100.00			1528	100.00	26,177.7	100.00		

taken by fish. A scarce amount of these organisms eaten suggests that they are a random element in the food of *Cottus gobio*.

It seems that there is food preference in *Cottus gobio* since larvae of Simulium, being abundant in the streams constitute little above 1% of food.

Differences in percentage incidence of numbers of organisms of different taxonomic groups in the food of *Cottus gobio* from the two localities and also in percentage frequency of these organisms found in alimentary canals result probably from the size of animals constituting the food items. For example, larvae of *Sialis sp.* which were often eaten by bullheads from Kamienny Potok are encountered in low numbers (0.92%) but larvae of Tendipedidae which are smaller are eaten very often (93.3%) and in large numbers (80.6%).

Filamentous algae and sand particles were also found in stomachs of bullheads. They were most probably consumed haphazardly during catching the animals which live among agglomerations of algae on stones in the stream.

FECUNDITY OF FEMALES

The data on this subject are gathered in Table VIII.

Table VIII. Number of roe in *Cottus gobio* L. from Kamienny Potok dependently on:

Fish age			Fish weight			Fish length		
Age	No. of roe	*M*	Weight (g)	No. of roe	*M*	Length (mm)	No. of roe	*M*
2	152-362	243	5.2-7.2	152-359	255	78-82	152-362	272
3	303-368	336	7.3-9.2	215-362	299	83-86	317	317
4	341-395	361	9.3-11.2	341	341	87-90	303-368	335
5	347-650	458	11.3-13.2	368	368	91-94	341	341
			13.3-15.2	347-426	380	95-98	347-395	371
			15.3-17.2	347	347	99-102	347	347
			17.3-19.2	514-650	582	103-106	365-514	432
						107	650	650

Number of roe in ovaries ranged from 150 to 650 eggs, 370 on the average. There were 30.8 eggs per one gram of body weight.

The roe of *Cottus gobio* is orange-yellow, its diameter being 1.7 to 2.2 mm.

The coloration of *Cottus gobio* during the spawning period is different from that in the other periods of life. Males are then darker and had a clearer pattern on the skin then females which are brighter in colour. A typical characteristic of males in this period is an orange margination of the first dorsal fin and the presence of sexual process.

5. DISCUSSION

In the streams under study the distribution of *Cottus gobio* differs from that reported by Dyk (1954), Dobšik, Libosvárský (1955), Müller (1960), Starmach (1965), Libosvárský, Lelek (1966). According to these authors, the upper sections of the Salmon region are inhabited by *Cottus poecilopus* Heckel, but *Cottus gobio* L. occurs in the lower course of a stream. In Kamienny Potok and Dzika Orlica, *Cottus gobio* occurs in the zone near the sources of these streams.

The bullheads from the two streams were larger and heavier than those from other waters, e.g. from Brothay stream (Smyly 1957), from the rivers: Lule älv (Müller 1960), Moor Hause (Crisp 1963) and Raba (Starmach 1965). This would indicate the advantageous food conditions in the streams under study.

Our own observations concerning sexual dimorphism of *Cottus gobio* L. confirm those reported by Oliva (1956), Starmach (1965) and Čihar (1969).

Cottus gobio L., being a less mobile fish, is especially vulnerable to formation of local forms conditioned by environmental factors and insolation, which is reflected by meristic and plastic features. This was also stated by Starmach (1965) and Koli (1969). Although the mechanism of formation of meristic features in *Cottus gobio* was not investigated, it is supposed that such formation occurs similarly as in other fish (Tåning 1952, Orska 1964). The analysis of meristic features permitted to conclude that although the local conditions affect the number of rays in the second dorsal fin, the number of vertebrae and rays in anal fin (A) show no significant differences within the catchment area.

The results of analysis of alimentary canals in the populations of *Cottus gobio* under study differ inconsiderably from the results of other authors. Pliszka (1956) reported that larvae of Tendipedidae formed over 50% of weight of the food consumed by *Cottus gobio* from the upper Vistula River. Trichoptera, Simulium, and Ephemeroptera are also significant food elements. Smyly (1957) is also of a similar opinion. Only the data given by Hartley (1948) on the food of *Cottus gobio* from the Cam River and Shepreth Brook differ considerably from the above discussed. Insecta formed there 58%, Oligochaeta — 3%, Mollusca — 1%, and Crustacea (Gammaridae) — 39% of the diet.

Schulze (1890), Staff (1950), Dyk (1952), Żukov (1965) reported that besides aquatic invertebrates, *Cottus gobio* consumes also roe and fry of salmonids. No fry of other fish was found in the food of *Cottus gobio* from Dzika Orlica and Kamienny Potok since there were no bullheads caught during the spawning of Salmon.

Cottus gobio reaches sexual maturity in the second year of life (cf. Żukov 1965).

An average of 370 eggs was found in the ovaries. This number is similar to those given by other authors. Smyly (1957) counted 50 to 250 eggs, Starmach (1965) — an average of 570 eggs, Żukov (1965) 100 to 300 eggs, but Schulze (1890) and Dyk (1952) each about 1000 eggs. The diameter of eggs of *Cottus gobio* from Kamienny Potok was greater than that reported by Smyly (1957).

Acknowledgements

I wish to thank Docent Dr. Z. Kozikowska for her kind help in elaboration of this work.

6. SUMMARY

Morphological analysis is given of *Cottus gobio* L. from Kamienny Potok (catchment area of the Oder River) and Dzika Orlica (catchment area of the Elba River) in Lower Silesia.

Estimation was made of diagnostic usefulness of meristic and plastic features of this fish basing on the author's own data and the data of other authors. Such factors as food composition, fecundity, sexual dimorphism, and places of occurrence in the streams were analysed.

It was found that number of rays in the first dorsal fin (D I) is most constant but in the second dorsal fin (D II) — most variable, different for each locality. The number of vertebrae and rays in the anal fin (A) showed small variability within each catchment area.

Among plastic features, the dimensions of the head and the lengths of some fins are very variable and thus they are of a little diagnostic value.

The food of *Cottus gobio* consists mainly of larvae of aquatic organisms: Tendipedidae, Megaloptera, Ephemeroptera, Trichoptera, Plecoptera and Diptera. No Salmon roe was found in the diet.

Males of *Cottus gobio* from the two localities examined were heavier and had almost all linear dimensions larger than females.

There were 370 eggs on the average in the ovaries, with a diameter of 1.7–2.2 mm.

7. STRESZCZENIE

Podano analizę morfologiczną *Cottus gobio* L. z Kamiennego Potoku (zlewisko Odry) i Dzikiej Orlicy (zlewisko Łaby) z Dolnego Śląska.

Oceniono wartość diagnostyczną cech merystycznych i plastycznych tej ryby w oparciu o materiały własne i innych autorów. Zestawiono dane dotyczące pokarmu, płodności, dymorfizmu płciowego oraz miejsca bytowania w danych potokach.

Stwierdzono, że ilość promieni w pierwszej płetwie grzbietowej (D I) jest najbardziej stała, zaś w drugiej płetwie grzbietowej (D II) najbardziej zmienna, różna dla każdego stanowiska. Ilość kręgów i promieni w płetwie odbytowej (A) wykazuje małą zmienność w obrębie tego samego zlewiska.

Wśród cech plastycznych stwierdzono, że wymiary dotyczące głowy i długości niektórych płetw są bardzo zmienne, a przez to mniej diagnostyczne.

Pokarm *Cottus gobio* stanowiły głównie larwy owadów wodnych: Tendipedidae, Megaloptera, Ephemeroptera, Trichoptera, Plecoptera, i Diptera. W pokarmie nie stwierdzono natomiast narybku pstrąga.

Samce *Cottus gobio* z obu badanych stanowisk miały większą wagę oraz charakteryzowały się prawie wszystkimi większymi wymiarami liniowymi niż samice.

W jajnikach samic stwierdzono średnio 370 ziaren ikry o średnicy 1,7–2,2 mm.

8. REFERENCES

Balon, E. 1956. K ichtyofaune Oravy pred naplenenim udolnej Nádrže [Zur Ichthyofauna des Orava-Flusses]. *Zool. Listy*, 5, 325–337 [German summ.].

[Berg, L. S.] Берг, Л. С. 1949. Рыбы пресных вод СССР и сопредельных стран [The freshwater fishes of the USSR and surrounding countries]. Moskva, Izdat. Akad. Nauk SSSR.

Čihar, J. 1969. Taxonomical and ecological notes on *Cottus gobio* Linnaeus, 1758, and *Cottus poecilopus* Heckel, 1836 (Osteichthyes: Cottidae). *Vest. čsl zool. Spol.*, 33, 102–110.

Crisp, D. T. 1963. Preliminary survey of brown trout (*Salmo trutta*) and bullheads (*Cottus gobio*) in high altitude becks. *Salm. Trout Mag.*, 167, 45–59.

[Chaban, A. P., Bogdanov, G. A.] Чабан, А. П., Богданов, Г. А. 1960. О нахождении подкаменщика (*Cottus gobio* Linee) в бассейне Иртыша [On a finding of *Cottus gobio* Linee in the Irtysh basin]. *Zool. Ž. Mosk.*, 39, 1102 [Engl. summ.].

Dobšik, B., Libosvárský, J. 1955. Prispevek k hodnouceni hospodársky duležitych ryb v řece Moravicy [A contribution of the valution of economically important fish in the River Moravice]. *Sb. vys. Šk. zemed. les. Fak. Brno*, 3, 253–268 [Engl. summ.].

Dorier, A. 1942. La ponte du Chabot de rivière (*Cottus gobio* L.) et l'evolution morphologique de l'alevin. *Trav. Lab. Hydrobiol. Piscic. Univ. Grenoble*, 79–86.

Dyk, V. 1952. *Naše ryby* [Our fishes]. Praha, Zdrovotnicke Nakladelstvi.

Dyk, V. 1954. The bullheads of the river Bystriče. *Sb. Sluko, I A*, 183–184.

[Fyodorov, V. G.] Федоров, В. Г. 1962. Нахождение бычка-продкаменщика (*Cottus gobio* L.) в западной Сибири [On the ocurrence common sculpin *Cottus gobio* L. in Western Siberia]. *Vop. Ihtiol.*, 2 (1), 89.

[Gundrizer, A. N.] Гундризер, А. Н. 1966. Нахождение подкаменщика (*Cottus gobio L.*) в бассейне реки Катуни (Центральный Алтай) [*Cottus gobio* L. in the basin of River Katunia (Central Altai] *Zametki po fauna i flora Sibiri*, 19, 37–40.

Hartley, P. H. T. 1948. Food and feeding relationships in a community of fresh-water fishes. *J. Anim. Ecol.*, 17, 1.

Jaśkowski, J. 1962. Materiały do znajomości ichtiofauny Warty i jej dopływów [Materialen zur Kenntnis der Fischfauna der Warta und ihrer Nebenflüsse]. *Frag. faun.*, 9, 449–499 [German summ.].

Kaj, J. 1954. Projekt rezerwatu dla ryb w dolnym odcinku rzeki Wełny [Project of fish preserve in lower sector of the River Wełna]. *Chrońmy Przyr. ojcz.*, 10, 43–46.

Koli, L. 1969. Geographical variation of *Cottus gobio* L. (Pisces, Cottidae) in Northern Europe. *Annls zool. Fenn.*, 6, 353–390.

Kux, Z., Weisz, T. 1960. Prispěvek k poznáni ichtyofauny Dunajce, Popradu, Váhu a Hronu [Ein beitrag zur Kenntnis der Ichthyofauna der nord-und mittelslowakischen Flüsse Dunajec, Poprad, Vah und Hron]. *Čas. morav. Mus. Brno*, 45, 203–240 [German summ.].

Libosvárský, J., Lelek, A. 1966. Výskyt ryb v říčce Loučce [Occurrencè of fishes in Loučka Creek]. *Zool. Listy*, 15, 47–54 [Engl. summ.].

Lohniský, K. 1963. Stáři a rust pstruha obecného formy potoční (*Salmo trutta m. fario* Linnaeus, 1758) v hornim povodi Divoké Orlice [Das Alter und Wachstum der Bachforelle (*Salmo trutta m. fario* Linnaeus, 1758) in Gewässern des Gebietes des oberen Flusses Divoká Orlice (Nordostböhmen)]. *Pr. kraj. Mus. Hradci Králové*, 5, 169–197 [German summ.].

Müller, K. 1960. Beitrag zur Systematik und Verbreitung von *Cottus gobio* L. und *Cottus poecilopus* Heckel. *Forh. K. Fysiogr. sallsk. Lund*, 30, 57–66.

Nybelin, O. 1969. Om stensimpans, *Cottus gobio* L. och bergsimpans *Cottus poecilopus* Heckel, utbrednigshistotia i sodra och mellersta Sverige [On the immigration of *Cottus gobio* L. and *Cottus poecilopus* Heckel into southern and middle Sweden]. *Acta R. Sci. Litt. Gothoburgensis, Zoologica*, 4, 1–53 [Engl. summ.].

Oliva, O. 1956. Prehled našich vranek (Cottus L.) [A review of our sculpins (Cottus L.)]. *Přirodov. Sb. ostrav. Kraje*, 17, 188–195 [Engl. summ.].

Orska, J. 1964. The influence of temperature on the development of meristic characters of the skeleton in Salmonidae. Part II. Variations in dorsal and anal

fin ray cout correlated with temperature during development of *Salmo irideus* Gibb. *Zool. Pol.*, 13, 49–76.

Paschalski, J. 1959. Pokarm naturalny głowacza pręgopłetwego (*Cottus poecilopus* Heckel) z potoku Poroniec [Food of the bullhead (*Cottus poecilopus* Heckel)]. *Pol. Arch. Hydrobiol.*, 6, 125–131 [Engl. summ.].

Penczak, T. 1968 a. Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Cz. I a. Hydrografia i rybostan Bzury i jej dopływów [The ichthyofauna of the rivers of the Łódź Upland adjacet areas. Part I a. The hydrography and fishes of the Bzura River basin]. *Acta Hydrobiol.*, 10, 471–497 [Engl. summ.].

Penczak, T. 1968 b. Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Cz. I b. Hydrografia i rybostan Pilicy i jej dopływów [The ichthyofauna of the rivers of the Łódź Upland and adjacet areas. Part I b. The hydrobiography and fishes of the Pilica River basin]. *Acta Hydrobiol.*, 10, 499–524 [Engl. summ.].

Pliszka, F., Backiel, T., Dziekońska, J., Kossakowski, J., Włodek, S. 1951. Badania nad odżywianiem ryb w Wiśle [Investigation into the food feeding habits of fish in the River Vistula]. *Roczn. Nauk roln.*, 57, 205–236 [Engl. summ.].

Pliszka, F. 1956. Znaczenie organizmów wodnych jako pokarmu ryb w świetle badań polskich [Importance of the aquatic animals as a food base on fish after Polish investigations]. *Pol. Arch. Hydrobiol.*, 3, 429–458 [Engl. summ.].

[Pravdin, I. F.] Правдин, И. Ф. 1966. Руководство по изучению рыб [Guide of fish investigation]. Moskva, Izdat. Piščevaja promyslennost'.

Schulze, E. 1890. Fauna Piscium Germaniae. Jahrbuch der Naturwiss. Verlag zu Magdeburg für 1889.

Skořepa, V. 1967. Zur Systematik der Böhmischen West-Groppen (*Cottus gobio* L.) (Cottidae, Osteichthyes). *Vestn. čsl. zool. Spol.*, 31, 260–273.

Smyly, W. J. P. 1957. The life-history of the bullhead or Millers thumb (*Cottus gobio* L.) *Proc. zool. Soc., Lond.*, 128, 341–353.

Spilmann, Ch. J. 1961. Poissons d'eau douce. In: P. de Beauchamp, L. Chopard [Dir.] *Faune de France*. 65, Paris, Librairie de la Faculté des Sciences.

Staff, F. 1950. *Ryby słodkowodne Polski i krajów ościennych* [The freshwater fishes of Poland and surrounding countries]. Warszawa, Trzaska, Ewert i Michalski.

Starmach, K. 1956. Rybacka i biologiczna charakterystyka rzek [Characteristic of rivers from biological and fishery point of view]. *Pol. Arch. Hydrobiol.*, 3, 307–332 [Engl. summ.].

Starmach, J. 1965. Koppen in den Karpathenflüssen. II. Antreten und Charakteristik der Buntflossenkoppe (*Cottus poecilopus* Heckel) und weisslossigen Koppe (*Cottus gobio* L.) in Raba Flussgebiet. *Acta Hydrobiol.*, 7, 109–140.

Tåning, A. V. 1952. Experimental study of meristic characters in fishes. *Biol. Rev.*, 27, 169–193.

Vladykov, V. 1931. Poissons de la Russie, Sous-Carpathique (Tschecoslovaquie). *Mém. Soc. zool. fr.*, 29, 217–374.

[Zinoviev, E. A.] Зиновьев, Е. А. 1963. Подкаменщик средней Камы [*Cottus gobio* L. of middle Kama]. *Izv. Inst. Perm. Univ.*, 14, 93–104.

[Żukov, P. I.] Жуков, П. И. 1965. Рыбы Белоруссии [Fishes of Belorussia]. Minsk, Izdat. "Nauka i technika".

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	421–435	1972

J. A. SZCZERBOWSKI

FISHES IN THE ŁYNA RIVER SYSTEM

Inland Fisheries Institute, University College of Agriculture, Olsztyn-Kortowo
Poland

ABSTRACT

The paper characterizes the species composition of fish fauna in the running waters of the catchment area of the Łyna River. Catches were made at 22 stations using a current-generating aggregate. The occurrence of 26 species of fish was ascertained. No clear correspondence was found between the division of rivers into the fish regions and the species of fish captured in them.

1. INTRODUCTION

A total length of rivers and larger streams that flow in the Olsztyn District amounts to 1900 km. Many of them meet all the requirements for clean waters and only about 5% is deprived of fish. The largest number of lakes in Poland is situated in the Mazurian Lakeland, and perhaps for this reason the investigations carried out in this region concern mainly the fish fauna of still waters. The paper by Backiel (1964) is the only one concerned with the fish populations occurring in rivers; it deals with the Drwęca River system and there are also some fragmentary studies done by Draganik, Szczerbowski (1963) and Szczerbowski et al. (1968), carried out in the stream Kośno.

The aim of this paper was to characterize the fish species composition of the running waters of the Łyna River system. This work will broaden the knowledge of the actual distribution of fish and also will form a basis for the fish management in this region.

2. MATERIAL AND METHODS

The material was gathered in 1970 and 1971. It consisted of major environmental factors (gradient, width) and of description of the fish fauna. Catches were made at 25 stations, 4 of which of the Guber River were pooled together to form station No. 22, on account of a high pollution of this river (Table I). In general 31 catches were made using the electric aggregate of type PAP, generating the constant current of 220 V and 2–4 A. The fish were caught from a boat that flew downstream. One person operated a special net which was at the same time the anode and two other persons directed the boat. After each catch the fish were specified, counted, and their mean weight obtained. In order to describe the affectiveness of the electrofishing, the catches were repeated at some stations several times a day and at other stations they were carried out 3 times a year (spring, summer, autumn). For the comparative reason, several anglers fished with fishing rods for about 10 days close to station 10 on the Symsarna River, gathering data on the species composition and on the weight of fish.

Table I. Sampling stations in the Łyna River system

River	No. of station	Place of station	Date of catch
Łyna	1	Kurki	19.X.1971
Marózka	2	Waplewo	19.X.1971
	3	Szwaderki	19.X.1971
Kośno	4	Kośno	10.VIII.1971
Pisa	5	Between affluents	10.VIII.1971
		Dobrąg and Wałpusza	18.X.1971
Dadaj	6	Behind Lake Dadaj	9.VIII 1971
Symsarna	7	Ustnik	19.V.1971
	8	Above weir Blanki-Symsar	19.V.1971
			18.V.1971
	9	Below weir Blanki-Symsar	9.VIII 1971
	10	Kłębowo	18.X.1971
			18.V.1971
Elma	11	Deksyty	17.V.1971
	12	Piaseczno	
	13	Koniewo	
Ryn	14	State Farm Nisko	16.X.1970
Sajna	15	Pleśno	16.X.1970
	16	Głowity-Kaskajmy	15.X.1970
Dajna	17	Pilec	7.VIII.1970
			14.X.1970
	18	Niewodnica	7.VIII.1970
	19	Niewodnica	5.VIII.1970
	20	Smokowo	15.X.1970
	21	Biedaszki	6.VIII.1970
Guber	22	Kotek, Pomnik, Sątoczno, Sępolno	6–7.VIII.1970

3. DESCRIPTION OF THE STUDY AREA

The Łyna River is the largest river in Warmia and Mazury. It is the left tributary of the Pręgoła River, to which it flows in USSR. The Łyna River is 289 km long and its catchment area covers 7126 km². The river starts from sources situated near the village Łyna, north of Nidzica, at 175 m a.s.l. There are many lakes of different limnological type in the river drainage area. Just before the Lake Łańsk, situated in the upper course of the river, the first left-bank affluent — Marózka — joins the Łyna River (Fig. 1). This affluent carries clean waters especially below lakes Mielno and Maróz. The total gradient is rather high (1.45‰) and the particular gradients reach in places to 5‰ (Fig. 2). Fast flow of water makes this affluent similar to a submontane stream. Below Olsztyn, at 52nd km from the sources the Wadąg River joins the Łyna River. The Wadąg River gathers a number of smaller tributaries which include the Pisa River, changing its name in the upper course to Dadaj and Dymer as well as Kośno and Kiermas. Somewhat farther Łyna forms two dam reservoirs and flows into the natural Lake Mosąg. A large portion of pollution enters the river in Olsztyn. This together with pollution from lower situated towns (Dobre Miasto, Lidzbark Warmiński, Bartoszyce) bring about a regular lowering the purity class of water. From Wadąg to Lidzbark Warmiński only smaller and periodically drying up streams enter the Łyna River. In the town Lidzbark the Symsarna River joins on the right the Łyna River; this tributary introduces pure waters due to flowing through lakes (Blanki, Symsar). At a distance

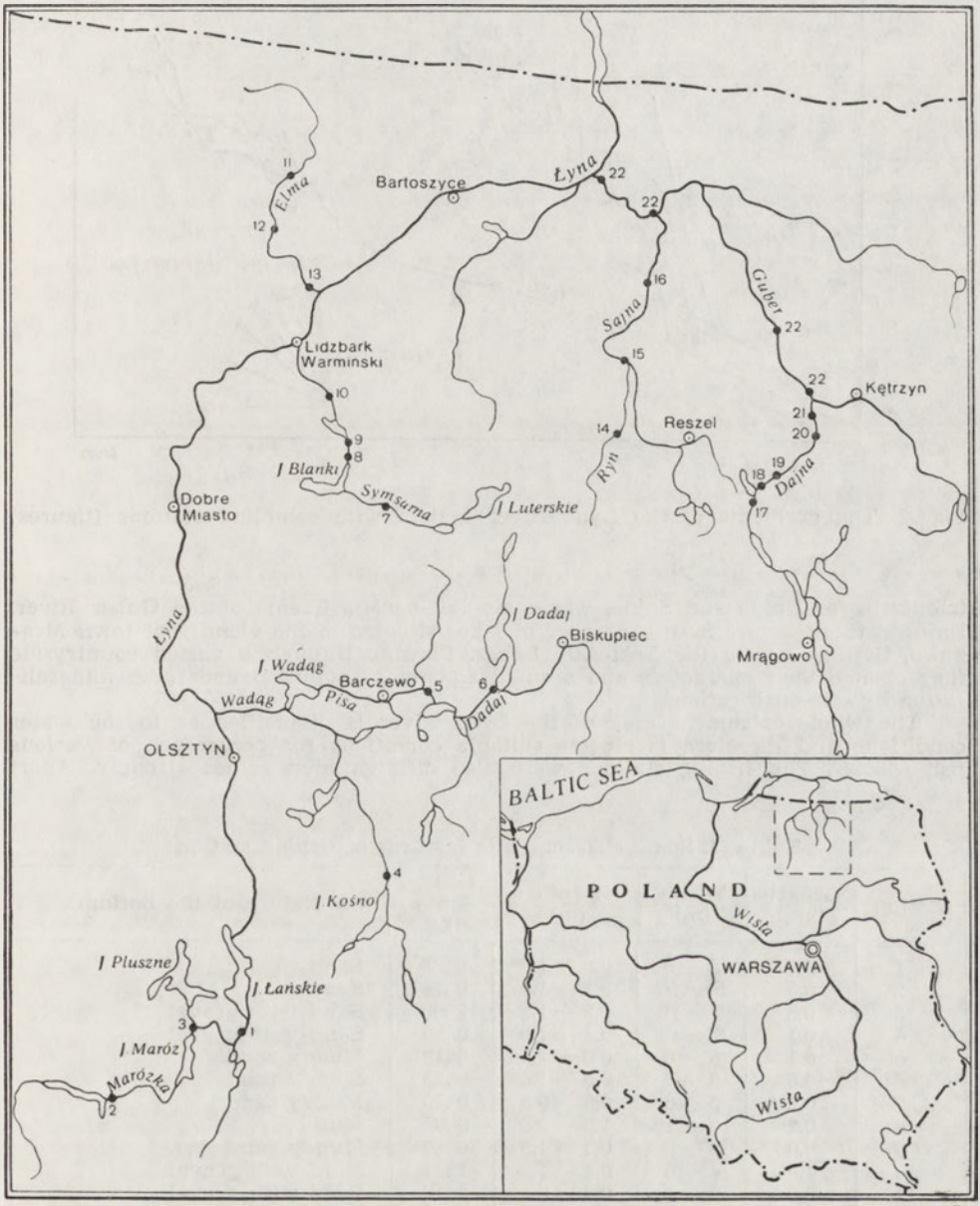


Fig. 1. Sampling stations in the Łyna River system. 1–22 — stations

of about 10 km down the river another River Elma enters on the left bank and still farther, over 10 km from the country boundary, the biggest left-bank affluent Guber flows into the river. In the town Kętrzyn, Guber becomes highly polluted, especially at the period of sugar campaign, when it becomes a typical sewage. The fish can occur in it only for short periods due to pure waters of such

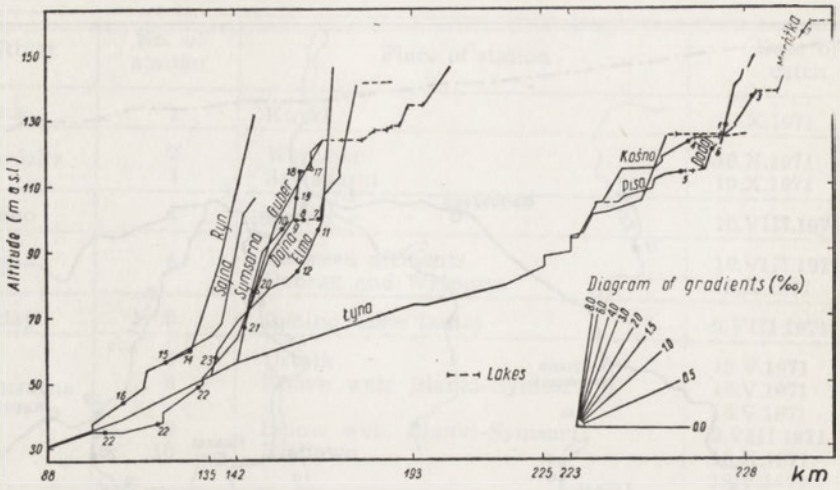


Fig. 2. The gradients of the Łyna River system with sampling stations (figures)

tributaries as Dajna and Sajna, which are left-bank affluents of the Guber River. Dajna gathers waters from a number of lakes situated in the vicinity of town Mrągowo, Sajna — from the Legińskie Lakes. Flowing through a varied countryside they change their characters and a small amount of pollution undergoes mineralization by self-purification.

The whole catchment area of the Łyna River is diversified as to the water conditions and therefore it creates suitable conditions for occurrence of various fish species. The fishing stations were also differentiated rather strongly. Apart

Table II. Some characteristic features of fishing stations

Station	Length (km)	Width (m)	Depth (m)	Gradients (‰)	Nature of the bottom
1	0.6	5 — 6	0.8 — 1.0	0.25	Sand
2	0.5	5 — 10	0.3 — 1.0	0.75	Sand
3	0.3	5 — 10	0.2 — 1.5	2.00	Sand with gravel
4	0.1	6 — 12	0.4 — 1.0	0.95	Sand with gravel
5	0.1	5 — 8	0.6 — 1.5	0.18	Muddy sand
6	0.5	6 — 8	0.5 — 1.0	0.25	Muddy sand
7	0.2	5 — 6	0.3 — 0.5	0.10	Muddy sand
8	0.2	10 — 15	1.0 — 2.0	0.08	Mud
9	0.9	3 — 6	0.3 — 1.5	0.20	Muddy sand
10	1.5	4 — 6	0.4 — 1.6	1.31	Sand with gravel
11	0.8	2 — 4	0.2 — 0.5	2.10	Sand with gravel
12	0.2	4 — 6	0.3 — 0.4	1.12	Sand
13	0.6	4 — 10	0.2 — 1.0	2.50	Stones and gravel
14	0.3	3 — 5	0.3 — 0.8	0.34	Sand
15	1.0	6 — 8	0.5 — 1.5	0.56	Muddy sand
16	1.5	5 — 10	0.4 — 1.5	0.79	Sanded gravel
17	0.6	5 — 10	0.3 — 1.2	1.00	Stones and gravel
18	0.4	10 — 15	0.5 — 1.5	0.25	Mud
19	0.5	3 — 10	0.4 — 1.5	8.12	Stones and gravel
20	0.5	4 — 5	0.5 — 0.7	2.97	Sand with gravel
21	1.3	4 — 7	0.3 — 1.0	8.65	Sanded gravel
22	0.5 — 2.5	4 — 12	0.3 — 1.5	1.22	Sand with gravel

from their lengths, ranging from 0.2 to 2.5 km which depended on the possibility of introducing and withdrawing the boat with the aggregate from the river, their widths ranged from 2 to 15 m and depth — from 0.2 to 2.0 m. The river gradients change with stations, in slowly running sections they amounted to 0.08‰, in the fastest section (Dajna, station 19) to 8.12‰ (Table II). It should be mentioned that often, within a short section of the river, its character changes abruptly because of numerous lakes situated in its course or artificial damming. The bottom was most often covered with sand-gravel and sand-mud sediments.

4. RESULTS

A total of 5057 fish were caught with the highest incidence of roach (21.2%). Gudgeon, dace, white bream, chub, pike and perch were also caught in large numbers (Table III). They all represented 8 families. Most numerous, both in numbers and in species, was the carp family. A total of 26 species were caught, 60% of which were cyprinids.

Table III. The species composition of fishes in Łyna system river

Species	No. of fishes	% of the total	Frequency		Average weight (g)
			n	%	
Salmo trutta m. fario L.	3	0.1	1	4.5	593
Salmo trutta m. lacustris L.	1	0.0	1	4.5	1770
Esox lucius L.	354	6.9	19	86.8	268
Tinca tinca Cuv.	47	0.9	8	36.4	266
Gobio gobio (L.)	640	12.6	20	90.9	13
Barbus barbus (L.)	2	0.0	1	4.5	480
Carassius carassius (L.)	7	0.1	4	18.2	154
Abramis brama (L.)	85	1.6	5	22.7	129
Blicca bjoercna (L.)	481	9.5	10	45.5	31
Leuciscus leuciscus (L.)	660	13.1	18	81.8	100
Leuciscus cephalus (L.)	482	9.5	17	77.3	250
Phoximus phoximus (L.)	1	0.0	1	4.5	2
Vimba vimba (L.)	7	0.1	1	4.5	131
Chondrostoma nasus (L.)	13	0.3	1	4.5	686
Aspius aspius (L.)	2	0.0	1	4.5	70
Alburnus alburnus (L.)	251	5.0	9	40.9	12
Alburnoides bipunctatus (Bloch)	83	1.6	9	40.9	7
Rutilus rutilus (L.)	1081	21.2	18	81.8	47
Scardinius erythropthalmus (L.)	18	0.4	4	18.2	71
Nemachilus barbatulus (L.)	66	1.3	7	31.8	7
Cobitis taenia (L.)	12	0.2	5	22.7	3
Anquilla anquilla (L.)	78	1.5	11	50.0	278
Lota lota (L.)	282	6.1	13	59.0	106
Lucioperca lucioperca (L.)	2	0.0	2	9.1	305
Perca fluviatilis L.	375	7.5	17	77.3	32
Acerina cernua (L.)	3	0.1	1	4.5	6
Cottus gobio L.	21	0.4	6	27.3	6

Of salmonid family 4 individuals were encountered, representing two forms: rainbow trout (*Salmo trutta morpha fario*) and lake trout (*Salmo trutta morpha lacustris*). They were found to occur in the upper course of the Łyna River (station No. 1) and in Marózka (station No. 3). The lake trout, weighing 1770 g occurred in the section similar in terms of environmental conditions to the Wda River near Loryńc (S a k o w i c z

1961 b), where were natural spawning places of this species. The rainbow trout occurred in the habitat typical for this species, i.e., the most similar to the stream at the boundary of trout and barbel regions.

Pike, *Esox lucius*, was commonly found. It was caught at 19 stations (86.8%) (Table III), being next to gudgeon in this respect. Its numbers ranged from 2 to 74 individuals per sample (Table IV) and percentage incidence of all the fish caught ranged from 0.5% (station 18) to 38.7% (station 15). Most of them were small individuals with average weight of about 268 g (Table III).

Among cyprinids, the typical representatives of running waters (rheophilous) and stagnophilous fish were encountered. Of the rheophilous forms greater attention deserve: barbel (*Barbus barbus*), minnow (*Phoximus phoximus*), silver bream (*Vimba vimba*), and nose (*Chondrostoma nasus*). Barbel was caught only in the Elma River (two individuals), minnow — only in one station of the Sajna River, silver bream and rainbow trout — in the Marózka River; a ciprinid *Chondrostoma nasus*, which is not mentioned in the literature for the catchment area of the Łyna River — was caught in one station (No. 16) of the Sajna River. Besides these rare species, the rheophilous species such as dace (*Leuciscus leuciscus*), and chub (*Leuciscus cephalus*) were very often encountered. Of these two species 1142 individuals were caught which formed 22.7% of the total number of fish captured. The following species of a common occurrence were often encountered: gudgeon (*Gobio gobio*) at 20 stations, bleak (*Alburnus alburnus*), sperlin (*Alburnoides bipunctatus*) at 9 stations, and roach (*Rutilus rutilus*) at 18 stations. Of the typical representatives of the carp family occurring in the slowly running waters the following were caught: *tench* (*Tinca tinca*), crucian carp (*Carassius carassius*), bream (*Abramis brama*), white bream (*Blicca bjoercna*), and rudd (*Scardinius erythrophtalmus*). They occurred in the sections of slowly running waters in the proximity of lakes. The family of Cobitidae was represented by stone loach (*Nemachilus barbatulus*) and spined loach (*Cobitis taenia*), the former being found only in sections with higher gradient of the rivers: Dajna, Sajna, and Elma.

The common eel (*Anguilla anguilla*) was represented by 78 individuals. They occurred in different habitats, starting from stations with salmon to typical lowland sections of the rivers. The percentageous incidence in the samples ranged from 0.5 to 19.6 (stations No. 7 and 1), the average weight being 278 g.

The burbot (*Lota lota*) was encountered in 13 stations (282 individuals) of the average weight of 106 g. The highest number of burbot (128) was captured in the Symsarna River, in the section between lakes Blanki and Symsar, where they formed 14.2% of the total number of fish captured. During 3 catches (spring, summer, autumn) the smallest number of fish of this species was caught in the first catch.

Table IV. Results of electrofishing

Species	Station																					Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Salmo trutta m. fario L.			3																				3
Salmo trutta m. lacustris L.	1																						1
Esox lucius L.	17	6		2	51	4			26	13	25	1	11	2	74	25	46	2	2	15	2	30	354
Tinca tinca Cuv.	1					6	9		26				1					1	1			2	47
Gobio gobio (L.)	4	6	72		2		3	1	190	26	14	2	5	26	15	5	178	12	4	33	10	32	640
Barbus barbus (L.)													2										2
Carassius carassius (L.)		1					2		2			2											7
Abramis brama (L.)					24		1	1	10													49	85
Blicca bjoercna (L.)	1			10	98	7	88	1	36				1				33	206					481
Leuciscus leuciscus (L.)		113	9		3	2			126	99	57	12	8	18	7	55	7	1	19	75	13	36	660
Leuciscus cephalus (L.)	2	25	1	13	18				82	55			13	47	2	60	92	4	5	48	7	8	482
Phoximus phoximus (L.)															1								1
Vimba vimba (L.)			7																				7
Chondrostoma nasus (L.)																13							13
Aspius aspius (L.)																						2	2
Alburnus alburnus (L.)					57		4	16	44	83			20				12			1		14	251
Alburnoides bipunctatus (Bloch)									11	16					1	7	3	1		35	5	4	83
Rutilus rutilus (L.)	14	1	8	40	242	58	60	64	162	2	11			4	70	6	100	164	10			65	1081
Scardinius erythropthalmus (L.)				1	12		2															3	18
Nemachilus barbatulus (L.)											22	3	14	13	8	5					1		66
Cobitis taenia (L.)				1	1				6									3				1	12
Anquilla anquilla (L.)	10	20	7	1		14	1	1	12	5							4	3					78
Lota lota (L.)	1		7	20	39	2			128	18	38		1		6		17	3				2	282
Lucioperca lucioperca (L.)									1													1	2
Perca fluviatilis L.		11	3	33	70	25	23	6	41	2		3	1		7		45	44	30		2	29	375
Acerina cernua (L.)									3														3
Cottus gobio L.										2			11			1			2	3	2		21
Total	56	183	117	121	617	119	193	90	906	321	167	23	88	110	191	177	537	444	73	210	42	278	5057

Pericidae were represented by perch-pike (*Lucioperca lucioperca*), perch (*Perca fluviatilis*) and ruff (*Acerina cernua*). Perch-pike and ruff were caught only in two stations; perch, on the other hand, was encountered in 17 stations.

The only representative of the cottid family in the Łyna River catchment area was bullhead (*Cottus gobio*). It was found to occur in 6 stations of the rivers: Symsarna, Elma, Sajna, and Dajna, where a total of 21 individuals was caught.

In general, the fish captured can be divided into: 1. species that occur often (in more than 13 stations), to which belong: pike, gudgeon, white bream, chub, roach, and perch; 2. those occurring in an average number of stations (7—13), to which belong: tench, white bream, bleak, sperlin, stone loach, common eel, burbot; and 3. rare species (less than 7 stations) to which belong the remaining fish.

4. DISCUSSION AND CONCLUSIONS

The investigations aiming at defining the qualitative and quantitative comparison of the fish fauna are met with difficulties mainly due to the lack of representative sample. Only by catching all the fish by draining up the water body, one can be certain that the relationships thus ascertained reflect the true situation in the water body. This, however, is most often not possible and that is why the authors of many papers characterizing the fish fauna tried to define the degree to which their materials are representative for the real situation (Backiel 1964, Larsen 1955, Penczak 1969 b, c, Pratt 1952, Szczerbowski et al. 1968). Such representativeness depends on many factors, mainly on the type of reservoir, the way of fishing, and the species of fish caught. Most often the chance of depicting a real situation decreases with increasing surface of water and the depth of water body. Selective sampling depends on the size of fish. The application of the current-generating aggregate for catching the fish in the water bodies with the depth below 1 m increases the chance of catching a representative sample; such catching depends also on parameters of the current applied and on the skillfulness of the team which handle the apparatus. Taking into account the above mentioned reasons, a trial was also made to define to what a degree the use of aggregate PAP reflects the real situation. The rivers Ryn and Dajna were sampled. The depth of the Ryn River ranged from 10 to 60 cm (30 cm on the average), the width — about 5 m. The Dajna River at the sampling station had a mean depth of 1.2 m, and width ranged from 10 to 15 m. The effectiveness of catching the fish

as related to numbers of fish present in this section was expressed by the following formula:

$$E = \frac{C_1 - C_2}{C_1}$$

where C_1 and C_2 denote number of fish caught in the first and second catch, E equals 0.41, which means that the application of the aggregate even in a shallow stream does not ensure catching the majority of fish.

In the Dajna River, the results of catches were so variable (more fish were caught in the second catch than in the first one) that it was difficult to obtain the proportion of captured fish.

The test catches which allowed to define the occurrence of various species were carried out in the same section of the Ryn River during one day, as well as in the Symsarna River from damming with Lake Blanki to Lake Symsar three times a year (spring, summer, autumn). The index of similarity of the effects of subsequent catches was calculated according to Steinhaus' formula (Perkal 1958):

$$W = \frac{2c}{a+b}$$

where: a — number of species in the first catch, b — number of species in the second catch, c — number of species that are common in the first and second catch.

The index calculated for the two subsequent catches in the Ryn River amounted to 0.91. It should be added that there was one species (pike) more in the first catch than in the second one. The index of similarity for the Symsarna River between the first catch (spring) and the second catch (summer) was 0.85, but between the first catch and the third catch (autumn) 0.76.

These values were high and the differences in the species composition among catches in the Symsarna River could result due to the seasonal changes in the composition of fish fauna (migration of fish) or due to very low numbers of such species as perch-pike and ruff, some individuals of which were caught not earlier than in the last catch.

The applied aggregate turned out to be much less selective than the fishing rods used sometimes for gathering the data on the fish fauna. When using the aggregate, 321 fish were caught of a total weight of 44.95 kg, representing 11 species (Fig. 3) in station 10, and when angling along the banks in the section of several kilometers below the station 10, 407 fish were caught of a total weight of 19.2 kg and they represented only 6 species.

In the catches done with the aggregate the following species predo-

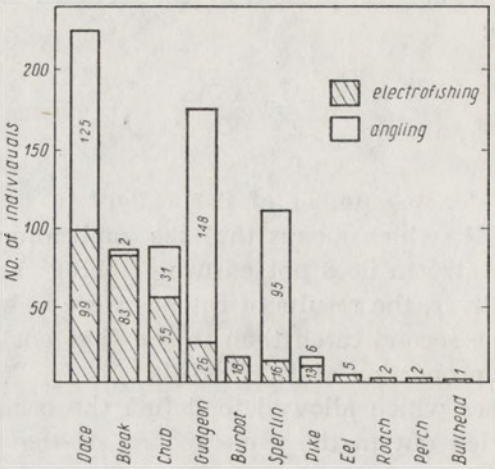


Fig. 3. Comparison of electrofishing and angling effects on ten stations

minated: dace, bleak, chub, whereas in the angling catches gudgeon, dace and sperlin were prevailing. Burbot, common eel, roach, perch and bullhead were not caught with fishing rods at all.

The obtained results permit to conclude that the aggregate applied in the chosen rivers was accurate enough to gather the data describing the species relationships in the investigated habitats.

The total numbers of fish caught in various stations ranged from 23 to 906 individuals and in 86% of cases it exceeded the lower limit of 60 individuals, reported by B a c k i e l (1964). According to him, a sample above this limit is sufficient to represent the fish fauna at a given station.

The sections that were fished were also longer than the lowest limit for sections of small rivers and streams reported by P e n c z a k (1969 b).

Most of the authors describing the fish fauna of running waters (S t a r m a c h 1956 and others) connect the occurrence of various species with the water gradient. Basing on this, S t a r m a c h (1956) divided a river into four regions for which the unit gradients range as follows: the trout region — 80 — 1.5‰, the grayling region — 8 — 1.0‰, the barbel region — 4 — 0.25‰, the bream region — 1.5 — 0.0‰.

It is rather easy to distinguish this regions, but on account of a frequent lack of the leading species in them, the other authors (H o c h m a n 1957, M i š i k 1959, Ž a d i n 1951) proposed another scheme of division into the following regions: 1. source, 2. montane, 3. sub-montane, 4. lowland, 5. estuarial.

In the rivers of the Łyna catchment area, the gradients ranged from 0.45 to 2.90‰ and for some sections of 1 km long were even higher (Fig. 2). This permits to distinguish in them the regions of grayling,

barbel, and bream, or sub-montane and lowland regions, however it should be mentioned that they occur not in a chronological way. In many cases, sections of the same river show high variability and sometimes the region of bream (lowland) is situated above the region of grayling or barbel (sub-montane). For example, a short section of the Dajna River below the Dejnowo Lake, showing a gradient up to 0.25‰ precedes the section of a stream included to the grayling region with unit gradient somewhat higher than 8‰.

This variability was reflected in the species composition of the fish. In the region of grayling (stations 3, 10, 13) it is difficult to distinguish the leading species probably on account of small surface of the section. In the region of barbel, which is represented by 7 stations, the dace was most numerous in 5 cases and in the two remaining — gudgeon and chub. In the region of bream roach was predominant in 5 stations, pike and white bream in 2 stations and gudgeon, dace and chub in the 3 remaining stations. In this region roach was the second in terms of numbers among the fish captured in 5 stations (Table V).

Table V. Number of occupied places (1, 2, 3) by most frequent fishes

Species	Fish zone of								
	Grayling (st. 3, 10, 13)			Barbel (st. 10, 11, 12, 16, 17, 20, 21)			Bream (st. 1, 2, 4—9, 14, 15, 18, 22)		
	1	2	3	1	2	3	1	2	2
Pike						2	2		
Gudgeon	1			1	1			1	1
Bream								1	
White bream							2	1	
Dace		2		5	1		1		2
Chub			1	1	1	3	1	1	
Bleak	1				1			1	
Sperlin						1			
Roach			2		1		5	5	
Stone loach		1			1				
Eel									3
Burbot					1				2
Perch	1					1		2	4

The occurrence or lack of various species is not a sufficient basis to define the regions whose distinguishing is understood as a full chance of development for the fish from which they took their names. As a matter of fact, this refers to pure waters since the fishery classification in the polluted waters in useless. In the Łyna catchment area which is rather pure, such classification rendered difficulty in several stations of the Guber River. Practically there was no fish in a section of about 30 km below Kętrzyn, and the river in its full length had water unsuitable for any usage. The fish captured in it most often gathered around the

inlets of pure waters flowing into the Guber River or on its estuarial section, and their composition ranked this river to the bream region. The Elma River was also relatively polluted but the typical representatives of pure waters were present in it (e.g. barbel). The remaining rivers, Łyna above Olsztyn included, are less polluted and they can be numbered to waters good for culturing the salmonids.

The species composition of the fish fauna was very differentiated between various stations in spite of a great similarity of gradients. Using the following formula for differentiation:

$$W = \frac{c}{a+b-c}$$

(where a and b are numbers of species in two stations, and c is the number of species common for the two stations) it was found that the most close were stations 10 from the Symsarna River and 17 as well as 18 from the Dajna River, where 75% of species of the total number of species caught were common. The majority of stations were more differentiated and the number of common species ranged from 26 to 50%. Most different from the remaining stations were stations No. 11 and 12 of the Elma River and No. 7 and 8 of the Symsarna River, where less than an average number of fish species were caught (Fig. 4).

In general, there is no clear division of the fish fauna in the fish regions in spite of a rather high differentiation both of habitats and the fish in the rivers of the Łyna catchment area. These regions can be distinguished only on the basis of their general character, gradients mainly, which are decisive for other factors (Backiel 1964). It seems more appropriate to distinguish the occurrence of dominant species in the Łyna River, as had been done by Backiel (1964), Penczak (1969 b), and Żarnecki, Kołder (1956) for other environments. In the barbel region, as defined by gradients, dace was most numerous and in the bream region roach as well as white bream and pike were predominant. In the whole catchment area of the Łyna River no connection was found between commonly used classification of rivers and the leading species. This suggests some inadequacy of such classification, especially for the regions of lesser vertical differentiation and in distinguishing the regions of grayling, barbel and bream. A total number of captured fish does not reflect thoroughly all the fish present in the catchment area of the Łyna River. Neither typically lake species were found nor such as threespine-stickleback (*Gasterosteus aculeatus* L.), sheat-fish (*Silurus glanis* L.), and ide (*Leuciscus idus*), whose occurrence was mentioned by a number of anglers. Nevertheless the fish fauna is diversified and with these latter is smaller by 3 species than that in the

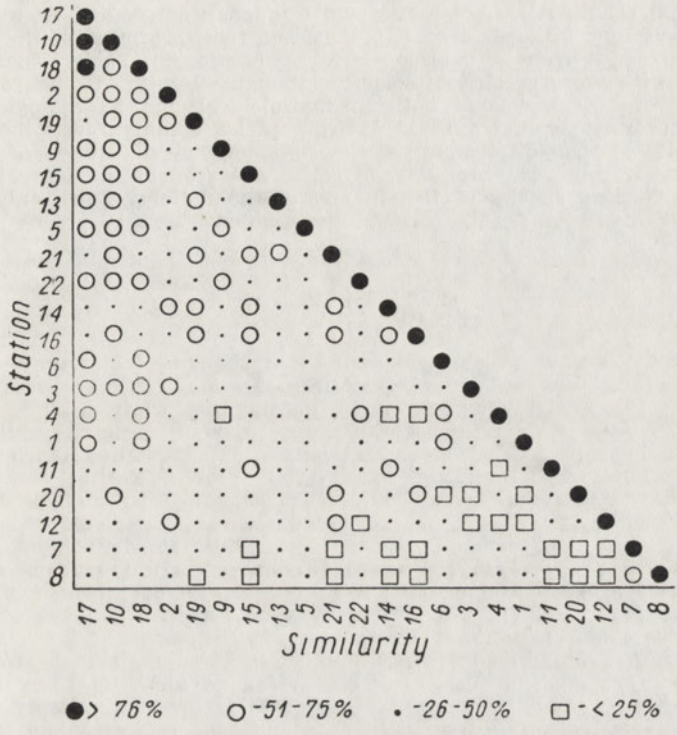


Fig. 4. Similarity of stations on account of met species

adjacent catchment area of the Drwęca River, where sunbleak (*Leuciscus delineatus*), ninespine-stickleback (*Pongitius pongitius*), thunder fish (*Misgurnus fossilis*) and bitterling (*Rhodeus sericeus amarus*) were additionally caught when using an aggregate. Number of species was also somewhat smaller than that in the catchment areas of the rivers: Bzura, Pilica, and Warta (P e n c z a k 1968 a, b, 1969 a), however, it surpassed the number of species encountered in the submontane rivers or in other upland rivers (S a k o w i c z 1961 a). On the basis of the collected material it should be stated that the Łyna River is a lowland river and in the majority of cases it can be classified together with its affluents as the region of barbel and bream.

5. SUMMARY

Characteristics of the fish fauna inhabiting the running waters of the catchment area of the Łyna River was the aim of this paper (Fig. 1). The materials were gathered in 1970 and 1971 (Table I), taking into account the main hydrological properties (Table II and Fig. 2), and the fish fauna captured by means of current-

-generating aggregate and the fishing rods. In the catches done with the aggregate at 22 stations, 5057 individuals were caught, among which most numerous were the following: roach (*Rutilus rutilus* L), dace (*Leuciscus leuciscus* L.), and gudgeon (*Gobio gobio* L.) (Table III). Considering the frequency of occurrence, gudgeon was the first followed by pike (*Esox lucius* L), dace and roach. Number of the fish captured in stations ranged from 23 (station 12) to 906 (station 9) (Table IV). The catches with the aggregate were much less selective than those with fishing rods (Fig. 3). The stations were differentiated both in hydrological and ichthiological aspects. The majority of stations had only 26–50% of species common for other localities (Fig. 4). In spite of such differentiation the commonly used division of rivers into the fish regions was not the case for the catchment area of the Łyna River since there were no leading species of fish in any of the regions. Thus, only dominant species were distinguished for the stations investigated (Table V).

6. STRESZCZENIE

Celem przedstawionej pracy było scharakteryzowanie ichtiofauny zamieszkującej wody bieżące zlewiska rzeki Łyny (Fig. 1). Materiały do pracy zbierano w latach 1970 i 1971 (Tab. I), uwzględniając podstawowe właściwości hydrologiczne (Tab. II, Fig. 2) oraz ichtiofaunę pozyskaną przy użyciu agregatu prądotwórczego i wędek. W połowach prowadzonych agregatem na 22 stanowiskach pozyskano 5057 ryb, wśród których największy udział miała płotka (*Rutilus rutilus* L), następnie jelec (*Leuciscus leuciscus* L.) i kiełb (*Gobio gobio* L.) (Tab. III). Pod względem częstotliwości występowania na pierwszym miejscu wymienić należy kiełbia, następnie szczupaka (*Esox lucius* L.), jelca i płoć. Ilość złowionych na poszczególnych stanowiskach ryb wahała się od 23 (stanowisko 12) do 906 (stanowisko 9) (Tab. IV). Połowy agregatem były o wiele mniej selektywne niż połowy na wędkę (Fig. 3). Poszczególne stanowiska były zróżnicowane zarówno pod względem hydrologicznym jak i składu ichtiofauny. Najwięcej z nich posiadało tylko 26–50% gatunków wspólnych z innymi stanowiskami (Fig. 4). Pomimo takiego zróżnicowania powszechnie przyjęty podział rzek na krainy rybne w zlewisku Łyny nie znalazł wyraźnego odzwierciedlenia, bowiem w żadnej krainie nie występowały ryby przewodnie. Z tego względu na poszczególnych stanowiskach wyróżniono więc tylko gatunki dominujące (Tab. V).

7. REFERENCES

Backiel, T. 1964. Populacje ryb w systemie rzeki Drwęcy [Fish populations in the Drwęca River system]. *Roczn. Nauk roln., Ser. B*, 84, 193–214 [Engl. summ.].

Draganik, B., Szczebowski, J. A. 1963. Wpływ odłowów elektrycznym agregatem prądotwórczym na liczebność drapieżnych ryb w potoku Kośno [The influence of catches by means of an electric shocker on the number of predatory fish in Kośno stream]. *Zesz. nauk. WSR Olszt.*, 16, 73–77 [Engl. summ.].

Hochman, L. 1957. Ichtyologicky vyzkum řeky Moravice [Ichtyology ivestigation of the Moravice River]. *Sb. vys. Sk. zeměd. les. Fac. Brno, Ser. A*, 1, 83–117.

Larsen, K. 1955. Fish population analyses in same small danish trout streams by means of DC electrofishing. *Medd Danm. Fisk-og Havunders., Ny Ser.*, 1 (10) 1–62.

Mišik, V. 1959. Ichtyofauna rieky Kysuce [Ichthyofauna of the Kuysuca River]. 7–40, Bratislava, Vyd. Slov. Akad. Nauk.

Penczak, T. 1968 a. Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Cz. I a. Hydrografia i rybostan Bzury i jej dopływów [The ichthyofauna of the rivers of the Łódź Upland and adjactent areas. Part I a. The hydrography and fishes of the Bzura River basin]. *Acta Hydrobiol.*, 10, 471–497 [Engl. summ.].

Penczak, T. 1968 b. Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Cz. I b. Hydrografia i rybostan Pilicy i jej dopływów [The ichthyofauna of the rivers of the Łódź Upland and adjactent areas. Part I b. The hydrography and fishes of the Pilica River basin]. *Acta Hydrobiol.*, 10, 499–524 [Engl. summ.].

Penczak, T. 1969 a. Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Cz. I c. Hydrografia i rybostan Warty i jej dopływów [The ichthyofauna of the

rivers of the Łódź Upland and adjacent areas. Part I c. The hydrography and fishes of the Warta basin]. *Acta Hydrobiol.*, 11, 69–118 [Engl. summ.].

Penczak, T. 1969 b. Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Cz. II. Ekologia [The ichthyofauna of the rivers of the Łódź Upland and adjacent areas. Part II. Ecology]. *Acta Hydrobiol.*, 11, 313–338 [Engl. summ.].

Penczak, T. 1969 c. Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Cz. III. Przegląd i charakterystyka gatunków [The ichthyofauna of the rivers of the Łódź Upland and adjacent areas. Part III. A review and character of species]. *Acta Hydrobiol.*, 11, 339–360 [Engl. summ.].

Perkal, J. 1958. *Matematyka dla przyrodników i rolników* [Mathematics for scientists and agronomists]. Warszawa PWN.

Pratt, V. S. 1952. A measure of the efficiency of alternating and direct current fish shockers. *Trans. Am. Fish. Soc.*, 81, 63–68.

Sakowicz, S. 1961 a. Ichtiofauna potoku Trzebiocha [Ichthyofauna of Trzebiocha Stream]. *Roczn. Nauk roln., Ser. D*, 93, 365–386.

Sakowicz, S. 1961 b. Rozród troci (*Salmo trutta morpha lacustris* L.) z jeziora Wdzydze [Propagation of trout (*Salmo trutta morpha lacustris* L.) from the Lake Wdzydze]. *Roczn. Nauk roln., Ser. D*, 93, 501–556 [Engl. summ.].

Starmach, K. 1956. Rybacka i biologiczna charakterystyka rzek [Characteristic of rivers from biological and fishery point of view]. *Pol. Arch. Hydrobiol.*, 3, 307–332 [Engl. summ.].

Szczerbowski, J. A., Grudniewski, Cz., Draganik, B. 1968. Efektywność odłowu ryb przy pomocy agregatu prądotwórczego w potoku Kośno [Intensivität der Befischung mit einem Stromerzeugenden Aggregat im Kosnobach]. *Zesz. nauk. WSR Olszt.*, 24, 509–520 [German summ.].

[Žadin, V. I.] Жадин, В. И. 1951. Опыт гидробиологической классификации рек СССР и деление рек на участки [Attempt of hydrobiological classification of rivers in USSR and rivers division on classis]. *Trudy Karelo-Finskogo otd. vses. naučno-issled. Inst. Ozern. rečn. ryb. Hoz.*, 231–243.

Żarnecki, S., Kołder, W. 1956. Ichtiofauna Wisły Śląskiej [Ichthyofauna of the Silesian Vistula]. *Biul. Zakł. Biol. Stawów PAN*, 3, 19–45 [Engl. summ.].

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	437–450	1972

O. MATLAK

SODIUM SALT (2,4-D) RELATED TO EMBRYO AND LARVAL DEVELOPMENT OF CARP

Experimental Farm of Laboratory of Water Biology, Polish Academy of Sciences, Gołysz, P.O. Chybie, Cieszyn district, Poland

ABSTRACT

Toxic influence of sodium salt 2,4-D upon embryo and larval development of carp was studied. Delayed hatchings in both herbicide concentrations were observed; larvae from 50 mg/l concentrations were of the smallest body length. The toxic influence of the 50 mg/l concentration was remarkable in the larval stage. On the fifth day since the hatching, mass mortality was observed among larvae in the 50 mg/l aquarium. In dead larvae hydrocoele embryonalis, deformations of the spinal chord and a swollen yolk sac were observed. 40 carp larvae and *Daphnia magna* survived the toxic impact of the herbicide in the concentration of 50 mg/l.

1. INTRODUCTION

Among herbicides being commonly used now, there are derivates of 2,4 dichlorophenoxyacetic acid. Sodium salt in solid form for preparing water solutions and ester solutions in organic solvents are its most often used forms.

Investigations of derivates of 2,4-D were initiated in the United States in 1941–1942 by Pokorny (chemical composition), and Zimmerman, (influence on physiological processes) (Herbicide handbook, 1967). These combinations, called also synthetic auxines, are, according to many authors, not very toxic for warm-blooded animals because of their close chemical relationship to natural plant material (Kurth 1963, Reimann 1969). Their damaging influence may be revealed as a secondary effect of the decay of plants destroyed by them (formation of H_2S, oxygen deficit — Reimann 1969). Although derivates of 2,4-D are, in general, not very toxic (Schlüter 1963, Ostrowski, Rola 1964), humans are likely to be exposed in professional contacts or by consumption of contaminated products in consequence of the increasing application of chemicals in agriculture (Stanosz 1971). Moreover, some of the discussed herbicides, e.g. esters, are more toxic (Solski 1968) and their impact can be further enhanced as a result of synergetic action of some preparations (Bandt 1957, Kurth 1963, Łakota 1970a, b, Okołotowicz 1971). For those reasons it is important, especially in experiments, to designate accurately what form of preparate has been used (Alabaster 1967). However, most of the herbicides are treated as practically innoxious agents when used in prescribed amounts and in not too shallow water reservoirs (Bauer 1961).

Derivates of 2,4-D are widely used in agriculture for destroying dicotyledonous weeds in growings of winter and spring corns, maize, fodder grasses, as well as in forestry. One of the preparates produced by Polish industry is "Pielik", the sodium salt of 2,4-D. The period of spring application of "Pielik" may coincide with the period of carp reproduction, when fishery shallow ponds are active (warmponds, spawning ponds, fry = nursery ponds). In some regions of Poland

it is also a period of more intensive rainfalls of stormy origin. Therefore "Pielik" may be dangerous, the more so as increasing amounts of pesticides are applied in Poland and the treated area is extending (Kołaczkowski 1971). Up to 1960 this area was only about 10% of corn growings (excluding rye), whereas during the following decade it has increased to more than 40%, and by 1970 it was already 65%. The highest amounts of applied herbicides were recorded in the western regions of the country (Rola 1972).

In 1971 the Laboratory of Water Biology of Polish Academy of Sciences in Cracow, and the Department of Bioenergetics and Bioproductivity of Nencki Institute of Experimental Biology in Warsaw started a project which has been carried out in Experimental Farm of PAS, Gołysz, Cieszyn district. Its aim was to examine the damaging influence of two solutions of the sodium salt 2,4-D on embryo and larval development of carps incubated in artificial conditions (the present paper), and to measure respiration and calorific value (Kamler 1972). The discussed investigations were carried out in cooperation, though they are reported in separate publications.

Among Polish authors it was only Solski (1968) who performed similar investigations, on carp eggs obtained from natural spawning-grounds, with jelly on them and exposed to various concentrations of atrazine. Among foreign authors, Veselov et al. (1965), Vladimirov, Sabodaš (1968) and Podoba (1968 a, b) investigated the youngest stages of carp. Others used fry or two-year old carps for their tests (Sushchenya 1956, Kurth 1963, Bandt 1957, Łakota 1966, 1970 a, b, Trzebiatowski 1968 and others). Most of biotests were performed on more vulnerable fishes than Cyprinidae, e.g. on species of the families of Salmonidae, Percidae, Esocidae (Kurth 1963, Alabaster, Abram 1965, Alabaster 1967, Łakota 1970 a, b, Okołotowicz 1971, Łysak, Marcinek 1972 and others).

Toxicity of derivates of 2,4-D was also investigated to determine the admissible concentrations in surface waters not used for municipal purposes (Okołotowicz 1971), to discover the biochemical processes occurring in water (Piątkowska 1971), to assess its influence on warm-blooded animals (Stanosz 1971), and from other points of view (Stanisławska 1971, Baltbarzdis et al. 1968, Solski 1968).

2. MATERIAL AND METHOD

A ready for use preparation was used, containing 85% of the sodium salt of the 2-chlorophenoxyacetic acid (2,4-D) manufactured by Nadodrzańskie Zakłady Przemysłu Organicznego Rokita at Brzeg Dolny, trade-marked "Pielik". The test solutions contained 5 and 50 mg/l of active substance. Solutions were made in water from the inlet flowing near the incubation pond (total hardness 4.0 German degrees); they were administered continuously, according with the amount of water flowing through the incubation vessels (3 l/min through each of them). The test solutions were dosed from 10 l scaled bottles placed over the vessels. The bottles were connected with them by glass tubes passed through the bottles' corks, with gum hoses on them, attached at the opposite sides to metal tubes placed in the middle part of net circles in the lower part of each appliance. Fresh solutions were prepared every 10 hr.

Spawn for tests was taken from 5 year old carp spawners grown in the farm Ochaby from fry originating from the voivodship of Białystok (Knyszyn farm). The females weighed 5.0–5.5 kg; the males were smaller. The females had well developed gonads and their efficiency was 600 g of eggs. The eggs were fertilized with sperm of three males. They were freed from jelly (the temperature of water and of other liquids was from 20.9 to 21.5°C, Matlak 1970), carefully mixed together and then distributed again into three portions, 300 g each, and placed into the three incubators. The eggs were incubated in heated water, in Weiss incubating vessels, 7 l of capacity. Every 2 hr water temperature was gauged in the incubators, in the supplying reservoir and in the aquaria. Water for incubation and for aquaria tests was drawn from the inlet mentioned above; it was magazined in a basin outside of the incubation place. Before being supplied into the incubators, water was passed through a filtering chamber with gravel and activated coal. Only when roiled up stormy water flew in, tap water from the Goczałkowice reservoir was used.

To sterilize water and to avoid mildew development on unfertilized or damaged

eggs, it was radiated with a bacteriocide lamp (type L-18) and the eggs were bathed twice in methyl blue for 10 min (Matlak 1970).

Embryo development of eggs from both solutions of 2,4-D and from the control were observed under a microscope in vivo in a water drop, drawn with eggs. The consecutive stages of embryo development were photographed (Photos 3 M,V–X and 4 by the author, the others by M. Janik).

Investigations were continued in aquaria (90×50×55 cm) filled with 35 cm of water column. Larvae development in aquaria occurred in continuously aerated water, at 21.0–22.4°C, at 7.04–7.68 mg/l of oxygen contents. In each aquarium there were 10,000 carp larvae transported from the vessels after incubation. Three days after the transportation plankton was added as food for the larvae. The plankton was drawn from experimental ponds at Gołysz or from laboratory cultures (*Daphnia magna* of various size and other species).

The herbicide was dosed to the aquaria daily by exchanging 80 l of water and introducing fresh test solutions. After incubation, samples of larvae were drawn from each vessel (60 individuals from each) to measure their lengths. Measurements were taken on material conserved in 2% formaline solution.

3. RESULTS

EMBRYO DEVELOPMENT

The investigations covered the embryo (74—88 hr) and larval (144 hr) stages of development of carp, about 10 days in total. Eggs were fecundated on May 28, at 3 p.m. and cleaned of jelly by 5 p.m.; whereupon they were placed in Weiss vessels. At this moment the eggs were at the stage of 4 blastomers. From this point until hatching they have been developing in a continuously intoxicated environment (vessel No. 1 — control, No. 2 — 5 mg/l solution of the herbicide, No. 3 — 50 mg/l). In Figures 1 and 2 are presented the results of continuous water temperature measurements in Weiss vessels and in aquaria. During the experiments there were damages caused by a change of the source of water feeding the incubators. Table I and Figure 3 present the stages of embryo development, mean daily water temperatures and the number of degree-days (D°).

Embryo development of eggs in both herbicide solutions and in the control was in general similar. Only in the stage of gastrula a difference

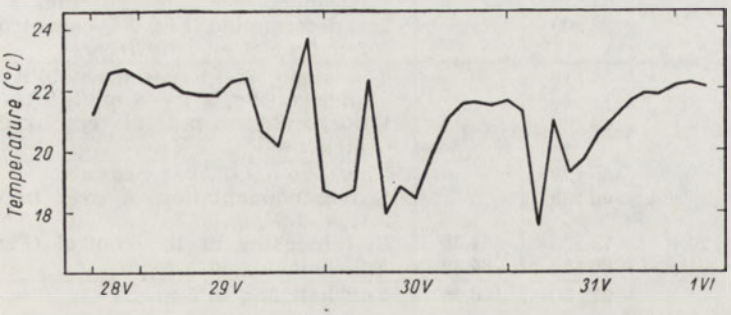


Fig. 1. Water temperature during incubation of eggs in Weiss incubating vessels

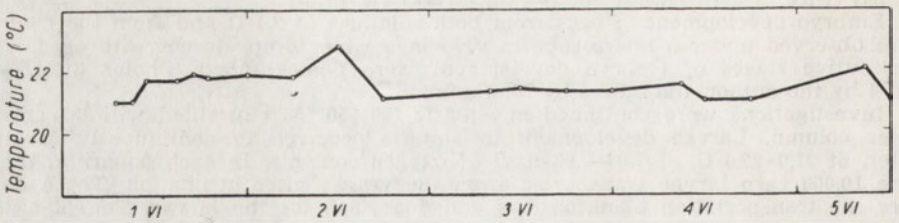
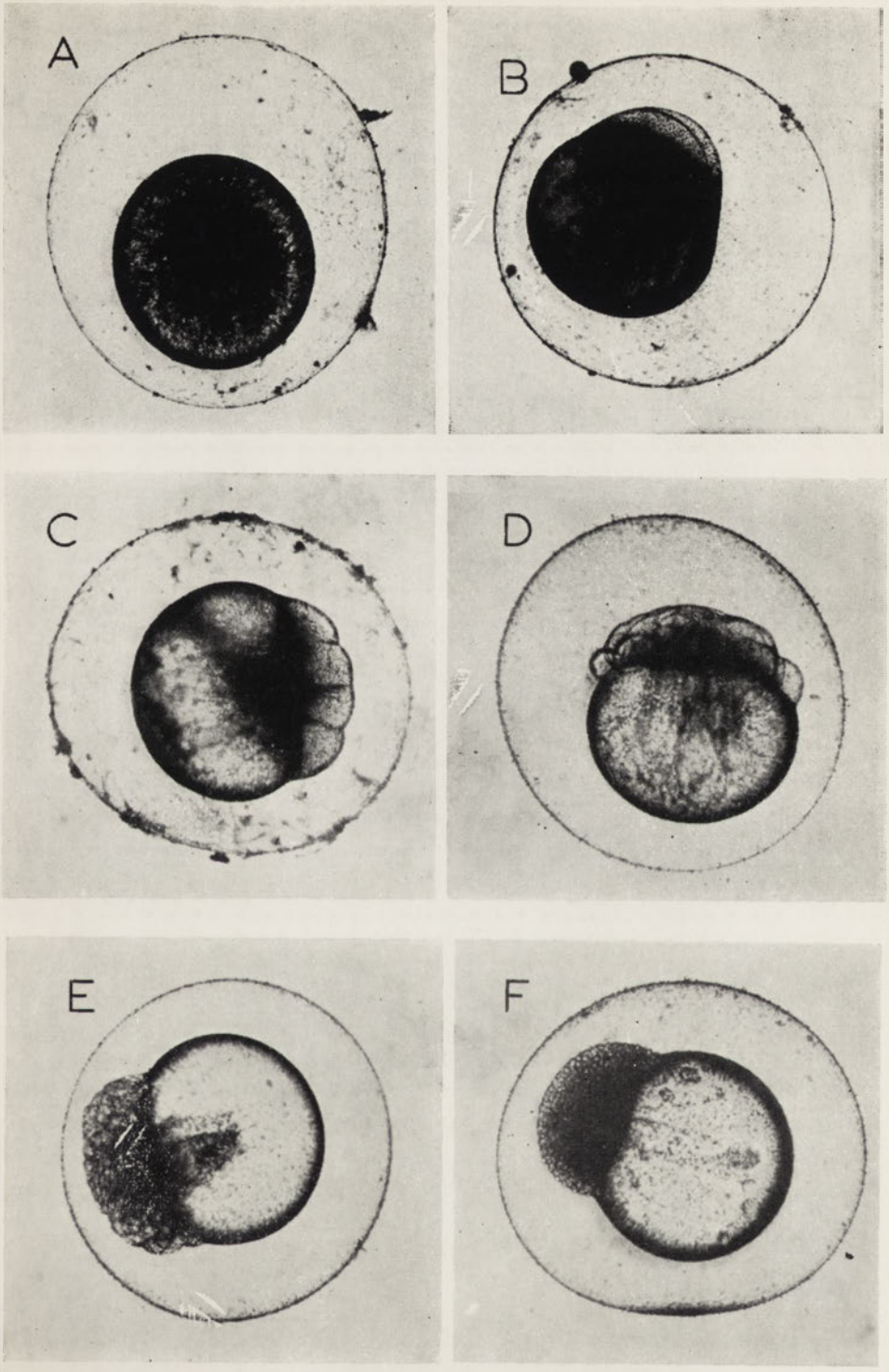
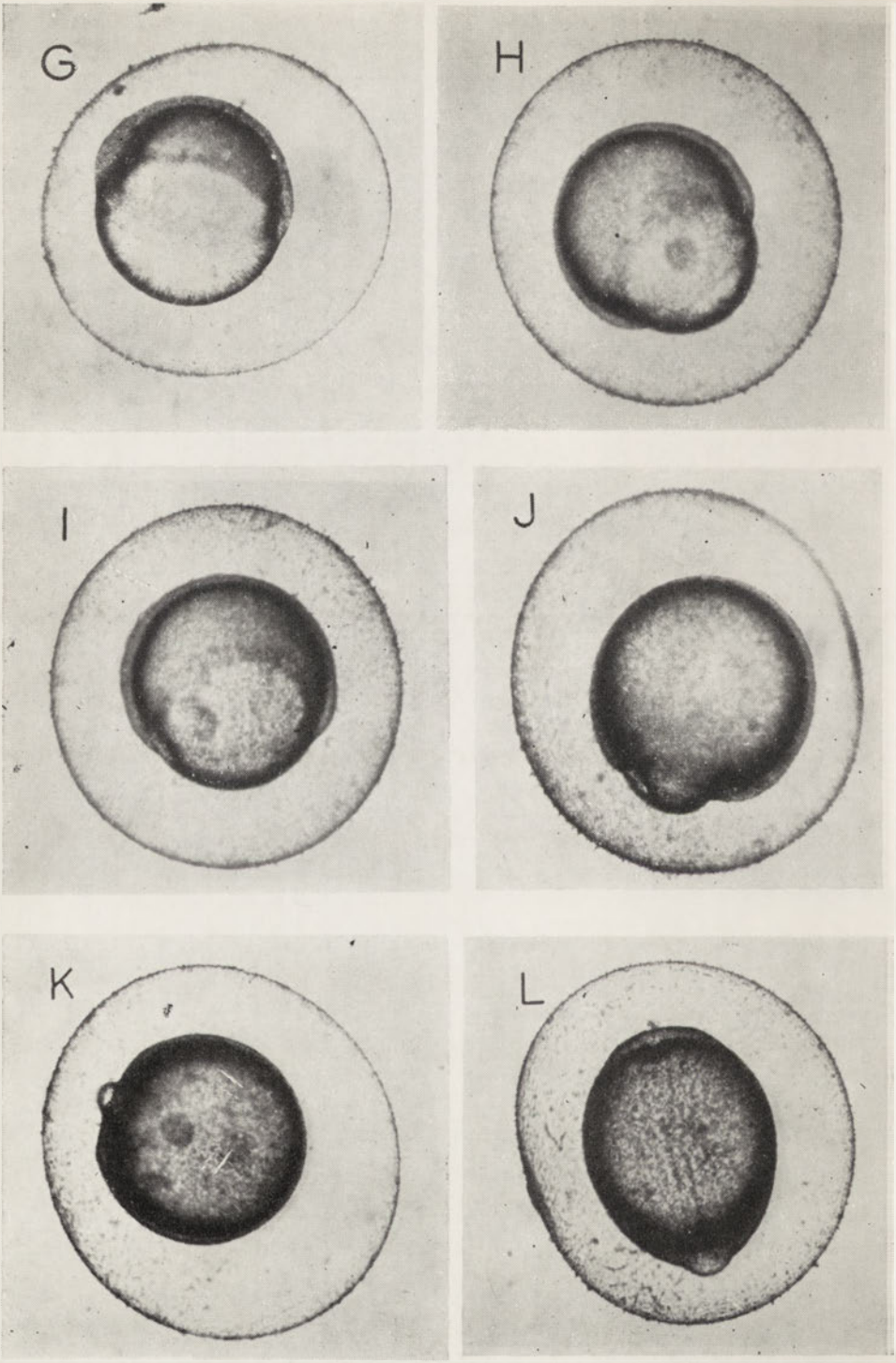


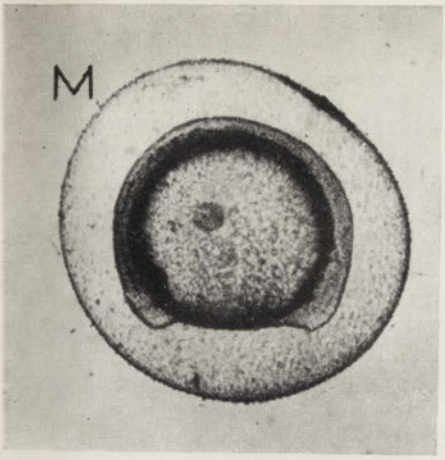
Fig. 2. Water temperature during development of larvae in aquaria

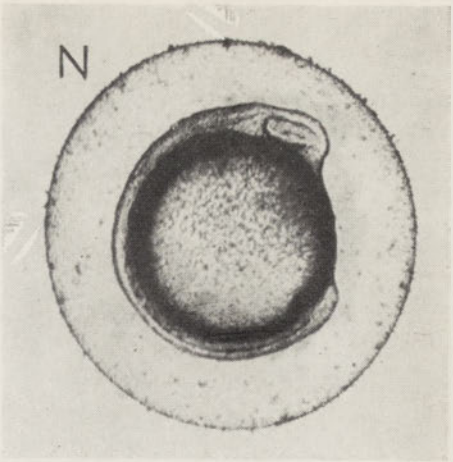
Table I. Embryo development of carp. Fecundation on May 28, 1971, 3 p.m.

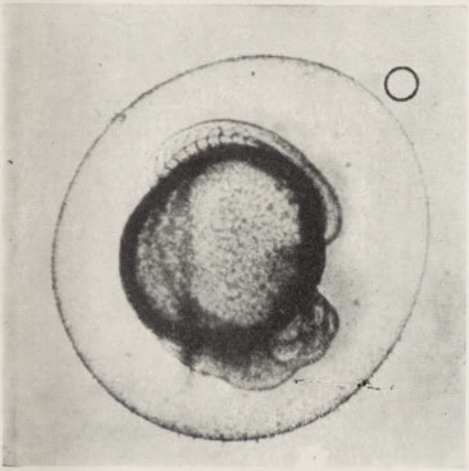
Date	Medium daily temp. (°C)	Time since fecundation hr min	Degree-days	Stage of development
		1 : 30		Formation of the space around the yolk and surface currents of plasma (Fig. 3 A)
		1 : 40		The 2 blastomers stage (Fig. 3 B)
		2 : 15		The 4 blastomers stage (Fig. 3 C)
		2 : 50		The 32 blastomers stage (Fig. 3 D)
May 28	22.2	3 : 15		Further stages of egg division
		4 : 30		Multicell morula (Fig. 3 E)
		6 : 00		Microcell morula (Fig. 3 F)
		7 : 30		The stage before gastrulation
		10 : 00		The stage of early gastrula (Fig. 3 G — control, 3 H — 5 mg/l, 3 I — 50 mg/l)
		12 : 40		Gastrula with a large yolk plug (Fig. 3 J)
		13 : 05		Gastrula with a small yolk plug (Fig. 3 K)
		15 : 00		Neurula formed up (Fig. 3 L)
		17 : 00		Embryo formation stage; eyes begin to emerge (Fig. 3 M)
May 29	21.6	20 : 45		Somite formation, elongated eye stage (Fig. 3 N)
		23 : 30		Consecutive stages of embryo formation (Fig. 3 O)
		27 : 30		First motions of embryo (Fig. 3 P)
		33 : 00		Tail deformation (Fig. 3 Q — control, 3 R — 5 mg/l, 3 S — 50 mg/l)
		34 : 15		The stage of increasing mobility of the embryo (Fig. 3 T — 5 mg/l, 3 U — 50 mg/l)
May 30	20.1	49 : 00		Embryo able to pull the egg by energetic motions (Fig. 3 V)
		65 : 20		The first few larvae recorded
		66 : 35		Strong pigmentation of eyes in embryos (Fig. 3 W)
May 31	20.4	73 : 50	84.38	Full hatching in the control (Fig. 3 X)
		80 : 50	84.89	Full hatching in 5 mg/l
June 1		87 : 50	106.95	Full hatching in 50 mg/l

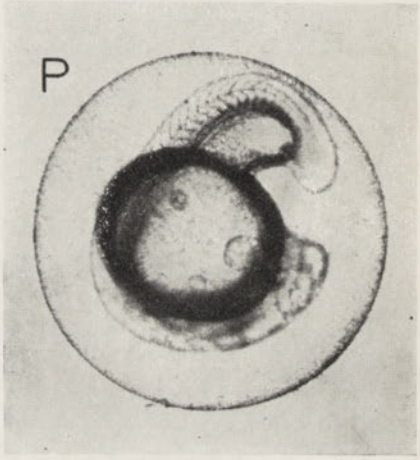


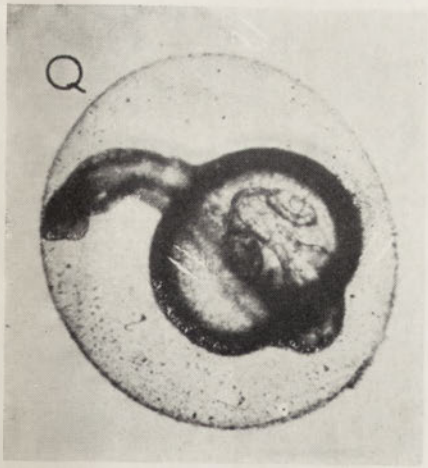


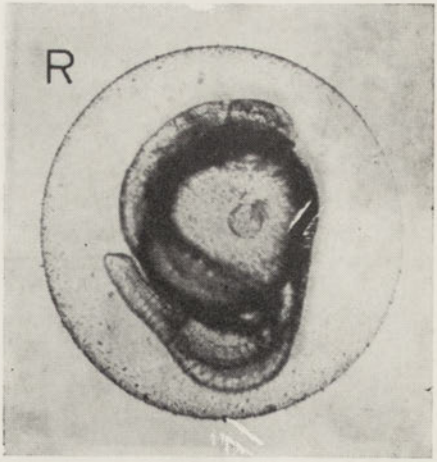


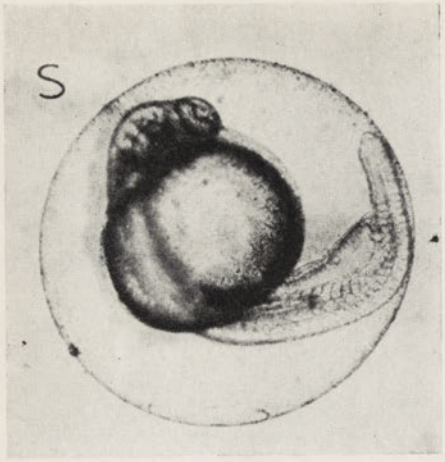


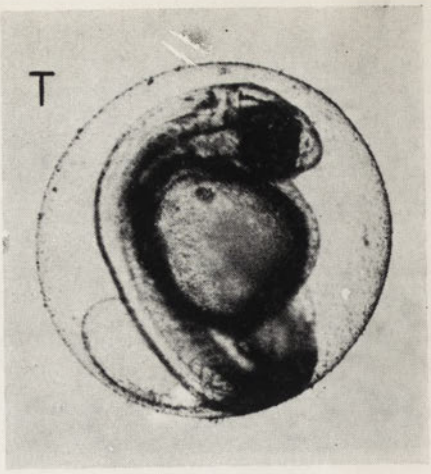


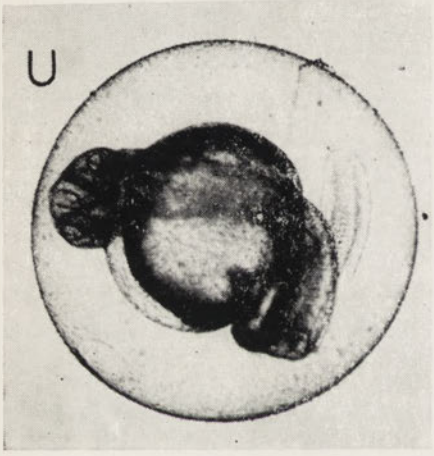


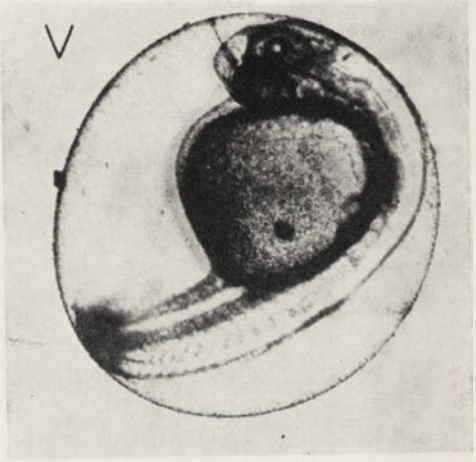


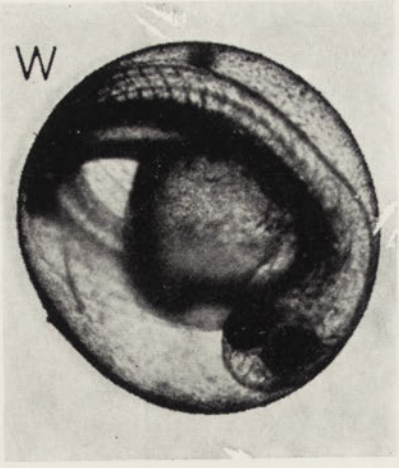












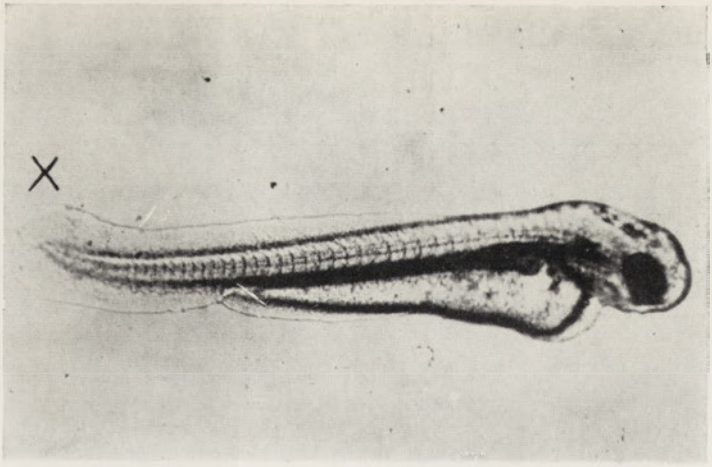


Fig. 3. Embryo development of carp (Compare Table I)

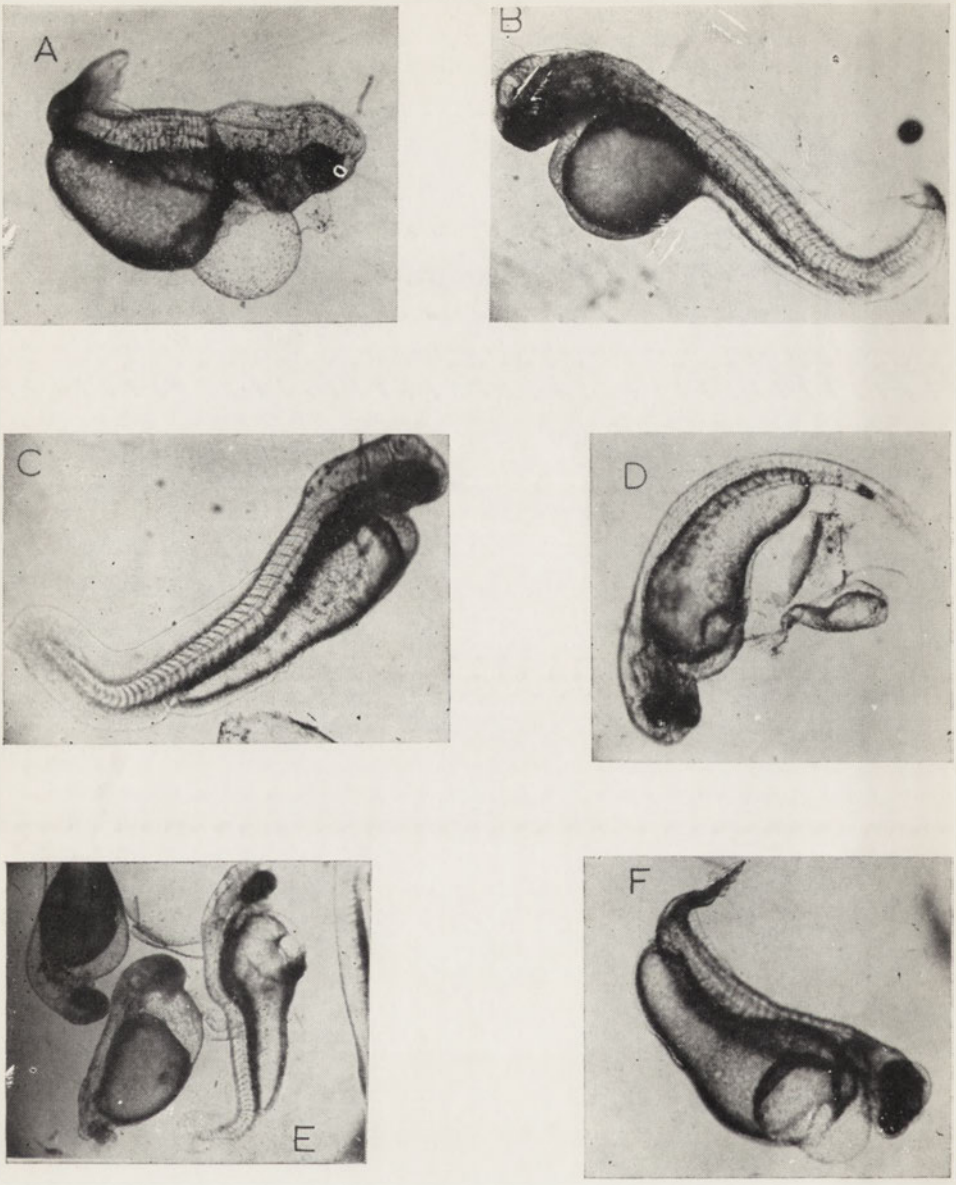


Fig. 4. Body deformations of carp larvae growing in sodium salt 2,4-D (50 mg/l)

was noticed, consisting in a slightly higher advancement of eggs from both herbicide solutions in comparison with the control (Fig. 3 GHI).

After 33 hr since fecundation a deformation of the tail part was noticed in some embryos in all incubators. The end part of the tail was narrowed and the skin fold was lacking (Fig. 3 QR). Since the deformation was noticed in all the three incubators, it cannot be ascribed to the herbicide's influence.

During the embryo development there were also differences in the losses between the Weiss vessels with different solutions and at the several stages of embryo development (Table II). The losses during the

Table II. Embryo development of carp in various concentrations of sodium salt 2,4-D and in the control

Specification	Control (%)	5 mg/l (%)	50 mg/l (%)
Unfecundated eggs	8.3	12.9	7.9
Fecundated eggs	91.7	87.1	92.1
Eggs dead until May 30 (fecundated)	24.1	34.7	24.4
Eggs dead until the hatching	36.7	14.8	45.3
Dead eggs, total (excluding unfecundated ones)	60.8	49.5	69.7
Alive hatched larvae	30.9	37.6	22.4
Normal larvae among those hatched alive	21.4	17.4	18.4
Deformed larvae	9.5	20.2	4.0
Total losses of eggs and larvae (including unfecundated and deformed ones)	78.6	82.6	81.6

whole observed period were almost identical in both vessels with herbicide solutions, while they were by only 3–4% lower in the control.

Hatching was observed earliest in the control vessel, 7 hr later in the vessel with 50 mg/l of 2,4-D, and 14 hr later in the vessel with the lower herbicide concentration (Table I).

Deformed larvae were noticed during their transport after hatching from the incubators to the aquaria. As it can be seen in Table II, there were most of them in vessel No. 2 (5 mg/l), in which disturbances, mentioned above, occurred shortly after the eggs had been placed in it. This kind of deformations were ascribed by Lieder (1955 a) to oxygen deficits during incubation. Analogous deformations were also observed by the author as a result of a prolonged chilling of water during embryo development of carps (Matlak 1969 b), and thus they should not be associated with the effects of the herbicide.

LARVAL DEVELOPMENT

After hatching, the larvae were transported to aquaria numbered 1, 2, 3 (control, 5, 50 mg/l, respectively). Most of them were vertically attached to the walls of the aquaria; others dwelled in water at various

depths or on the bottom. The most numerous clusters of larvae were placed near the back walls and their corners.

Symptoms of the noxious influence of the higher herbicide concentration could be observed in aquarium No. 3 at the moment of transport of the larvae. It was expressed in their behaviour which could be called, after Schäperclaus (1954) and Bauer (1961), as the stage of an initial anxiety. Similar symptoms were observed in this aquarium during two consecutive days, while in the other two aquaria the larvae behaved normally.

On the second day after hatching a few dozens of dead larvae were found in all the three aquaria. These were the deformed individuals, or damaged by manipulations at transport.

More conspicuous symptoms of the toxic effect of the sodium salt of 2,4-D in the concentration of 50 mg/l could be observed after 70 hr since the hatching; the larvae were more vulnerable and their balance was distorted. On the next day, June 5 on 7 a.m. most larvae in aquarium No. 3 dwelled over or on the bottom in lateral positions, many of them with symptoms of paralysis. Some larvae tried to jump up and swim, but they were falling to the bottom again, only to continue the same movement after a moment of "rest". In the two other aquaria the larvae behaved normally.

On the sixth day of observation, at 8 a.m., only 40 larvae were healthy in aquarium 3. The others, dead or ill, dwelled on the bottom. In the sample containing 60 larvae drawn at that time, various deformations were observed in 51 individuals; the most frequent was hydrocoele embryonalis, ventral or spinal deformations of the spinal chord or of the end part of tail only; in many individuals an enlarged and round yolk sac evidenced difficulties in resorbtion. In most cases all deformations were observed in the same individuals (Fig. 4).

In *Daphnia magna* no toxic influence was found in aquaria, though a technical preparation was applied, more toxic than the chemically pure substances. Daphniae dwelled on various levels in aquaria water; they were most numerous near the surface. Many Daphniae had sporing eggs.

On June 6, from 11 a.m. to 4 p.m., there was a break in electric current supply, caused by a network damage. The aquaria failed to be aerated during five hours and larvae died in great numbers (aquaria 1 and 2). At 11 a.m. the experiment was ended.

In samples drawn from aquaria 1 and 2 (40 pieces each), hydrocoele embryonalis was observed in a few individuals, most of them weak or deformed ones. In the sample from aquarium 3 this disorder was observed in all larvae. A few dozens of larvae from aquarium No. 3 survived the toxic influence of 50 mg/l concentration of 2,4-D, which

was an evidence of their great vitality and individual resistance. Beginning from the 7th day after the hatching they were growing quickly (Fig. 5) and their alimentary canals were well filled up.

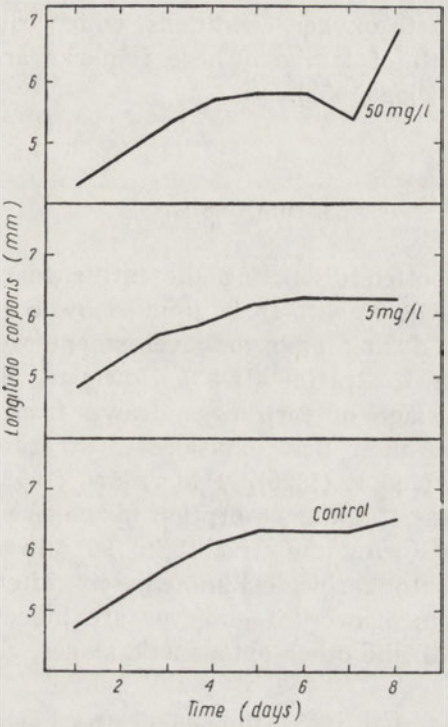


Fig. 5. Growth of carp larvae in aquaria

The measurements showed that the body lengths were smallest in individuals developed at 50 mg/l of herbicide concentration. The differences were found in larvae immediately after the hatching and they remained conspicuous until the end of the experiment (Table III). On June 5 and 6, the medium body length in the control was 6.3 mm and at 50 mg/l it was 5.8 mm. The differences were statistically highly

Table III. Results of measurements of body length (l.c.) of carp larvae after hatching

Date	Longitudo corporis (mm)		
	Control	5 mg/l	50 mg/l
May 31	4.7		4.3
June 1		4.8	
June 3	5.7	5.7	5.3
June 4	6.1	5.9	5.7
June 5	6.3	6.2	5.8
June 6	6.3	6.3	5.8

significant in both cases ($p<0.001$). From the third to the sixth day after the hatching, the growth curve of larvae was broken down in relation with the shift from embryo to branchial respiration (Fig. 5). It is possible that this shift involves an impairement of respiration and a greater vulnerability to oxygen conditions, which can have a negative effect upon the growth of larvae. (Those remarks are consistent with the findings by Pliszka 1953).

4. DISCUSSION

No works were spotted discussing the influence of 2,4-D solutions upon carp embryo development. It is implied by Solski's (1968) experiments that carps during embryo development were vulnerable to various concentrations of atrazine (H-53). However, Solski failed to define precisely the stage of carp eggs drawn from spawning places and at the moment of their first exposure to atrazine. It can be seen from data by Bogucki (1928), Pliszka (1953) and Matlak (1969 a) that the process of water absorption to the space around the yolk is the most intensive during the first 15 to 90 min after fecundation. Exposure of embryos to herbicides immediately after the fecundation of eggs can provide an answer, if embryos are indeed the most resistant in comparison with the other ontogenetic stages, as Solski (1968) suggested.

It can be expected that the influence of the herbicide would have been even more clear, if it had been applied at the moment of fecundation, and during separation of eggs from the jelly, rather than after 2 hr delay; this point ought to be considered in subsequent experiments.

Opinions about deprivation of jelly are not consistent. Dziekońska (1956) suggested that the separation from jelly stimulates embryo development, but handicaps the process of hatching. Pliszka (1953) stated that the lack of jelly improved the conditions of respiratory and gas metabolism of an embryo and thereby facilitated the hatching. In our opinion, the technique of separation of eggs from jelly must be improved in order to eliminate the influence of mechanical factors.

Cases of unsynchronic development of carp eggs were observed. They were also found by Kokurewicz (1970) in tench and, less frequently, in roach, hatched in identical temperature and simultaneously.

The fact that after 33 hr from the fecundation and after the hatching, deformed embryos and larvae were found in all the vessels, excludes the influence of both concentrations of 2,4-D and suggests that the deformations have been caused by the disturbances, mentioned above, involved by the shift of the source of water supply, when muddy, stormy

water flowed into the feeding pipeline. Lieder (1955 a, b), Domurat (1956), Dziekońska (1956), Gottwald (1960), Kokurewicz (1969, 1970) and Matlak (1969 b) explain the cases of anomalies, retarded development and death of embryos by oxygen deficits and oscillations of temperature. According with Trifonova (1949), there are three critical periods in embryo development, during which even slight deviations from normal conditions (a slight change in the pH value, a minor increase of temperature, a decrease in oxygen contents, an increase in CO_2 concentration) are lethal for embryos. Sometimes early embryo damages can have genetic background (Schäperclaus 1954).

Hydrocoele embryonalis, recorded on mass scale in aquarium 3 (50 mg/l) and sporadically in the other two, is considered to be an illness to which individuals weakend by exposure to negative conditions are vulnerable. In our study, such a negative factor turned out to be the influence of the higher of the two applied herbicide concentrations. Early writers, like Fiebiger (1903) and Dieterich (1938) considered hydrocoele embryonalis to be a symptom of a general disease of young fish. According with Schereschewsky (1935), it is a symptom of pathological processes related to the state of hypertrophy of the thyroid gland and to "the stil ignored errors in artificial hatching methods". Gottwald (1960) observed that if the level of water oxidation during the development of eggs is continuously low, embryo mortality rate increases and their growth is disturbed; hydrocoelis appears in mass scale. Solski (1968), too, found this disorder in carp larvae as a result of exposure to different concentrations of atrazine. Many symptoms recorded by the present author are consistent to the diagnosis of this disease given by Wolf (Greenberg 1969).

It turned out in our investigations that carps in embryo and larval development were more sensitive test organisms than *Daphnia magna*. Thus our observations confirm the results of Alabaster (1967), Solski (1968), Trzebiatowski (1969) and Łakota (1970 a, b) who reported toxic doses for those invertebrates as two to five times greater than for carp larvae and fry. It can be said that *Daphnia magna* can be used for prolonged tests, but it is not a very sensitive organism, at least with respect to the herbicide applied in our experiment.

It also turned out that test organisms belonging to the same family revealed various degrees of vulnerability to the influence of the same preparation. For example, there were a few dozens of carp larvae which survived the toxic influence of the higher concentration of the herbicide owing to their individual vitality and resistance, and after the toxic agent was withdrawn, they were marked by very quick growth (Fig. 5, the upper part of the 50 mg/l curve).

Acknowledgements

The study reported here was undertaken on the initiative of Prof. dr. S. Wróbel, head of the Laboratory of Water Biology of the Polish Academy of Sciences in Cracow. The author is thankful to him for his many instructive remarks and advices. She also expresses her gratitude to Mrs. M. Janik, S. Lewkowicz, B. Sc., K. Srokosz, B. Sc., and to the personnel of the Experimental Farm at Gołysz for their extensive aid in the experiments.

5. SUMMARY

Toxicity of two concentrations of sodium salt of the phenoxyacetic acid 2,4-D was studied. A manufactured product with the trade mark "Pielik", produced by Nadodrzańskie Zakłady Przemysłu Organicznego Rokita at Brzeg Dolny was used.

The study was carried out on de-jellied carp eggs incubated in Weiss vessels and on carp larvae in aquaria. 900 g of carp spawn and 30,000 carp larvae were used in the experiments. The experiment was made a single time, its pattern included a control, 5 and 50 mg/l concentrations AS. The test solutions were administered beginning from 2 hr after fecundation, at the stage of 4 blastomers, and it was continued during 10 days. It is suggested that in subsequent experiments, herbicides should be administered beginning from the moment of fecundation and during the withdrawal of jelly, i.e. at the stage of the "swelling" of eggs, rather than after they are swelled up, as in the present study.

Only once during the whole embryo stage, at the stage of gastrula, eggs from both toxic solutions were more advanced in development than in the control.

Hatching were retarded, in comparison with the control, by 7 hr in the vessel with 50 mg/l concentration and by 14 hr in the vessel with 5 mg/l herbicide concentration.

Body length of larvae after the hatching and during 6 days of the experiment was the least in aquarium with 50 mg/l concentration.

Losses during the whole embryo period were almost identical in both vessels with herbicide solutions, and only by 3 to 4% lower in the control.

Anomalies discovered in some embryos after 33 hr since the fecundation should not be associated with the herbicide's influence, as they appeared in all the vessels. They were caused by disturbances related to the shift of the source of water supply (circulation of water in the vessels was disturbed and eggs remained in their lower parts).

Toxicity of the herbicide during larval development was revealed only in the higher concentration. After the larvae had been placed in the aquaria, symptoms of anxiety and increasing vulnerability were observed during the first three days, disturbances of balance on the fourth day, symptoms of paralysis by the end of the fourth and on the fifth day and mortality early on the fifth day.

In paralysed and dead larvae, hydrocoele embryonalis, deformations of the spinal chord, mostly lordotic or only of the end part of the tail, and a big rounded yolk sac witnessing disturbances in yolk resorbtion were observed. Beside the large swollen sac, in many larvae there were round bladders in the front part of the body, below the head, and the following anomalies: a shortening of the head, eye globes shifted towards the front, a characteristic roundness of the mouth profile and closed mouth. In individuals with hydrocoele, the alimentary canal was rarely on its proper place, but in its medium part it was withdrawn from the organs which it normally touches. In many cases it was even torn.

40 carp larvae and *Daphnia magna* of various sizes, added to the aquaria as food for larvae, survived the toxic influence of sodium salt 50 mg/l 2,4-D.

6. STRESZCZENIE

Badano toksyczność dwóch stężeń pochodnych kwasu fenoksyoctowego soli sodowej 2,4-D, preparatu fabrycznego o nazwie "Pielik", produkowanego przez Nadodrzańskie Zakłady Przemysłu Organicznego Rokita w Brzegu Dolnym.

Badania prowadzono na pozbawionych śluzu jajach karpi wylęganych w aparatach Weissa i na larwach karpi, w akwariach. Do doświadczeń użyto 900 g ikry karpi i 30.000 larw karpi. Badania wykonano w jednym powtórzeniu: kontrola, stężenie 5 mg/l AS i 50 mg/l. Dozowanie roztworów testowych rozpoczęto po 2 godz. od zapłodnienia, od stadium 4 blastomerów i kontynuowano przez ok. 10 dób. W przyszłych badaniach dozowanie herbicydów należy rozpocząć w momencie zapłodniania jaj i podczas pozbawiania jaj śluzu, a więc w stadium „pęcznienia" jaj a nie po ich napęcznieniu, jak to miało miejsce w przedstawionych badaniach.

W stadium gastruli tylko jeden raz w ciągu całego rozwoju zarodkowego stwierdzono wyższy stan zaawansowania rozwoju jaj pochodzących z obu stężeń, w porównaniu z kontrolą.

W porównaniu z kontrolą zanotowano opóźnienie wylęgów o 7 godz. w aparacie z koncentracją 50 mg/l i o 14 godz. w aparacie z koncentracją 5 mg/l soli sodowej 2,4-D.

Długość ciała — longitudo corporis — larw po wylęgu i w ciągu 6 dni doświadczenia była najmniejsza w akwarium ze stężeniem 50 mg/l.

Straty przez cały okres zarodkowy były prawie identyczne w aparatach z obu stężeniami herbicydu, a tylko o 3–4% niższe w aparacie kontrolnym.

Stwierdzonych po 33 godz. od zapłodnienia i po wylęgu anomalii u niektórych zarodków i larw nie należy łączyć z działaniem herbicydu, z uwagi na to, że wystąpiły we wszystkich aparatach. Spowodowały je zakłócenia związane z zamianą źródła wody zasilającej (zakłócenia w cyrkulacji wody w aparatach, zaleganie jaj w dolnej części aparatu).

Toksyczność stosowanego herbicydu w stadium rozwoju larwalnego ujawniła się tylko w wypadku wyższego ze stosowanych stężeń. Po przeniesieniu larw do akwarium zanotowano objawy zaniepokojenia i zwiększającej się wrażliwości w ciągu pierwszych trzech dni, zachwiania równowagi w czwartym, objawami paraliżu pod koniec czwartego i w piątym, śmiertelnością z początkiem piątego dnia po wylęgu.

U larw porażonych i martwych zanotowano masowo puchlinę wodną woreczka żółtkowego (Hydrocoele embryonalis), deformacje kręgosłupa, przeważnie lordotyczne lub tylko końcowego odcinka części ogonowej, i duży kulisty woreczek żółtkowy świadczący o zaburzeniach w resorbcji żółtka. Prócz dużych rozmiarów puchlinowego worka, u wielu larw stwierdzono w przedniej części ciała (poniżej głowy) kuliste, gazowe pęcherze oraz następujące anomalie: skrócenie głowy, względne przesunięcie do przodu gałek ocznych, charakterystyczne zaokrąglenie profilu pyska i zamknięcie pyska. U osobników z puchliną, przewód pokarmowy rzadko znajdował się na zwykłym miejscu, lecz w swym środkowym odcinku był przeważnie odsunięty od organów, z którymi normalnie się styka. W wielu wypadkach był on nawet porozrywany.

Toksyczne działanie 50 mg/l soli sodowej 2,4-D przeżyło 40 larw karpi i różnej wielkości *Daphnia magna*, dodane do akwarium jako pokarm dla larw.

7. REFERENCES

Alabaster, I. S. 1967. Estimating the effect of herbicides on Fish. European Weed Research Council Symposium. 1–3, Ministry of Agriculture, Fisheries and Food, London.

Alabaster, I. S., Abram, F. S. 1965. Estimating the toxicity of pesticides to fish. *Pestic. Abstr., Sect. C*, 11 (2), 91–97.

[Baltbarzdis, Z. Ja., Slava, E. E., Sausa, V. K.] Балтбарздис, З. Я., Слава, Э. Э., Сауса, В. К. 1968. Изучение действа некоторых гербицидов как структуроразрущающих факторов [Investigation of some herbicides as structure destroying factors]. In: Sanitarnaja gidrobiologija i vodnaja toksikologija. Mat. XI Konf. po izučeniju vnutrennyh vodoemov Pribaltiki. 2, 11–17, Riga, Izdat. "Zinatne".

Bandt, H. J. 1957. Über die Giftwirkung von Herbiziden auf Fische. *Z. Fisch., N. F.*, 6, 121–125.

Bauer, K. 1961. Studien über Nebenwirkungen von Pflanzenschutzmitteln auf Fische und Fischnährtiere. *Mitt. biol. Bund Anst. Ld-u Forstw.*, 105, 1–72.

Bogucki, M. 1928. Badania nad przepuszczalnością błon oraz ciśnieniem osmotycznym jaj ryb łososiowatych [Recherches sur la perméabilité des membranes et sur la pression osmotique des oeufs des Salmonides]. *Acta Biol. exp.*, 2, 19–46 [French summ.].

Dieterich, E. 1938. Die Hydrocoele embryonalis (Dotterblasenwassersucht) der Salmoniden. *Z. Fisch.*, 36, 605–642.
Domurat, J. 1956. Rozwój embrionalny troci (*Salmo trutta* L.), szczupaka (*Esox lucius* L.) i płoci (*Rutilus rutilus* L.) w środowisku bezwodnym [Embryonic development of trout (*Salmo trutta* L.), pike (*Esox lucius* L.) and roach (*Rutilus rutilus* L.) in the environment deprived of water]. *Pol. Arch. Hydrobiol.*, 3, 167–173 [Engl. summ.].
Dziekońska, J. 1956. Badania nad wczesnymi stadiami rozwojowymi ryb. I. Rozród i rozwój embrionalny leszcza (*Abramis brama* L.) w Zalewie Wiślanym [Studies on embryonic development of fish. I. Observations on the spawning and the embryonic development of bream in the Vistula Lagoon]. *Pol. Arch. Hydrobiol.*, 3, 291–305 [Engl. summ.].
Fiebiger, J. 1903/4. Über Dotterblasenwassersucht der Salmoniden. *Z. Fisch.*, 11, 124–131.
Gottwald, S. 1960. Niska zawartość tlenu w wodzie przyczyną puchliny woreczka żółtkowego (Hydrocoele embryonalis) u larw pstrąga tęczowego (*Salmo irideus* Gibb.) [Low oxygen level as a cause of blue-sac disease (Hydrocoele embrionalis) of rainbow trout alevins (*Salmo irideus* Gibb.)]. *Roczn. Nauk roln., Ser. B*, 75, 259–271 [Engl. summ.].
Greenberg, D. B. 1969. *Forellenzucht.* Aufl. 3. Hamburg, Paul Parey.
Herbicide handbook of the weed society of America. 1967. 1 st ed. Genoa, N. Y., W. F. Humphrey Press.
Kamler, E. 1972. Bioenergetical aspects of the influence of 2.4-D-Na on the early developmental stages in carp (*Cyprinus carpio* L.). *Pol. Arch. Hydrobiol.*, 19, 451–474).
Kokurewicz, B. 1969. The influence of temperature on the embryonic development of the perches (*Perca fluviatilis* L. and *Lucioperca lucioperca* L.). *Zool. Pol.*, 19, 47–67.
Kokurewicz, B. 1970. The effect of temperature on embryonic development of *Tinca tinca* (L.) and *Rutilus rutilus* (L.). *Zool. Pol.*, 20, 317–337.
Kołaczkowski, S. 1971. Zagrożenie środowiska wodnego pestycydami [Pesticides as a menace for aquatic milieu]. Materiały sesji naukowej „Wpływ pestycydów na wody powierzchniowe". Kraków 1970, 7–32, Warszawa, Komitet Hydrobiol. PAN.
Kurth, H. 1963. *Chemische Unkrautbekämpfung.* Aufl. 2. Jena, G. Fischer Verl.
Lieder, U. 1955 a. Über die Temperaturempfindlichkeit von Fischeiern. *Dt Fisch. Ztg, Radebeul*, 12–15.
Lieder, U. 1955 b. Über Schädigungen von Fischeiern durch Sauerstoffmangel. *Dt Fisch. Ztg, Radebeul*, 308–311.
Łakota, S. 1966 Badania nad toksycznym działaniem Krzewotoksu na ryby [Studies of toxic effect of "Krzewotox" on fishes]. *Zesz. probl. Post. Nauk roln.*, 60, 181–184.
Łakota, S. 1970 a. Badania nad działaniem niektórych herbicydów na ryby i inne organizmy wodne [Investigations on the influence of some herbicides on fishes and other water organisms]. *Biul. Przem. Organicz.*, 3, 13–42.
Łakota, S. 1970 b. Badania nad trwałością niektórych herbicydów w środowisku wodnym [Untersuchungen über die Dauerhaftigkeit einiger Herbizide im Wassermilieu]. *Gosp. ryb.*, 22, 12–13.
Łysak, A., Marcinek, J. 1972. Multiple effect of simultaneous action of some chemical substances on fish. *Roczn. Nauk roln. Ser. H*, 94, 00–00.
Matlak, O. 1969 a. Künstliche Erbrütung von Karpfeneiern und Betrachtungen über Wachstum und Entwicklung der Larven und des Striches. *Acta Hydrobiol.*, 11, 201–222.
Matlak, O. 1969 b. Relatiile termice in timpul incubări icrelor si cresterii larvelor de carp în holesteielě de predezvoltare ale statiunii Gołysz in anul 1968 [Thermic relations in the egg incubation and carp larvae growing period in the predevelopment ponds of the Gołysz Station (Poland) in 1968]. *Bul. Inst. Cerc. Protect. pisc.*, 28, 61–73 [Engl. summ.].
Matlak, O. 1970. Wylęganie karpia w warunkach sztucznych [Artificial hatching of carp]. Instytut Rybactwa Śródlądowego, Samodzielna Pracownia Postępu, Olsztyn-Gołysz, 42, 1–20.
Okołotowicz, G. 1971. Badania nad ustaleniem dopuszczalnych stężeń pestycydów w wodach powierzchniowych [Investigations of safe concentrations of pesticides in surface waters]. Materiały Konferencji „Ochrona wód przed zanieczyszczeniem". 10, 1–6, Warszawa, Inst. Gosp. Wodnej.
Ostrowski, J., Rola, J. 1964. Toksykologiczno-pozostałościowe aspekty sto

sowania herbicydów [Toxicological and residual aspects of herbicides application]. *Post. Nauk roln.*, 5, 51–62.

Piątkowska, B. 1971. Wpływ herbicydów pochodnych kwasu fenoksyoctowego na wybrane procesy zachodzące w wodzie [Influence of herbicides, phenoxynacetic acid derivates on some processes in waters]. Materiały Konferencji „Ochrona wód przed zanieczyszczeniem". 14, 1–7, Warszawa, Inst. Gosp. Wodnej.

Pliszka, F. 1953. Rozród i rozwój certy (*Vimba vimba* L.) [Reproduction and development of *Vimba vimba* L.]. *Pol. Arch. Hydrobiol.*, 1, 137–163 [Engl. summ.].

[Podoba, Z. P.] Подоба, З. П. 1968 a. Влияние никеля и кобальта на эмбриональное развитие рыб [Influence of nickel and cobalt on embryonic development in fish]. In: *Sanitarnaja gidrobiologija i vodnaja toksikologija.* Mat. XI Konf. po izučeniju vnutrennyh vodoemov Pribaltiki. 2, 145–148, Riga, Izdat. "Zinatne".

[Podoba, Z. P.] Подоба, З. П. 1968 b. Влияние химически очищенных сульфатных сточных вод на рыб и их эмбриональное развитие [Influence of chemically purified sulphate wastes on fishes and their embryonic development]. In: *Sanitarnaja gidrobiologija i vodnaja toksikologija.* Mat. XI Konf. po izučeniju vnutrennyh vodoemov Pribaltiki. 2, 149–152, Riga, Izdat. "Zinātne".

Reimann, K. 1969. *Verhalten und Toxikologie von Pestiziden in Wasser und Abwasser.* München, R. Oldenbourg.

Rola, J. 1972. Stan i perspektywy chemicznego odchwaszczania zbóż w Polsce [The actual state and perspectives of chemical weed control in Poland]. Tezy do referatów i komunikatów 12 Sesji Naukowej Instytutu Ochrony Roślin w Poznaniu, 10–11.

Schäperclaus, W. 1954. *Fischkrankheiten.* Berlin, Akademie Verlag.

Schereschewsky, H. 1935. Die „Kinderkrankheiten" der Salmoniden (Dotterblasenwassersucht und Dotterblaseneinschnürung). *Z. Fisch.*, 33, 473–501.

Schülter, M. 1963. Zur chemischen Bekämpfung von Wasserpflanzen in der Binnenfischerei. *Z. Fisch.*, 11, 333–343.

Solski, A. 1968. Ocena herbicydów dla celów rybackich [Evaluation of herbicides for fishing purposes]. *Zesz. przyr.*, 8, 61–126 [Engl. summ.].

Stanisławska, J. 1971. Kształtowanie się zespołów organizmów w wodach zanieczyszczonych pestycydami [Formation of communities in water bodies polluted with pesticides]. Materiały Konferencji „Ochrona wód przed zanieczyszczeniem", 26, 1–7, Warszawa, Inst. Gosp. Wodnej.

Stanosz, S. 1971. Niektóre aspekty toksyczności herbicydów [Investigation on the toxicity of herbicides]. Szczecińskie Towarzystwo Naukowe, Wydz. Nauk Lek., 17 (3), 1–40.

[Sushchenya, L. M.] Сущения, Л. М. 1956. Действие гербицидов ПХФ и ИФК на некоторые пресноводные животные [The influence of pentachlorphenol and IPC on some freshwater animals]. *Učen. Zap. beloruss. gos. Univ., Ser. biol.*, 26, 226–242.

[Trifonova, A. N.] Трифонова, А. Н. 1949. Критические периоды эмбионального развития [Critical periods in embryonic development]. *Usp. sovrem. Biol.*, 28, 154–168.

Trzebiatowski, R. 1968. Przydatność Dalaponu i Simazyny do zwalczania twardej roślinności wodnej w stawach karpiowych [Use of Dalapon and Simazine for control of emerged water plants in carp ponds]. *Zesz. nauk. WSR Olszt.*, 24, 569–588 [Engl. summ.].

Trzebiatowski, R. 1969. Oddziaływanie niektórych herbicydów na *Daphnia magna* i larwy *Tendipes plumosus* [Effects of some herbicids on *Daphnia magna* and *Tendipes plumosus* larvae]. *Roczn. Nauk. roln., Ser. H.* 90, 749–763.

[Veselov, E. A., Pomazovskaja, I. V., Remezova, E. I., Čerianov, S. E.] Веселов, Е. А., Помазовская, И. В., Ремезова, Е. И., Черианов, С. Е. 1965. Токсическое действие гексахлорана на рыб и водных беспозвоночных [Toxic action of hexachlorane on fishes and aquatic invertebrates]. In: Voprosy gidrobiologii, I S'ezd vses. gidrobiol. Obšč., Moskva 1965. Tezisy dokladov 65, Moskva, Izdat. "Nauka".

[Vladimirov, V. I., Sabodaš, V. M.] Владимиров, В. И., Сабодаш, В. М. 1968. Влияние сульфатного цинка на развитие и жизнестойкость личинок карпа [Influence of zinc sulphate on the development and vitality of carp larvae]. In: Sanitarnaja gidrobiologija i vodnaja toksikologija. Mat. XI Konf. po izučeniju vnutrennyh vodoemov Pribaltiki. 2, 18–22, Riga, Izdat. "Zinātne".

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	451–474	1972

E. KAMLER

BIOENERGETICAL ASPECTS OF THE INFLUENCE OF 2,4-D-Na ON THE EARLY DEVELOPMENT STAGES IN CARP (*CYPRINUS CARPIO* L.)

Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

ABSTRACT

The influence of the formulated product "Pielik" on the embryo and early larval development of carp was investigated. The active ingredient of "Pielik" is 2,4-D-Na, the concentrations of 5 and 50 mg A.I./l were tested and compared with a control (0 mg/l). The mass mortality of larvae in 50 mg/l was observed in the 200th hour after eggs fertilization. No changes in respiration, weight, and calorific values, were observed in the embryos developing in the test solutions as compared to the control. On the other hand, respiration, weight, and calorific values of larvae which were treated with herbicide were for the most part lower than in the control. The results obtained seemed to indicate the significance of glycolysis in the embryo development of both control and treated animals; they also showed that the aerobic processes in larvae seemed to be handicapped by the herbicide.

1. INTRODUCTION

The aim of the present work was to find out in what way the herbicide "Pielik" affects the energy expenditure in carp in the course of its embryo and early larval development, i.e. in the yolk utilization period. "Pielik" is a formulated product, its active ingredient contains 85% of 2,4-D-Na (sodium salt of 2,4 dichlorophenoxyacetic acid) and up to 3% of chlorophenols and some other admixtures. This product was selected for use in this research, as it was considered that from the practical point of view the first priority is to measure direct effect of products as applied in the field. "Pielik" is a herbicide most commonly used in Polish agriculture. It is classified within the third class toxicity range among noxious compounds, ref. *Monitor Polski* No. 28, 156 (1965). Its concentrations used in the present work corresponded to those applied in farming operations in order to destroy the undesired vegetation growing in carp ponds. The time of the application of "Pielik" in the field coincides with the carp reproductive period. It is a well known fact that in Poland, fish obtained from carp ponds constitutes the main part of the freshwater fish yield. Simultaneously, heavy rainfalls very frequent in the spring season, in our climate, may cause the danger of herbicide penetration into the waters. Sometimes its concentrations may be very high and then they might cause fish mortality. However, besides the examination of the effect of lethal concentration, it seems none 'the less to be a matter of great importance to carry on investigations on the influence of lower concentrations of that herbicide upon the course of physiological processes. It is generally known that the conditions under which the early period of postembryonic development of fish occurs, are crucial for them (e.g. Olifan 1949). For that reason, it seemed to be well-adviced to undertake the present studies.

2. MATERIAL AND METHODS

Research was carried out in 1971, at the Experimental Farm of the Polish Academy of Sciences, Gołysz, Cieszyn District, at the initiative of the Asst. Prof. Dr S. Wróbel, the manager of that institution. Material used in experiments, consisting of carp eggs and larvae, has been prepared by Dr. O. Matlak for her own, parallel studies on the influence of "Pielik" upon fish development, mortality, morphopathology, and behaviour (Matlak 1972). Owing to those circumstances, it is possible to refer directly the results obtained in the present work to the results issuing from observations of other phenomena.

Subject to study were fertilized carp eggs obtained by means generally applied in the fish-breeding farms and described by Matlak (1970). A detailed description of the manner and conditions under which the actual experiments were performed is presented in the work by Matlak (1972), whereas here we will restrict ourselves to just an outline of the procedure. Treatment with hypophysis in spawners was performed on May 27, at 6.30–7.30 p.m. The artificial spawning was carried out on May 28, at 3 p.m. This moment will be mentioned further on as "0 hour" of development. About 2 hours later (May 28, 5 p.m.) the fertilized eggs were put into Weiss apparatuses in 0, 5, and 50 mg/l concentrations of herbicide solutions (full particulars about the preparation of solutions are given below). The hatched larvae were transferred into aquaria. At the 145th hr of the development, the larvae were supplied with plankton as a food. The whole experiment lasted for 10 days.

"Pielik" is produced by Chemical Works — Nadodrzańskie Zakłady Przemysłu Organicznego Rokita, Brzeg Dolny, Wołów District.

During the time of embryo development the applied flow of water was 3 l/min (±0.3 l/min) through each of the three Weiss' apparatuses. In addition, there was a supplementary inflow of concentrated solutions of "Pielik" into two apparatuses, with the rate of flow 1 l/hr (±0.1 l/hr). During the whole period of experiment the utilized "Pielik" was taken out from a single container, exlusively. Fresh solutions were prepared every 10 hours. In the three Weiss apparatuses there were the following concentrations of "Pielik" solutions: 0 (control), 5.89 mg/l, and 58.90 mg/l. The herbicide concentrations expressed in pure 2,4-D-Na amounted, thus, to 5 mg/l and 50 mg/l, those values will be referred to later on in this paper. There was no water flow through the aquaria, where larvae were grown, nor through the respirometers; consequently, the concentration values were constant. In this case fresh solutions were prepared once a day. Trzebiatowski (1969 a) recommends a dosage of the mixture of "Antyperz" (38% TCA-Na) with "Pielik" herbicide 120–150 kg/ha in weight proportion "Antyperz" : "Pielik" — 10 : 1, as effective means against water plant growth in the ponds, which for ponds 1 m deep in conversion to active ingredient of "Pielik" corresponds to concentration of approximately 10 mg of 2,4-D-Na/l. In his further work (Trzebiatowski 1969 b) he qualifies that dosage as harmless for carp.

Unfortunately, it was not possible to keep up a stable temperature during the embryo development in Weiss' apparatuses (Fig. 1; see also Matlak 1972). During that time temperature was measured every 2 hours. Fluctuations of water temperature in aquaria with larvae were lesser (Fig. 1), measurements were taken 2–4 times a day. Nearly all the time temperature was almost identical in the three concentrations of solution. Respiratory measurements were carried out in a constant-temperature bath in 23 ± 0.1°C.

Oxygen contents in the Weiss' apparatuses were not observed; however, it was expected to be approximate to air saturated. Aquaria containing larvae were ventilated with compressed air. Oxygen contents in all the three aquaria were measured during a period of three days and amounted to 7.04 up to 7.68 mg O_2/l (mean 7.37 mg O_2/l), which amounted to approximately from 83 to 90% of air saturation (the mean approximately 86%) with the average temperature in that period of time of 21.3°C and the average atmospheric pressure of 735 mg Hg.

Oxygen consumption was measured in constant pressure microrespirometers described in Klekowski (1968). Glass respirometric chambers had the shape of an overturned mushroom, of 35 mm diameter and 12.5 ml volume, the total air volume in the respirometric chambers + absorber + arms system amounted to approximately 20 ml. In the initial 70 hr of fish development, measurements were taken in 0.5 ml then in 1.0 ml of solution, with adequate concentration of herbicide.

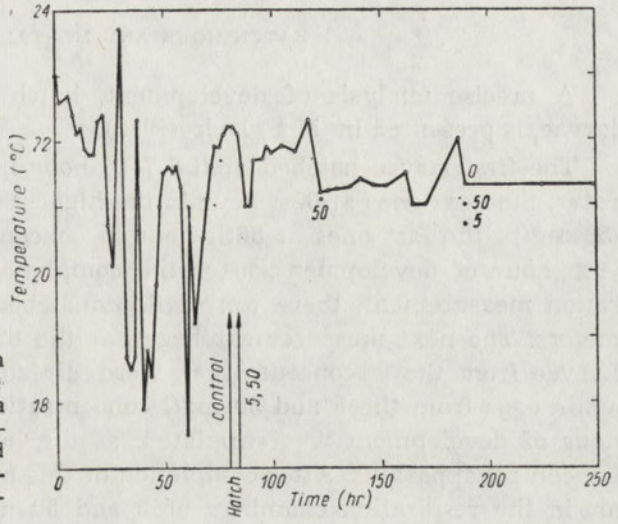


Fig. 1. Water temperature in Weiss apparatuses (embryo development) and in aquaria (larval development). Line — water temperature in 0, 5, and 50 mg/l; points — temperature different in given concentration

Up to the 14th hour of development 25 individuals were placed into each respirometer, up to the 68th hr — 15, up to the 139th hr – 10, and then until the end of measurements — 7 individuals per 1 respirometer. Altogether 185 measurements of oxygen consumption were carried out; 9 respirometers were used parallelly (3 concentrations × 3 repeats), information about measurements frequency is presented in Fig. 2. Since it was proved earlier (K a m l e r 1972) that Q_{10} values for young carp were close to the values expected from Krogh's "normal curve", a method given by W i n b e r g (1956) based on the corrected "normal curve" was applied for conversion of the oxygen consumption measured at the fixed measurement temperature (23°C) into the oxygen consumption at the cultures temperature (Fig. 1). *RQ* measurements were carried out in the same microrespirometers in accordance with indications suggested by K l e k o w s k i (1968). Taking into consideration the necessity of great frequency of oxygen consumption measurements during the period of the embryo development, *RQ* measurements were performed twice and only on larvae, viz. in the range from 114th to 120th hour of development and from 139th to 142nd hour of development (2×3 concentrations × 3 repeats; 18 measurements in total). After the end of measurements, wet weight of individuals, dried on a filter paper, was determined. Then, material was dried up to constant weight at 50°C in exsiccators over NaOH. Weight exactness amounted to 0.01 mg.

The calorific value of 1 mg dry weight was determined in a microbomb calorimeter described in P h i l l i p s o n (1964), modified by K l e k o w s k i, B ę c z k o w s k i (in prep.) applying the technique recommended by P r u s (1968). Calibration and determination of exactness of the apparatus was performed by burning 25 benzoic acid pellets. Heat sensitivity of the apparatus amounted to 0.6346 cal per 1 mm of galvanometer deflection, the coefficient of variation was 4%. The calorific value of 39 samples was determined. The total number of burned pellets was 234, of 5–15 mg weight each. Measurements of the calorific values of animals, cultivated in 50 mg/l, were carried on only during the initial 115 hours of development, due to the scarcity of material in the time following that period. Data on frequency of sampling for the calorific values determinations is presented in Fig. 7.

Thirteen determinations of ash content were carried out in a muffle furnace at 550°C. Ignition in a muffle furnace requires a great quantity of material, hence ash contents could not be determined in all samples with that method. The ash remaining after the burning of pellets in the microbomb calorimeter was always weighed — data derived from there were treated as an aproximate estimate of ash content.

3. RESULTS

HATCH HOUR AND MORTALITY

A precise analysis of development, hatch hours, and mortality of larvae, is presented in Matlak (1972).

The first larvae hatched at the 74th hour in the control Weiss apparatus, the next ones at 81st hour in the highest concentration of herbicide (50 mg/l), the last ones at 88th hour in concentration of 5 mg/l. At the 79th hour of development, after the completion of a subsequent respiration measurement, there were still unhatched eggs in all the respirometers. The next measurement began at the 82nd hour of development. Larvae from the 0 concentration were already put into respirometers, while eggs from the 5 and 50 mg/l concentrations were not; so the 80th hour of development was stipulated, as the hatch hour of larvae from the control apparatus. After completion of this measurement, at the 85.5th hr, in the respiration chambers of 5 and 50 mg/l concentrations, there were new-hatched larvae, hence the 84th hr of development was defined as the hatch hour of larvae in both concentrations of herbicide.

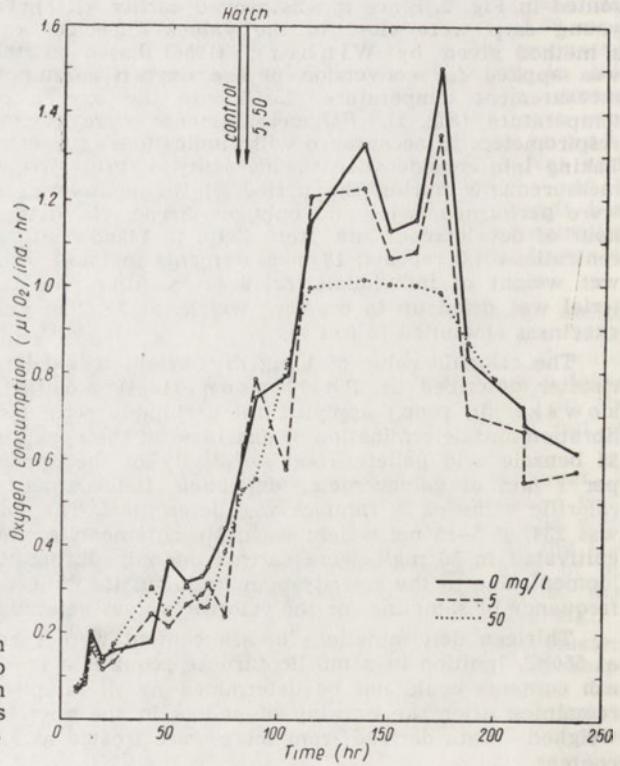


Fig. 2. Oxygen consumption at 23±0.1°C, in carp embryo and larval development in 0, 5, 50 mg/l. Each point marks the mean value of three measurements

Matlak (1972) ascertained that in the course of duration of the experiment, larvae did not consume any exogenic food given to them.

At the 200th hour of development a mass mortality of larvae in the 50 mg/l concentration was observed.

RESPIRATION

Changes in oxygen consumption in the course of embryo and early larval development are shown in Fig. 2. The general picture of oxygen consumption variations is rather similar in all the concentrations used in the experiment. In the course of embryo development the oxygen consumption increases, though irregularly. An increase in the oxygen consumption was observed at approximately 14th hour of development; according to Matlak (1972) it is the period of the formation of neural tube. Another increase in the oxygen consumption was observed about 50th hour; according to Matlak (1972) it is a period of the intensified mobility of embryo. Oxygen consumption increases very intensively during the period directly preceding the hatching time and in the course of the hatch itself; in the time between the 114th and 179th hour of development it is high. During this time there is a very significant temporary decrease in respiration rate between 151th and 163th hour of development (distinctly noticeable in the control larvae and those from 5 mg/l concentration) and then a new increase at 174th hour of development. Then again the oxygen consumption falls down. This corresponds to the deflection ascertained by Matlak (1972), of the larvae growth curve connected with the transition from the embryonic into branchial respiration. The general means of oxygen consumption by embryos and larvae in the period of the first 200 hours of development were compared, since the results from all concentrations were available only for that period of experiment. In the control the mean amounted to 0.529 μlO_2/ /ind. · hr (95% confidence interval 0.413—0.645), in 5 mg/l concentration — 0.472 (0.368—0.576) and in 50 mg/l concentration — 0.449 (0.355— —0.543). Thus, together with the increment of the herbicide dose the general mean of the oxygen consumption has slightly decreased, however, those differences are statistically nonsignificant.

As it can be seen in Fig. 2, differences between the oxygen consumption rate in each particular concentration of herbicide are set differently in various periods of development. For evaluation of significant differences between any two groups of results the criterion of non-parametric statistics for independent data was used (Wilcoxon-Mann--Whitney-Festigner Test, in Walsh 1965, Gubler, Genkin 1969). The oxygen consumption rates, between the 0—5, 0—50, and 5—50 mg/l concentrations of the herbicide, were compared. The comparisons were

carried out separately for each particular period of development, characterized by diverse oxygen consumption (Fig. 2, Table I). Thoroughout

Table I. Differences in oxygen consumption (μl O_2/ind. · hr, 23.0±0.1°C) by carp eggs and larvae in three concentrations of herbicide. N–$p>5\%$, S–$p<5\%$, SS–$p<1\%$, n — number of observations compared

Concentrations compared (mg/l)	Time (hr)							
	8-49	n	50-113	n	114-179	n	180-237*	n
0– 5	N	35	S	40	N	29	S	24
0–50	N	35	SS	38	S	29	S	16*
5–50	N	36	N	42	S	30	SS	16*

* 200 hr only in 50 mg/l.

the greater part of the embryo development (up to the 49th hour of development), no differences in the respiration of animals in various concentrations of herbicide have been observed. Nevertheless, at the end of the embryo development, in the period of hatching, and in the course of the first 1.5 days of the larval life (50th — 113th development hour), the respiration rates in the control were higher than they were in both series supplemented with herbicide; between those two no differences were recorded. During the period of time of high oxygen consumption (114th — 179th development hour), the oxygen consumption in the 50 mg/l concentration was distinctly lower than in the control or the 5 mg/l concentration, between them no differences were found. After the 180th hour the oxygen consumption in the control was higher than in the 5 mg/l concentration. It the 50 mg/l concentration the oxygen consumption was at that time the highest, but pretty soon, at approximately 200th hour, all these larvae died.

Thus, in some phases of the development the herbicide caused a substantial lowering of the level of oxygen consumption and in general, the stronger was the concentration of herbicide, the higher degree of lowering of consumption, except the case of a higher oxygen consumption by larvae in the 50 mg/l concentration, in their premortal time.

As it is shown in Fig. 2, the oxygen consumption up to the 114th hour of development, although in Table I it is arbitrarily separated in two sections, can be approximated by the exponential equation, as it was previously applied to fish embryo development, e.g. by Devillers (1965): $QO_2 = a \cdot e^{kt}$, where: t is time, e is 2.71828..., and a and k are constants. Calculation results obtained by least squares method are presented in Fig. 3. In the 5 mg/l concentration the a constant is distinctly lower than in the control, while the k constants are quite similar, whereas in the 50 mg/l concentration the a constant is higher than in the control, while the k constant is distinctly lower. Those formulas enable the estimation

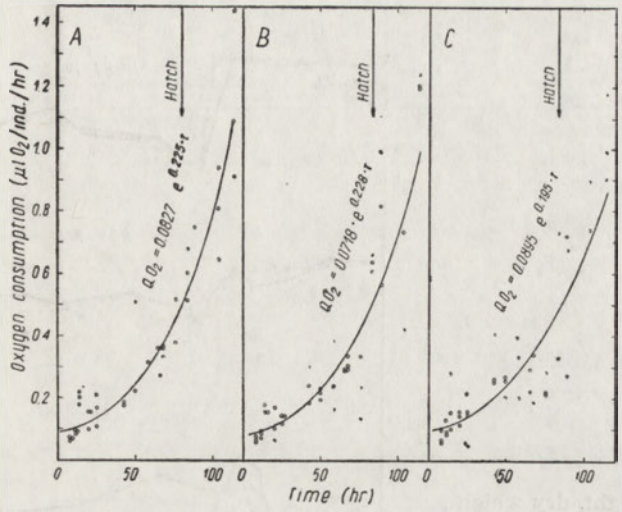


Fig. 3. Oxygen consumption at 23±0.1°C, during carp embryo and early larval development up to 114th hr of development. A — control ($n = 38$), B — 5 mg/l ($n = 43$), C — 50 mg/l ($n = 41$). Points — individual measurements, lines — approximated oxygen consumption calculated from formulas

of oxygen consumption in the period of 0—8 hours of development, when due to technical reasons measurements were not carried out.

The *RQ* values, measured in larvae in periods between 114—120 and 139—142 hours of development, were low and very slightly variable: the mean 0.617, S.E. 0.014, 95% confidence interval 0.587—0.647. Due to the reasons described below, the calculated oxy-calorific coefficient, $4.74 \cdot 10^{-3}$ cal/µl O_2 was not based on the measured *RQ* value.

LIVE WEIGHT, DRY WEIGHT, ASH CONTENTS

Live weight of an egg (Fig. 4) amounts to about 4 mg, during embryo development it almost does not change and is very similar in all concentrations. In the hatching time it diminishes fourfold, in the control it is attained earlier than in animals cultivated in the presence of herbicide. The live weight of larvae after the hatch increases slightly and it is also very similar in all concentrations.

The dry weight is a particularly important element in the present considerations (Fig. 4). In the course of the embryo development dry weight of eggs drops slightly from approximately 0.33 to approximately 0.28 mg. The dry weight of newly hatched larvae is about a half lower than the weight of the dry mass of an egg. Later on, a slight increase of the dry mass of larvae is observed. The general mean dry mass of individuals, in the period of the initial 200 hr of development (for that period

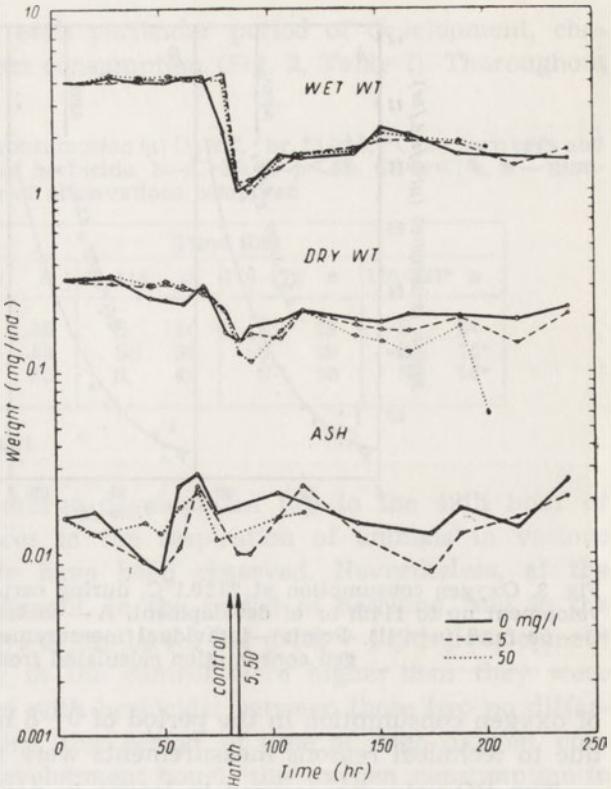


Fig. 4. Wet weight, dry weight, and ash content during carp embryo and larval development in 0, 5, 50 mg/l. Wet and dry weights — mean values from three measurements, ash content — calculated from dry weight of one individual and from percentage of ash in dry weight determined in microbomb calorimeter

there are data obtained from all the concentrations), amounted in the control to 0.240 mg (95% confidence interval 0.220—0.260 mg), in the 5 mg/l concentration — 0.236 mg (0.216—0.256), and in the 50 mg/l concentration — 0.223 mg (0.195—0.251). As it can be seen, together with the increase of the concentration of herbicide the dry mass of individuals decreased inconsiderably, however, those difference are statistically nonsignificant. The dry weight of animals cultivated in the 0, 5, and 50 mg concentrations of herbicide/l was compared; the comparison was carried out separately for the embryo and larval development (Table II). As it can be seen, the supplement of herbicide did not have any effect on the changes in the dry weight of eggs, whereas the dry weight of larvae was so much lower, the higher was the concentration of herbicide but only the differences between the control and the 50 mg/l concentration were statistically significant.

Changes of dry weight contents in live weight are presented in Fig. 5. In the course of the embryo development this ratio lowers somewhat (from over 8% down to approximately 7%); this would indicate that the loss of dry weight is made up by water. During the hatch there occurs a loss of fluids and this is expressed by a sudden rise in the curves in

Table II. Dry weight of carp eggs and larvae in three concentrations of herbicide

Concentration (mg/l)	Mean weight (mg)	
	Eggs	Larvae
0	0.263	0.202
5	0.268	0.183
50	0.268	0.153
Concentrations compared (mg/l)	Differences (N—p>5%, SS—p<1%)	
	Eggs	Larvae
0— 5	N	N
0—50	N	SS
0—50	N	N

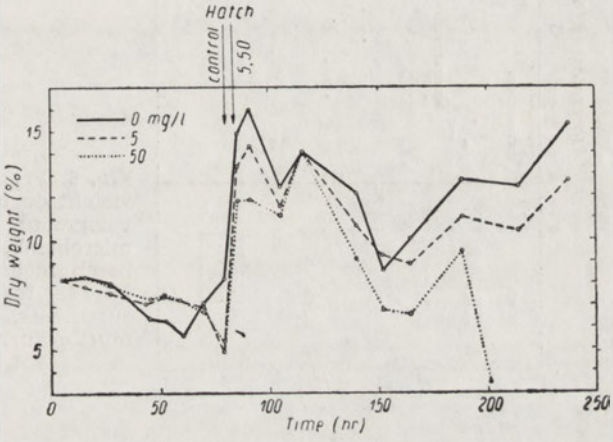


Fig. 5. Percentage of dry weight contents in live weight, during carp embryo and larval development in 0, 5 and 50 mg/l

Fig. 5. In the first half of the investigated part of larval development (80th—149th hour of development) the dry weight percentage in live weight is slightly higher than in the second half (150th—237th hour of development). In the control it amounts respectively to 13.7 and 11.9%, in the 5 mg/l — 12.1 and 10.6%, in the 50 mg/l concentration — 11.1 and 6.6%. As it can be seen, together with the increase in the concentration of herbicide the percentage dry weight in live weight diminishes, though the differences between the control and the 5 mg/l concentration are insignificant ($p > 5\%$), whereas between the control and the 50 mg/l concentration they are significant ($p < 5\%$), while between the 5 and 50 mg/l concentrations — significant only in older larvae ($p < 1\%$) — U-test (cf. also Fig. 5).

Ash contents in one individual was in the range from 0.01 to 0.02 mg (Fig. 4). It was not possible to state here any correlations either with the development stages or with the concentration of herbicide. It should be pointed out that the applied method used for the determination of ash

remaining in a microbomb was very inaccurate. It is distinctly evident in Fig. 6, where the changes in the percentages of ash contents in dry weight determined in a microbomb and in a muffle furnace are compared. Variation of the results obtained by burning in the bomb is quite considerable,

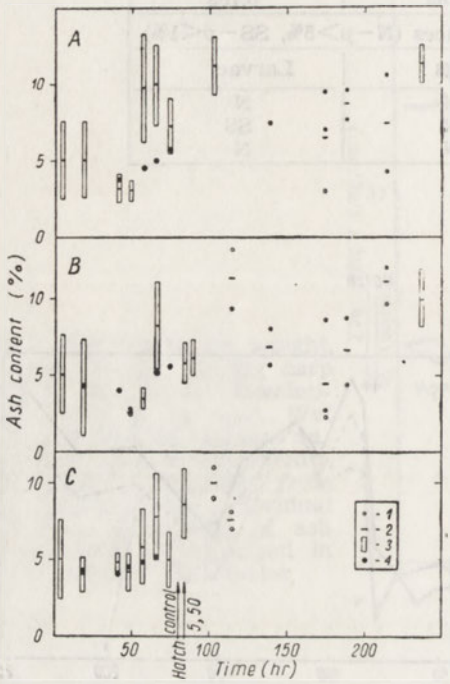


Fig. 6. Percentage of ash contents in dry weight during carp embryo and larval development. 1 — individual measurement in microbomb, 2 — mean values from microbomb measurements, 3 — 95% confidence interval for results from microbomb measurements, 4 — individual measurements in muffle furnace. A — control, B — 5 mg/l, C — 50 mg/l

but the values obtained from the muffle furnace are, in general, within the range of confidence limits of results obtained from the microbomb. It seems that the percentage of ash contents in the dry weight increases in the course of the development.

CALORIFIC VALUES

Changes in the calorific value of 1 mg of dry weight are presented in Fig. 7A. During the embryo development, calorific value decreases, and directly after the hatch it lowers abruptly. In consequence of a rather scanty amount of material, measurements of larvae, cultivated in the 50 mg/l concentration after the 115th hour of development, were not carried out. At the end of the investigation period the colorific value of 1 mg of dry weight in the control and in the 5 mg/l concentration has slightly increased. The general means of calorific values in eggs and larvae, in the course of the initial 115 hours of development (up to this time data from all concentrations are available), amounted to 5.84 cal/mg dry weight

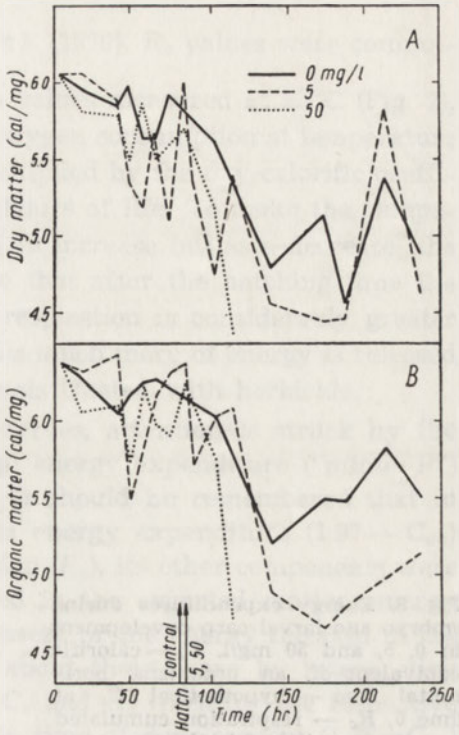


Fig. 7. Calorific value of 1 mg dry matter (A) and of 1 mg organic matter (B) during embryo and larval carp development in 0, 5 and 50 mg/l

(95% confidence interval 5.75—5.93) in the control, 5.56 (5.43—5.69) in the 5 mg/l concentration, and 5.66 (5.54—5.78) in the 50 mg/l concentration. It means that the calorific value of 1 mg of dry mass of animals cultivated without the supplement of herbicide is significantly higher than in animals cultivated in both concentrations of herbicide; between them no differences have been remarked. Moreover, what is noteworthy, is the fact of greater variation of the calorific value in animals cultivated in herbicide as compared with the control animals.

The pattern of changes in the calorific value of 1 mg of organic matter (i.e. ash-free dry matter) is rather similar (Fig. 7 B).

ENERGY EXPENDITURES

The term "energy expenditure" is understood in the present work as a definition covering all kinds of energy, discharged by an organism, in whatever form, e.g. heat energy released in the process of metabolism, or chemical energy contained in excreted or rejected matter. Energy expenditures in the course of embryo and larval development of carp are

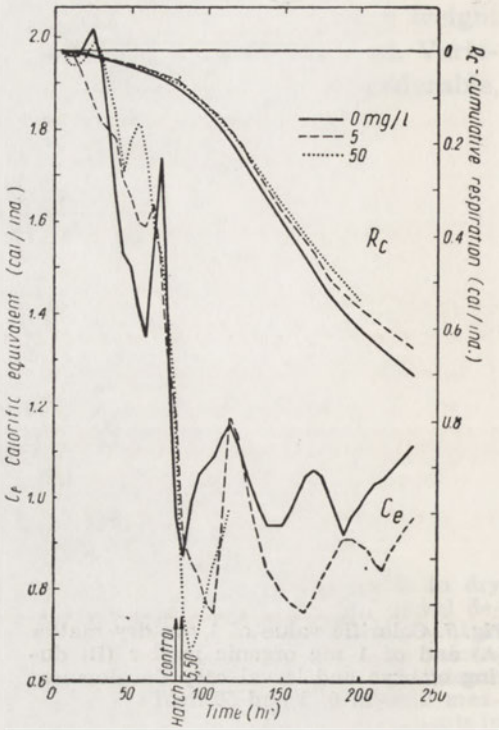


Fig. 8. Energy expenditures during embryo and larval carp development in 0, 5, and 50 mg/l. C_e — calorific equivalent of an organism, horizontal line — hypothetical C_e at time 0, R_c — respiration cumulated from time 0 to time n

presented in Fig. 8. On the left side scale, changes in the calorific equivalent of an organism (C_e) are shown, computed on the basis of the measured dry weight of an organism and the calorific value of 1 mg of dry weight. In the course of the embryo development, C_e value lowers gently; in the hatch time a sudden fall of the value was observed. Thoroughout approximately 36 hours after the hatch the C_e increases slightly again, without reaching, however, the level from the time before the hatching period. The dynamics of those changes is fairly similar in all the three series of experiments, yet, the C_e of larvae cultivated in the herbicide solutions are lower than in the control. In Figure 8, there is shown vertical line, 1.97 cal/ind.; it represents the C_e in the fifth hour of development. Due to the lack of earlier measurements, it has been assumed that it corresponds to the calorific equivalent at 0 hour of development. Using this line, one can make out, by how much the calorific equivalent, in the n-time (C_{en}), is lower than the initial one. The value ($1.97 - C_{en}$) indicates the total energy expenditure; in this case it is equal to the negative production ("minus P").

Figure 8 also presents the respiration cumulated from time 0 to time n $R_c \approx \sum_{\tau_o}^{\tau_n} R_i$, in the sens of K l e k o w s k i (1970). R_c values were computed on the basis of oxygen consumption values measured at 23°C (Fig. 2), which were subsequently converted to oxygen consumption at temperature of cultures presented in Fig. 1, then multiplied by the oxy-calorific coefficient and cumulated to the successive hours of life. To make the camparison easier, the R_c is presented not as an increase but as a decrease (the right side scale in Fig. 8). One can see that after the hatching time the energy expenditure in the process of respiration is considerably greater than before the hatch. In control animals much more of energy is released in the respiration process than in animals treated with herbicide.

While comparing the two sets of curves, attention is struck by the fact that only a minor part of the total energy expenditure ("minus P") is released in the respiration process. It should be remembered that in the present work, apart from the total energy expenditure ($1.97 - C_{en}$) and one of its components, viz. respiration (R_c), its other components were not examined, as e.g., energy contained in the excreted matter, energy contained in rejected matter (e.g. egg cases), or the energy released in the anaerobic way. One can get an idea about their share by interpreting (see Fig. 8) the distance between the C_e and R_c lines for the respective concentrations of herbicide in the given time of development ($1.97 - C_{en}$) $- R_{cn}$. The proportion of respiration to the total energy expenditure is different in various developmental stages, it has particularly low values during the hatch: in the control animals, at the 80th hour of development it amounts to 8%, in the animals treated with 5 and 50 mg of herbicide/l, in the 85th hour of development — 7 and 6.5% respectively.

4. DISCUSSION

In the published works there are given results from acute experiments on mortality of various animals under the influence of 2,4-D-Na. Contrary to the present work, up to this time for the most part, pure chemical compounds have been investigated. Basing on the results obtained by K l e k o w s k i, Z v i r g z d s (1971), it can be inferred that the median lethal concentration (24 hr) of pure 2,4-D-Na preparation for *Simocephalus vetulus* (Cladocera) is higher than 7.5 mM, this concentration being 38 times stronger than the strongest one used in the present work (50 mg/l). Analogous data quoted for fish *Rasbora heteromorpha* (A l a b a s t e r 1969) and *Lepomis macrurus* (K o ł a c z k o w s k i 1970) amount to 1160 and 350 mg of 2,4-D-Na/l. In the highest of the presently examined concentrations, 50 mg of herbicide/l, mortality of larvae occurred at about 200th hour of development. In comparison with the works cited above

concentrations applied in the present work were low, but one should bear in mind that all those authors have used a purified substance, whereas in the present work use was made of a formulated product — "Pielik", containing highly toxic admixtures such as e.g. chlorophenols, therefore it is clear that the direct comparison of the obtained results is not possible. However, Trzebiatowski (1969 b) studied, similarly to this work, the effect of the formulated product — "Pielik" on carp. He applied "Pielik" in mixture with "Antyperz" (38% TCA-Na), as well as "Antyperz" alone. He found out that "Antyperz" concentration of 190 mg of active substance/l is safe for carp, whereas the same concentration with odmixture of "Pielik" 42.5 mg 2,4-D-Na/l caused 33.4% mortality in one-year old carps, in the autumn season, at about 20°C temp., after 104 hours of treatment. Thus, despite the differences that were to be expected in result of the different age of fish and the synergistic action of both herbicides in the experiments of Trzebiatowski (1969 b), one can recognize that results issuing from the present work are in general in accordance with results obtained by Trzebiatowski, which stands to reason, as in both works the formulated product was used.

Although a detailed analysis of the changes in the time of hatching under the influence of the examined herbicide was not carried out, nevertheless it was ascertained that the 5 and 50 mg/l concentrations delayed the hatch of carp. Korde, Zvirgzds (1971) observed in *Misgurnus fossilis* a delay in hatching under the influence of 2,4-D-Na. They observed delays longer than one hour occurring in concentrations in the range from 5 mM (=approximately 1250 mg/l). As it can be seen, the two works are only "qualitatively" compatible (delay of hatching caused by herbicide), whereas "quantitatively" the results differ from one another. Korde, Zvirgzds (1971) noticed shorter retardations under the influence of greater concentrations; this again is easily comprehensible, as they have applied a pure compound.

In a comprehensive treatise, based on a wide material, about the embryo development of fish and some other vertebrates, Trifonova (1949) distinguished three critical periods with decreased resistance against external factors. Those are the periods of intensive differentiation of tissues, the first — in the time of early cleavage, the second — preceding the gastrulation, the third — the embryo formation time and the formation of axial organs. During the critical periods, oxygen consumption increases and the anaerobic processes are less intense (production of lactic acid falls down). The first result from the examination of oxygen consumption was obtained at the 8th hour of development, so inferring from the data of Matlak (1972) the first two critical periods were missed in the present work. Anyhow, the presently stated increase in oxygen consumption at the 14th hour of development (Fig. 2), i.e. according to

Matlak (1972), the time of formation of the nerve channel, would correspond to the third critical period, as in Trifonova (1949). According to her, the critical periods (=periods of differentiation) are separated by periods of great resistance against the external agents; those are periods of growth of the previously differentiated tissues; the oxygen consumption is at that time lower and intensivity of the anaerobic processes increases. A slightly different picture of glycolysis in *Migurnus fossilis* embryos developing in aerobic conditions was obtained by Milman (1965). He stated high lactic acid content before the cell division, then it decreased and remained on a steady, low level up to approximately 30th hour of development; afterwards it increased again up to the hatch time, which occurred at approximately 48th hour of development. In fact, Milman (1965) did not compare his results with the results of Trifonova and her followers, but judging from the diagram, he performed the determination of the lactic acid content every 5—10 hr though the development period lasted a short 50 hr, so that he could have overlooked some changes in its content. Milman (1965) also examined the lactic acid production by embryos exposed in anaerobic conditions. Basing on the obtained results, and on the oxygen consumption data taken from literature he carried out the evaluating calculations. He demonstrated that the lactic acid production was so great that it warranted respiration by means of transitional products of the carbohydrates transformation with twofold or threefold surplus.

Thus, it is obvious that during its development a fish embryo gets a part of energy by anaerobic way. In the present work, neither the *RQ* for embryos nor the lactic acid production were investigated, therefore we will confine our considerations to the conditions under which the energy was acquired by the developing embryos. It is well known (e.g. Pliszka 1953, Blaxter 1969), that at the moment of the hatching of larvae, their blood is colourless and the circulation and respiration systems are poorly developed. So it can be assumed that in the period of embryo development the importance of the system of oxygen transport is inconsiderable. According to Matlak (1970), the diameter of a developing egg is of about 2.3 mm. To calculate the partial oxygen pressure required to provide the necessary oxygen supply in a sphere (so that oxygen reaches zero just in the centre) a formula given by Kleiber et al. (1943) was used:

$$C = \frac{QO_2 \cdot r^2}{6D}$$

where: C — partial O_2 pressure (atmoshere), QO_2 — oxygen consumption rate (µl O_2/mm^3 of tissue per hour) (1 mm^3 of tissue $\approx$ 1 mg wet weight), D — diffusion rate (mm^3 per mm^2 for 1 mm of diffusion distance, 1 atm of partial pressure difference for oxygen, and 1 hr), r—radius (mm).

Krogh (1919) measured diffusion constants for water and for muscle tissue of mature animals: 0.34 and 0.16 (ml O_2 per cm^2 diffusion area for micron diffusion distance per min, 1 atm of partial pressure difference for oxygen). These constants converted to the units as shown in formula are: $D=0.204$ for water and $D=0.096$ for muscles. Oxygen probably diffuses faster through developing fish egg than through muscles of mature animals, therefore those two diffusion constants were used alternatively in the present considerations.

Three stages of embryo development in the control group (without herbicide treatment) were taken under consideration: at 8.3th hour of development $QO_2=0.0154$ µl/mg wet wt. · hr, at 42.5th hour $QO_2=0.0302$, and at 76.3th hour $QO_2=0.1960$. The partial O_2 pressure required to provide the necessary oxygen supply in eggs (C, atm · 10^{-3}) would be about:

	8.30 hr	42.5 hr	76.3 hr
D for water:	17	33	212
D for muscles:	35	71	450

Assuming that water in respirometric chambers was balanced with air, then in the cultures and at measurement temperature (close to 23°C), the oxygen pressure in the immediate surrounding of eggs would amount to approximately $6 \cdot 10^{-3}$ atm, i.e. much too little to meet the requirements for oxygen in any of the three situations, mentioned above. In reality, the oxygen pressure in the immediate environment of eggs is probably even lower than assumed above, which indicates, all the more, the partial release of energy through anaerobic way. If measurements of oxygen consumption had been carried out not in water but in air environment, then the oxygen pressure would have been of the order of $200 \cdot 10^{-3}$ atm. In that case, the oxygen requirement would be satisfied completely, provided that the diffusion rate *D* for water is valid for that tissue. Winnicki (1968) stated that oxygen consumption by Salmo embryos in moist air was higher than in water.

A high increase in oxygen consumption was observed in the period immediately preceding the hatch and in the course of hatching. Its high values were noticed during 114th — 179th hours of development (Fig. 2). Subsequently (on the fifth day after the hatch) the oxygen consumption falls down. Olifan (1949) investigating fish growth (including carp) in the early period of the post-embryo development found that in the course of first 4—6 days of larval life (period I) the larval growth was intensive and at that time their most important vital functions depended on the yolk sac. Next (period II) intensivity in the growth of larvae decreases, which is connected with the reabsorption of the yolk sac; at that time larvae not adapted to the exogenous food are, more or less, starving. Though Olifan (1949) failed to measure the respiration, he suggested

that in effect of the intensive growth of organisms in period I the respiratory rate should be high; thereafter, in the course of about fifth day after the hatch — it should lower down. The present results are in accordance with those surmises but their interpretation ought to be completed. It seems that the high level of oxygen consumption observed in the hatching time and lasting up to the fifth day of larval development may be also partially caused by the repayment of oxygen debt, i.e. the oxidation of glycolysis products accumulated during the time of embryo development. It is possible, owing to the improved conditions of oxygen diffusion (acc. to Matlak (1966) the body length of carp larvae shortly after the hatch exceeds its height 6—7 times) and the formation of systems transmitting oxygen. No references have been spotted in the literature, mentioning the capability of eliminating glycolysis products by larvae. Both Trifonova (1949) and Milman (1965) indicated high lactic acid contents in the embryos before hatching. At present, it has been stated that the *RQ* in larvae at 114th—142th hour of development was very low (the mean value 0.617). It would confirm the hypothesis of the repayment of oxygen debt in that period of time. The decrease in oxygen consumption observed on the fifth day after the hatch may occur not only in connection with the slowing down of the rate of growth of the animals, but also with the ending of the repayment of oxygen debt.

In most works on bioenergetic there are mentions about difficulties relating to the *RQ* measurement and interpretation, as concerns the evaluation of proportions of fat and carbohydrate in the katabolized substance with the view to compute the oxy-calorific coefficient of the consumed O_2. In brief, those difficulties may stem from different causes: 1. there may be a difference between the CO_2 produced in a given time interval and the CO_2 exhaled during that time (retention or washout of CO_2 from an organism), 2. although there is a possibility of measurement of the N-free *RQ*, yet, other substances than carbohydrates, such as fat and proteins may be also katabolized; 3. synthesis of fat from carbohydrate (high *RQ);* or gluconeogenesis from fat (low *RQ*). If water animals are tested there are moreover two additional factors of doubt: 4. retention of CO_2 in the medium; and 5. as water animals may often be exposed to the conditions of low oxygen contents in the environment, the *RQ* values may be affected by intensified glycolysis (high *RQ*), or the repayment of oxygen debt, (low *RQ*), as it happened, in all probability, in the present case. On the other hand, if those stipulations were disregarded and if be taken for granted that the measured *RQ* values showed true proportions of katabolized carbohydrate and fat, then, at the extreme *RQ* values 0.707 and 1.00, the oxy-calorific coefficients would amount to 4.686 and 5.047 cal/ml O_2, respectively (Harrow, Mazur 1958). The difference comes to 7%, which is a fairly small error in comparison with the errors usually committed at the determination of the oxygen consump-

tion. Therefore it is inadvisable to use the measured *RQ* data for calculation of the energy expenditure; Swift, French (1954), Kamler (1970), and Jezierska (unpublished) have also called attention to this fact. The choice of the oxy-calorific coefficient, used in the present paper, was based on the following presumptions: Stroganov (1962) reported that fish eggs contained large quantities of proteins and fats (in carp 24 and 2% respectively). Approximate protein and fat contents are given by Blaxter (1969); moreover, he points out that carbohydrate is present in small quantities (<0.3%) and its synthesis occurs continuously. According to Blaxter (1969), fat is undoubtedly used as a fuel, however, the proportion of protein used for energetic purposes is not easy to determine, since egg retains some nitrogeneous metabolites, nevertheless it is considered that approximately 40% of the original protein may be metabolized. Kleiber (1961) reports that heat production of fasting animals katabolizing only fat and protein amounts to 4.7 — 4.8 kcal/l O_2. Thus, the use of the determined oxy-calorific coefficient $4.74 \cdot 10^{-3}$ cal/µl O_2 seems to be quite justified.

Generally, oxygen consumption by larvae, measured in the present work at 23°C was ranging from approximately 0.5 to 1.5 µl/ind. · hr (Fig. 2) which in conversion to 20°C amounts to approximately 0.4—1.2 µl/ind. · hr. An average wet weight of one individual amounted to 1.5 mg. Winberg, Hartova (1953) and Kamler (1972) studied dependence of oxygen consumption upon young carp weight, at temperature of 20°C. Formulas obtained by them were almost identical, in approximation: $QO_2 = 0.6 \cdot W^{0.98}$. Oxygen consumption by one individual weighing 1.5 mg, calculated by this formula, amounts to 0.9 µl O_2/ind · hr, thus, it is in accordance with the result obtained here.

Assuming that the growth of animals during the embryo period can be determined, in approximation, by the exponential equation (Winberg, Pechen 1968) and according to Devillers (1965), such a formula was used for the obtained results of the oxygen consumption (Fig. 3), disregarding, in this case, the changes connected with development phases. It can be seen that in the control, the oxygen consumption increase during the embryo period was about sixfold. Abramova et al. (1965) studied the mechanism causing an increase in oxygen consumption in the embryo development of *Misgurnus fossilis*. They have shown in the examinations in vitro that the oxygen consumption increase occurs mainly in the fraction of mitochondrial structures closely related to the yolk granules. Furthermore, the oxygen consumption increase is also connected with some yet unaccountable factors which are active within the whole embryo and perish after its homogenization.

Oxygen consumption by embryos did not change under the influence of the applied doses of herbicide (Table I, Fig. 2). This means that chorion gives sufficient protection against herbicide in the investigated concen-

trations; nevertheless, one ought to remember that fertilization and swelling of eggs took place in an environment free from herbicide. At the end of embryo development, during the hatch and larval development, there was observed, on the whole, a decrease of oxygen consumption under the influence of herbicide. The inhibition of oxygen uptake by the blue liver mitochondria in the presence of 2,4-D-Na was reported by Hiltibran (1969). Metelev (1971) reports that after the poisoning with 2,4-D (amine salt), glycogen contents in fish liver decreased 3.4 times. Klekowski, Zvirgzds (1971) stated a statistically significant decrease in *Simocephalus vetulus* (Cladocera) respiration at 1.25 mM concentration of chemically pure 2,4-D-Na, as compared with the control. Zvirgzds et al. (1971) found that under the effect of the 1 mM concentration of chemically pure 2,4-D-Na, oxidative phosphorylation of mitochondria in carp liver decreased, as compared with the controls. The ATP-ase activity increased at this concentration, but decreased at the higher ones. Thus, one can believe that this herbicide is particularly dangerous for young larvae even in small doses, which do not produce a direct lethal effect since it increases the share of glycolysis in the release of energy. Since this process is less effective, it might cause a quicker exhaustion of larvae energetic reserve and this may have an unfavourable effect on their further development. Only in the premortal period a greater oxygen consumption was observed in animals cultivated in the 50 mg/l concentration, as compared with the control. This fact is familiar (e.g. Gyllenberg 1969) and is probably due to incomplete regulation of cell respiration.

Comparison of ash contents, as determined by "bombing" and by burning in a muffle furnace, revealed no significant differences. A similar result was obtained in an earlier work (Kamler in print) by determining organic matter content in *Perlodes intricata* (Plecoptera) larvae. However, variation of results obtained by the microbomb is so great that they can be used only as a rough estimation merely, when small quantities of material absolutely cannot be burned in a muffle furnace. The stated increase of the ash percentage in dry weight results from utilization of an animal's own organic substance.

Similarly as in the case of oxygen consumption, the applied doses of herbicide did not bear any effect, during the embryonic period, on other elements under investigation: dry weight and calorific values did not differ from one another in the control animals and those cultivated in the two concentrations of herbicide. Likewise, the percentage of eggs wasted away (without unfertilized eggs) according to Matlak (1972) is not distinctly higher in the herbicide, as compared with the control. However, it has to be reminded, again, that eggs were put into test solutions only after their swelling. In that situation the protective role of chorion proved to be sufficient. The influence of herbicide begins to bear

its effect only at the hatching period: the dry weight of larvae cultivated in herbicide is smaller than in the control ones (Fig. 4, Table II); the calorific value of 1 mg of larvae dry mass is also smaller (Fig. 7). Similarly Solski (1968), while putting carp eggs which probably had been already swelled, into atrazine solution, stated that they were not affected by those doses which had been noxious to larvae.

In the time of hatching, the calorific equivalent of an organism (C_e in Fig. 8) drops abruptly. It is, in all probability, connected with the rejecting of egg cases and with an intensification of metabolic processes. While comparing the control fish with fish cultivated in herbicide solutions, one can observe a phenomenon which is apparently paradoxical: a lower oxygen consumption (R_c for the 5 and 50 mg/l concentration in Fig. 8) and simultaneously a more intensive fall in the calorific equivalent (C_e for 5 and 50 mg/l concentration in Fig. 8) in the treated fish. This result would confirm the above presented data, by other authors and obtained by different methods, as to the increase of glycolysis share in the process of energy release under 2,4-D-Na influence.

A slight increase of the calorific equivalent was observed in the course of about 1.5 days after the hatch (85th—115th hour of development —Fig. 8). It might result from a measurement error, or else from the partial using up of materials cast off during the hatch, as nutriment. No description of that phenomenon occurring in fish has been spotted, but on the other hand, Jura, George (1958) report that young snails, when they emerge from egg capsules, utilize the jelly as food for a few days.

Afterwards (approximately at 115th — 190th hour) the calorific equivalent decreases slightly. The effect of herbicide (exclusively the 5 mg/l concentration, there are no data available for the 50 mg/l concentration) is expressed in lower C_e values in treated fish, as compared with the control ones. It is quite significant that at that time the increment of the calculated R_c (approximately 0.1 cal/20 hr) is approaching or perhaps even greater than the decrease of the calorific value in one individual (approximately 0.1 cal/30 hr). This would indicate the continued repayment of oxygen debt. The effect of herbicide in 50 mg/l concentration is evident in the decrease in the repayment of oxygen debt; this in turn, could result from the slowing down of the oxidative phosphorylation rate.

Oxygen consumption is, in general, considered to be a good integrating indicator of the physiological state of an organism. However, results of the present work indicate that it is not always right, and that, in bioenergetic studies on embryo development and also in researches on the effect of substances restraining oxidative phosphorylation, not only oxygen consumption but other factors, as well, should be taken into account, i.e. changes in energy contained in excreted and rejected matter and in calorific energy released in the anaerobic way.

Acknowledgements

I wish to thank Miss E. Duszak for her active help during all the stages of the preparation of this work. Dr. O. Matlak has made the necessary material available to me and all the time shared her experience with me. Elaboration of this work was made possible thanks to the initiative and valuable help of Ass. Prof. Dr. S. Wróbel. Miss H. Sztrantowicz and Mr. K. Srokosz, M. Sc. were of great assistance in the laborious measurements.

5. SUMMARY

A thorough study was made of the effect of the commercial formulated product "Pielik", with active ingredient consisting of 2,4-D-Na, on the embryo and early larval development of carp. Animals developing without herbicide as well as animals treated with 5 and 50 mg A. I./l were observed. Changes in respiration rate, live and dry weight, ash, and calorific values of 1 mg of dry weight were measured; moreover, total energy expenditure was compared with the energy released in the respiratory processes. On the whole, it has been stated that embryos placed into test solutions two hours after fertilization were not affected by the applied doses; nevertheless, in some phases of larval development there was an appreciable effect of herbicide manifested by a decrease in respiration rate, dry weight, and calorific values. After 200 hr of development mass mortality of larvae occurred in the 50 mg/l concentration. The obtained results seem to point out to the significance of glycolysis in the embryo development of the control animals and of the other ones developing in test solutions, and also to indicate the handicap of aerobic processes in larvae under the influence of herbicide. As it was demonstrated by comparison with other papers, the commercial product "Pielik", examined at present, applied in practice is more toxic than pure 2,4-D-Na. Processes occuring during early stages of development of fish treated with herbicides are very complex and they call for wide and complex studies, including determinations of chemical composition of body and rejected matters. The knowledge of oxygen consumption processes, alone, does not suffice to judge about the physiological state of an organism at that kind of problems. The knowledge of *RQ* itself does not allow for determination of the oxy-calorific coefficient, though *RQ* may be still useful, as it has been, as an auxiliary, general indicator for the observed processes, especially when the other elements are examined parallelly.

6. STRESZCZENIE

Prześledzono wpływ preparatu handlowego, "Pielika", którego substancję aktywną stanowi 2,4-D-Na, na rozwój embrionalny i wczesne stadia rozwoju larwalnego karpia. Badano zwierzęta rozwijające się bez dodatku herbicydu i pozostające pod ciągłym wpływem stężeń 5 i 50 mg A. S./l. Mierzono zmiany oddychania, żywej i suchej wagi, popiołu i wartości kalorycznej 1 mg suchej masy, oraz porównano ogólne wydatki energii z energią wydawaną w procesie respiracji. Ogólnie stwierdzono, że zarodki umieszczone w roztworach testowych po 2 godz. od zapłodnienia, nie są wrażliwe na stosowane dawki, natomiast w niektórych fazach rozwoju larwalnego zaznacza się wpływ herbicydu, prowadzący do obniżenia respiracji, suchej wagi i wartości kalorycznej. Po 200 godz. nastąpiła masowa śmiertelność larw w stężeniu 50 mg/l. Uzyskane wyniki wydają się wskazywać na znaczenie glikolizy w rozwoju embrionalnym zwierząt kontrolnych i rozwijających się w roztworach testowych, oraz na upośledzenie procesów aerobowych u larw pod wpływem herbicydu. Jak wykazały porównania z innymi pracami, badany obecnie preparat handlowy stosowany w praktyce, jest bardziej toksyczny od czystego 2,4-D-Na. Procesy zachodzące podczas działania herbicydów na wczesne stadia rozwojowe ryb są bardzo skomplikowane i wymagają szerokich badań kompleksowych, z uwzględnieniem zmian składu chemicznego ciała i materii odrzucanej. Sama znajomość zużycia tlenu nie pozwala sądzić o fizjologicznym stanie organizmu, przy takich, jak obecne, badaniach. Sama znajomość *RQ* nie pozwala na określenie współczynnika oxy-kalorycznego, natomiast *RQ* nadal może być przydatny, jako pomocniczy, ogólny wskaźnik zachodzących procesów, wówczas gdy badane są równolegle i inne elementy.

7. REFERENCES

[Abramova, N. B., Likhtman, T. V., Neifakh, A. A.] Абрамова, Н. Б., Лихтман, Т. В., Нейфах, А. А. 1965. Исследование механизмов увеличения интенсивности дыхания в эмбриональном развитии рыб [Investigation of mechanisms responsible for the rise in respiration with embryonic development in fish]. *Ž. evoljuc. Biochim. Fiziol.*, 1, 227–233 [Engl. summ.].

Alabaster, J. S. 1969. Survival of fish in 164 herbicides, insecticides, fungicides, wetting agents and miscellaneous substances. *Int. Pest Control*, 11 (2), 29–33.

Blaxter, J. H. S. 1969. Development: Eggs and larvae. In: W. S. Hoar, D. J. Randall [Eds] *Fish physiology*. III, 177–252, New York, Academic Press.

Devillers, C. 1965. Respiration et morphogenèse dans l'oeuf des téléostéens. Année biol., 4, 157–186.

[Gubler, E. V., Genkin, A. A.] Гублер, Е. В., Генкин, А. А. 1969. Применение критериев непараметрической ститистики для оценк различии двух групп наблюдений в медико-биологических исследованиях [Application of nonparametric statistics to evaluation of differences between two groups of observations in medical-biological investigation]. Moskva, Izdat. "Medicina".

Gyllenberg, G. 1969. The energy flow through a *Chorthippus parallelus* (Zett.) (Orthoptera) population on a meadow in Tvärminne, Finnland. *Acta zool. fenn.*, 123, 1–74.

Harrow, B., Mazur, A. 1958. *Textbook of biochemistry*. VII ed. Philadelphia, W. B. Saunders Co.

Hiltibran, R. C. 1969. Oxygen and phosphate metabolism of bluegill liver mitochondria in the presence of 2,4-dichlorophenoxyacetic acid derivatives. *Trans. Ill. St. Acad. Sci.*, 62, 175–180.

Jezierska, B. [unpubl.] Elements of energy balance of *Perca fluviatilis* L.

Jura, C., George, J. C. 1958. Observation on the jelly mass of the eggs of three molluscs, *Succinea putris*, *Limnaea stagnalis*, and *Planorbis corneus* with special reference to metachromasia. *Proc. K. ned. Akad. Wet., Ser. C*, 61, 590–594.

Kamler, E. 1970. The main parameters regulating the level of energy expenditure in aquatic animals. *Pol. Arch. Hydrobiol.* 17, 201–216.

Kamler, E. [in print.]. Some data on ecological physiology and bioenergetics of a cold-water stenotherm *Perlodes intricata* (Plecoptera). *Vehr. int. Ver. Limnol.*, 18, 00–00.

Kamler, E. 1972. Respiration of carp in relation to body size and temperature. *Pol. Arch. Hydrobiol.*, 19, 325–331.

Kleiber, M. 1961. *The fire of life, An introduction to animal energetics*. New York, J. Wiley.

Kleiber, M., Cole, H. H., Smith, A. H. 1943. Metabolic rate of rat fetuses in vitro. *J. cell. comp. Physiol.*, 22, 167–176.

Klekowski, R. Z. 1968. Constant-pressure microrespirometer for terrestrial invertebrates. In: W. Grodziński, R. Z. Klekowski [Eds] *Methods of ecological bioenergetics*. 67–86, Warszawa-Kraków, Pol. Acad. Sci.

Klekowski, R. Z. 1970. Bioenergetic budget and their application for estimation of production efficiency. *Pol. Arch. Hydrobiol.*, 17, 55–80.

Klekowski, R. Z., Zvirgzds, J. 1971. The influence of herbicide 2,4-D-Na on respiration and survival of *Simocephalus vetulus* (O. F. Müller) (Cladocera). *Pol. Arch. Hydrobiol.*, 18, 393–400.

Klekowski, R. Z., Bęczkowski, J. [in prep]. A new modification of microbomb calorimeter.

Kołaczkowski, S. T. 1970. Zagrożenie środowiska wodnego pestycydami [Pesticides as a menace for aquatic milieu]. Materiały sesji naukowej „Wpływ pestycydów na wody powierzchniowe". Kraków 1970, 7–32, Komitet Hydrobiol. PAN.

[Korde, B. A., Zvirgzds, J. K.] Корде, Б. А., Звиргздс, Ю. К. 1971. Влияние 2,4 D-Na на выживаемость икры и темп раннего эмбрионального развития вьюна [The effect of 2,4 D-Na on the survival of fertilized eggs and the rate of the early embryonic development of loach (*Misgurnus fossilis* L.]. In: *Eksperimental'naja vodnaja toksikologija*. 2, 31–42, Riga, Izdat. "Zinātne".

Krogh, A. 1919. The rate of diffusion of gases through animal tissues with some remarks on the coefficient of invasion. *J. Physiol., Lond.*, 52, 391.

Matlak, O. 1966. Wzrost narybku dwóch rodzin karpi hodowanych w Gołyszu [Zuwachs der Karpfenbrut von zwei Karpfenfamilien aus Gołysz]. *Acta Hydrobiol.*, 8, suppl. 1, 255–291 [German summ.].

Matlak, O. 1970. Wylęganie karpia w warunkach sztucznych [Artificial hatching of carp]. Instytut Rybactwa Śródlądowego, Samodzielna Pracownia Upowszechniania Postępu, Olsztyn-Gołysz, 42, 1–20.

Matlak, O. 1972. Sodium salt 2,4-D related to embryo and larval development of carp. *Pol. Arch. Hydrobiol.*, 19, 437–450.

[Metelev, V. V.] Метелев, В. В. 1971. Токсичность пестицидов для рыб, механизм действа и методы индикации [Toxicity of pesticides for fish, mechanism of action and methods of indication]. In: *Eksperimental'naja vodnaja toksikologija.* 2, 104–122, Riga, Izdat. "Zinātne" [Engl. summ.].

[Milman, L. S.] Мильман, Л. С. 1965. Гликолиз в эмбриональном развитии вьюна *Misgurnus fossilis* [Glycolysis in the embryonic development of loach]. *Ž. obšč. Biol.*, 26, 237–240 [Engl. summ.].

[Olifan, V. I.] Олифан, В. И. 1949. Циклический характер роста рыб на ранних этапах их постэмбрионального развития [Cyclic character of fish growth in early stages of their postembryonic development]. *Dokl. Akad. Nauk SSSR*, 66, 1211–1214.

Phillipson, J. 1964. A miniature bomb calorimeter for small biological samples. *Oikos*, 15, 130–139.

Pliszka, F. 1953. Rozród i rozwój certy (*Vimba vimba* L.) [Reproduction and development of *Vimba vimba* L.] *Pol. Arch. Hydrobiol.*, 1, 137–163 [Engl. summ.].

Prus, T. 1968. Measurement of calorific value using Phillipson microbomb calorimeter. In: W. Grodziński, R. Z. Klekowski [Eds] *Methods of ecological bioenergetics.* 15–32, Warszawa-Kraków., Pol. Acad. Sci.

Solski, A. 1968. Ocena herbicydów do celów rybackich [Evaluation of herbicides for fishing purposes]. *Zesz. przyr.*, 8, 61–126 [Engl. summ.].

[Stroganov, N. S.] Строганов, Н. С. 1962. Экологическая физиология рыб [Ecological fish physiology]. I. Moskva, Izdat. Mosk. Univ.

Swift, R. W., French, C. E. 1954. *Energy metabolism and nutrition.* Washington, Scarecrow Press.

[Trifonova, A. N.] Трифонов, А. Н. 1949. Критические периоды эмбрионального развития [Cristal periods in embryonic development]. *Usp. sovrem. Biol.*, 28, 154–168.

Trzebiatowski, R. 1969 a. Zastosowanie herbicydów do zwalczania makrofitów w stawach karpiowych [Application of herbicides for destroying macroplants in carp ponds]. *Roczn. Nauk roln., Ser. H*, 90, 713–729 [Engl. summ.].

Trzebiatowski, R. 1969 b. Oddziaływanie herbicydów (Antyperzu i mieszaniny Antyperzu z Pielikiem) na narybek i kroczki karpia (*Cyprinus carpio* L.) [Effects of some herbicides (Antyperz and mixture of Antyperz and Pielik) on one and two year old carp (*Cyprinus carpio* L.)]. *Roczn. Nauk. roln., Ser. H*, 90, 731–747 [Engl. summ.].

Walsh, J. E. 1965. *Handbook of nonparametric statistics.* II. Princeton, D. van Nostrand Co.

[Winberg, G. G.] Винберг, Г. Г. 1956. Интенсивность обмена и пищевые потребности рыб [Rate of metabolism and food requirements of fish]. Minsk, Izdat. Belgosuniversitet.

[Winberg, G. G., Hartova, L. E.] Винберг, Г. Г., Хартовн, Л. Е. 1953. Интенсивность обмена у мальков карпа [Rate of metabolism in carp fry]. *Dokl. Akad. Nauk SSSR*, 89, 1119–1122.

[Winberg, G. G., Pechen, G. A.] Винберг, Г. Г., Печень, Г. А. 1968. Рост, скорость развития и плодовитость в зависимости от условий среды [Growth, rate of development and fecundity depending on the environmental conditions]. In: G. G. Winberg [Red] Metody opredelenija produkcii vodnych životnyh. 45–77, Minsk, Izdat. „Vyšejšaja Škola".

Winnicki, A. 1968. Respiration of the embryos of *Salmo trutta* L. and *Salmo gairdneri* Rich. in media differing in gaseous diffusion rate. *Pol. Arch. Hydrobiol.*, 15, 23–38.

[Zvirgzds, J. K., Lāce, Z. M., Grundule, M. V., Zujka, A. A.] Звирздс, Ю. К., Лаце, З. М., Грундуле, М. В., Зуйка, А. А. 1971. Влияне 2,4 D-Na на окислительное фосфорилирование, механохимические изменения и аденозинтрифосфатазную активность митохондрий печени карпа [Effect of 2,4 D-Na on oxidative phosphorylation, mechanochemical changes and adenosintriphosphatase activity of mitochondria of carp liver]. In: *Eksperimental'naja vodnaja toksikologija.* 2, 12–25, Riga, Izdat. "Zinātne" [Engl. summ.].

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	19	4	475–504	1972

R. Z. KLEKOWSKI and J. W. ZAJDEL*

CAPACITY ELECTROLYTIC RESPIROMETER KZ-CER-01T** WITH REVIEW AND DISCUSSION OF ELECTROLYTIC RESIROMETRY

Department of Bioenergetics and Bioproductivity, Nencki Institute of Experimental Biology, Pasteura 3, Warszawa, Poland

ABSTRACT

A review of development of electrolytic respirometers as well as analyses of systems applied in them are given. The new capacity electrolytic respirometer KZ-CER-01T is described in which a battery of condensers is used as a source of current, and electronic system ensuring a stability of generated portions of oxygen during subsequent "impulses" and automatic counting and recording systems are applied. Thus the measurements in the respirometer can be carried out continuously for many days at a constant concentration of oxygen in the vessel holding the object investigated.

CONTENTS

---

* Department of Experimental Minicomputers, Institute of Mathematical Machines, Skrońskiego 8, Warszawa, Poland.

** The patents pending.

1. INTRODUCTION

In recent decades, and especially in the years when the International Biological Programme has been in action, the investigations on ecological physiology and especially on ecological bioenergetics developed considerably. The latter become especially a necessity since they supply key data and methods for understanding the mechanisms of energy flow through ecosystems, the estimation of production at different trophic levels, and modelling the dynamic energy budget of a total ecosystem (cf. Phillipson 1962, Duncan, Klekowski in print, Klekowski, Duncan in print, Klekowski 1970, Mann 1969, Winberg 1962).

In such investigations, the measurement of respiration transferable to so-called "cost of maintenance" of an organism, population, or a whole community, plays an important role since the amount of liberated energy in the respiratory process constitute often a large share in the total amount of energy assimilated by an organism (cf. Kamler 1970).

Thus, needless to say that there is a constant and increasing need for measuring the respiration, done at small time intervals, for a relatively long period, with a considerable accuracy. Especially diel measurements are lacking, or carried out for several diel cycles which would inform about the instantaneous respiratory rate and its diel rhythm as well as the cumulative respiration, informing about the cost of maintenance of an organism since its birth to each, randomly chosen, moment of its life. Such requirements were met with when starting the construction of the respirometer KZ-CER-01T, described in this paper. The measurement in this respirometer is done in gaseous phase, which predisposes it for measuring gaseous metabolism of animals respiring with the atmospheric oxygen. However, the respiration of aquatic animals is often, in present practice, measured in gaseous phase (volumetric respirometers, manometric respirometers of Warburg type, Cartesian divers, and others) since the measurement of respiration in fluid phase is much more difficult. The Winkler method, commonly used, both for respirometry in closed vessels and in flowing respirometers is time-consuming and unsuitable, in spite of many trials, to automation and registration of results. In each case when measurements should be taken, for any reasons, at fluid phase, one can easily use electrometric methods (galvanic and polarographic: cf. Mackereth 1964, Klekowski, Kamler 1968, 1971, Teal 1971).

The model of the respirometer proposed in this paper permits, according to us, to carry on long-lasting (for many days) measurements of respiration, both in aquatic animals and in those respiring with the atmospheric oxygen, with a simultaneous recording the results and maintaining the constant concentration of oxygen in the environment in which stays the object investigated.

2. DENOTATIONS OF SYMBOLS

a.	— logical signal
A	— Anode
AM, AM$_A$, AM$_B$, AM$_C$	— Amplifiers
A(RE)	— Amplifier (Relay)
AP	— Air pump
b.	— logical signal
c.	— logical signal
C	— Cathode
C$_1$, C$_2$...	— Capacitors
C-CH	— Compensating chamber
CE$_1$, CE$_2$	— Control electrodes
CO	— Comparator
CV	— Compensating vessel
d.	— logical signal
DA	— Drying arm
DC	— Decade counter
DU	— Detecting unit of O_2-consumption in *R-CH*
ε.	— Error signal
EC	— Electric clock
EU	— Electrolysis unit

F_1, F_2 — Fuse
i, i_1, i_2... — current intensity
ID — Index drop
k. — logical signal
L_A, L_B, L_C — Control lamps (L_A — charge, L_B — impulse, L_C — ready)
LM — Level meter
M — Manometer
MB — Measuring burette
MS — Main switch
N_A, N_B, N_C, N_D — NOR logical gates
NDA — Nonlinear Detector Amplifier
OG — Oxygen generator
OSG — "One shot" generator
p — pressure in R-CH
p_o — pressure in C-CH
p. — logical signal
P_1, P_2... — Potentiometer
Pd — palladium cathode
PU — Programming unit
R, R_1, R_2... — Resistors
RE, RE_A, RE_B, RE_C — Relays
$1RE_A$, $2RE_A$... — Contacts 1, 2 etc of relay RE_A
R-CH — Respiratory chamber
RU — Recording unit of oxygen generator
s — Signal
S_1, S_2... — Switches
SCH_1, SCH_2 — Schmitt trigger
SCU — Steering and computing unit
SO_2 — Junction on the panel
SO_1 — Junction on the respiratory gasometric unit
SPS — Stabilized power supply
St_1, St_2, St_3, St_4 — Stabilizators (Zener-diode)
SU — Steering unit of oxygen generator
SWO — Square wave oscillator
T_1, T_2... — Transistors
TM — Timing mechanism
Tr — Transformer
τ — Time delay (time of capacitor C_1 charging)
T — Time delay unit
U_1, U_2 — reference voltage
U_B — battery voltage
U_{C1} — voltage on the capacitor C_1
$u(t)$ — alternating voltage source
U_S — supply voltage

3. HISTORICAL SURVEY OF METHOD DEVELOPMENT

The earliest example of known respirometers in which the O_2 consumption is compensated by means of the electrolytic method is the type proposed by Fernandes (1923) (Fig. 1 A). In this respirometer the oxygen is produced in the electrolysis unit *EU* from the solution of NaOH. Anode-vessel *A* is connected to the respiratory chamber *R-CH* and manometer *M* is open to the atmosphere; the manometer serves as the zero instrument to control the speed of O_2 production. The speed is manually adjusted by means of varying the intensity of the electrolysis current. Cathode-vessel *C* is connected to the measuring burette *MB* in which hydrogen is collected. The closed air circuit, forced by the air pump *AP* is connected to the respiratory chamber through $Ba(OH)_2$ solution in which CO_2 is absorbed. The quantity of O_2 produced in the anode-vessel equals half the volume of H_2 collected in the measurement burette. Carbon-dioxide absorbed by $Ba(OH)_2$ is determined by titration with HCl. To calculate the consumed O_2 in *STP*-conditions, except for the corrections for temperature, barometric pressure and water vapour pressure, the corrections for the possible changes in the barometric pressure and hydrostatic pressure in the burette during the experiment are also necessary. Fernandes (1923) measured gas exchange of the germinating peas.

De Boer (1929) (Fig. 1 B) adopted Fernandes respirometer but introduced essential changes; he connected the second arm of the manometer *M* to the compensating chamber *C-CH* and closed hermetically the electrolysis unit *EU* and thus he become independent of the changes in the barometric pressure during the measurements. He also added the compensating vessel *CV* joined with the burette *MB*

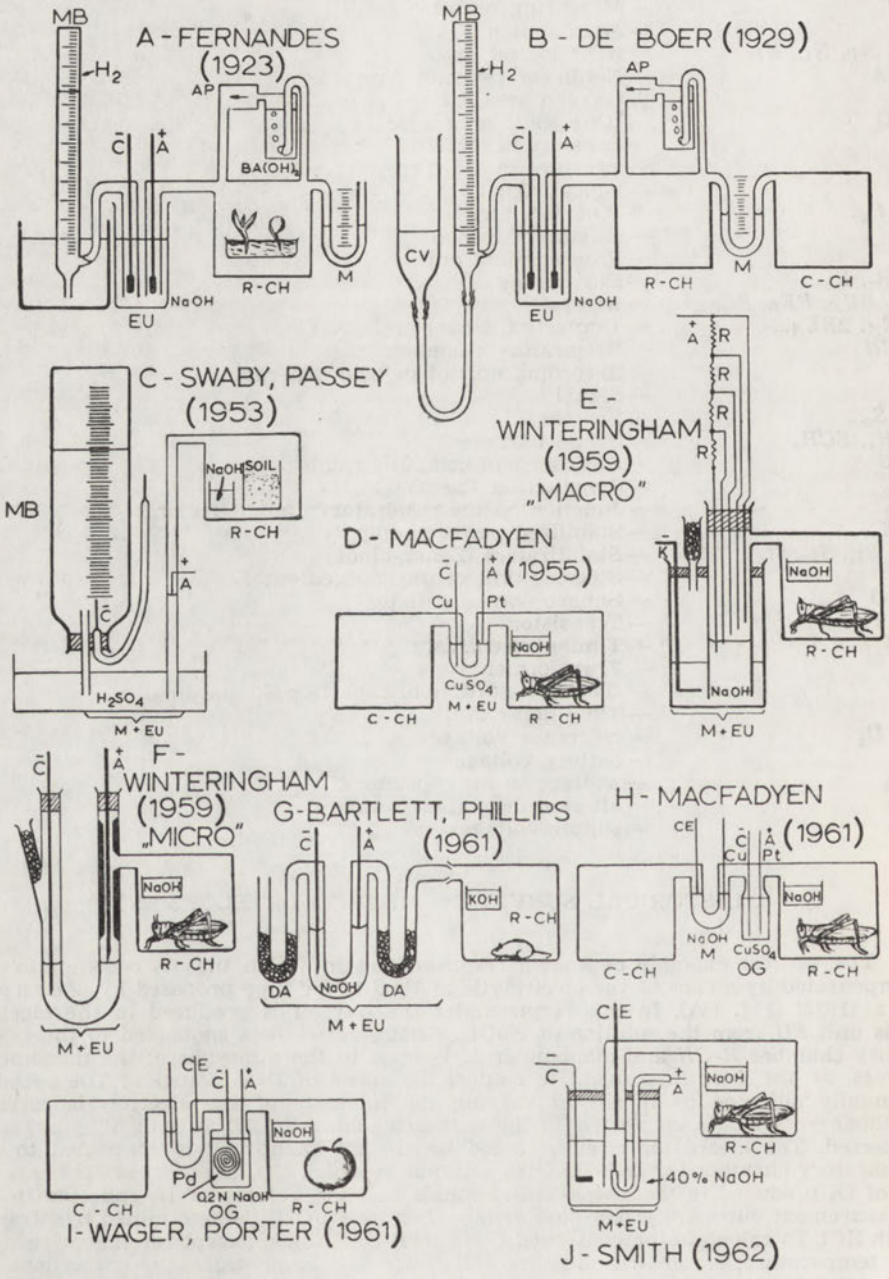


Fig. 1. Development of electrolytic respirometers

to compensate the variations of the hydrostatic pressure in the burette. De Boer (1929) measured the respiration of Phycomycetes in his respirometer.

Swaby, Passey (1953) (Fig. 1 C) like their predecessors measured volumetrically the quantity of H_2 collected in the measurement burette *MB;* the solution of H_2SO_4 was used as the electrolyte. The construction is simplified, since the cathode *C* is located directly in the burette. An important improvement is the introduction of feed-back in the automatic control of the electrolysis time by placing the anode *A* in a kind of manometer open to the atmosphere. The consumption of O_2 in the respiratory chamber *R-CH* results in the contact of the anode with the electrolyte and following discharging of the compensating volume of O_2 this contact is broken. The lack of compensating chamber makes it necessary to introduce the corrections for the barometric pressure. Swaby, Passey (1953) measured the gas exchange of the soil.

Capraro (1953) gives only a very short description of the principle. The solution of NaOH was subject to electrolysis and the current intensity of the electrolysis was controlled by the pressure changes in the respiratory chamber. The current intensity, which through integration in the time function was converted into the amount of produced oxygen, was measured.

In the respirometer of Winteringham (1959) (Fig. 1 E) in the "macro" model, the current intensity of the electrolysis fluctuate in time, since as a result of application the system of 5 anodes *A* and resistors *R* the current intensity increases or decreases step by step together with the increase or decrease of the oxygen uptake in the respiratory chamber *R-CH*. The principle of operation of the automatic control by connecting the anode to the manometric system is the same as in Swaby, Passey (1953). In the "micro" model (Fig. 1 F) the anode is single. Since the cathode part of the electrolysis unit does not have a compensating chamber but is connected directly to the atmosphere, the changes in the atmospheric pressure during the measurements were considered in the corrections for calculations. The intensity of the electrolysis current was integrated by the planimetring of the field under the curve on the record in the "macro" model or by adding the series of peaks in the "micro". The amount of discharged oxygen was calculated from the Faraday's laws of electrolysis and additionally calibrated directly by means of a mercury burette attached to the respiratory chamber; the burette "simulated" the consumption of the known volumes of O_2.

Bartlett, Phillips (1961) (Fig. 1 G) apparently not knowing or not taking into account the models available at that time (e.g. Winteringham 1959) constructed an electrolytic respirometer based on a completely different principle. The electrolysis unit *EU* with 1 n NaOH as the electrolyte compensates automatically the oxygen consumption in the respiratory chamber *R-CH* in a way very similar to that of Swaby, Passey (1953). The amount of produced oxygen is calculated gravimetrically as the 16/18 of the loss in weight of the electrolysis unit caused by the electrolytic degradation of water.

MacFadyen (1955) in his first model (Fig. 1 D) followed De Boer (1929) and used the compensating chamber *C-CH* and the time of electrolysis was adjusted automatically in the same way as in the Swaby, Passey (1953) respirometer. An essential modification was the use of the copper sulphate solution as the electrolyte which does not cause the discharge of gaseous hydrogen on the cathode as in the previous respirometer but results in deposits of metallic copper. A further improvement was the introduction of the two electrical systems in MacFadyen's (1961) next model (Fig. 1 H): lst the pressure-charge detecting circuit located in the manometer *M* connecting the respiratory *R-CH* and compensating chambers *C-CH* with the NaOH solution as the electrolyte; in this circuit there is very small flow of alternating current whose interruption — by the triode or pentode electronic amplifier — switches on the direct current to the 2nd oxygen generating circuit flowing through the $CuSO_4$ solution in a separate oxygen-generator *OG* connected to the respiratory chamber. A timing mechanism tests the pressure-change detecting circuit at regular cycles (e.g. every 10 sec); if the latter is open the oxygen-generating current is switched on for the period of one cycle, providing a "dose" of oxygen. One such "dose" is constant for a given run of measurements and is pre-set depending upon the oxygen required by the given object. The amount of oxygen is calculated from the current intensity and the time of flow. A recorder takes down each dose of oxygen. The same recorder and the timing mechanism may work with 6 respirometers. The accuracy of the system depends on the stability of the oxygen generating current and the accuracy with which this current and its duration time are measured.

Chronologically subsequent[1] model of the electrolytic respirometer is described by Wager, Porter (1961). The main diagram is similar to that of MacFadyen (1961): respiration *R-CH* and compensation *C-CH* chambers; independent detecting and oxygen-generating circuits; the oxygen "doses" of the amount determined by the oxygen generating current and its duration; recording of the number of "doses"/time unit. In the oxygen generator the NaOH solution is subject to electrolysis and the hydrogen is absorbed by the large palladium cathode Pd surface.

Smith (1962) developed and improved the system of Winteringham (1959). The introduced separate detecting and oxygen generating circuits (like MacFadyen 1961, Wager, Porter 1961) controlled electronically. Assuming that the electrolysis current intensity is constant, the periods of electrolysis for each respirometer are added by an electric clock. Since there is no compensating chamber the corrections for the differences in the barometric pressure are necessary.

The Phillipson (1962) model of the respirometer does not in fact differ from the Winteringham (1959) system. The use of two respirometers (out of the set of 10) as the thermo-barometers makes the corrections possible without recording the changes of the barometric pressure. The recorder only takes down the electrolysis duration, with the assumption that the electrolysis current intensity is not subject to any changes.

Slight changes in the MacFadyen (1961) model were introduced by Fourche (1964) and Byzov et al. (1967). Fourche (1964) used the refinements of the pressure-detecting system, electrodes and electronics and also the photographic recording of the number of oxygen "doses". Byzov et al. (1967) used as a pressure detector in the horizontal manometer an index drop whose location is controlled by a photodiode.

MacFadyen's (1961) concept of a respirometer was also adopted in the commercially available "Sopromat" respirometer produced by Voith and designed for determination of BOD (biological oxygen demand) of sewages and polluted waters.

4. COMPARISON OF ELECTRICAL SYSTEMS USED TILL NOW

Table I shows properties of electrical systems of the respirometers mentioned in the previous chapter. Apart from the first two (Fernandes 1923, De Boer 1929) they are systems with a feed-back between the detecting unit of oxygen consumption in the respiratory chamber and the oxygen generator controller. The recording unit does not have to be electrical but may be of a different design. Figure 2 shows the block diagram of a typical electrolytic respirometer system.

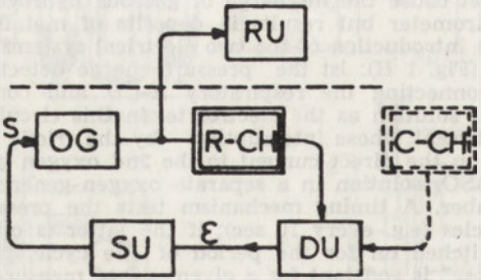


Fig. 2. General block diagram of an electrolytic respirometer

[1] Taking into account the receipt dates of manuscripts.

Table I. Characteristics of various systems used in electrolytical respirometers

Author	Detection of oxygen consumption					Oxygen generat			Measurement of oxygen generation							Calibration
	Detecting circuit						Process on the cathode		Non-electric	Electric						
	Feed-back control of oxygen generation	Compensating chamber	Common: Anode of O_2 generator functions as control electrode	Independent: separate control electrode	Two-stage	Multi-stage	Electrolyte	Generation of gaseous H_2	Generation of gaseous H_2 and absorption	Copper generation	Gasometric-volumetric	Gravimetric	Measurement and integration of function $\Sigma Q = \int i(t)dt$ $i \neq \text{const.}$ $\Delta t \neq \text{const.}$ $Q \neq \text{const.}$ I	Measurement of time electrolysis $\Sigma Q = \Sigma \Delta t \cdot i$ $i = \text{const.}$ $\Delta t \neq \text{const.}$ $Q \neq \text{const.}$ II	Counting of switchings (N) $\Sigma Q = N \cdot \Delta t \cdot i$ $i = \text{const.}$ $\Delta t = \text{const.}$ $Q = \text{const.}$ III	Counting of switchings (N) $\Sigma Q = N \cdot Q$ $i \neq \text{const.}$ $\Delta t \neq \text{const.}$ $Q = \text{const.}$ IV	Calculation from Faraday's laws	Empirical: imitation of O_2 consumption
Fernandes (1923)							NaOH	+		+			+					
De Boer (1929)		+					NaOH	+		+	+		+					
Swaby, Passey (1953)	+		+		+		H_2SO_4	+		+	+		+					
Capraro (1953)	+	?	+?	?	+		NaOH	+?		+	+		+				+?	+
MacFadyen (1955)	+	+	+		+		$CuSO_4$	+		+	+		+				+	+
Winteringham (1959)	+	+	+		+	+	NaOH	+		+	+		+				+	+
Bartlet, Phillips (1961)	+	+	+		+	+	NaOH	+		+	+		+				+	+
MacFadyen (1961)	+	+	+	+	+		$CuSO_4$	+		+	+		+		+		+	+
Wager, Porte (1961)	+	+	+	+	+	+	NaOH	+		+	+		+		+		+	+
Smith (1962)	+	+	+	+	+	+	NaOH	+		+	+		+	+	+		+	+
Phillipson (1962)	+	+	+	+		+	NaOH	+		+	+		+	+	+		+	+
Fourche (1964)	+	+	+	+	+	+	$CuSO_4$	+		+	+		+	+	+		+	+
Byzov et al. (1967)	+++	++	+	+++	+++	+	$CuSO_4$, $CuSO_4$, $CuSO_4$	+ + +		+ + +	+ + +		+ + +	+ + +	+ + +		+ + +	+ + +
„Sapromat"		+	+	+	+	+									+			
Present work	+	+	+	+	+	+									+		+	+

*and empirical: voltametric.

OXYGEN CONSUMPTION DETECTORS

As seen from Table I the earlier respirometers did not have separate detection systems of oxygen consumption and generation. It was only MacFadyen's (1961) model and a number of the successive ones which had a separate detecting unit. They were mostly manometers in which one arm connected to the respiratory chamber had an electrode operating as a level sensor. A typical circuit is shown in Fig. 3 A. The alternating voltage source (because of the electrolysis of liquid in the manometer) is supplied through a contact electrode to the input of the amplifier *A* or a very sensitive relay (*RE*).

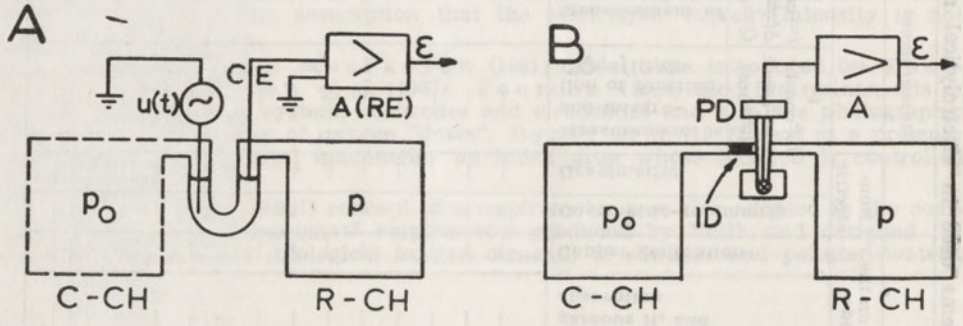


Fig. 3. Electric diagram of the detection unit of oxygen consumption in the respiratory chamber. A — manometric unit (MacFadyen 1961, Wager, Porter 1961), B — volumetric unit with photodiode (Byzov et al. 1967)

Such detector circuit gives the error signal (ε) in the form of two levels:

$$\varepsilon = 0 \text{ when } p \geqslant p_o, \qquad \varepsilon \neq 0 \text{ when } p_o > p$$

where: p_o — pressure in the compensating chamber *C-CH*, p — pressure in the respiratory chamber *R-CH*.

A volumetric variant of the oxygen consumption detecting system was applied by Byzov et al. (1967) (Fig. 3 B). The oxygen consumption in the respiratory chamber *R-CH* causes the shift of the index drop of liquid *ID* in the horizontal capillary connecting the respiratory *R-CH* chamber to the compensating chamber *C-CH*. The shifting index drop of dyed liquid covers and uncovers the photodiode *PD* which results in the signal ε taking two above mentioned levels.

OXYGEN GENERATORS

Solutions of NaOH, H_2SO_4 and $CuSO_4$ were used as electrolytes (Table I). When the first two are used, the gaseous hydrogen is discharged

on the cathode and collected in the measuring burette (Fernandes 1923, De Boer 1929, Swaby, Passey 1953) or released into the atmosphere (Winteringham 1959, Barlett, Phillips 1961, Smith 1962, Phillipson 1962). The discharge of gaseous hydrogen does not permit the use of compensating chamber on the cathode side of the manometer. An original solution but one which makes the operation of the respirometer complicated was found by Wagner, Porter (1961) who used a large surface palladium cathode which absorbed hydrogen. It was only through introduction of the $CuSO_4$ electrolyte by MacFadyen (1955) which causes the discharge of the negligently small volume of copper on the cathode that the use of both compensating chamber and a separate oxygen generator with its own electrical circuit was possible.

GENERATION CONTROL AND MEASUREMENTS OF THE QUANTITY OF PRODUCED OXYGEN

Table I shows that in the first two respirometers (Fernandes 1923, De Boer 1929) there is no automatic feedback control of oxygen generation. In those two and also in the model of Swaby, Passey (1953) in which the oxygen generation control was already applied the measurement of its amount is done in a nonelectrical way. The volume of the discharged hydrogen is measured and half of this volume is the discharged oxygen. Also in the Barlett, Phillips (1961) respirometer there is an automatic control of O_2 generation, but the measurement of its amount is nonelectrical. The weight loss of the whole electrolysis unit is measured here; 16/18 of this loss is the weight of the produced oxygen.

In all other models (Table I) the amount of generated oxygen is measured electrically. For this purpose it is necessary to know the electric charge which was moved through the electrolyte in a given time interval. Then the equivalent amount of discharged oxygen for such a charge may be calculated from the Faraday's laws of electrolysis. For certain known and reiterating charges, unmarked in electrical units, it is also possible to determine the oxygen equivalent in the empirical way.

The systems that control generation and measure the amount of produced oxygen may be classified by taking into account the following parameters occurring in the process of electrolysis:

i — intensity of the electrolysis current,

Δt — time duration of the electrolysis (in the extreme case the electrolysis may be continuous),

Q — electrical charge passing through the electrolyte in Δt.

The intensity of the electrolysis current may always assume the same value (i=const.) or it may be different both in the particular periods ("doses") of electrolysis and in a single period ($i \neq$ const.). The electrolysis duration may be the same (Δt=const.) or it may be of a different length ($\Delta t \neq$ const.). In the same way the charges moved through the electrolyte in the successive period of electrolysis may be the same (Q=const.) or they may be different from each other.

In Table I the respirometers with electrical measurement of the amount of generated oxygen were divided into four groups depending on the constancy or inconstancy of the mentioned above parameters: i, Δt, Q.

Group I (Fig. 4 A) includes respirometers of direct operation, that is, such in which there are no separate circuits of oxygen consumption and generation detection; thus the same electrode (A — anode) is both detecting and generating. The current flows in those time intervals ($\Delta t \neq$ const.) when the anode contacts the electrolyte. The intensity of the electrolysis current changes depending on the battery condition and the properties of electrodes and electrolyte ($i \neq$const.). The measurement of the amount of produced oxygen is done by means of recording the current intensity (i) in the time function and integration of Q for the chosen periods, e.g. graphic method (Winteringham 1959). Direct integration of Q by means of voltameter, e.g. silver voltameter (MacFadyen 1955) is also possible.

Group II (Table I, Fig. 4 B) consist of the respirometers differing from these in group I by stabilization of the electrolysis current (i=const.). In such a respirometer it is enough to record the time of electrolysis (Δt) which can be done by means of an electric clock (Smith 1962) or a chronograph (Phillipson 1962). Such stabilization of the intensity of electrolysis current, however, requires the use of the detecting circuit independent of the oxygen generation circuit (Smith 1962). The current i is switched on by a relay RE for certain (unequal) periods of time, depending on the signal from the detecting unit DU. The current intensity is constant due to the applications of the high value voltage source and the resistance R. Intensity of the polarization of electrode $U_p \ll U_B$ and thus is of small influence on the current intensity i. Phillipson's model (1962) does not have an independent detecting system which can make the stabilization of the current intensity of electrolysis more difficult.

The intention of the designers of respirometers belonging to group III (Table I, Fig. 4 C) was the use of stabilized electrolysis current (i=const.) and constant and recurrent length of the particular periods of electrolysis, that is "impulses" (Δt=const.). Thus in turn causes that the charges are constant (Q=const.) and so the portions of the

generated oxygen are of the same size. It is also very essential that the system controlling the oxygen generator always ensures the generation of the whole amount of oxygen corresponding to the electric charge (Q) independent of the signal changes (e.g. emersion of the control electrode) within the impulse (Δt) duration. Sufficient repetition of the impulse time and good current stabilization are crucial for the operation of such respirometers. The latter proves to be rather difficult due to the character of the electrolysis process and particularly the processes of electrode polarization. In the respirometers of group III the number of impulses in the time function is recorded by means of graphic or photographic recorders. Most probably the forerunner of such a respirometer is MacFadyen's model (1961). The basic elements of the system appear in the remaining respirometers of this group: Wager, Porter (1961), Fourche (1964), Byzov et al. (1967). The last two mentioned above are a direct development of MacFadyen's concept. In all these models the detection circuit is independent of the oxygen generation circuit. The source of the alternating voltage $u(t)$ (Fig. 4 C) is fed to the amplifier input *AM* through

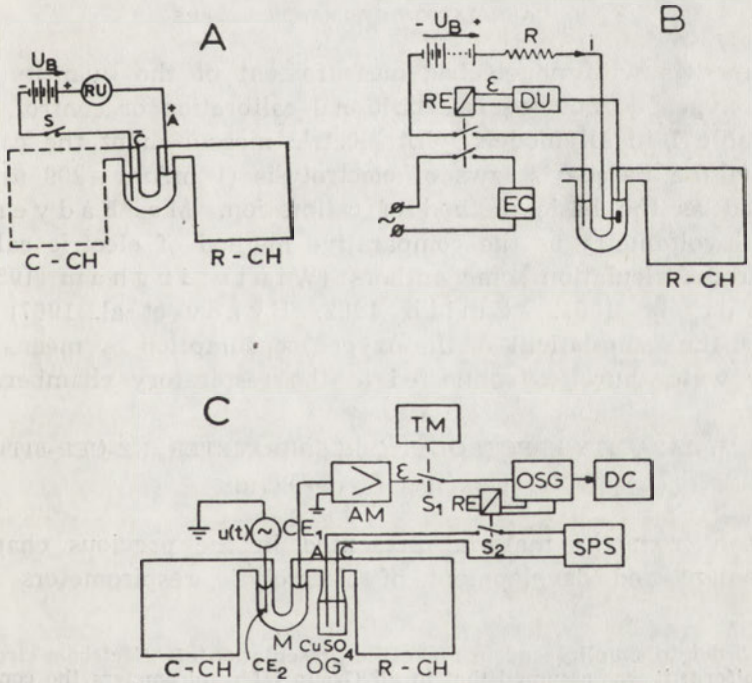


Fig. 4. Electric diagrams of respirometers (compare Table I). A — Group I (Caprаro 1953, MacFadyen 1955, Winteringham 1959), B — Group II (Smith 1962, Phillipson 1962), C — Group III (MacFadyen 1961, Wager, Porter 1961, Fourche 1964, Byzov et al. 1967, "Sapromat")

the control electrode CE_1 [2]. The signal ε comes to the snap-action switch S_1 of the timing mechanism *TM* which shuts the S_1 for a short time at regular intervals. If at the moment of closing of S_1 the signal $\varepsilon \neq 0$ then the "one shot" generator *OSG* is switched on which in turn, by means of relay *RE* and switch S_2 supplies current i=const. from the stabilized power supply *SPS*; the current flows in the oxygen generator *OG* during Δt=const. After Δt the switch S_1 is switched on again; if the signal a is still not equal to 0 then the "one shot" generator *OSG* switches on the current i for the next Δt. The impulses are repeated so long as $\varepsilon \neq 0$, that is the control electrode CE_1 is submerged in the electrolyte in the manometer. Each impulse is counted in the decade counter *DC* by means of a pen-recorder, or photographic registrator or digital printer, etc.

The respirometer proposed by the authors of the present work belongs to a new group IV (Table I) in which the constant charge (Q=const.) is obtained without the stabilization of the intensity of the electrolysis current ($i \neq$ const.) and without the stabilization of impulse time ($\Delta t \neq$ const.). Chapter 5 contains the description of the respirometer and its comparison with those discussed so far.

CALIBRATION OF RESPIROMETERS

The models with nonelectric measurement of the quantity of generated oxygen do not require additional calibration or control. As seen from Table I in all models with electric measurement the calculation based on the Faraday's laws of electrolysis (1 mA/hr=209 µl $O_{2(STP)}$) was used as the basic method of calibration. MacFadyen (1955) proposed voltametry as the comparative method of electric calibration. Apart from calculation some authors (Winteringham 1959, Wager, Porter 1961, Smith 1962, Byzov et al. 1967) used as a control the "simulation" of the oxygen consumption by means of mercury or water burettes connected to the respiratory chamber.

5. CAPACITY ELECTROLYTIC RESPIROMETER (KZ-CER-01T)

PRINCIPLE OF OPERATION

As seen from the material presented in the previous chapter the improvement and development of electrolytic respirometers in their

[2] In order to simplify and to make the presentation of electronic circuits' diagrams uniform it was assumed that in all Group III respirometers the control electrode is in the manometer's arm from the side of the respiratory chamber, that is, closing of the detection circuit is a signal for switching on the oxygen generator. In fact, in the respirometers of MacFadyen (1961) and Fourche (1964) such signal is breaking of the detection circuit (the control electrode is on the side of the compensating chamber). However, this difference is not essential for the principle of operation of Group III respirometers.

electric aspect went in the direction of stabilizing the parameters, that is: i — intensity of the electrolysis current, Δt — electrolysis duration, and as a results of that: Q — stabilized electric charge passing through the electrolyte in Δt. The measurement consists in counting the number of Q, practically the number of times the electrolysis unit is switched on. This causes certain difficulties, the most significant of which are connected with obtaining the real stabilization of current and with the accuracy of circuits stabilizing the electrolysis duration during individual switchings.

One of the proposed objectives by us is also to obtain the stabilization of the electric charge Q, without the necessity, however, of stabilizing the other parameters: i, Δt which can change during the successive "impulses". Such dosing of the charge we obtain through the application of capacitance. The capacitor stores the charge Q expressed by the formula:

$$Q = U \cdot C$$

where: U — voltage, C — capacitance.

Since in the process of electrolysis it is impossible to discharge the capacitor to $U=0$ because of the polarization of electrodes, the discharging is carried to certain voltage U_2 at which the capacitor is disconnected from the circuit to which the charge is released. A device performing dosing of the charge by means of transferring it through charging and discharging of the capacitor will be called here a "capacity pump". It will be described later.

GENERAL DESCRIPTION OF THE RESPIROMETRIC UNIT

A volumetric respirometer described earlier by Klekowski (in print) has been used as a model for the general diagram of the glass parts of the capacity electrolytic respirometer. The apparatus (Fig. 5) consists of two identical vessels, one of which (1) plays the role of the respiratory chamber and the other (2) of the compensating chamber. The upper parts of both vessels (3) connected to the lower ones by ground joint act as CO_2 absorbers. Each of them contains in the circular cavity 25% of NaOH solution (4) which saturates cylindrically shaped Whatman filter paper (5). Both absorbers are connected by ground joint to the respirometer arms (6, 7). The arm (6) connected to the respiratory chamber has an additional branch which by means of ground joint is connected to the oxygen generator (8), where in the $CuSO_4$ solution saturated in 0.5% of H_2SO_4 (9) platinum anode (10) and cathode (11) are submerged. The external terminals of electrodes are in short tubes in which they are connected to the cables by means of Wood alloy. The joints and cables are protected by rubber tubings slipped

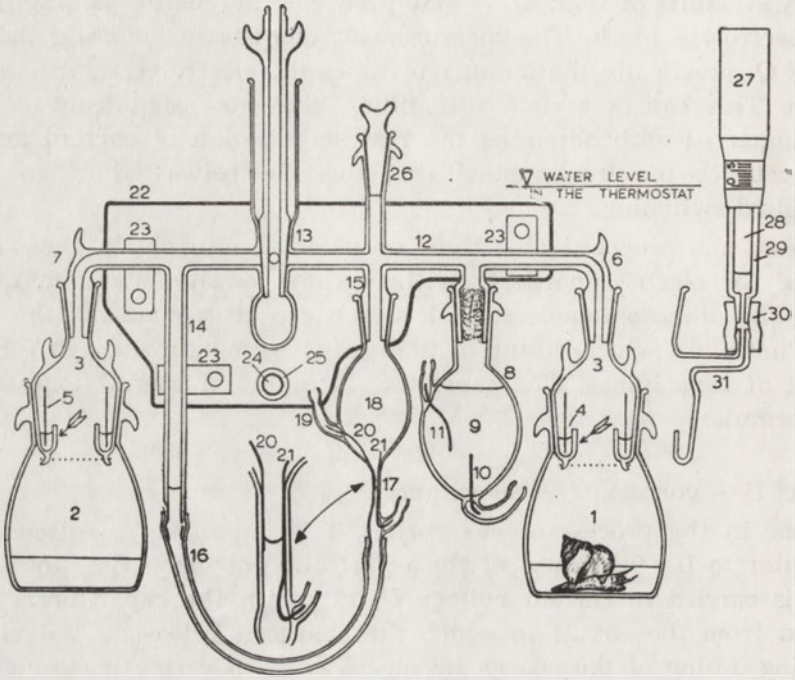


Fig. 5. Capacity electrolytic respirometer — respiratory gasometric unit. 1 — respiratory chamber, 2 — compensating chamber, 3 — CO_2 absorber, 4 — 25% NaOH solution, 5 — filter paper, 6, 7 — respirometer arms, 8 — oxygen generator, 9 — saturated $CuSO_4$ solution, 10 — anode, 11 — cathode, 12 — horizontal capillary, 13 — tap, 14, 15 — branches of the horizontal capillary, 16 — tygon tubing, 17 — capillary of the control electrodes holder, 18 — bulb of the control electrodes holder, 19 — protecting tubing of the control electrode, 20 — control electrode CE_1, 21 — control electrode CE_2, 22 — perspex supporting plate, 23 — brass holders, 24 — brass outtriggers, 25 — attaching screw, 26 — ground stopper, 27 — micrometer gauge, 28 — piston of micropipette, 29 — cylinder of micropipette, 30 — pressure-tubing joint, 31 — branches of CO_2 absorber. Arrow — surfaces greased with vaseline

on the glass tubes. The tubings protect the places where electrodes are connected to the cables and come out above the surface of the water in the thermostat. The cables are connected to the hermetic socket. The respirometer arms (6, 7) are joined together by a horizontal capillary (12). In its middle part the horizontal capillary may be closed by a specially constructed tape (13), after the tape is closed, both parts of the capillary are cut off from each other and from the atmosphere; after it is open, both parts are joined and join the atmosphere. On both sides of the tape (13) the horizontal capillary has branches (14, 15) directed downwards. The branch of this part the horizontal capillary which is connected to the compensating chamber has an olivary at the end to which tygon tubing (16) is fixed. The other branch (15) of the

horizontal capillary between the tape (13) and a branch connected to the oxygen generator (8) is short and terminated with a ground joint. The control electrode holder is mounted on this joint. In its lower part it has a capillary of 1.5 mm diameter (17) widening into a bulb (18) in its upper part. The bulb has the capacity of ca. 10 ml. The lower part of the capillary has an olivary to which tygon tubing (16) is fixed. Half-way the bulb height there is a tube (19) with the upper end of the control electrode CE_1 (20). This electrode is a 0.15 mm platinum wire. The lower end of the wire goes as far as approximately 1/3 of the capillary length. This part which is in the capillary is sealed by means of the oxygen flame to the capillary wall. The other control electrode CE_2 (21) has one end in the tube branching out at the lower 1/3 length of the capillary and is sealed to its wall (opposite to the control electrode CE_1 (20)). The upper end of CE_2 (21) reaches the bulb. The cables connected to the electrodes by means of Wood alloy and protected by rubber tubing reach the hermetic socket.

The respirometer is mounted on the perspex supporting plate (22) by means of brass holders (23). This plate (22) is fixed rotary to the

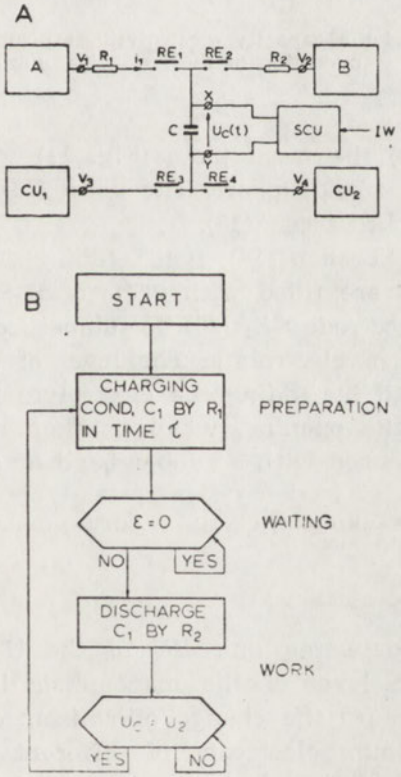


Fig. 6. Principles of functioning of the "capacity pump". A — unit diagram, B — flow diagram

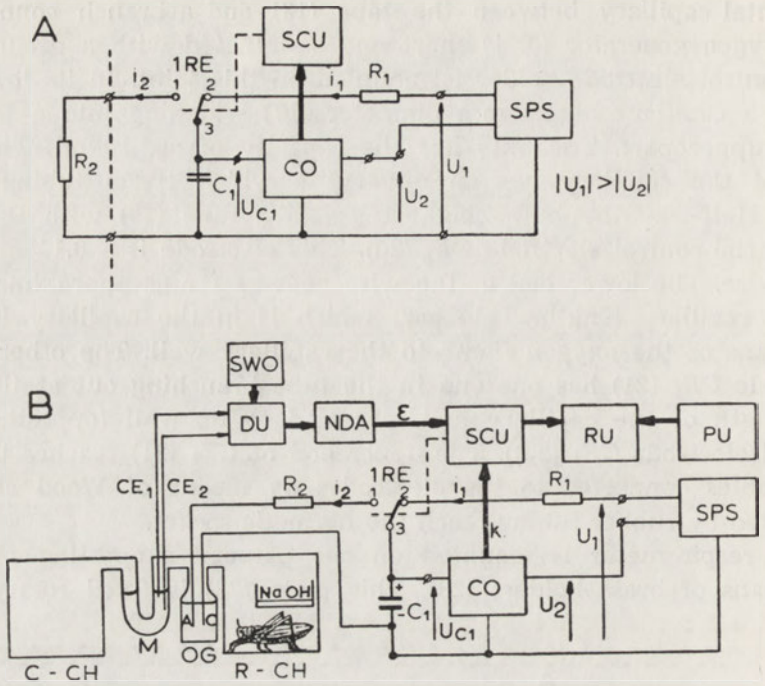


Fig. 7. Block diagram of the capacity electrolytic respirometer (*CER*) showing the operation of the "capacity pump"

pin (24), attached to the basal plate (Fig. 11) which is suspended in the thermostat on a steel support. The plate (22) may be immobilized by tightening the nicked screw (25).

The lower part of branch (14), tygon tubing (16) and the lower 2/3 of the capillary (17) are filled with 0.5% of H_2SO_4 so that the lower end of the control electrode CE_1 (20) be submerged — with the tape (13) open — in 3—5 mm of electrolyte. The level of electrolyte in the capillary (7) is adjusted by adding the electrolyte through a very small funnel inserted in the opening, which is then closed with a ground glass stopper (26) secured with a rubber band or a stainless spring.

DESCRIPTION OF THE ELECTRIC CIRCUITS

Detecting unit

The manometric detecting unit *DU* of the U-pipe type was used. The changes in the level of the manometric liquid which is 0.5% solution of H_2SO_4 cause the change of resistance of the system consisting of two platinum electrodes of different length CE_1 and CE_2 (20 and 21 in Fig. 5). The electric unit detecting the changes of resistance is a sensitive conductometer which detects whether the re-

sistance of the electrolyte column between the electrodes is higher or lower than the accepted minimal critical value R_m. R_m is chosen depending on the used electrolyte and electrode arrangement. In our detecting unit it is possible to set this value in the 1 to 8 kΩ range. The value of 3 kΩ was accepted; at this value the shorter electrode is submerged to the depth of ca. 0.5 mm in the 0.5% H_2SO_4 electrolyte and the distance between the electrodes is about 1.2 mm. The detecting unit's operating point is adjusted by means of a potentiometer P_4 (Fig. 8) on the front panel. Since the detecting unit must be able to react to very small changes of resistance occurring on the level of the accepted value of electrolyte resistance (3 kΩ) it must have very large relative sensitivity (below 1%). The amplifier of non-linear threshold characteristic was used.

The detecting unit operates in the following way (Fig. 7, 8, 9): square wave oscillator *SWO* of 5 kHz [3] operating in the multi-vibrator circuit (transistors T_{17} and T_{18}) supplies through the capacitor C_7 the detecting unit *DU* formed by a divider: resistance of electrolyte between electrodes CE_1 and CE_2 — resistors R_{52} and R_{11} and diode D_{22}. The detecting unit signal is fed to the nonlinear amplifier (transistor T_9, resistor R_9) and then to the shaper SCH_1 (Schmitt trigger) consisting of transistors T_5 and T_6. Amplifier and shaper are denoted on the block diagram (Fig. 7 B) by a symbol *NDA*.

If the input signal amplitude on the resistor R_{52} is lower than the amplifier threshold the amplifier gain is 0 and there is no signal at the output. When the input signal amplitude exceeds the threshold value which happens when the resistance between the control electrodes decreases sufficiently enough due to the increase of the electrode submersion CE_1 a sequence of pulses (reinforced "peaks" of input signal) appear at the amplifier output (resistor R_9). These pulses will be later formed in the shaping unit (SCH_1 in Fig. 8).

Thus the error signal is a sequence of pulses which occur when the pressure in the respiratory chamber decreases due to the oxygen consumption of an object in the chamber. It should be stressed that the parameters of the detecting unit should be chosen so that it reacts to the changes of gas quantity on the respiratory chamber smaller than the oxygen amount generated by 1 pulse. In other words: a good detecting unit provides the oxygen doses following 1 pulse and not in series with long intervals. This was accomplished in the above described detecting unit by an original arrangement of control electrodes, large relative sensitivity below 1%, and suitably chosen capillary diameter (17 in Fig. 5) containing the control electrodes.

[3] This frequency has been chosen empirically. Lower frequencies proved unsuitable as they caused electrolysis on the control electrodes and generation of gas bubbles.

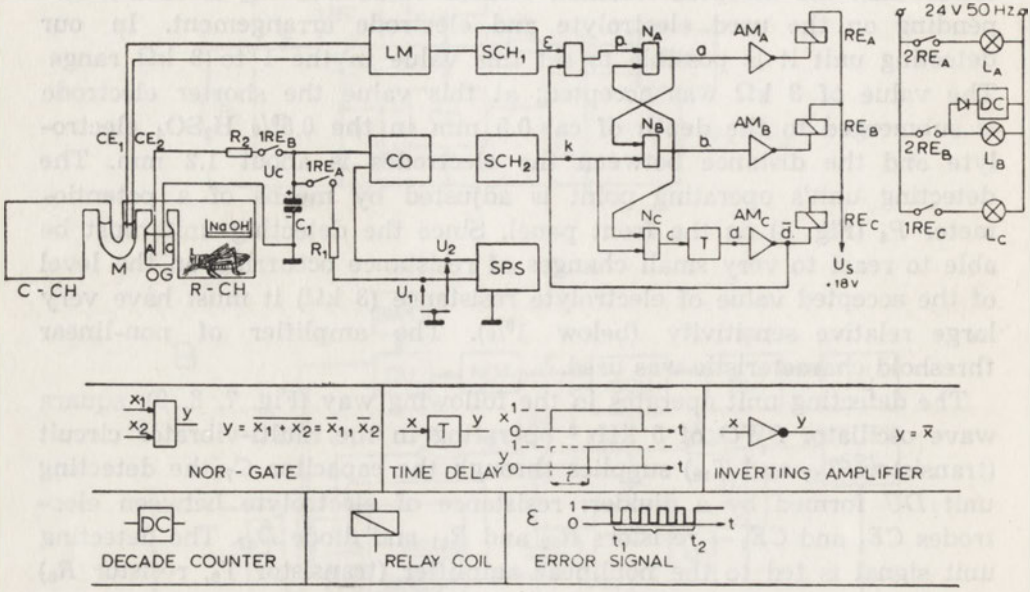


Fig. 8. Logical diagram of the capacity electrolytic respirometer. "0" — 0–2 V (false), "1" — 9–18 V (true), *p.* — a signal beginning the charge dosing phase, *k.* — output signal of the comparator: "1" when $U_c < U_2$, *a.*, *b.*, *c.*, *d.* — NOR output signals N_A, N_B, N_C, N_D

Charge dosing unit ("capacity pump")

The unit operates in the following way:

1. Preparatory cycle: relay contact 1*RE* (Fig. 7 A) is in such a position that it connects points 2 and 3. The capacitor C_1 is charged until the voltage has the value of U_1. The duration of the cycle has to be chosen in such a way that the capacitor be sufficiently charged. The minimum time depends on the time constant R_1C_1. Either the operating cycle or the idle cycle may follow charging while the capacitor is connected to the voltage source U_1.

2. The operating cycle: may follow the preparatory cycle. In the operating cycle the relay contact 1*RE* connects points 1 and 3. The capacitor C_1 is discharged through the output circuit R_2. Discharging continues until the voltage on the capacitor is decreased to the value of U_2. This value is detected by the comparator *CO* and signalled to the steering and computing unit *SCU*. Then the relay 1*RE* is switched and the preparatory cycle begins. Switching must be fast enough in comparison with the speed of voltage changes on the capacitor C_1 to

ensure sufficiently small dynamic error. Since the capacitor was discharged from voltage U_1 to U_2 the following charge was moved through the output circuit:

$$Q=(U_1-U_2)\cdot C$$

where: C is capacitance.

The accuracy with which the charge is dosed depends on the stability of the reference voltages U_1 and U_2, speed of switching, comparator sensitivity, and the quality of the capacitor C_1. To obtain the sufficient speed of switching one can use the sufficiently fast electromechanical contact relays or contactless electronic relays. In our respirometer the hermetic relays (contactrons) were used as the switching elements which ensure the sufficient speed of switching.

The steering and computing unit *SCU* realizes a definite cycle: [preparatory cycle → waiting (idling) → operating cycle] or [preparatory cycle → operating cycle] (see Fig. 6 B), counts the number of charge doses and doses the charge which depends on the way in which the device is used. A considerable flexibility of charge dosing is possible due to the application of the steering unit with a suitable algorythm.

In our respirometer the steering unit doses the charge keeping up with the varying in time demand. Figure 7 B shows the using of "capacity pump" in respirometry. The unit operates in the following way: oxygen consumed by an animal causes the pressure drop in the respiratory chamber *R-CH* and changes the level of the electrolyte in the manometer *M* which in turn causes that the electrode CE_1 is submerged more and the electrolyte resistance between the electrodes is lowered. The decrease of resistance is detected by the detecting unit *DU*, amplified in the nonlinear detector amplifier *NDA* and in the form of signal ε sent to the steering and computing unit *SCU*. The steering and computing unit *SCU* controls the charging and discharging of the capacitor C_1 by means of the relay *1RE*. In the charging phase when the contacts (2) and (3) are connected in the relay *1RE*, capacitor C_1 is charged by resistor R_1 in time τ chosen in such a way that the voltage on the capacitor increases to the level U_1. After time τ the unit is ready for the operating cycle. If signal ε equals 0 then the unit is kept in the waiting (idle) phase and the capacitor C_1 is all the time connected to the voltage source U_1. When the signal ε does not equal 0, the operating cycle is begun where contacts 1 and 3 are connected in *1RE* and the current i_2 flows through the resistor R_2 and the oxygen generator *OG*. Capacitor C_1 became discharged under control of the comparator *CO* which compares the voltage U_{C1} on the capacitor C_1 with the voltage of reference U_2.

When both these voltage become equal ($U_{C1}=U_2$) comparator *CO* supplies a signal to the steering and computing unit *SCU*, relay $1RE$ connects points 2 and 3 and thus the next phase of charging the capacitor C_1 is begun. This phase, like the previous one, lasts for time τ and increases the voltage on the capacitor to U_1.

N.B. the operating cycle once begun lasts till the end; also in the case when, within the cycle, signal ε stops.

The described above method of dosing the charge by the capacity pumps ensures that equal portions of oxygen are generated in the oxygen generator.

Successive operating cycles are counted by the pulse counter incorporated in the steering and computing unit *SCU*. The meter readings are recorded by the recording unit *RU* which is periodically triggered by the programming unit *PU*. In our respirometer a set of (15) telephone calls counters was used as the pulse counter. The camera (type Robot-Recorder 18 M) with the programming unit (Stenergerät IIa) was used for photo-recording.

The logical diagram of the respirometer

The steering unit was based on the elements of the *NOR* (sum negation) logical function (Fig. 8). The N_A and N_B make the static trigger controlled by means of logical signals *p.* and *k.* Signal *p.* is the logical product of negation of the signal ε and the readiness signal *d.* This product is produced on the N_D element. Since signal ε is a sequence of "0" pulses (see: explanation to Fig. 8) in case of oxygen demand in *R-CH* and the continuous signal "1" in case of the lack of demand we receive a sequence of "1" pulses on the N_D output when there is oxygen demand and when the unit is in the readiness state (charged capacitor C_1). The first pulse of the sequence will put the trigger (N_A-N_B) into such a state that $a.=0$ and $b.=1$. Signal $b.=1$ will stimulate the relay RE_B through the amplifier AM_B. The contact $1RE_B$ of the relay will then be closed and the discharge current of the capacitor C_1 will begin to flow through resistor R_2 and oxygen generator. Since the capacitor C_1 was charged signal *k.* had "0" value at the moment of switching the (N_A-N_B) trigger. After switching the trigger (N_A-N_B) signal *b.* assumes the value of "1" which will cause the signal *c.* to have the value of "0", and thus the readiness signal *d.* will become "0" too. The effect of the readiness decay is the fact that the signal *d.* assumes the "0" state. The phase of charge dosing lasts since the appearance of $b.=1$. When the capacitor C_1 discharges to the value of the lower threshold (voltage U_2) it will be detected by the comparator *CO* and will cause the change of signal *k.* from state "0"

into state "1". The trigger $(N_A - N_B)$ will be adjusted so that $a.=1$ and $b.=0$. The relay RE_B will stop operating whereas the contact $1RE_B$ will be opened thus completing the charge dosing phase. A moment later[4] relay RE_A will start operating, stimulated by an amplifier AM_A whose contact $1RE_A$ will close the charging circuit of the capacitor. Then signal $k.$ will again become "0" since the voltage on the capacitor will reach the value of $U_c > U_2$. Since charging is taking place $b.=0$ and thus signal $c.=1$ will appear on the output of the N_C element. After time τ the readiness signal $d.$ will appear and only then the trigger $(N_A - N_B)$ will be in the charge dosing phase ($a.=0$, $b.=1$). It was mentioned earlier in this paper that time τ is chosen in such a way that charging capacitor C_1 to the full value of voltage U_1 is ensured.

To check the correct operation the device was equipped with three control lamps which show the phases of operation: L_A — charging, L_B — charge dosing, L_C — readiness state.

Schematic diagram of the electronic circuit

Figure 9 shows the schematic diagram two parts of which can easily be isolated: the stabilized power supplies and proper steering unit.

The power supply consists of a 220V 50 Hz transformer Tr protected with a fuse F_1, rectifier $D_{1\text{-}4}$, filter C_3 C_4 C_1 and the stabilizing element. The stabilizer's circuit with a series control element (transistors T_1 and T_2 in the Darlington circuit) was needed. The comparing elements is made up of transistors T_3 and T_4 and the resistor divider R_3, R_4, R_5, P_1. The reference voltage is generated on the stabilizer St_1. The steering unit can be divided into sub-units which perform functions shown in the block diagram (Fig. 7) and the logical diagram (Fig. 8). Individual sub-units will be discussed below.

Level meter LM was discussed in detail in the part on "Detecting unit". It is an electric part of a detecting unit which includes apart from LM, the manometer M and the electrode system $(CE_1 - CE_2)$. The level meter can be divided into three parts: square wave oscillator SWO, detecting unit DU and a part of the threshold amplifier NDA. This part of NDA which does not belong to LM is the Schmitt shaper shown in the logical diagram (Fig. 8) as a sub-unit SCH_1 because it is the matching element between the level meter and the logical elements. The SCH_1 system is made of transistors T_5 and T_6.

[4] The characteristic of the relays used here are such that the time from the moment the voltage is applied to the coil to the moment of closing the contact is longer than the time between the moment of removing voltage from the coil and that of opening the contacts. Due to this property relays RE_A and RE_B are never switched on at the same time.

Comparator *CO* is a simple one-transistor T_{10} system. The reference voltage U_2 is generated on the stabilizer St_2. The input signal from the comparator is shaped by the SCH_2 system consisting of transistors T_7 and T_8. The SCH_2 system matches the comparator's input signal to the signal requirements in the used logical elements *NOR*. In the block diagram (Fig. 7) the shaper was shown as the component part of the comparator CO.

NOR logical elements. They are two-input resistor-diode-transistor circuits. The element N_A consists of transistor T_{14}, diode D_{17}, collector resistor R_{43}, resistor R_{42} and the input resistors R_{40} and R_{41}. The elements N_B, N_C, N_D are made of transistors T_{15}, T_{16} and T_{19}, respectively.

Inverting amplifiers *AM*. They are one transistor circuits which, instead of a collector resistor, use circuits composed of a relay coil, a series resistor and a diode protecting against overloading. The logical "1" on the amplifier input causes the relay work and the appearance of the logical "0" on the collector of the transistor. It is, therefore, the inverter for logical signals. The amplifier AM_A is composed of the elements T_{11}, R_{21}, RE_A, D_{11}, R_{20}, R_{19} and D_{14}. Amplifiers AM_B and AM_C are built of transistors T_{12} and T_{13}.

Time delay circuit *T* is composed of the elements R_{51}, R_{27}, C_6, P_2, St_4. The logical "1" on the output of the element N_C — transistor T_{16} — causes charging of capacitor C_6 through resistors R_{51}, P_2 and R_{27}. Transistor T_{13} will be driven only when the voltage reaches the proper value. The time delay adjustment is performed by means of a potentiometer P_2. When the logical "0" appears on the output of N_C the capacitor C_6 is quickly discharged through R_{27} and transistor T_{16} ($R_{27} \ll R_{27} + R_{51}$).

Controlling and computing circuit. Control lamps *L* are supplied with alternating current from a separate transformer winding *Tr* through the contacts of respective relays. The decade counter *DC* is supplied by means of a rectifier composed of diodes $D_{5\text{-}8}$ and capacitor C_5.

CALIBRATION

Table I shows that calibration of systems is necessary in all models of electrolytic respirometers in which the measurement of O_2 generation is done electrically. As a rule calculation is based on the Faraday's laws of electrolysis from which it appears that for example 1 μAh gives 298.45 mg O_2 through electrolysis of e.g. $CuSO_4$. In respirometers in groups I, II, and III (Table I) the results of measurements are finally converted into Ah which is enough to convert it into the amount of generated oxygen (=consumed in the respiratory chamber). Some authors

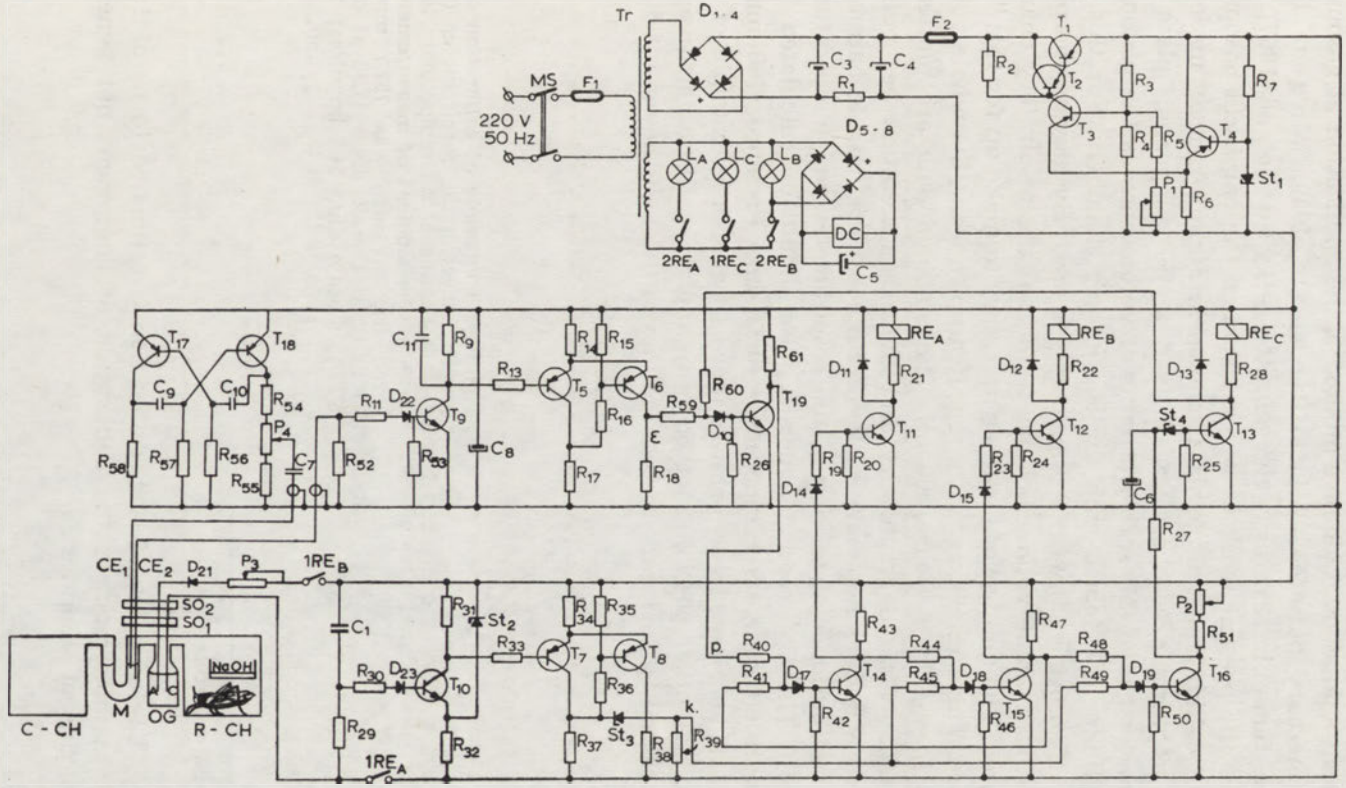


Fig. 9. Schematic diagram of the electronic circuit of the capacity electrolytic respirometer (*CER*)

use additional control of calibration. For this purpose MacFadyen (1955) uses voltameter. A simpler method is the application of gasometric devices (mercury burette — Winteringham 1959, Wager, Porter 1961, Smith 1962 or water burette — Byzov et al. 1967). The authors of the present work in general applied the empirical gasometric method of calibration. The device is shown in Fig. 5. A ready made element of titrating microburette was fixed to the supporting plate (22). A micrometer gauge (27) scaled in units of volume moves the piston (28) in the cylinder (24) joined with the respiratory chambers. For this purpose a CO_2 absorber (3) with a branch (31) is used. By moving the piston back by known same volumes of gas its loss in the respiratory chamber is simulated. The number of impulses necessary to make up for this "loss" is recorded. The same "losses" are repeated up to using the whole volume of micropipette (in our case 500 µl). The obtained data are plotted on the diagram averaged graphically and then O_2 generation per pulse is calculated. N.B. It is necessary to take into consideration the barometric pressure at the moment of closing the respirometer and temperature of water bath. The O_2 production per 1 pulse should be calculated in µl $O_{2(STP)}$, that is Standard Temperature (0°C) and Pressure (760 mmHg) (details in Klekowski in print). Figure 10 gives the example of such calibration. Another possible method is to apply a ballistic galvanometer and to measure by its means the charge moved through the circuit during

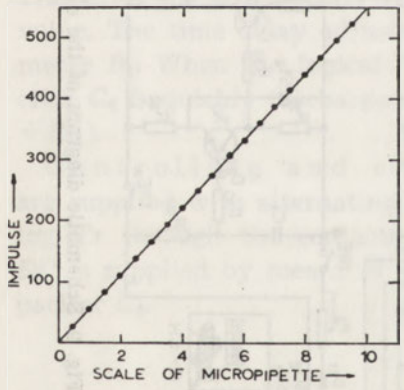


Fig. 10. An example of calibration of the capacity electrolytic respirometer (*CER*). 1 scale division of micropipette = 19.8 µl. Since at the moment of measurement the barometric pressure was 752.7 mm Hg (19°C) thus: 1 scale div. = 17.98 µl of gas (*STP*); 1 scale div.=54.5 impulses; 17.98 : : 54.5=3.329 µl $O_{2(STP)}$/imp.

one pulse. The accuracy of such calibration is estimated to be of 1–2%. This last method requires the application of equipment not generally used in biological laboratories.

DESCRIPTION OF THE *CER* UNIT

CER assembly consists of the following elements: 1. water thermostat in which 15 respirometers (such as shown in Fig. 5) are suspensed

Each unit has a hermetic socket (SO_1 in Fig. 9). From the socket a 4-wire cable[5] with hermetic plugs leads to a proper panel in the control and measurement rack (SO_2 in Fig. 9). In three upper sections[6] of the rack[7] there are 15 panels[8] of 5 to a section. Each panel consists of a set of electronic circuits (as in Fig. 9) designed for cooperation with one respirometric unit. There are on each panel: 1 — a socket for the cable connecting the respirometric unit; 2 — control lamps indicating: charging of capacitors, readiness state, operation cycle — discharging through oxygen generator, 3 — control of operation point of detecting unit *DU*, 4 — control of duration of operation cycle, 5 — 0.5 A fuse in the *d.c.* circuit, 6 — 0.5 A fuse in the primary winding of the mains transformer, 7 — main on-off switch.

The lower part of the contr.-means. rack contains 15 batteries of capacitors. One battery consists of polyester capacitors of the MKSE-011 type having the total capacitance of 500 μF. For proper selection of pulses the capacitance of 100, 250 and 500 μF may be used from each battery of capacitors.

A recording unit is located in the upper part of the rack. It consists of: 1 — a block of 15 impulse counters[9]. A clock is situated near their windows. 2 — "Robot" photographic camera (Recorder 18 M), "Robot"-device (Stenergerät IIa) programming the frequency of photographs and conditions of exposure, 3 — flash lamp. Figure 12 shows two frames with the recorded indications of the counters. Depending on the used cartridges it is possible to take 80 to 3000 photographs without changing the cartridge. And so, for example, with 4 photographs per hour continuous recording may last up to 30 days.

OPERATIONAL INSTRUCTION

The way of procedure with a single gasometric unit *GU* (Fig. 5) will be given below. *GU* is located outside the water bath on a stand. The tap (13) is open. Place a cylinder of Whatman's filter paper (starchless) of equal size in both CO_2 absorbers (3) and grease the surfaces marked by arrows with vaseline in order to prevent creeping of NaOH solution. Grease slightly the ground joints of absorbers with vaseline-caoutchouc mixture (or good silicone non-toxic grease for biological purposes). Soak filter paper in both CO_2 absorbers with an equal amount of 25% NaOH,

---

[5] Two wires in the cable led to the electrodes CE_1 and CE_2 are screened in order to eliminate external disturbances which may disturb the operation of a very sensitive detecting circuit.

[6] Type B-2, produced by POLON, Poznań, Poland.

[7] Type C-25, produced by POLON, Poznań, Poland.

[8] Type A-1, produced by POLON, Poznań, Poland.

[9] Telephone calls meters, produced by TELFA Manufacturing Co., Bydgoszcz, Catalogue No. 8-4467-002-5.

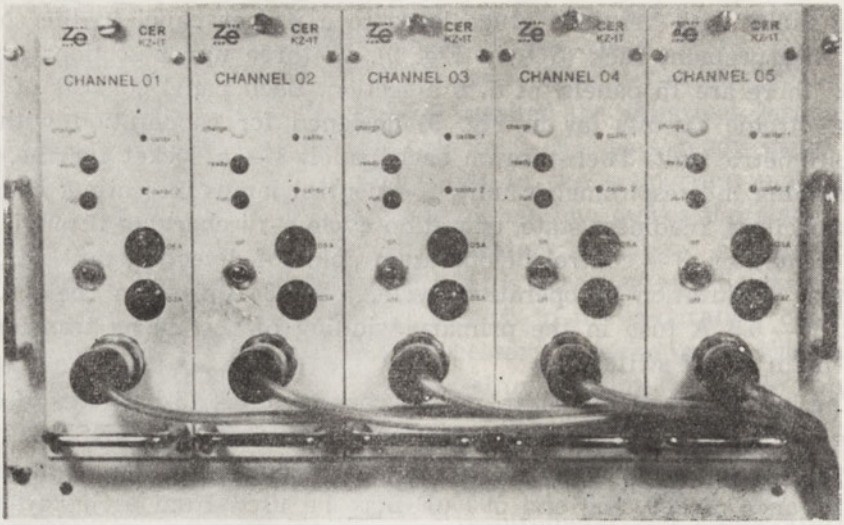


Fig. 11. General view of the capacity electrolytic respirometer *(CER)*

then attach the absorbers to the respirometer's arms and secure with springs or rubber rings. Place the animal(s) in the respiratory chamber, then attach both chambers to the ground joints of appropriate absorbers, greased earlier, and secure with spring or rubber rings.

Place the gasometric unit on a support in water bath. Adjust the position of supporting plate (22) to set up the right electrolyte level in capillary (17), fix by means of nicked screw (25). Connect the cable which goes between the gasometric unit and the appropriate panel in the control and measurent rack. Switch on the main switch on the panel so that oxygen generation starts and the H_2SO_4 solution in the oxygen generator becomes saturated with oxygen. Wait ca. 10 min until temperature is equalized. Close the tap (13) and switch on the recording device.

Whenever a larger number of respirometers is used at the same time, each experimentator must make his own compromise about the succession of operations with individual instruments.

EXAMPLE OF THE MEASUREMENT OF OXYGEN CONSUMPTION

Figure 13 shows two examples of the measurement of oxygen consumption by cockroaches (*Blatella germanica* L.) Figure 13 A shows the frequent measurement, made ca. every 5 min (20°C, animal wet weight 36 mg). Section 1 was made at the time when after closing the valve (13 in Fig. 5) the pulses succeded each other continuously until the me-

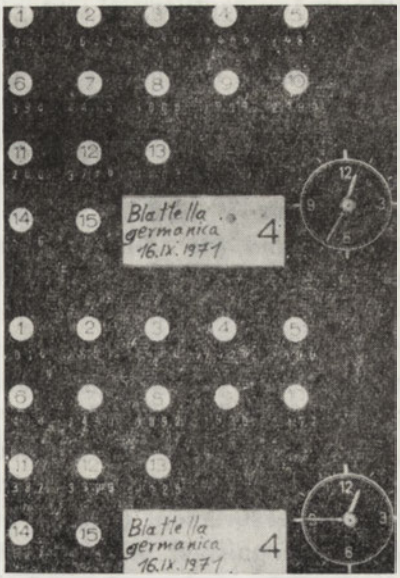


Fig. 12. A frame from the film recording the reading of the pulse counters

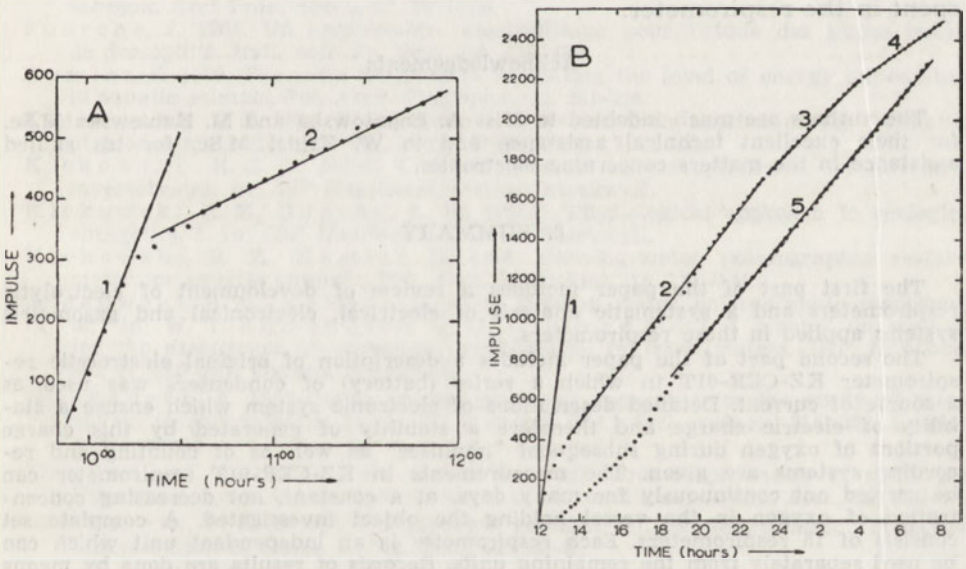


Fig. 13. Examples of measuring the oxygen consumption by *Blattella germanica*. A — measurements every 5 min. 1 — maximum oxygen generation in respirometer, 2 — respiration of animal. B — measurements every 15 min. 2, 3, 4 — decrease of respiration from thirst and hunger, 5 — constant respiration in presence of food and water

niscus of the electrolyte in the capillary (17 in Fig. 5) was lowered to the working position. Thus, this section allows for the calculation of the maximum oxygen generation in the oxygen generator at the given capacity of the battery of capacitors and the regime of charging and discharging. In our case: 776 imp/hr that is 4.6 sec/imp. From previous calibration (compare Fig. 10) it was known that 1 imp.$=0.329$ µl $O_{2(STP)}$, thus the maximum generation of $O_2=776 \cdot 0.329=255$ µl O_2/hr. With the capacity of $C_1=500$ µF and the full operation cycle 1.5 sec (2400 imp/hr), the maximum efficiency of the described respirometer equals 790 µl $O_{2(STP)}$/hr. It may be added that such efficiency corresponds to the continuous electrolysis with the current of 3.8 mA.

Section 2 shows respiration of *Blattella germanica* (Insecta). The calculation: 154 imp/hr : $154 \cdot 0.329=50.7$ µl O_2/hr.

Figure 13 B shows two measurements of cockroach's respiration (20°C, animal wet weight 130.5 mg) within 20 hours with the recording done every 15 min. In the first measurement (upper curve) three sections of the curve (2, 3, 4) were distinguished for which similar calculations to those in the previous example were performed. They were: 1 — 43.6, 2 — 34.6, 3 — 23.0 µl $O_{2(STP)}$/hr · ind. The decrease of oxygen consumption after 10 hr in the respirometer resulted probably from thirst and hunger. In the second measurement (lower curve) food and water were added. Oxygen consumption (34.6 µl $O_{2(STP)}$) did not decrease with time spent in the respirometer.

Acknowledgements

The authors are much indebted to Miss A. Łopatowska and M. Kaniewska M.Sc. for their excellent technical assistance and to W. Zajdel M.Sc. for his skilled assistance in the matters concerning electronics.

6. SUMMARY

The first part of the paper includes a review of development of electrolytic respirometers and a systematic analysis of electrical, electronical and gasometric systems applied in these respirometers.

The second part of the paper includes a description of original electrolytic respirometer KZ-CER-01T in which a series (battery) of condensers was used as a source of current. Detailed descriptions of electronic system which ensure a stability of electric charge and therefore a stability of generated by this charge portions of oxygen during subsequent "impulses" as well as of counting and recording systems are given. The measurements in KZ-CER-01T respirometer can be carried out continuously for many days, at a constant, not decreasing concentration of oxygen in the vessel holding the object investigated. A complete set consists of 15 respirometers. Each respirometer is an independent unit which can be used separately from the remaining units. Records of results are done by means of set of counters of impulses and automatic photographical registration.

7. STRESZCZENIE

W pierwszej części pracy dokonano historycznego przeglądu rozwoju respirometrów elektrolitycznych i dokonano systematycznej analizy układów elektrycz-

nych, elektronicznych i gazometrycznych jakie były stosowane w tych respirometrach.

Druga część pracy zawiera opis oryginalnego respirometru KZ-CER-01T, w którym zastosowano jako źródło prądu elektrolizy baterie kondensatorów. Podano szczegółowy opis układów elektronicznych, które zapewniają stałość ładunku elektrycznego, a co za tym idzie, stałość generowanych przez ten ładunek porcji tlenu w czasie poszczególnych „impulsów" oraz układu zaliczającego i rejestrującego. Pomiary w respirometrze KZ-CER-01T mogą być prowadzone w sposób ciągły przez wiele dni, przy stałym, niemalejącym stężeniu tlenu w naczyniu zawierającym badany obiekt. Komplet urządzeń składa się z 15 respirometrów. Każdy respirometer stanowi samodzielny układ, który może być wykorzystywany niezależnie od pozostałych. Rejestracja wyników odbywa się za pomocą zespołu liczników impulsów i automatycznego układu fotograficznego.

8. REFERENCES

Bartlett, R. G., Phillips, N. E. 1961. Observations on a device for measuring metabolic gaseous exchange with electrolytic oxygen production. *Quart. J. Fla Acad. Sci.*, 24, 162–168.

De Boer, S. R., 1929. Respiration of Phycomycetes. *Recl Trav. bot. néerl.*, 25, 117–240.

[Byzov, A. L., Byzova, Ju. B., Geodakyan, V. A., Golubtzov, K. V., Trusova, N. N.] Бызов, А. Л., Бызова, Ю. Б., Геодакян, В. А., Голубцов, К. В., Трусова, Н. Н. 1967. Автоматический микрореспирометр для продолжительного измерения потребления кислорода [The automatic microrespirometer for prolonged measurement of the oxygen]. *Zool. Ż., Mosk.*, 46, 1856 [Engl. summ.].

Capraro, V. 1953. A new method of measuring oxygen consumed in the metabolism of small animals. *Nature, Lond.*, 172. 15.

Duncan, A. Klekowski, R. Z. [in print]. Parameters of an energy budget and their efficiencies. In: *IBP Handbook*, Oxford, Blackwell.

Fernandes, D. S. 1923. Aerobe und anaerobe Atmung bei Keimlingen von *Pisum sativum*. *Recl Trav. néerl.*, 22, 107–256.

Fourche, J. 1964. Un respiromètre électrolitique pour l'étude des pupes isolées de drosophile. *Bull. biol. Fr. Belg.*, 98, 475–489.

Kamler, E. 1970. The main parameters regulating the level of energy expenditure in aquatic animals. *Pol. Arch. Hydrobiol.*, 17, 201-216.

Klekowski, R. Z. 1970. Bioenergetic budgets and their application for estimation of production efficiency. *Pol. Arch. Hydrobiol.*, 17, 55–80.

Klekowski, R. Z. [in print]. Constant-pressure microrespirometer for terrestrial invertebrates. In: *IBP Handbook*, Oxford Blackwell.

Klekowski, R. Z., Duncan, A. [in print]. Physiological approach to ecological energetics. I. In: *IBP Handbook*, Oxford, Blackwell.

Klekowski, R. Z., Kamler, E. 1968. Flowing-water polarographic respirometer for aquatic animals. *Pol. Arch. Hydrobiol.*, 15, 121–144.

Klekowski, R. Z., Kamler, E. 1971. Flowing water polarographic respirometer. In: W. T. Edmondson, G. G. Winberg [Eds.] *A manual on methods for the assessment of secondary productivity in fresh waters. IBP Handbook No. 17*, 280–287, Oxford, Blackwell.

MacFadyen, A. 1955. Notes on two pieces of apparatus for the study of metabolism in soil animals. In: D. K. Kevan [Ed.] *Soil zoology*. 315–332, London, Butterworth.

MacFadyen, A. 1961. A new system for continuous respirometry of small air-breathing invertebrates under near-natural conditions, *J. exp. Biol.*, 38, 323–341.

Maskereth, F. J. H. 1964. An improved galvanic cell for determination of oxygen concentration in fluids. *J. Sci. Inst.*, 41, 38–41.

Mann, K. H. 1969. The dynamics of aquatic ecosystems. In: J. B. Cragg [Ed.] *Advances in ecological research*. 6, 1–81.

Phillipson, J. 1962. Respirometry and the study of energy turnover in natural systems with particular reference to harvest spiders (Phalangiida). *Oikos*, 13, 311–322.

Smith, H. W. 1962. An electrolytic respirometer with microlitre sensitivity. *Lab. Pract.*, 11, 741–746.

Swaby, R. J., Passey, B. I. 1953. A simple microrespirometer for studies in soil microbiology. *Austr. J. agric. Res.*, 4, 334–339.

Teal, J. M. 1971. Respiration an energy flow. In: W. T. Edmondson, G. G. Winberg [Eds.] *A manual on methods for the assessment of secondary productivity in fresh waters. IBP Handbook No. 17*, 270–276, Oxford, Blackwell.

Wager, H. G., Porter, F. A. E. 1961. An apparatus for the automatic measurement of oxygen uptake by electrolytic replacement of the oxygen consumed. *Biochem. J.*, 81, 614–618.

[Winberg, G. G.] Винберг, Г. Г. 1962. Энергетический принципл трофических связей и продуктивности экологических систем [Energetic principle in investigation of trophic relations and productivity of ecological system]. *Zool. Z., Mosk.*, 41, 1618–1630 [Engl. summ.].

Winteringham, F. P. W. 1959. An electrolytic respirometer for insects. *Lab. Pract.*, 8, 372–376.

NOTICE TO AUTHORS

Polskie Archiwum Hydrobiologii publishes papers in every field of hydrobiology. Only works not submitted elsewhere are accepted.

Length. Succinctness of style is a prerequisite. In general, papers are limited to 16 printed pages, including illustrations, references etc.

Languages. Papers are published in the official congress languages of Societes Internationalis Limnologiae, i.e., English, French, German and Italian. Works submitted in Polish will be translated into English by the Editors. The Authors are required to quote English equivalents of all the special terms they employ, according to the usage current in English and/or American scientific publications. A translation will be supplied to the Author for a verification. The final touch must be made at that phase. No amendments other than purely technical will be allowed in proofreading.

Paper arrangement. A paper should be arranged as follows: 1. ABSTRACT — a brief report of the topic, results and conclusions, not exceeding 100 words, 2. INTRODUCTION — containing a formulation of the subject, a statement on its actual stage of elaboration, and a clear definition of the aim of the paper, 3. MATERIAL and METHODS — the description must be sufficiently detailed to enable a repetition of the procedure by other researchers, 4. RESULTS — only the results attained in the work should be dealt with under this heading, 5. DISCUSSION — a comparison of the results brought by the paper with results of other works, an elucidation of theoretical and logical aspects, deductions and conclusions, etc., 6. SUMMARY — presenting the main results and conclusions in no more than 200 words. The summary will be printed in the author's native language and in the language of the main text.

Tables. They should be typed on separate sheets, numbered with Roman numerals, with a brief title above each table. Large tables exceeding the size of a printed page should be avoided.

Figures. Pencil drawings on profile paper (mm) are accepted. Only contrast photographs can be reproduced. The size of figures should not exceed the size of a sheet of normal typescript. Each figure must bear an Arab numeral and the name of the author. Titles of figures and their descriptions ought to be presented on a separate sheet of typescript, common for all of them. Figures should not contain information already cited in tables, and vice versa.

Bibliography. It should contain all the references cited in the text. The list should be arranged in the following manner: surnames of the authors (in alphabetical order), initials, year of publication, title of paper, title of journal (abbreviated according to the World List of Scientific Periodicals), volume, pages of reference (first and last); see the examples below.

When papers published in non-congress language are quoted, summaries in a congress language (if any) should be mentioned (see example No. 2). If the title is stated in a congress language, it should be quoted (see example No. 2). Names of non-Latin authors should be transliterated into the Latin alphabet according to the ISO-Recommendations, unless the cited author himself prefers another transliteration of his own name.

Books should be cited as in examples 4 and 5.

1. Reynoldson, T. B., Young, J. O., Taylor, M. C. 1965. The effect of temperature of the life-cycle of four species of lake dwelling triclads. *J. anim. Ecol.*, 34, 23-43
2. Solski, A. 1962. Mineralizacja roślin wodnych. I. Uwalnianie fosforu i potasu przez wymywanie [Mineralization of the aquatic vegetation. I. Liberalization of phosphorus and potassium salts by leaching]. *Pol. Arch. Hydrobiol.*, 10, 107-196 [Engl. summ.].
3. [Imsheneckij, A. A.] Имшенецкий, А. А. 1949. Оптимальные питательные среды для анаэробных целлюлозных бактерий. [Optimal nutritional environments for anaerobic cellulose bacteria] *Mikrobiologia*, 18, 215—223.
4. Ekman, S. 1953. *Zoogeography of the sea.* London. Sidgwick and Jackson.
5. Beeton, A. M., Chandler, D. C. 1963. The St. Lawrance Great Lakes. In: Frey, D. C. [Ed.] *Limnology in North America*, 535-558, Madison, The University of Wisconsin Press.

In the text, references should be quoted by mentioning the author's name and date, e.g. (Bogucki 1953) or Bogucki (1953); when a work by more than two

authors is referred to, only the name of the first among them should be mentioned, followed by "et al.", e.g., Reynoldson et al. (1965); papers by the same authors published in the same year should be distinguished by small letters of the alphabet added after the year, e.g., Kamler, Riedel (1960 a).

Sometimes there is a need to cite works which have not yet been published in print. They ought to be set forth in the following way:

a. unpubl. (Not expected to be published at any definite time). To be mentioned in the text only.

b. in prep. (A work prepared but not submitted for print by its author). Quote the author's name and the work's title.

c. in print. State the author's name, title of the work and of the journal, and the year and volume whenever possible.

Marking of the typescripts. The authors of papers submitted in congress languages are requested to make no markings on their manuscripts.

Reprints. The authors receive 25 reprints of their papers free of charges. 75 more reprints is given for which the costs are covered from Author's fees or by his Institute if the formal order is delivered. It is emphasized that the number of reprints printed must be equal, for technical reasons, for all papers of a given issue, so the Authors are requested not to refuse that 75 reprints.

Cena zł 39,—

CONTENTS

Pol. Arch. Hydrob. T. 19, nr 4, s. 343—504, Warszawa 1972. Indeks 37335