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Manuscripts, of which the original and one carbon copy complete with tables and figures should be sent, should be typewritten (double-spacing) with a left-hand margin of 4 cm and about 30 lines per page. The title of the paper (in the language of the text and in the author's native language) should be indicated together with the author's name, address, name and address of the laboratory.

The article should be arranged as follows: 1. a brief introduction, 2. a section on methods, 3. the results, 4. discussion, 5. a summary, giving the main results, of not more than 200 words in the language of the text, 6. a summary, same text as in point 5 but in author's native language and 7. references. Together with the manuscript should be sent 2 copies of an abstract of not more than 150 words in the language of the text, which will be printed also on index cards.

POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	17 (30)	4	421-434	1970
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THE ELEMENTS OF ENERGY BALANCE IN GRASS CARP  
(*CTENOPHARYNGODON IDELLA* VAL.). PART I

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ABSTRACT

Energy changes were investigated in grass carp bred on plant food. The oxygen consumption, excretion of carbon dioxide and nitrogen, value of food ration and the basic energy indicators  $K_1$  and  $K_2$  and also  $U^{-1}$  were determined.

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1. INTRODUCTION

This is a part of research on energy changes in grass carp growing on various food in conditions of laboratory culture. The work has been carried out in order to determine the parameters of energy balance (oxygen consumption, excretion of carbon dioxide and nitrogen, food ration and growth) in fish fed with pure plant food. Then these parameters were used to calculate the energy budget using the method of indirect calorimetry.

2. MATERIAL AND METHODS

The experiments were carried out on fish from fish ponds in Żabieniec<sup>1</sup>, on one year old, weighing 20-70 g. The fish were fed with lettuce *Lactuca sativa*. In order to obtain optimal conditions, the lettuce was chosen as it is preferred by young grass carp (PENEZ and TÖLG 1966, JÄNICHEN 1967, FISCHER 1968). All cultures and measurements were carried out at temp. 23°C. Before the research the fish were kept about 2 months in laboratory condi-

<sup>1</sup> Institute of Inland Fishery, Żabieniec.

tions, in constant temperature, and were fed only with lettuce. During the measurements were taken (about 6 months) the fish were kept in 10 l glass jars with a constant flow, which were placed in a water thermostat having constant temperature. Everyday the fish were fed, and also the water was changed. The days on which the determinations of oxygen, carbon dioxide and nitrogen were made, the jars after change of water were tightly closed and the flow was switched on. The jars were supplied with settled water, well aerated and having the same temperature as the water in the thermostat with jars (Fig. 1). Each time the flow was measured. The scheme of experi-

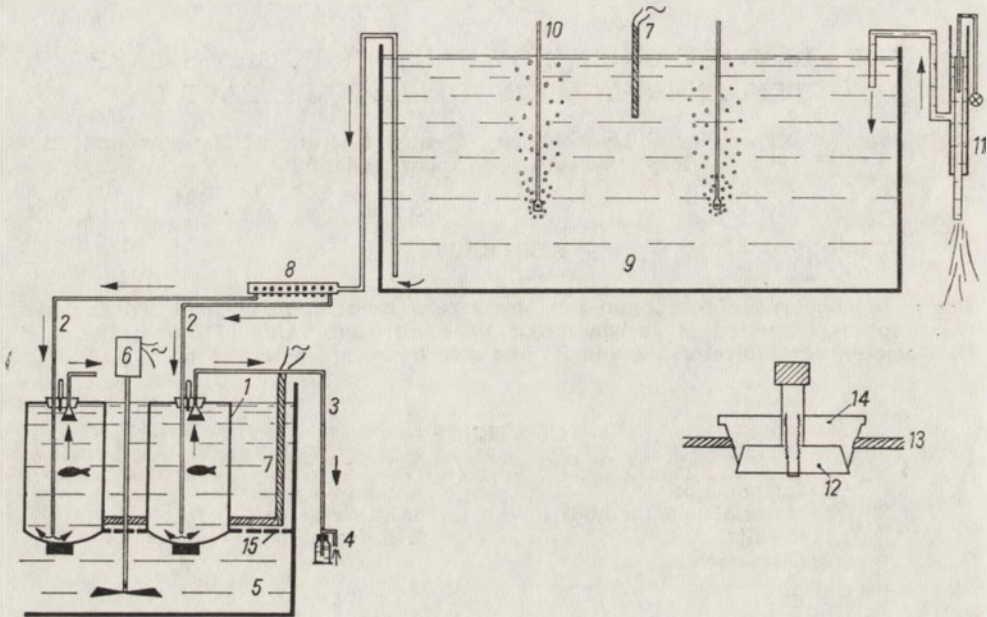


Fig. 1. Scheme of the experiment. 1—the jar with fish; 2—water inflow; 3—water outflow; 4—water sampling; 5—thermostat; 6—agitator; 7—heater; 8—dividers; 9—water reservoir; 10—air diffuser; 11—regulator of water level; 12—solid part of stopper; 13—rim of jar; 14—rubber part of stopper; 15—stand of respiration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

ment is shown in Fig. 1. The thermostat contains 10 experimental jars with fish, two heaters and one agitator placed in the middle. The jars are supplied with water from the container (9). The jar's outflow has a funnel covered with a net. The water container is placed above the thermostat with fish. In order to maintain a constant water level it has a permanent connection with the regulator (11). The regulator is a rather long pipe supplied with tap water. When the water level passes beyond the level in the container the water pours out. All parts of the apparatus are made of glass or PCV.

In the jars with fish no agitators were placed as it has been considered that the motion of fish itself is sufficient enough, and also that the outflow is at the top of the jar and the inflow at the bottom. The container had no agitator as it was well aerated.

One of the jars in the thermostat was always without fish but the same measurements were made as for other jars (that is flow regulation, water change) and it was treated as a control one.

Respective elements of the balance (food ration, respiration, excretion of  $\text{CO}_2$  and nitrogen) were measured as following:

Food intake was measured by giving the fish each time a weighed amount of lettuce and after 24hr the rest was taken out and weighed. Simultaneously one portion of weighed food was dried and then a part of it was burnt in PHILLIPSON'S microbomb calorimeter (1960). Whereas the second part was burnt in a muffle oven and the ash content was determined. In order to check whether the calorific value of plants did not change during the 24hr of exposure, one portion of food was placed in the control jar (without fish) then after 24hr was taken out and its calorific value was calculated. It was found that this value does not undergo any essential changes. The energy value of 1 mg of dry weight of food was multiplied by the amount of food consumed within 24hr and thus the food intake was calculated.

The fish increments were determined every two weeks by weighing. In order to calculate their calorific value 3 fishes from a parallel culture (the same food and temperature) were killed and their ash content as well as the calorific value of 1 g of dry weight were estimated by burning in bomb calorimeter<sup>2</sup>.

Respiration of fish was measured by the modified Winkler's method in a constant flow (about 2 l per hr). The jars were tightly closed by screwing home as on Fig. 1. The measurements of respiration as when determining the food intake were made 10 times on 9 fish. Apart from that daily measurements were made every one hour on 9 fish and in the control.

Excretion of carbon dioxide was measured by SOROKIN'S method (1958) comparing the production of carbon dioxide in jars holding fish and in the control one without fish. These measurements were also made in tightly closed jars in constant flow. During the research it was found that the results obtained by SOROKIN'S method are overestimated. Additionally 22 tests to estimate carbon dioxide were made simultaneously in the apparatus of SOROKIN and VAN SLYKE (modified by RODIER). These tests showed that SOROKIN'S apparatus in these conditions gives results 2.67 times overestimated (Standard Error of this average is  $\pm 0.084$  for  $n = 22$ ). A correction was made in the calculations.

Nitrogen excretion from the fish organism was estimated by determining the amount of excreted ammonia (PRCCHAZKOVA 1964). Despite several attempts the amount of excreted carbamide was not determined, therefore on the basis of literature (SMITH 1929, FRY 1957) it was assumed that urea nitrogen is 15% of ammonium nitrogen.

### 3. RESULTS

The calorific value of lettuce during the experiment varied from 3.0542 to 3.8696 cal/g dry weight. These variations were mainly the result of variations of the ash content from 14 to 24%. Also the lipid content was estimated.

<sup>2</sup> All estimations of the calorific value obtained by burning in bomb calorimeter of PHILLIPSON type were carried out under the guidance and help of Dr. T. PRUS, to whom I am very grateful.

It is variable but generally small as it hardly amounts to 1%. The value of food ration also varied considerably during the measurements and was from 1000 to 7000 cal/fish. This calculated per 1 g of fish body was about from 84.82 to 168.21 cal/g of fish body. The average food ration is  $126.728 \pm 8.526$  (S.E.) cal/g of fish at  $n = 10$ . The value of food ration depends to some extent on the weight of fish. This dependence is expressed by the regression formula:

$$C = 743.9 \cdot W^{0.4575} \text{ cal/24 hr}$$

where  $C$  — value of food ration,  $W$  — weight of fish (g) (Fig. 2).

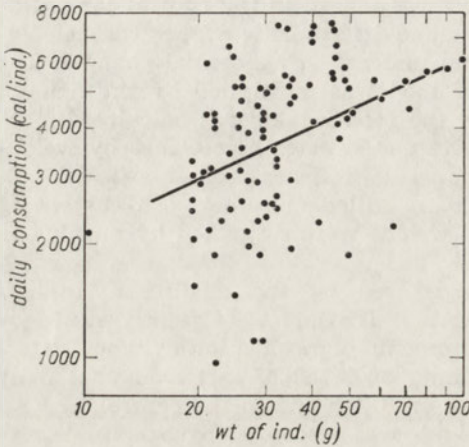


Fig. 2. Dependence of the daily food ration on the fish body weight

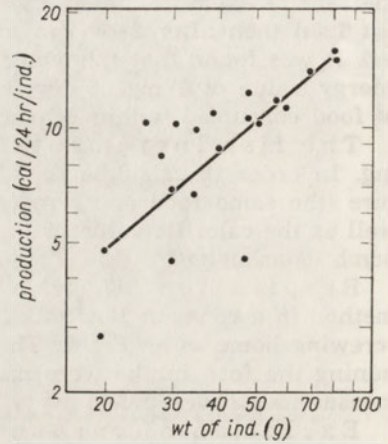


Fig. 3. Dependence of daily fish increments on its weight

The results obtained by burning the examined fish in bomb calorimeter are rather equal. 1 mg of fish dry weight has an average calorific value  $4.3383 \pm 0.11$  cal (S.E.) for  $n = 10$ . But average daily fish increments vary, depending on the weight, from 29 to 139 cal. So the average daily increment of 1 g of fish may be calculated and is 2.460 cal. The dependence of daily increment on fish weight is expressed by the regression formula:

$$P_g = 4.122 \cdot W^{0.83}$$

where  $P_g$  — increment (cal/24hr),  $W$  — weight of fish (g). This regression is presented on Fig. 3.

The oxygen consumption by fish at the time of measurements was remarkably variable. Depending on the weight of fish it varied from 1500 to 10,000  $\mu\text{l/hr}$ . Dependence of oxygen consumption on weight is expressed by formula:

$$R = 498 \cdot W^{0.5981}$$

where  $R$  — respiration  $\mu\text{l/hr}$ ,  $W$  — weight of fish (g). This dependence is presented in Fig. 4.

It may be presumed that these variations depend also on the activity of fish.

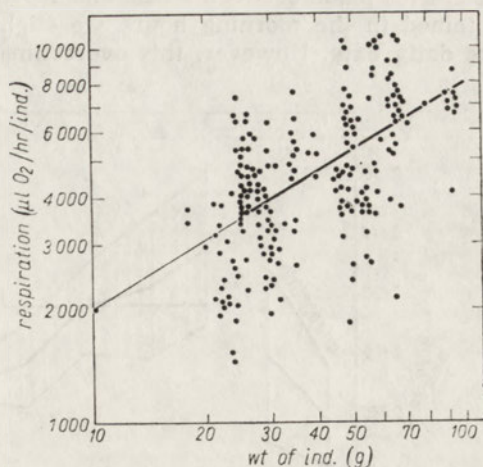


Fig. 4. Dependence of the respiration of an individual on body weight

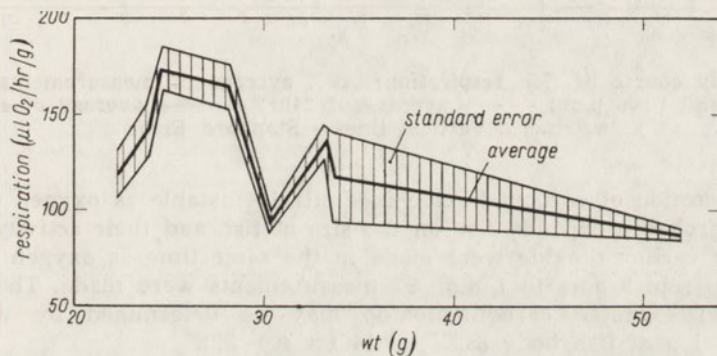


Fig. 5. Dependence of the respiration of 1 g of fish on body weight

Figure 5 presents the dependence of fish respiration on weight calculated per weight unit—g. This diagram is made according to the averages calculated from the measurements of fish of the same weight. 122 measurements were made. This diagram also shows the range of Standard Error. On the basis of these data the average for all measurements was calculated and it is  $130 \pm 4.57 \mu\text{l/g}\cdot\text{hr}$  for  $n = 122$ . In order to check how the data, obtained in our measurements, always taken between 9 a.m. and 1 p.m. are related to the daily respiration, daily measurements were once made: 9 fish were measured every hour. Figure 6 presents the results. This diagram also shows the average of measurements obtained in a determined period of time and the range of Standard Error. This range is relatively wide because of a small number of tests, which for technical reasons could be made only simultaneously. At the beginning of experiment an adaptation period was observed, which had not been taken into account in calculations. Line No. 2 represents the average of daily measurements and the line No. 3—the average of mea-

surements made between 9 a.m. and 1 p.m. Thus it may be stated that the data obtained in the morning hours are slightly overestimated as compared with the daily data. However, this overestimation is not big.

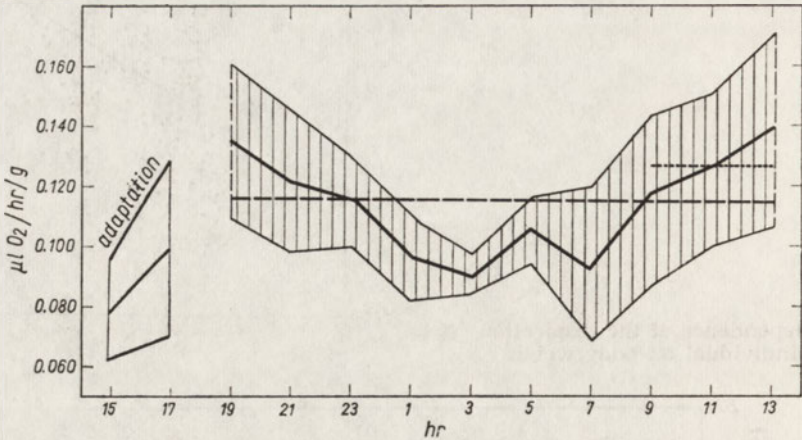


Fig. 6. Daily course of fish respiration: ..... average of measurements between 9:00 a.m. and 1:00 p.m.; - - - - average of 24hr; ——— average of each measurement. Vertical lines — Standard Error

The excretion of carbon dioxide is similarly instable as oxygen consumption, and probably depends also on the size of fish and their activity. Measurements of carbon dioxide were made in the same time as oxygen measurements, i.e. from 9 a.m. to 1 p.m. 92 measurements were made. The average quantity of excreted carbon dioxide may be determined by the value 117.4  $\mu\text{l/hr/g}$  of fish body (S.E. = 9.14 for  $n = 92$ ).

Estimation of the excretion of ammonium nitrogen gave results varying from 80 to 900  $\mu\text{g/hr}$  depending on the fish weight. The dependence of the amount of excreted nitrogen on the fish size is determined by the regression formula:

$$QN = 42.5 \cdot W^{0.545}$$

where  $QN$  — quantity of excreted nitrogen ( $\mu\text{g/hr}$ ), and  $W$  — weight of the examined object (g).

This dependence is presented in Fig. 7. When calculating these data per 1 g of fish, the averages for respective fish weights are obtained and are presented in Fig. 8. The range of Standard Error is indicated at each average with a vertical line. Data obtained by SMITH (1929) and quoted recently in literature as fundamental data are added to this diagram. This diagram shows that the amount of nitrogen excreted by 1 g of fish body during an hour decreases as the weight of fish increases. In the carried out experiment this value is average 5.77  $\mu\text{g}$  (S.E. =  $\pm 0.3082$  for  $n = 92$ ). Taking into consideration the correction for urea nitrogen (SMITH) the average quantity of nitrogen excreted per 1 g of fish body was assumed as 6.64  $\mu\text{g/hr}$ .

On the basis of thus obtained fundamental elements of energy balance, calculations of the energy balance of an average weight fish may be made



as well as on the basis of regression curves of any weight fish. Also using the method of indirect calorimetry the calculations of the value of protein, lipid and carbohydrate conversions can be made.

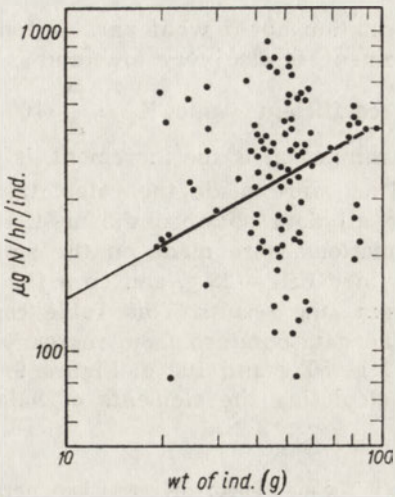


Fig. 7. Dependence of the quantity of excreted nitrogen on body weight

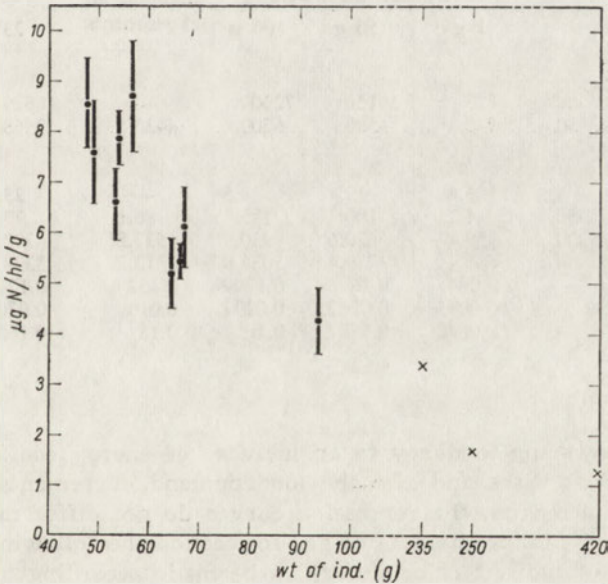


Fig. 8. Dependence of the quantity of excreted nitrogen by 1 g of fish body: — Standard Error; x SMITH'S (1929) data; • author's data

On the basis of pattern  $C = R + P + F$ , where:  $C$  — daily food ration,  $R$  — respiration,  $P$  — increment,  $F$  — faeces, the value of assimilation in calories was calculated (i.e.  $R + P$ ). This value for this experiment was on the average

623.3 cal per fish. The quantity of faeces can be calculated according to the equation  $F = C - A$ . This average value is 3812.2 cal. Assimilation:  $U^{-1} = \frac{A}{C} \cdot 100$  is on the average 13.2%. The coefficient  $K_1 = \frac{P}{C} \cdot 100$  providing information about what part of consumption is used up by the fish for the increment, is also very low, and is on the average 1.9, Standard Error 0.23. The coefficient value  $K_2 = \frac{P}{A} \cdot 100$  providing information about what part of assimilation is the increment, is on the average 13%.

Thus were made the calculations of energy balance on average values from all data obtained during the experiment. Apart from that analogous calculations were made on the single fish, which weighed the least of all examined fish — 23 g, and on a fish having a greatest weight — 52 g. Table I presents the results. This Table contains also theoretical calculations based on the data obtained from regression curves for the hypothetical fish weighing 1 g, 50 g and 100 g. Figure 9 presents how these three different ways of calculating the elements of balance and energy coefficients are related.

Table I. The most important energy indicators obtained from the experiment and regression curves

Indicator	Unit	Theoretical fish weighing			Average of experiments	Fish weighing	
		1 g	50 g	100 g		23 g	52 g
Calorific value of fish	cal	723	36150	72300	—	16629	37596
$C$	cal	748.9	4500	6200	4435.5	3868.83	4410.6
$C$ in % from the cal. value of fish	%	103.6	12.4	8.5	—	23.2	11.8
$P$	cal	4.2	100	185	86.1	77.92	147.6
$R$	cal	59.4	620.6	930.9	537.25	392.26	552.2
$F$	cal	685.4	3779.4	5084.0	3812.2	3398.48	3710.2
$A/C$	—	0.084	0.16	0.1799	0.132	0.1215	0.158
$K_1$	—	0.0055	0.0222	0.0297	0.019	0.020	0.028
$K_2$	—	0.0648	0.1387	0.1657	0.13	0.165	0.21

This Figure shows the tendency to an increase of energy coefficients as the growth of fish increases and also the food demand, increments and respiration. Data obtained from the regression curves do not differ much from the experimental data. Using the data of gas conversion the following calculations by the method of indirect calorimetry can be made according to the diagram of KLEKOWSKI (unpublished) and basing on the averages from the whole experiment:

$O_2$  consumption — 130.2  $\mu\text{g/hr/g}$  of fish body,

$CO_2$  excretion — 117.4  $\mu\text{l/hr/g}$  of fish body,

N excretion — 6.64;  $6.64 \cdot 6.25 = 41.5$   $\mu\text{g}$  of proteins.

The following indicators were assumed (HARROW and MAZUR 1966):

Food	Consumption	Production	RQ	Calorific value
1 g	O <sub>2</sub> (ml)	CO <sub>2</sub> (ml)		Kcal/g
Carbohydrates	828.8	828.8	1.00	4.2
Fats	2019.3	1427.3	0.71	9.5
Protein	966.3	773.9	0.80	4.3

thus burning of 41.5 µg of protein uses:

$$41.5 \cdot 0.9666 = 40.089 \mu\text{l O}_2$$

$$41.5 \cdot 0.774 = 32.121 \mu\text{l CO}_2$$

oxygen consumption for carbohydrates and fats:

$$130.2 - 40.089 = 90.111 \mu\text{l O}_2/\text{hr/g}$$

CO<sub>2</sub> excretion for carbohydrates and fats:

$$117.4 - 32.121 = 85.279 \mu\text{l CO}_2/\text{hr/g}$$

$$\text{thus } RQ = \frac{117.4}{130.2} = 0.9$$

$$\text{non-protein } RQ = \frac{85.279}{90.111} = 0.946 \approx 0.95$$

are

$$90.111 \mu\text{l O}_2 \cdot 1.01 = 91.012 \mu\text{g of carbohydrates}$$

$$90.111 \mu\text{l O}_2 \cdot 0.091 = 8.200 \mu\text{g of lipids}$$

$$41.552 \mu\text{g of proteins.}$$

Therefore the energy used by 1 g of fish body during an hour:

$$0.091 \cdot 4.2 \text{ cal} = 0.38822 \text{ cal (carbohydrates)}$$

$$0.0082 \cdot 9.5 \text{ cal} = 0.0779 \text{ cal (lipids)}$$

$$0.0415 \cdot 4.3 \text{ cal} = 0.1784 \text{ cal (proteins)}$$

$$\hline 0.6385 \text{ cal/hr/g}$$

what gives an energy consumption during 24hr by 1 g of fish body—15.324 cal, so by a fish weighing 35 g—536.34 cal. Thus it can be stated that the conversion of carbohydrates is 59.86%, of protein—27.94%, of lipids—12.20%.

#### 4. DISCUSSION

The values obtained from oxygen measurements are consistent with the literature data on carp and crucian carp. The fact that the oxygen measurements displayed great variation hence the standard error was quite big (Fig. 5) seems to be to a large extent the result of various activity of fish during the measurements. In quite big jars of 10 l capacity the fish have plenty of place. In such experiments this is an uncontrolled factor but undoubtedly not without significance. Fry (1957) gives the variations in oxygen consumption depending on the activity of fish, on the example of *Carassius*

*auratus* from about 100 to 260 ml/kg/hr, and of *Ameiurus nebulosus* from about 200 to 600 ml/kg/hr, that is about three times as much. It seems that

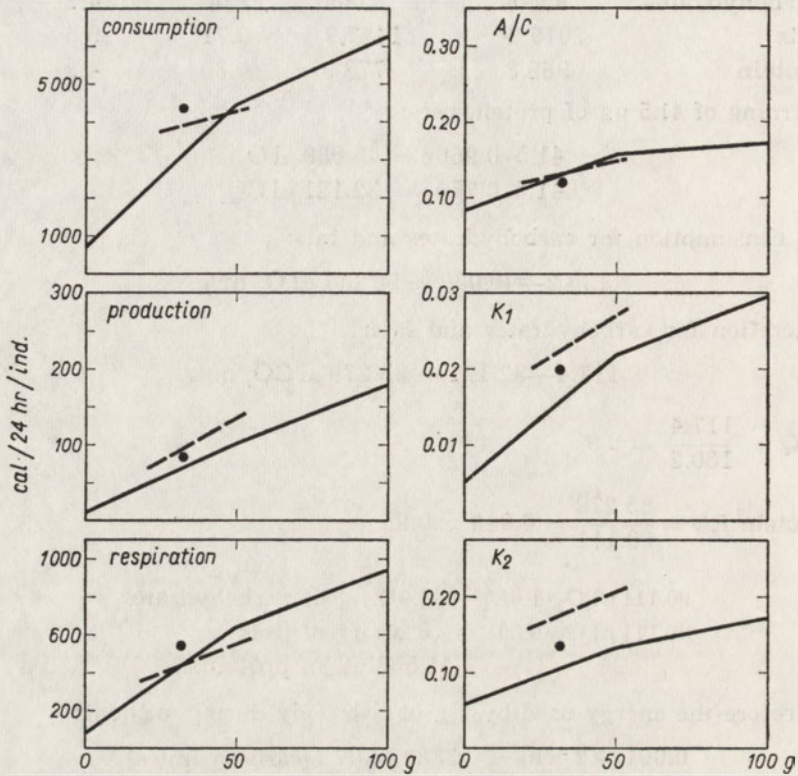


Fig. 9. Dependence of the studied energy indicators on fish body weight: ——— data from the regression curves; • average of experiments; - - - - data for 23 and 53 g fish

the excretion of carbon dioxide varies for the same reasons. The oxygen regression curve depending on weight shows a relatively small inclination as compared with the regression calculated by WINBERG for the respiration of fish in general. WINBERG assumes 0.84, while according to our calculations this value is 0.598. Value "a", i.e. the respiration of fish weighing 1 g, is several times greater in our experiments (it is 498). But comparing the regressions obtained in our experiments with the data of EDWARDS et al. (1969) it may be assumed that the differences in the course of regression curves of WINBERG and our's may be explained by the various activity of examined fish. EDWARDS et al. say that fish in the resting stage have the regression formula as following:

$$R_1 = 0.214 W^{0.721} \text{ ml/g} \cdot \text{hr}$$

for the same fish but active:

$$R_2 = 0.737 W^{0.590} \text{ ml/g} \cdot \text{hr}$$

Thus the value "a" increases and the index value decreases. Having these results it may be assumed that both the relatively high value "a" and the low value "W" in the regression formula obtained for grass carps may be the result of higher activity of fish in jars, than according to the data of WINBERG (1961). Chinese authors (YEH YE TSU 1959) also give some data on the respiration of the grass carp. These data were obtained by means of experiment. They are for a fish weighing 1.11 g  $R = 291.28 \mu\text{l/hr}$ , for a fish weighing 9.6 g  $R = 1860.39 \mu\text{l/hr}$ . If we calculate theoretically the respiration for these weights of fish according to WINBERG's regressions and the regressions obtained by us, the obtained data are differ from the real results of Chinese authors. These differences according to WINBERG's regressions are 43.03% and 45.39%; according to the regression obtained by us on grass carp weighing 1.11 g by 83.84%, whereas for the weight 9.6 g only by 4.7%. Therefore we may come to the conclusion that not only the variations in the respiration value, but also of the correlation between respiration and weight are quite considerable in grass carp. This most surely depends on the kind of food and also on the activity of investigated fish.

RQ of grass carp is high, typical for phytophagous fish. It varies from 0.92 to 1.00 (Table II). High RQ is often found among fattened animals, as

Table II. Energy balance of grass carp obtained by the indirect method for 1 g of fish per 1 hr

Indicator	Unit	Average of experiments weight 35 g	Maximum weight 52 g	Minimum weight 23 g
O <sub>2</sub>	$\mu\text{l}$	130.2	89.02	175.00
CO <sub>2</sub>	$\mu\text{l}$	117.4	78.20	167.67
N-protein	$\mu\text{g}$	41.5	27.312	39.06
RQ non-protein	—	0.95	0.92	1.00
Demand of carbohydrates	$\mu\text{g}$	90.012	54.47	169.11
Demand of lipids	$\mu\text{g}$	8.2	8.995	—
Demand of proteins	$\mu\text{g}$	41.562	27.312	39.06
Energy used by carbohydrates	cal	0.3822	0.2284	0.7102
Energy used by lipids	cal	0.0779	0.0845	—
Energy used by proteins	cal	0.1784	0.1173	0.1677
Sum of used energy	cal	0.6385	0.4302	0.8779
% of carbohydrates metabolized	%	59.86	53.09	80.9
% of lipids metabolized	%	12.2	19.64	—
% of proteins metabolized	%	27.94	27.27	19.1

in our experiments, where the fish were cultivated in conditions of food abundance. In such conditions according to PROSSER (1950) it may even exceed the value 1.00. Depending on the weight that is as the fish grows, the RQ decreases. This is consistent with the results obtained by NEDHAM (1950), who investigated the embryonic development of the crab *Carcinus*. The already quoted Chinese authors (YEH YE TSU) also count the RQ for grass carp and this value depending on its body weight varies from 0.88 to 0.93 — on the average they assume 0.91. This value is considerably lower than the one obtained in our experiments, but this is probably the result of the quantity and quality of given food.

The average value of excretion of carbon dioxide by 1 g of fish body is absolutely comparable with the same data obtained by the Chinese authors in their experiments with grass carp. Our data are  $118 \mu\text{l/g}\cdot\text{hr}$  for the size of fish about 35 g, while YEH YE TSU obtained the value  $187 \mu\text{l/hr}$  for a fish weighing 9.6 g.

Figure 8 presents the relations between the nitrogen excreted out of the fish bodies and their weight. Similar dependences were found by SMITH (1929). It seems, however, that the decreasing amount of excreted nitrogen depends on the weight of fish only indirectly, while there is a direct dependence between the excreted nitrogen and food ration, which in turn is strictly dependent on the body weight. Daily food ration per 1 g of the body of fish, weighing about 20 g, is 153 cal, whereas of the fish weighing 49 g—about 84 cal. Therefore it may be concluded that the protein conversion in the entire fish (not calculated per 1 g of body) is rather constant and is about 30%, regardless of the value of food intake.

It is commonly known that the value of daily food ration changes according to several factors such as, e.g. temperature, quantity and quality of food, and others. Therefore they were tried to be eliminated by constant temperature, food abundance and its quality. As it has been already mentioned the lettuce was chosen as the food preferred, because as it has been previously found (FISCHER 1968) the value of food ration of grass carp varies from 661 cal to 9786 cal according to the quality of food. The value of food ration as Fig. 2 shows increases together with the weight. But this increase is not too big, and so calculating per 1 g of fish body the amount of taken food increases quite considerably (at a weight increase from about 20 to almost 100 g the amount of taken food per 1 g of fish body increases from about 60 to 160 cal). A confirmation of these results may be found in general regularities of this type described by WINBERG (1956), KARZINKIN (1952).

Assimilative faculty of grass carp cultivated on pure plant food is very low and is on the average 13%. This is justified by the dominant amount of carbohydrates in the given food, which are generally assimilated in a small per cent—cellulose near to zero (not taking into consideration the cellulose bacteria and some protozoan species), while the remaining carbohydrates—usually within the range of 60–75%. Similar low values of food assimilated by the investigated organisms were obtained by PRUS (in prep.) in his paper on *Asellus aquaticus* (8.7%). It seems, that apart from the main factor having a repercussion on the assimilation value, i.e. the kind of food, the physiological state of the examined organism is also of some significance. An assimilation increase as the fish grows is clearly visible in Table II, and also in Fig. 9. However, despite the growth this value is several times smaller than in other fish, e.g. according to IVLEV (1939) the carp assimilates more than 40% of consumed food.

On the grounds of obtained results it can be stated that the grass carp fed only with plant food is of no special significance in water bodies as an energy converter, but its main significance is in the destruction of aquatic vegetation and thus fertilization of water bodies.

After calculating the obtained values of food ration into a per cent from the body calorific value (such coefficients are widely applied by Russian scientists—IVLEV, WINBERG, KARZINKIN) (Table I), this dependence is even more pointed out.

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## 5. SUMMARY

In order to learn about the energy changes taking place in grass carp cultivated on plant food, in conditions of laboratory experiment the following were investigated: oxygen consumption, excretion of carbon dioxide and nitrogen, increments and food ration of fish of a size from 20 to 60 g. It was found that 1 g of dry weight of fish body has a calorific value  $4.3383 \text{ cal} \pm 0.11$  (S.E.) for  $n = 10$  the food ration varies from 84.82 to 168.21 cal/g of fish. The respiration is on the average  $130 \pm 4.572 \text{ } \mu\text{l/hr/g}$  for  $n = 122$ , excretion of carbon dioxide  $117.4 \text{ } \mu\text{hr/g}$  for  $n = 92$ , excretion of ammonium nitrogen  $5.77 \pm 0.3082 \text{ } \mu\text{g/hr/g}$  for  $n = 92$ . "Non-protein" RQ is 0.95. On the basis of obtained data it was calculated that the protein conversion in the metabolism of grass carp is 27.94%, of lipids 12.20%, of carbohydrates 59.86%. Also low energy indicators were found  $K_1 = 19.6\%$ ,  $K_2 = 13.68\%$  and also the food utilization was low  $U^{-1} = 13.2\%$ .

Due to the experiments it may be assumed that the grass carp fed only with plant food is a poor energy converter in water bodies.

## 6. STRESZCZENIE

W celu poznania przemian energetycznych zachodzących u białego amura hodowanego na pokarmie roślinnym, przebadano w warunkach eksperymentu laboratoryjnego: zużycie tlenu, wydalanie dwutlenku węgla, azotu, przyrosty i racje pokarmową ryb ważących 20–60 g. Stwierdzono, że gram suchej masy ciała ryby posiada wartość kaloryczną  $4,3383 \text{ cal} \pm 0,11$  (Standard Error) dla  $n = 10$ , racja pokarmowa waha się od 84,82 do 168,21 cal/g ryby. Oddychanie średnio wynosi  $130 \pm 4,572 \text{ } \mu\text{l/godz./g}$  dla  $n = 122$ ; wydalanie dwutlenku węgla  $117,4 \text{ } \mu\text{l/godz./g}$  dla  $n = 92$ ; wydalanie azotu amoniakalnego  $5,77 \pm 0,3082 \text{ } \mu\text{g/godz./g}$  dla  $n = 92$ . RQ „niebiałkowe” wynosi 0,95. Na podstawie uzyskanych danych obliczono, że w metabolizmie białego amura przemiana białkowa wynosi 27,94%, lipidowa 12,2%, węglowodanowa 59,86%. Stwierdzono również niskie wskaźniki energetyczne  $K_1 = 19,6\%$ ,  $K_2 = 13,68\%$  oraz wykorzystanie pokarmu  $U^{-1} = 13,2\%$ . Na podstawie przeprowadzonych eksperymentów można przypuszczać, że biały amur karmiony wyłącznie pokarmem roślinnym jest słabym konwertorem energii w zbiornikach wodnych.

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## QUANTITATIVE CHANGES IN THE CIRCULATING BLOOD OF TENCH (*TINCA TINCA* (L.)) IN THE ANNUAL CYCLE

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### ABSTRACT

Morphological blood examinations on 320 tenches (162 males and 158 females) during 10 months proved the direct effect of reproduction cycle on the amount of erythrocytes and leucocytes in the circulating blood. Especially among the females, during the spawning-season a considerable decrease of the number of erythrocytes, hemoglobin and hematocrit's index takes place. Besides an intensive leucocytosis is observed during that period. In the other part of the year, with the exception of January, the number of erythrocytes is higher in males. Whereas other parameters such as mean volume blood cells, mean concentration and hemoglobin content in one erythrocyte are higher in females.

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### INTRODUCTION

The specific biological character and the variety of fish species makes the comparison of hematological indices exceptionally difficult. The dependence of hematological indices on physiological and environmental conditions and also on the age and sex of fish belong still to the controversial subjects in literature. Some authors do not find differences connected with sex dimorphism as e.g. SMITH (1930) for *Salmo trutta* L. and GLAZOVA (1967) for *Acipenser ruthenus* L. and *Acipenser stellatus* Pall. Others like LANGE (1919) and PAVLOV and KROLIK (1935) justify the quantitative changes in the composition of the circulating blood of males and females for optional months of the annual cycle. IVLEV (1955) observed even in winter similar correlations in the blood of *Lota lota* (L.), *Silurus glanis* L. and *Cyprinus carpio* L. Furthermore SCHLICHER (1927), PAVLOV and KROLIK (1935) state that the number of erythrocytes and hemoglobin is higher during the entire year in the blood of males than of females.

Other authors find the differences connected with the sex of fish only during the production of gonads and spawning. ROBERTSON et al. (1961) proves that the

hematological indices are considerably lower in the blood of females during the spawning migrations of *Oncorhynchus tshawytscha* (Walb). The results of ČERNIKOVA (1967) for *Coregonus lavaretus beari* Kassl. are approximate. But according to SCHLICHER (1927) the quantitative changes in some freshwater fishes during spawning are the same in the blood of males and females.

The variability of hematological parameters in different seasons of the year in connection with the phenomenon of spawning and feeding migrations is another controversial subject. KAWAMOTO (1929) described the seasonal differences of *Anguilla anguilla* (L.) thus proving the increase of the number of erythrocytes in winter months at a simultaneous decrease of total blood volume in the organism of these fish. ZAJCEVA (1967) obtained approximate results for the Baltic eel. But SCHLICHER (1927) is of the opinion that the level of erythrocytes of some freshwater fish he examined, does not depend on temperature, salinity and feeding, but exclusively on physiological processes connected with the spawning cycle. And on the contrary according to MOLNAR et al. (1960) the temperature is the factor regulating the number of erythrocytes and hemoglobin in *Abramis brama* (L.) and *Lucioperca lucioperca* (L.). ČERNIKOVA (1967) did not find any seasonal differences in the blood of *Rutilus rutilus* (L.), *Abramis brama* (L.) and *Lota lota* (L.). She says that there are slight variations between the spring and autumn in *Esox lucius* L. and *Perca fluviatilis* L., while the visible quantitative changes in *Coregonus lavaretus beari* Kassl. are connected with the spawning-season.

The research work aims at determining the proper hematological indices of the circulating blood of tench with consideration to the changes in the annual cycle.

## 2. MATERIAL AND METHODS

4-6 years old tenches were investigated. The length of fish was 25-38 cm and their weight 250-670 g. They were from Drwęckie and Łańskie Lakes (Olsztyn province), which have similar physiographical conditions. Apart from that the experimental determinations of the studied parameters showed a similar range of deviation.

The caught fish were taken to the laboratory, and before their examination they were kept for 16-20hr in oxygenated (on the average 7.0 mg/l O<sub>2</sub>) lake water. The temperature of water in the aquaria ranged from 11 to 14°C, and in winter from 5 to 7°C.

The blood for investigations was taken from the vena caudalis with Pasteur's pipette, previously rinsed with heparin. The time of the collection of blood samples since taking the fish out of water did not exceed 1 min. According to VINNICKIJ and ČISTOVA (1967) parallelly with the accepted method of bleeding fish after taking them out of water, a series of measurements of blood samples collected from fish kept under water were made. In this instance the blood was also sampled from vena caudalis by puncturing the tail above the water surface. Different methods of blood sampling did not affect the quantitative results and the variations in the number of erythrocytes are within the range of mean deviations of the sample (Table I).

The first blood drops were always used to calculate quantitatively the blood constituents and included:

1. Calculation of the number of erythrocytes and leucocytes in 1 mm<sup>3</sup>. The blood was diluted 200 times in Potain's blood count pipette with colour solution of violet with an addition of physiological fluid with the pH 6.8. The erythrocytes and leucocytes were calculated out of the diluted blood in Bürker's chamber according to the assumed principles.

2. Photocolorimetric estimation of hemoglobin value using the method of Drabkin in the modification of Green and Teal.

Table I. Hematological indices depending on the method of collecting blood samples

May	Blood samples collected					
	out of water			under water		
	red blood cells (mill/mm <sup>3</sup> )	hematocrit (%)	hemoglobin (g%)	red blood cells (mill/mm <sup>3</sup> )	hematocrit (%)	hemoglobin (g%)
Males	2.06	37.6	12.22	2.15	35.4	12.42
(n = 15)	±0.11	±2.7	±0.65	±0.19	±2.7	±0.62
Females	1.72	31.3	11.26	1.71	32.7	12.30
(n = 15)	±0.11	±2.4	±0.73	±0.09	±2.8	±0.48

3. Determination of the hematocrit's index with the help of Wintrobe's micropipette. Wintrobe's formulas were used in calculations of mean volume blood cells, mean concentration and mean hemoglobin content in 1 erythrocyte. Arithmetical means of the absolute values obtained in calculations from the successive monthly samples were introduced in the formulas. However, it should be pointed out that these calculations are approximate and when determining the hematological indices a correction including the oval shape of erythrocytes should be introduced. Also because of the uneven distribution of hemoglobin in cytoplasm conditioned by the presence of nucleus the values of mean hemoglobin concentration are also approximate.

Apart from that in order to check the state of health a detail parasitological dissection of tenches was made. Fish with an excessive amount of protozoans *Chilodonella cyprini*, *Ichthyophthirius multifiliis* and Trematode *Asymphylodora tincae* were eliminated. Also fish with symptoms of occult dropsy were eliminated: with fluid exudative in the body cavity, with changes in the viscera (swellings, hyperaemia, changes in colour) and most frequently with an intestinal catarrh.

On the whole 162 males and 158 females were examined.

### 3. RESULTS

Visible differences in the number of erythrocytes in the annual cycle are observed between the males and females of tenches (Table II, III; Fig. 1, curve A).

Apart from the short spawning-season the average numbers of erythrocytes in the circulating blood of males are approximate (about 2.0 mill./mm<sup>3</sup>). The number of erythrocytes considerably decreases during the spawning-season, and the average decrease is about 0.5 mill./mm<sup>3</sup> (Fig. 1, curve A).

The transmutations resulting from maturing and spawning and taking place in the organisms of females are much deeper. These processes cause significant quantitative variations of blood constituents. Since early spring (March) gradually towards June (Fig. 1, curve A) the number of erythrocytes in the circulating blood decreases; the lowest, mean annual value (about 1.4 mill./mm<sup>3</sup>) lasts during the entire spawning-season. In autumn, in normal

feeding conditions the number of erythrocytes gradually increases and attains values approximate to the average level of males.

The hemoglobin values within the year depend to a considerable extent on the reproduction cycle of tenches. The variations of the hemoglobin level and of the number of erythrocytes are more or less parallel.

Table II. Hematological indices in the circulating blood of tench (*Tinca tinca* (L.)) males in the annual cycle

Date	Number of males	Red blood cells (mill/mm <sup>3</sup> )	White blood cells/mm <sup>3</sup>	Hematocrit (%)	Hemoglobin (g%)
11-22.VI.1965	13	2.07 ±0.21	39,354 ±13,451 <i>t</i> =7.66	43.4 ±4.0 <i>t</i> =4.21	9.64 ±0.85 <i>t</i> =9.77
1.VII	5	2.13 ±0.20	28,640 ±10,779 <i>t</i> =1.86	41.0 ±4.4	9.51 ±0.59
24.VII	19	1.50 ±0.14 <i>t</i> =7.16	42,514 ±23,077	34.4 ±4.8 <i>t</i> =5.57	9.00 ±0.74
15-27.VIII	18	1.67 ±0.15 <i>t</i> =3.14	13,953 ±9,035 <i>t</i> =5.05	35.6 ±5.7	9.73 ±0.96
7-21.IX	12	2.01 ±0.21 <i>t</i> =6.64	15,953 ±8,525	38.6 ±5.2	10.63 ±1.39 <i>t</i> =4.27
12.X	10	2.03 ±0.14	13,911 ±3,988	33.8 ±3.8	10.16 ±1.54
9-29.XI	20	2.15 ±0.16	4,280 ±2,154 <i>t</i> =8.68	38.0 ±4.7	11.29 ±1.24 <i>t</i> =2.18
10.I.1966	7	2.05 ±0.09	4,171 ±2,159	33.9 ±2.4	12.60 ±0.80 <i>t</i> =2.62
4.III	12	2.16 ±0.13	5,546 ±1,509	37.5 ±2.7	12.45 ±0.77
20.IV	8	2.07 ±0.19	6,228 ±2,427	37.0 ±6.5	12.24 ±1.27
11.V	20	2.09 ±0.20	9,617 ±4,065	36.9 ±3.6	12.39 ±0.75
4.VI	18	1.93 ±0.18	32,950 ±11,011	32.3 ±2.7	11.21 ±0.46

The decrease of hemoglobin content during the spawning-season (Fig. 2, curves A, C) is proportional to the number of erythrocytes. Rather small hemoglobin concentration—7.87–8.97 g% for females and about 9.0 g% for males—corresponds with the smallest number of erythrocytes. As the number of erythrocytes systematically increases in autumn the hemoglobin content gradually increases in the blood of tenches. The maximum level took place in January of the year of investigations. The greatest annual value (about 12 g%) of hemoglobin is during a considerable part of the year, for males—January to the period before the spawning-season (June), for females—January to March. An earlier decrease of the hemoglobin content and of other parameters (Fig. 2) in the blood of females is caused by spawning changes.

Table III. Hematological indices in the circulating blood of tench (*Tinca tinca* (L.)) females in the annual cycle

Date	Number of females	Red blood cells (mill./mm <sup>3</sup> )	White blood cells/mm <sup>3</sup>	Hematocrit (%)	Hemoglobin (g%)
11-22.VI.1965	6	1.35 ±0.22 <i>t</i> = 5.10	40,200 ±28,058 <i>t</i> = 2.08	29.5 ±3.3 <i>t</i> = 2.21	7.87 ±0.78 <i>t</i> = 8.23
24.VII	20	1.41 ±0.14	45,029 ±27,899	32.4 ±4.1	8.97 ±0.85 <i>t</i> = 2.85
15-27.VIII	17	1.59 ±0.12 <i>t</i> = 4.96	12,700 ±7,500 <i>t</i> = 4.64	34.4 ±4.1	8.98 ±1.02
7-21.IX	17	1.69 ±0.15 <i>t</i> = 2.09	23,025 ±8,083 <i>t</i> = 3.87	35.5 ±5.8 <i>t</i> = 2.38	9.02 ±0.81
12.X	15	1.96 ±0.15 <i>t</i> = 4.74	13,677 ±5,138 <i>t</i> = 3.84	34.6 ±4.1	10.08 ±1.17 <i>t</i> = 3.02
9-29.XI	28	1.99 ±0.14	8,296 ±4,342 <i>t</i> = 3.64	34.8 ±4.0	10.79 ±1.10 <i>t</i> = 3.80
10.I.1966	10	2.04 ±0.16	6,320 ±3,798	35.0 ±4.6	12.43 ±1.36 <i>t</i> = 2.51
4.III	15	1.94 ±0.17	7,762 ±2,632	34.3 ±4.8	12.02 ±0.63
20.IV	10	1.90 ±0.21	8,000 ±4,017	33.7 ±3.3	11.86 ±1.05
11.V	14	1.73 ±0.11 <i>t</i> = 2.58	18,909 ±10,838 <i>t</i> = 2.70	32.5 ±3.4	11.68 ±1.02
4.VI	16	1.53 ±0.11	37,575 ±21,594	27.6 ±3.5	10.62 ±0.50

The hematocrit's index calculated on the basis of investigations during the annual cycle is on the average 34.8% for tenches. Considerable differences are noticed between the males (variations within the year 33.8-43.4%, on the average 36.8%) and females of tench (variations 29.5-35.5%, on the average 33.1%). The level of the hematocrit indicator of females of tench is even during the greater part of the year with the exception of the spawning-season (Table III). A decrease of hematocrit value in spawning-months partly determines the difference in the number of erythrocytes in a volume unit. These relations are not complete as there simultaneously an obvious increase of leucocytes is observed due to the spawning preparations and spawning of tench females.

In the same period, in the blood of males at a constant level of erythrocytes, an average increase of leucocytes — 29,700/mm<sup>3</sup> increases the value of hematocrit's index by 6.5% (*t* = 4.21). The hematocrit's index illustrates also the subsequent decrease of number of erythrocytes in blood during the spawning of males (Table II).

At a relatively even number of leucocytes the decrease of the number of erythrocytes by 0.63 mill./mm<sup>3</sup> in the second half of July lowers the hemato-

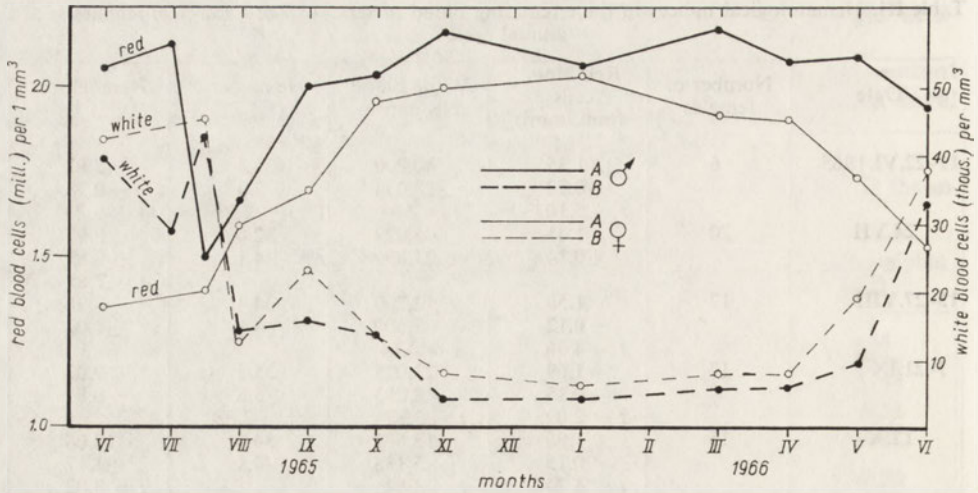


Fig. 1. Differences between the average number of erythrocytes (A) and leucocytes (B) in 1 mm<sup>3</sup> of the circulating blood of tench males and females

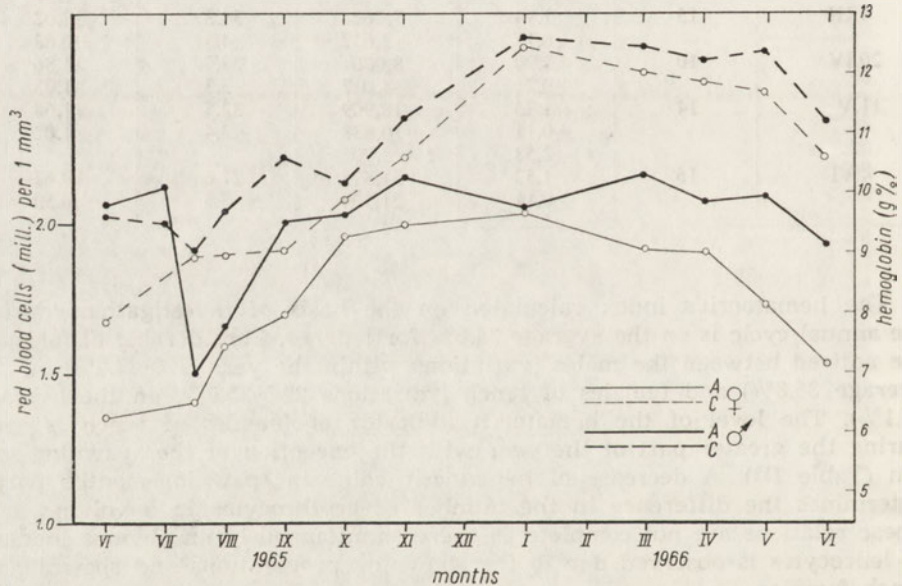


Fig. 2. Changes in the hemoglobin level (C) and of erythrocytes (A) in the circulating blood of tench males and females

crit's index by 6.6%. Successive, further variations of the hematocrit value in August and September are the result of levelled level of erythrocytes after the spawning of tenches.

At constant number of blood constituents in  $\text{mm}^3$ , the mean volume blood cells is approximate from October to May and is on the average  $175 \mu^3$  for tenches. For other months this result is not precise as the hematocrit value increases due to spawning leucocytosis.

Relative hemoglobin index (average hemoglobin content) calculated out of annual investigations is 55.0  $\gamma\gamma$  for males, and 59.9  $\gamma\gamma$  for females. In the instance of females the mean range of hemoglobin content in one erythrocyte is higher during the year (51.4–69.3  $\gamma\gamma$ ) than in the instance of tench males (44.6–61.3  $\gamma\gamma$ ). Maximal mean hemoglobin content takes place during spawning as a result of lower number of erythrocytes in a volume unit and in January at the greatest density of hemoglobin.

Similar results were obtained for the range of mean hemoglobin concentration in one erythrocyte; 25.4–38.4% females (on the average 41.1%), 22.2–37.1% males (on the average 29.7%), and maximal values in January.

The average numbers of leucocytes (Fig. 1, curve B) are ruled similarly in the blood of tench males and females during the year. The maximum number in the spawning-season is on the average 40,000 in  $\text{mm}^3$  (Tables II, III). But during that period the standard deviation is quite high and reaches  $\pm 23,077$  (males) and  $\pm 27,899$  (females) in this group of studied fish. This means that the preparations of fish to spawning differs and in the instance of females it may indicate spawning in portions.

The regulation of physiological processes, further equalization of metabolism in autumn and winter result in a decrease of the number of leucocytes in the circulating blood of tenches. The number of leucocytes in the period from October to March is relatively small and is hardly 4,171–5,546 for males, and 6,320–8,296/ $\text{mm}^3$  for females, while the standard deviation  $\pm 1500$  to  $\pm 4300$  respectively.

#### 4. DISCUSSION

Therefore, it has been proved that the physiological processes connected with the spawning cycle are the main factor forming the quantitative relations in the circulating blood of tench. The variable environmental conditions, however much slower, are of some significance.

The author's private observations and studies of SCHLICHER (1927), ROBERTSON et al. (1961) and ČERNIKOVA (1967) show that the number of erythrocytes in the circulating blood decreases during the spawning-season. SCHLICHER is of the opinion that the decrease of the number of erythrocytes takes place after spawning. In the instance of tench it takes place regularly in June or in unfavourable conditions even in the first ten days of July as it happened during the investigations in 1965. SCHLICHER did not observe any differences as regarding sex in blood constituents within the year. His opinion is that the curve of mean annual values is characterized by rhythmical decreases in the number of erythrocytes successively in June, September, October and in March and April. The author's own estimations showed a specific distribution of the number of erythrocytes, which is different for males and females during the year. In the blood of tench males a short-lasting decrease in the number of erythrocytes is observed only during spawning and is quickly compensated, while in the instance of females — during the

greater part of the year parallel with development and gonad resorption. Thus for the first time a division connected with sexual dimorphism takes place and in accordance with the results of SCHLICHER (1927), PUCKOV and FEDOROVA (1951) for other fish—the number of erythrocytes is higher in males. In the instance of tench this regularity is not true for the entire year as it is not valid for autumn and winter. In these periods the level of erythrocytes in the blood of tench males and females is approximate and sometimes even the same. Therefore in such a situation, at least for the tench males, the maximum annual number of erythrocytes cannot be estimated.

This shows that apart from the quantitative regulation there are also other means of retaining the balance in blood during the changes of environment. E.g. ČERNIKOVA (1967) and SCHLICHER (1927) observed an increase of blood cell surface in autumn and winter and bigger erythrocytes in the blood of females. The calculations of hematological indices for tenches show that other parameters such as mean volume of erythrocytes, mean concentration and hemoglobin content in one erythrocyte are greater in females.

It should be also assumed that in the successive seasons of the year the degree of hemoglobin condensation changes (SCHLICHER 1927, ANTIPOVA 1954, MOLNAR et al. 1960, ZAJCEVA 1967, KORZUEV and GLAZOVA 1967) as well as the oxygen bond ability. These problems are not sufficiently studied yet. The majority of estimations of hemoglobin content with the help of the method of Sahli cannot be used in interpretations. Therefore the literature available is insufficient to determine the differences in the hemoglobin level as related to seasonal variations. It seems that mainly the temperature regulates the hemoglobin condensation in erythrocytes, and this has been confirmed by the investigations of MOLNAR et al. (1960) and ZAJCEVA (1967). But the annual estimations of hemoglobin content in tenches are not univocal as the spawning of these fish takes place in the warmest season of the year. There are two parallel processes: spawning changes due to which the number of erythrocytes and hemoglobin decrease (Fig. 2, curves A, C), and the action of temperature in changed oxygen system. Therefore it is difficult to conclude about the extent to which the thermic conditions affect the quantitative relations during summer, but undoubtedly the changes of temperature cause the hemoglobin condensation in erythrocytes in autumn and winter.

A similar process of increasing hemoglobin level in that period was observed by SCHLICHER (1927), MOLNAR et al. (1960) and others. At the same time KIRSIPUU (1963) and ZAJCEVA (1967) consider the autumn-winter hemoglobin increase as an ostensible phenomenon as the total blood volume in the organism of fish decreases. The analyses described here do not allow to state this for tench. And contrariwise the levelled level of erythrocytes in the blood of males, system of hematocrit values and the character of changes of total protein (EINSZPORN-ORECKA 1970) exclude the possibility of greater differences in the blood volume in the organism of tench during winter preparations.

Quantitative variations of leucocytes in a volume unit as distinguished from the previous ones determine univocally the changes resulting out of seasonal differences of metabolism of tenches. The hematopoietic processes are the quickest in spring and during spawning of these fish (EINSZPORN 1964). IVLEV (1965) even thinks that the spring regeneration of hematopoietic processes does not require the resumption of active feeding. Therefore the



maximum annual number of leucocytes in  $\text{mm}^3$  of the circulating blood takes place during the spawning of tenches. These phenomena, in opposition to the interpretation of SCHLICHER may be called spawning leucocytosis. But the minimal numbers of leucocytes are the result of lower activity of fish in autumn and winter.

Numerical data of SCHLICHER (1927) are considerably different from those in this paper. SCHLICHER calculated much greater numbers of leucocytes in  $\text{mm}^3$  of the circulating blood (average level during 10 months of the year was 40,000–68,000/ $\text{mm}^3$ , maximum one 174,750/ $\text{mm}^3$ ). This difference is partly explained by the selection of research material, while in the investigations here the fish were selected from the parasitological point of view. This undoubtedly affected the estimation of the level of erythrocytes for tenches during the year. The range of the number of erythrocytes according to SCHLICHER is from 0.85 to 1.8 mill./ $\text{mm}^3$ , while in the investigations presented here from 1.5 to 2.16 mill./ $\text{mm}^3$  for males, and from 1.35 to 2.0 mill./ $\text{mm}^3$  for females.

## 5. SUMMARY

The quantitative estimations of blood constituents carried out on 162 males and 158 females of tench *Tinca tinca* (L.) showed that the reproduction cycle affects directly the number of erythrocytes and leucocytes. The preparation period and spawning-process cause a considerable decrease of the number of erythrocytes and hemoglobin in the circulating blood. Apart from this that period especially in the instance of females is characterized by intensive leucocytosis. The level of leucocytes is relatively even in the remaining part of the year and is the lowest in autumn and winter.

Therefore visible differences can be observed in the annual cycle in the number of erythrocytes in the circulating blood between males and females, and the number of these erythrocytes with the exception of winter is higher in males. Whereas other parameters such as mean volume of erythrocytes, mean concentration and hemoglobin content in one erythrocyte are greater in females.

## 6. STRESZCZENIE

Na podstawie oznaczeń ilościowych elementów krwi, wykonanych na 162 samicach i 158 samicach lina *Tinca tinca* (L.), ustalono, że cykl rozrodczy rzutuje bezpośrednio na kształtowanie liczby czerwonych i białych ciałek krwi. Przygotowanie i proces tarła powodują znaczne obniżenie ilości erytrocytów i hemoglobiny we krwi obwodowej, szczególnie u samic. Ponadto okres ten cechuje wzmożona leukocytoza. Poziom białych krwinek w pozostałej części roku jest stosunkowo wyrównany, najniższy w jesieni i zimie.

W cyklu rocznym zaznaczają się zatem wyraźne różnice między samcami i samicami w liczbie erytrocytów we krwi obwodowej, przy czym ilość tych krwinek, z wyjątkiem okresu zimowego, jest wyższa u samców. Natomiast inne parametry, jak średnia objętość czerwonych krwinek, średnie stężenie i zawartość hemoglobiny w jednym erytrocycie są większe u samic.

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T. EINSZPORN-ORECKA

SEASONAL VARIATIONS IN THE PROTEIN COMPOSITION  
OF BLOOD SERUM OF TENCHES (*TINCA TINCA* (L.))

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ABSTRACT

Analysis of protein composition was made on 238 tenches (106 males and 132 females). The differences in protein depending on basic life activities of fish: spawning cycle (connected with the spring-summer period) and autumn and winter, were determined. The level of total protein in the serum of tenches ranges during the year from 4.5 to 9.0 g%. Essential differences in the amount of total protein take place especially in spring (spawning period) and in winter. Similarly the greatest rearrangements within protein fractions take place during the spawning of tenches. In the instance of males this is mainly connected with the synthesis of globulin fractions, and in females — with the accumulation of considerable amounts of albumin in the serum.

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1. INTRODUCTION

Papers dealing with the distribution of serum proteins include various systematic groups such as Cyclostomata, Selachii or Teleostei (marine and freshwater). The protein composition was studied with regard to the biology of these fish, ecological factors, physiological condition and their species. The obtained results allow to determine many common features of separation of protein of fishes, but there are still difficulties in determining the functions of respective fractions, and especially of those from the globulin group.

The albumin level in *Salmo* (OSTROUMOVA 1967) and in *Acipenser sturio* L. (MAGNIN 1958, 1960) decreases together with the age of these fish. And also similarly as in other animals the number of albumin fractions depends on the nourishment of fish and usually decreases during winter because of endogenic nourishment (SORVACEV 1957a, b, KUZMINA 1966). RIEDMÜLLER'S (1965) opinion is that the albumins of carp regulate the oncotic pressure and are responsible for water fixation in blood. Whereas according to the results of KULIKOVA (1967) they are carriers and transporters of lipides in the serum of fish from the Gobiidae family.

KIRSIPUU (1964b) considers the  $\alpha$ -globulin fraction as very important during spawning. It takes part in the synthesis of proteins necessary for spawn production. KIRSIPUU suggests that  $\alpha_2$ -globulins may play the part of catalyst of egg maturation processes or of a transporter of lipides in blood. The latter is defined better by KULIKOVA (1967), who determined about 60%  $\alpha$ -lipoproteins in the serum of fish from the Gobiidae family during the maturation of gonads.

The following  $\beta$  fraction according to OFFHAUS et al. (1955) is characterized by great stability. The  $\beta$ -globulin content decreases together with the albumins in the blood of starved carps, *Carasius carasius* (L.) and *Abramis brama* (L.) (LIEBMANN et al. 1960, KUZMINA 1966, SORVACEV 1957b). Apart from that during spawning a new fraction appears in the  $\beta_2$ -globulin zone, in the blood of salmons, which as OSTROUMOVA (1967) assumes, is the ovovitine used for spawn production.

The occurrence of the last fraction from the globulin group usually denoted as  $\gamma$  and with which the transfer of antibodies is connected is still a matter for discussion. DEUTSCH and McSHAN (1949), ENGLE et al. (1958), DRILHON (1959) and DRILHON et al. (1961) say that the serum of Teleostei fishes unlike the Selachians does not contain  $\gamma$ -globulins as none of the fractions pointed out in the gel medium does not migrate towards the cathode zone. ENGLE connects the absence of  $\gamma$ -globulins of Teleostei fishes with the lack of plasmatic cells in the spleen, which similarly as in the instance of mammals are responsible for the synthesis of proteins from the  $\gamma$  fraction. Simultaneously OFFHAUS et al. (1955) and RIEDMÜLLER (1965) obtained two various types of electrophorograms of normal serums of carp with a low participation of  $\gamma$ -globulins and abundance of these proteins attaining even 97%.

The paper presented here is a successive attempt to show the differences in serum proteins of tench males and females within the year.

## 2. MATERIAL AND METHODS

Similarly as in the previous paper (EINSZPORN-ORECKA 1970) investigations were carried out on 4-6 year old tenches (25-38 cm long, 250-670 g of weight). The quantitative determinations of blood constituents, level of total protein and fractionizing of serum proteins were made on the same fish, which were investigated from the point of their state of health. The value of hematological indices was determined in the first blood sample, whereas the serum was obtained from the following ones from the vena caudalis. On the average about 3 cm<sup>3</sup> of serum was centrifuged out of 8-10 cm<sup>3</sup> of blood.

The level of total protein in serum was estimated by colorimetric biuret method. The separation of serum proteins was made with the help of paper electrophoresis. The electrophoresis was made in buffer barbital of a pH 8.6, ionic strength 0.12, in room temperature, on stripes of filter-paper Whatmann 1 (3.5×36 cm), at a constant voltage 150 V. The time of total separation was 19.5hr. The stripes were dried in room temperature, and then they were coloured with bromophenol blue for 17hr. When the colouring was finished the stripes were washed three times for 2 min in 2% aqueous solution of acetic acid. After drying, the particular parts of stripes with colour lines of successive fractions were dissected and eluted in 6 ml of methanol with 2% aqueous solution of sodium carbonate. The elution lasted 2hr. The estimations were made in the photoelectric colorimeter using a filter of a wave length 595 m $\mu$ . The sum of extinctions was assumed as 100%, and the particular extinctions of sections were expressed in per cents of this value. The standard deviation from the arithmetical mean and the nominal statistical degree of observed changes were calculated.

## 3. RESULTS

The mean, annual value of total protein in serum, calculated on the basis of estimations for 162 tench males and 158 females, is about 6 g% (separation of serum proteins was made for 106 males and 132 females). Characteristic deviations are noticed in the spring and winter periods (Fig. 1). In May the level of protein in the serum of females is hardly 4.61 g% ( $t = 11.17$ ). Similarly in the serum of males it decreases to 4.81 g% ( $t = 4.64$ ). A following decrease, preceded by a short-lasting equalization of level, takes place during spawning. Also then the protein amount is 4.56 g% ( $t = 3.39$ ).

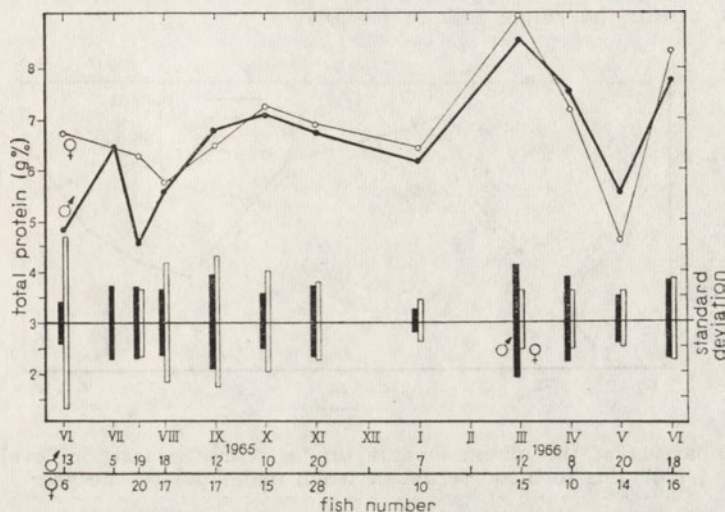


Fig. 1. Seasonal variations in the level of total protein in blood serum of tench males and females

In the second part of the year (from August to January) the amount of connected with the production of albumins during the maturation of spawn. The protein level increases then in the optimal spawning conditions up to about 8.35 g%.

In the second part of the year (from August to January) the amount of protein in the serum of tenches is relatively even. At the end of the wintering period of fishes the protein level rapidly increases to 8.57 and even 9.05 g% ( $t = 5.52$ ;  $t = 13.37$ ). The rapid increase of protein in March is most certainly not the only one, which is proved by similar or even higher protein level in the blood serum during the wintering period than in autumn in November.

Under the influence of paper electrophoresis the proteins of blood serum of tench are on the average separated into 5 fractions, which according to the accepted nomenclature (but without identifying their function) were: albumins,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\gamma$ -globulins (Fig. 9). Some of these fractions from group  $\alpha_1$  or  $\gamma$ -globulins, depending on the investigated period may separate into two, and in the instance of  $\alpha_1$  even into three subfractions. At a similar separation of fraction proteins the arrangement of stripes in the group of

globulins is more marked and more in the direction of albumins (Fig. 9B, C, E). But smaller amounts of  $\gamma$ -globulins do not separate well enough from fraction (Fig. 9D).

#### A. ANALYSIS OF ALBUMIN VALUE

The fraction value of albumin in fish, as opposed to mammals, is considerably lower. The percentage of albumin in the serum of tench males ranges within the annual cycle from 18.5 to 27.7‰ (in a single instance to 30‰), while the absolute value calculated in proportion to the variable level of total protein is within the range 1.99 to 1.0 g‰.

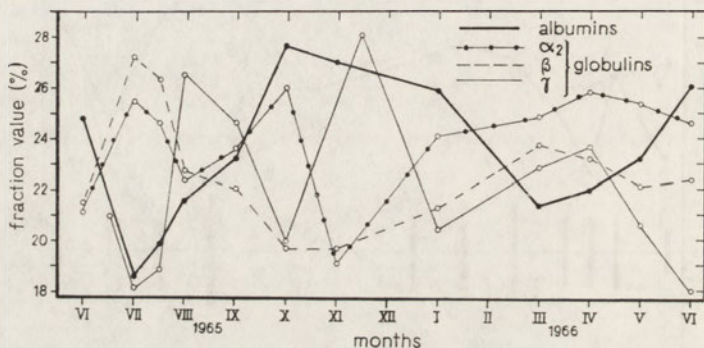


Fig. 2. Comparison of the albumin value (in ‰) with the fraction level of  $\alpha_2$ ,  $\beta$ ,  $\gamma$ -globulins in blood serum of tench males during the year

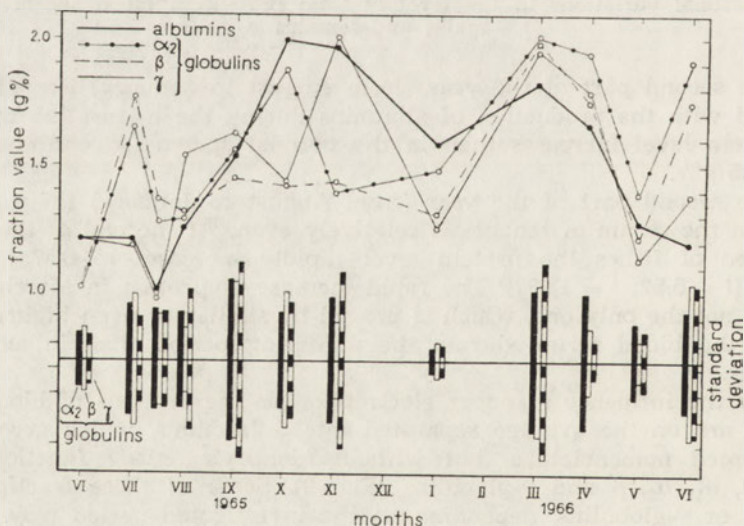


Fig. 3. Comparison of the value (g‰) of albumin fraction and  $\alpha_2$ ,  $\beta$ ,  $\gamma$ -globulins in blood serum of tench males

The course of curves of annual percentage values and absolute albumins (Fig. 2, 3) is the same as that of the cycle of seasonal changes of the energy of tench males. Apart from that these two systems compared with the curve of the total protein value slightly differ. A visible convergence of the level of total protein and albumins (in g%) can be observed in successive stages of the annual cycle (Fig. 4). This regularity is not observed only in the pre-spawning period, because the increase of protein level depends then chiefly on the amount of globulins.

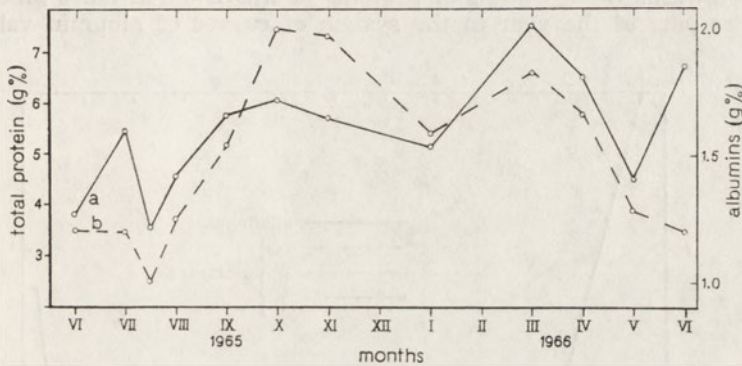


Fig. 4. Dependence between the amount of total protein (a) and the albumin value (b) in blood serum of tench males

The system of curves of annual values of albumin fractions of blood serum of males consists of several stages strongly connected with the character and seasonality of these processes. (Fig. 2, 3).

The first of these stages is in spring and is characterized by changes in the composition of serum proteins and mainly albumins are formed. The amount of this fraction increases to 24.8% ( $t = 3.68$ ) and even 26.2%. But simultaneously with the decrease of the level of total protein after the winter period the participation of albumins is relatively low in the total protein balance, and is about 1.2 g%.

The next period includes the spawning of males. The amount of albumin fractions decreases in the blood serum (Fig. 2), and therefore the absolute level of these proteins decreases to 1.0 g% ( $t = 3.53$ ) (Fig. 3). The limitation of albumin transport takes place even during the spawning preparations. The increase of the level of total protein (Fig. 4) then is caused by transmission of proteins from the globulin group to blood. Since August to November the reproduction processes become stable and the period of intensive feeding follows. Intensive synthesis of albumins is expressed by greater participation of these proteins in blood serum. At the end of autumn the albumin level in proportion to the remaining globulin fractions is 27.7% ( $t = 4.58$ ). This increase is directly connected with the increased level of total protein in the circulating blood (Fig. 4). The annual maximal amount of albumins is about 2.0 g% ( $t = 4.80$ ) (Fig. 3).

The tench disposes in a specific way of the organism reserves during the wintering period. In relation to the maximum values of the previous period the amount of albumins decreases slightly during winter, i.e. from 1.97 to

1.69 g<sup>0</sup>/<sub>0</sub> (the lowest value in January is 1.58 g<sup>0</sup>/<sub>0</sub> ( $t = 2.81$ ). This level is maintained till May, despite the percentage decrease of albumins in proportion to other fractions (27-21%<sub>0</sub>;  $t = 3.45$ ).

The variations of albumins in blood serum of females are considerably greater than in males. Within the year the percentage value of albumins is from 46 to 8.58%<sub>0</sub>, and calculated in g<sup>0</sup>/<sub>0</sub>—3.7 to 0.49 g<sup>0</sup>/<sub>0</sub> (Fig. 5, 6). In the separation of proteins of blood serum the processes connected with the gonad development are more visible than the effect of environmental factors. The tendency towards the synthesis of proteins of this fraction takes place during the greater part of the year in the system of curves of albumin values. The

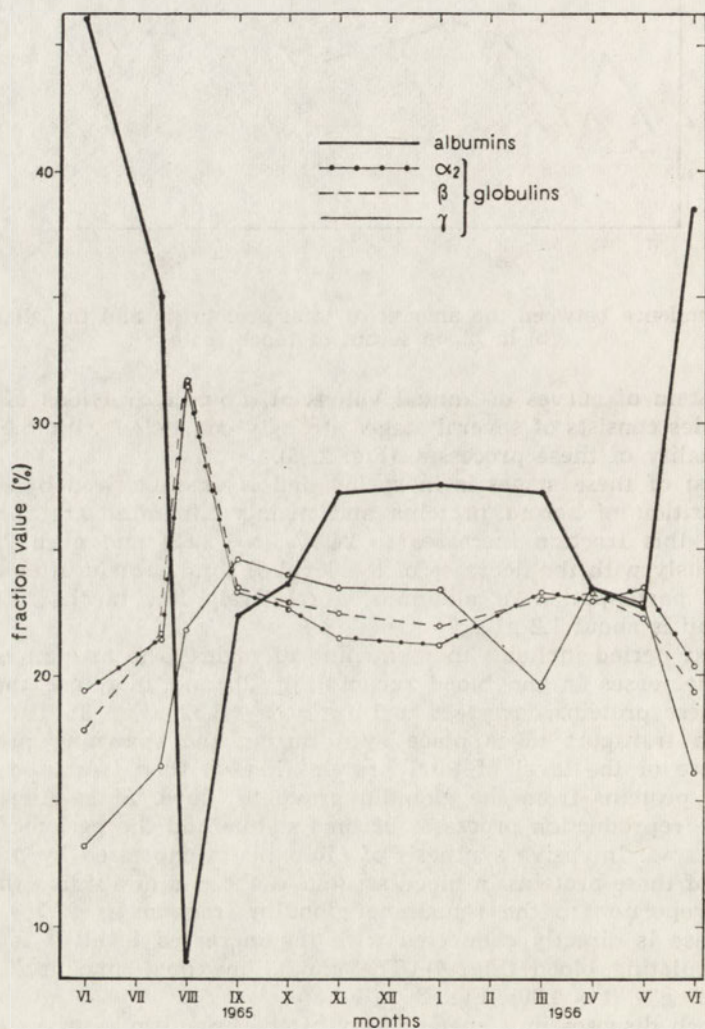


Fig. 5. Comparison of the albumin value (in %<sub>0</sub>) with the fraction level of  $\alpha_2$ ,  $\beta$ ,  $\gamma$ -globulins in blood serum of tench females during the year



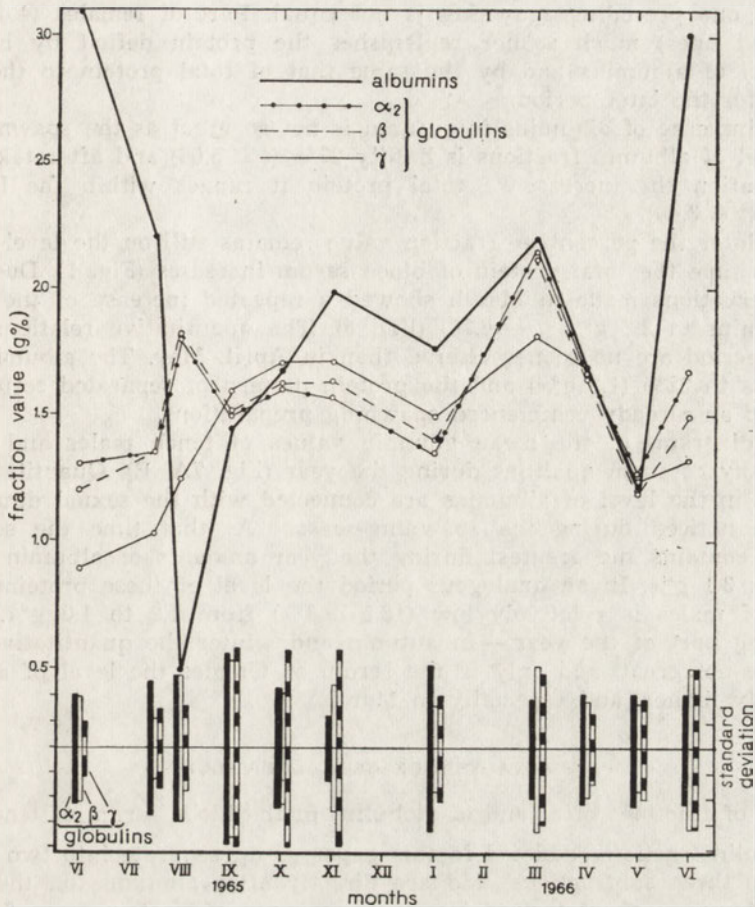


Fig. 6. Comparison of absolute values (g%) of albumin fraction and  $\alpha_2$ ,  $\beta$ ,  $\gamma$ -globulins in blood serum of tench females

intensity of this process is marked by the increase of the absolute value of albumins during spawning, autumn and wintering period (Fig. 6).

An increase of the amount of albumins in blood serum due to spawning takes place in early spring, and in unfavourable temperature conditions at the beginning of May. Additional observations showed that the albumins for spawn production are supplied without the renewal of active spring feeding. The accumulation of these proteins in blood serum is usually very rapid. The level of albumins as compared with April and May increases at least twice from 23 to 46.1% ( $t = 9.23$ ), and in absolute values increases from 1.2 to 3.0 g% ( $t = 5.47$ ). The standard deviation amounting to  $\pm 7\%$  in the early period of spawning preparations proves about the uneven course of the process or even probably about spawning in portions.

The maximum level of albumins is maintained in the blood serum during June and July, and then rapidly decreases to 8.58%, i.e. 491 mg% ( $t = 7.56$ )

(Fig. 5, 6). However, the separation of examined serums both in that period and the one preceding spawning is not equal. Part of females (4 out of 9 examined ones) much sooner replenishes the protein deficit by balancing the level of albumins and by the same that of total protein to the values typical for the later period.

The increase of albumins in autumn is not so great as the spawning one. The level of albumin fractions is hardly 27% ( $t = 5.06$ ) and after taking into consideration the increase of total protein it ranges within the limits of 2.0 g% ( $t = 3.46$ ).

In winter the percentage fraction value remains still on the level of 27%. But this time the total protein of blood serum increases (Fig. 1). Due to this the observations made in March showed a repeated increase of the amount of albumins to 2.2 g% ( $t = 6.76$ ) (Fig. 6). The quantitative relations of the winter period are no sooner altered than in April, May. The albumin value decreases to 22% (1.2 g%) and the protein pattern of separated serum parts points to an already commenced spawning preparations.

The diagrams of the mean albumin values of tench males and females have many common qualities during the year (Fig. 7A, B). Quantitative differences in the level of albumins are connected with the sexual dimorphism and are noticed during the spawning-season. At that time the serum of females contains the greatest during the year amounts of albumin — about 46%, i.e. 3.1 g%. In an analogous period the level of these proteins in the serum of males is relatively low (18.5–19.8%) from 1.2 to 1.0 g%. In the remaining part of the year — in autumn and winter the quantitative variations are not great, and only in the serum of females the level of albumins is slightly higher, and especially in March.

#### B. ANALYSIS OF GLOBULIN VALUE

##### a. Level of fractions of $\alpha_1$ and $\alpha_2$ -globulins in the blood serum of tenches

Globulin fractions, denoted in this paper as  $\alpha_1$ , separate into two and rarely into three subfractions, and are directly after albumins on the phorogram. They are characterized by small amounts of protein from 2 to 5%, which do not display any determined seasonal differences.

$\alpha_2$ -globulins, the third fraction of this group as distinguished from the two previous ones is intensively dyed on the phorogram (Fig. 9). During the year the range of percentage variations of  $\alpha_2$ -globulins differs (Fig. 8A). It is 19 to 26% in the serum of males. Whereas in the serum of females the basic scatter is slightly smaller — from 18 to 23%. The maximum value 31.5% ( $t = 8.88$ ), which is considerably greater than the average annual level of  $\alpha_2$ -globulins is exclusively connected with the spawning season.

The spawning process is one of the three periods, during which there are the greatest rearrangements of globulin fractions. The spawning variations, especially visible in males, are preceded by a considerable protein consumption in June (or in May of the following year), which amounts to about 500 mg%. Then the lowest level of  $\alpha_2$ -globulins in the year (1965) takes place and is about 1.0 g% ( $t = 5.89$ ) (Fig. 3). These changes indirectly point towards an increased demand for proteins, and among others of those from the  $\alpha_2$  group, and are closely connected with spawning increase, which is for the males 1.64 g% ( $t = 6.65$ ).

The successive increase of the number of  $\alpha_2$ -fraction (Fig. 10) in autumn is more visible in the serum of males (26.0%, 1.88 g<sup>0</sup>/<sub>0</sub>;  $t = 4.00$ ,  $t = 2.74$ ) than of females (22.6%; 1.61 g<sup>0</sup>/<sub>0</sub>), but in both instances it takes place in October (Fig. 3, 6).

The changes in spring are more visible than those in autumn and during spawning. In March  $\alpha_2$ -globulins in the serum of females attain the maximum annual value 2.15 g<sup>0</sup>/<sub>0</sub> ( $t = 5.80$ ). Their level is the same in the males, but with the difference that the relative content of this fraction in serum proteins increases simultaneously to 24.98%. Proportionally to this increase the value of  $\alpha_2$ -globulin fraction remains on the level 1.95% till April (Fig. 3).

#### b. Level of $\beta$ -globulin fraction in blood serum of tenches

The proteins of the fourth fraction of globulin series — from  $\beta$ -group display a marked stability as compared with other fractions. Therefore the level of  $\beta$ -fraction is more even, and the basic variations remain in the limit of 5%, and during the year from 19 to 23.8%. Greater values are exclusively the result of spawning changes. In July the increase of  $\beta$ -globulins in the serum of males amounts to 26–27% ( $t = 8.25$ ), whereas in the serum of females after spawning it amounts to 31.6% ( $t = 9.72$ ) (Fig. 8B).

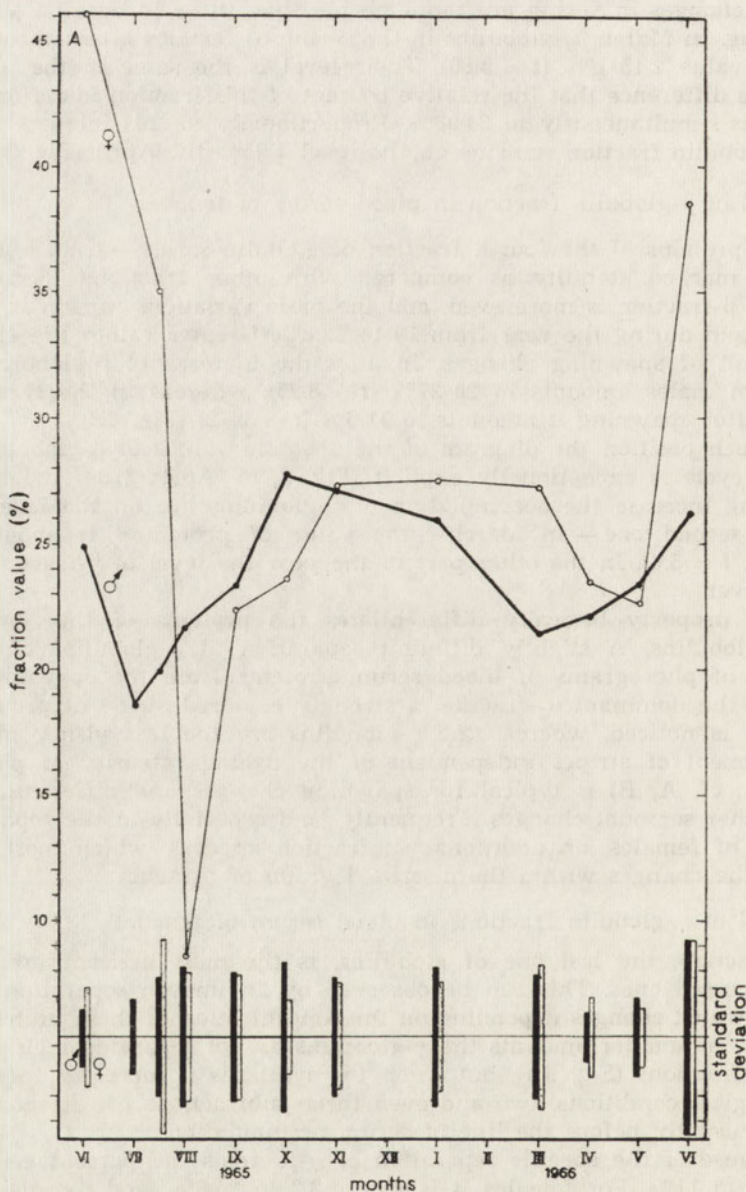
In such position the diagram of the absolute values of  $\beta$ -globulins in the annual cycle is exceptionally explicit (Fig. 3, 6). Apart from the mentioned spawning increase the accumulation of  $\beta$ -globulins are on the level 1.8 g<sup>0</sup>/<sub>0</sub>, in the second one — in March — the value of protein  $\beta$  is about 2.0 g<sup>0</sup>/<sub>0</sub> ( $t = 5.8$ ,  $t = 5.6$ ). In the other part of the year the level of  $\beta$ -fraction is relatively even.

This property basically differentiates the proteins of this group from other globulins. A slightly different separation of  $\beta$ -globulins changes the pattern of phorograms of blood serum proteins from the spawning period. Besides the dominant  $\alpha_2$ -fraction a strongly coloured stripe of proteins from  $\beta$ -group is noticed, whereas the  $\gamma$ -globulins are the less visible ones. Such arrangement of stripes independent of the dyeing intensity of phorograms (Fig. 11, cf. A, B) is typical for spawning changes and differentiates them from other seasonal changes. Frequently, and especially in the separations of serums of females, an additional subfraction appears, which most probably shows the changes within the discussed group of proteins.

#### c. Level of $\gamma$ -globulin fractions in blood serum of tenches

$\gamma$ -fraction, the last one of globulins, is the most hesitant group among the discussed ones. This can be observed by an uneven separation of  $\gamma$ -globulins, which changes depending on the concentration of these proteins. When they are in smaller amounts the  $\gamma$ -globulins do not separate precisely enough from  $\beta$ -fractions (Fig. 9A), but when the synthesis is increased, especially in pathological conditions, two and even three subfractions are formed (Fig. 9B, C) and usually before the line of serum accumulation.

Because of the specific separation of  $\gamma$ -proteins the percentage range increases to 11%. For females it is from 13.2 to 24.6%, and for males — from 17.9 to 28.1%, but the percentage variations of the latter have a continuous character and apparently undetermined (Fig. 8C). However, two periods of the value of  $\gamma$ -globulins may be assumed in general in the annual cycle: autumn-winter one with an increased number of  $\gamma$ -fractions, and the spring-



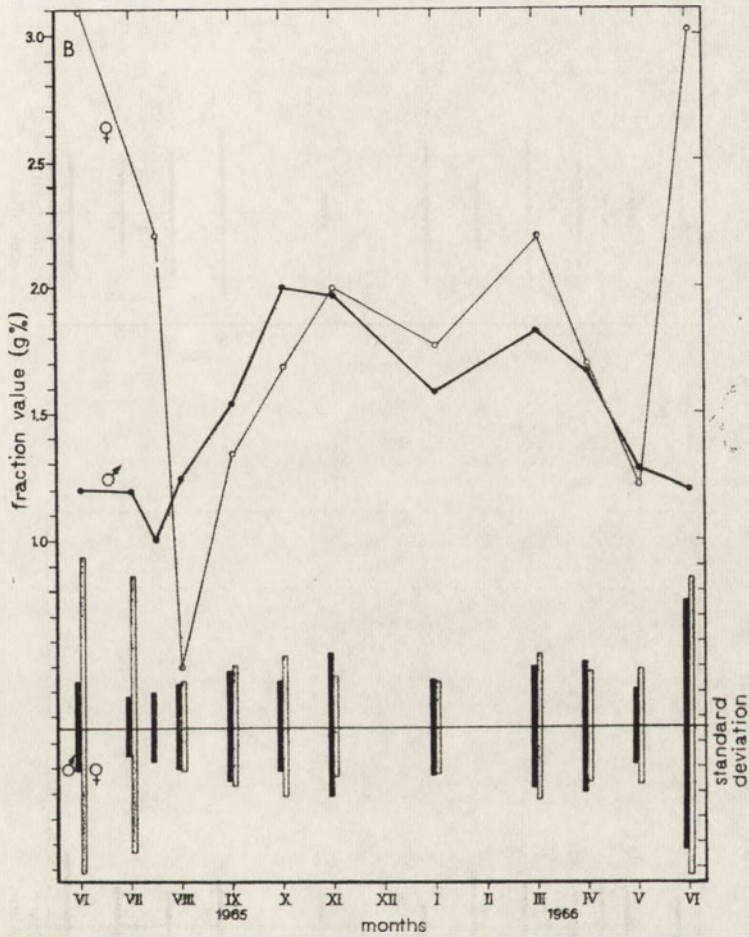


Fig. 7. Changes in the albumin level of tench males and females during the annual cycle. A — albumin value in ‰; B — albumin value in g‰

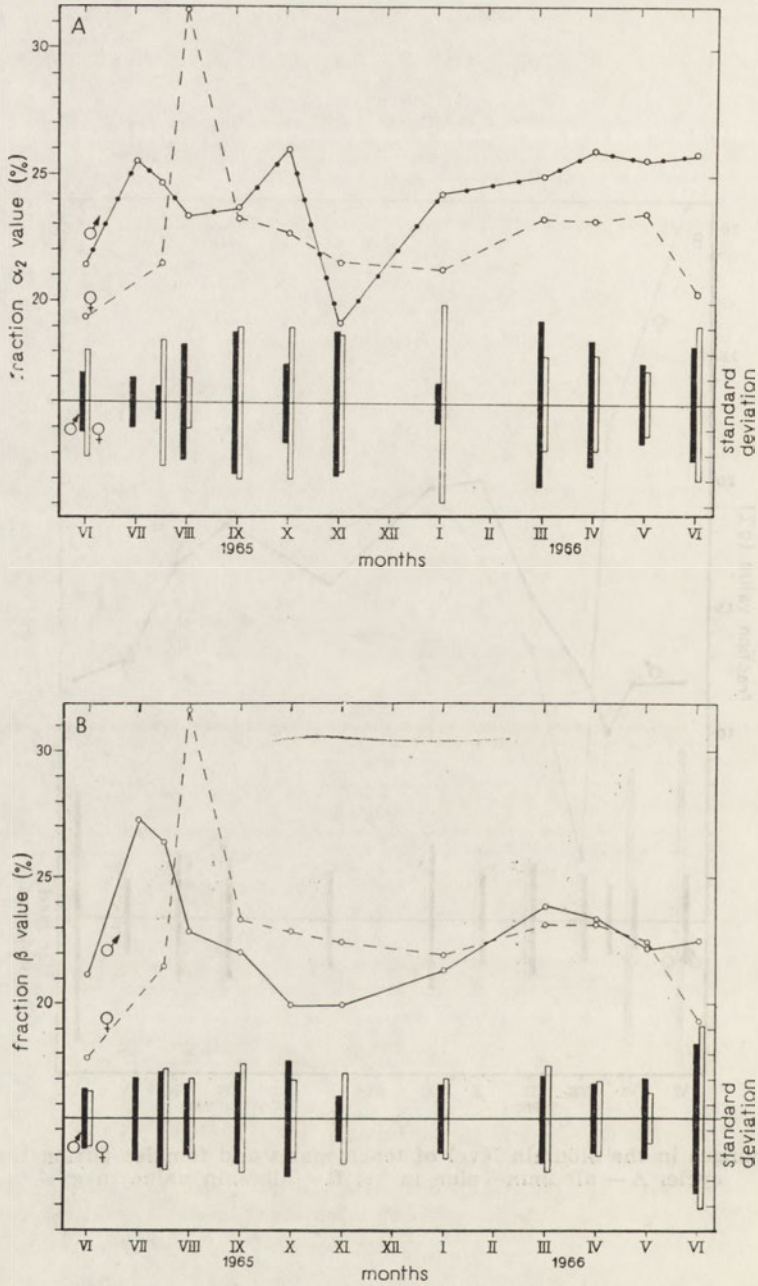
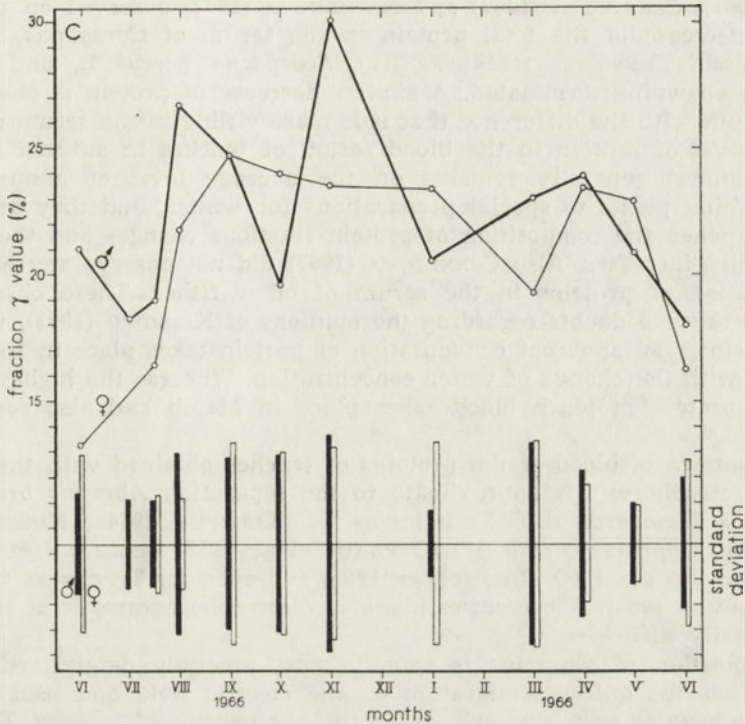


Fig. 8. Comparison of the fraction value (in %) of tench males and females during the year. A —  $\alpha_2$ -globulins; B —  $\beta$ -globulins; C —  $\gamma$ -globulins



-spawning one with a much lower level of these proteins. Lowering of the level of  $\gamma$ -globulins connected with spawning is noticed since May; the smallest amounts take place successively in the serum of females in the preparation period in June, while in males—in July. The decrease of the value of  $\gamma$ -fraction is in that period below 1.0 g% (Fig. 3, 6). After spawning, according to the general trend of changes the level of  $\gamma$ -globulins is balanced and the greatest amounts are observed in autumn (Fig. 8C). This increase thus remains almost during the entire wintering period of tenches.

#### 4. DISCUSSION

The fluctuations of the level of total protein in the serum of fishes is the relatively precise indicator of changes taking place in the organism. The greatest rearrangements within the globulin fractions, and by the same in the values of total protein take place during spawning. A quick increase of proteins in the serum of tench females connected with the maturation of spawn indicates that these proteins may be produced to a great extent by the reserves of the organism. Similar accumulation of proteins during spawning also takes place in the serum of males, but lasts shortly. However, it is difficult to explain these phenomena on the basis of these observations, and it is more difficult as during that period an increased protein level was not observed up to the present in other fishes. GOLOVKO (1964), ROBERTSON

et al. (1961), ČERNIKOVA (1967) and NAJDENOVA (1967) observed on the other hand a decrease of the total protein in the serum of *Coregonus lavaratus baeri* Kessl., *Trachurus trachurus* (L.), *Scorpaena porcus* L, and salmones after the spawning terminated. A similar decrease of protein is observed in tenches, but with the difference that it is more visible in the serum of males.

The level of protein in the blood serum of tenches in autumn (September–November) generally remains on the average level of annual value. Thus nothing points to special preparations for winter, and they are observed only when the composition of protein fractions changes and the amount of albumins increases. Also ČERNIKOVA (1967) did not observe any additional accumulation of proteins in the serum of other fishes. These observations give rise to some doubts regarding the opinions of KIRSIPUU (1963), who says that in winter an apparent condensation of protein takes place in fish serum together with the change of water concentration. Whereas the highest annual level of protein in tench blood takes place in March and also remains in April.

The pattern of blood serum proteins of tenches obtained with the help of paper electrophoresis is approximate to the separation *Abramis brama* (L.), *Lucioperca lucioperca* (L.), *Esox lucius* L. (KIRSIPUU 1964a, KIRSIPUU and PIHU 1965), *Cyprinus carpio* (L.) (OFFHAUS et al. 1955, LIEBMANN et al. 1960, ŁYSAK and WOJCIK 1960, RIEDMÜLLER 1965) and even for *Trachurus trachurus* (L.) (GOLOVKO 1964). A correct estimate of electrophorograms is at this stage exceptionally difficult.

The position of albumins is usually most precisely determined as this fraction has the quickest migration on the electric field and usually takes form of a regular spot, similarly as on the phorograms of tenches. The albumin level in the serum of tenches depends on the feeding of those fish and increases at the end of autumn. The albumin concentration in winter is similar to that in autumn. During that period the amount of albumins in the serum of females decreases hardly by 5% (300 mg%). A more marked decrease (about 400 mg%) takes place in early spring at the beginning of May (differences in dyeing of phorograms are showed on Fig. 12). The demand of females for albumins in winter is much greater than that of males and this is expressed by a higher level of proteins in the serum of females. Also in spring the level of this fraction decreases rapidly in April and May (decrease about 835 mg%).

Not much is known about the significance of albumins in the spawning process. In other animals this protein apart from its transport properties is also used for the development of organism. Analysis of blood serum of tench females proves the participation of these proteins in spawn-forming process. During the gonad development these albumins increase almost twice thus attaining a very high concentration 46%. But this is at the moment an isolated opinion. Other authors (KIRSIPUU and PIHU 1965) say the same about globulins ( $\alpha_2$ -fraction).

Shortly before spawning an opposite phenomena of accumulation of fraction proteins  $\alpha_1$  and  $\alpha_2$  takes place in the serum of males. Similar periods of more intensive transport of proteins of  $\alpha_2$  fraction take place in the remaining part of the year, i.e. in autumn and at the end of winter. Accumulation of greater amounts of  $\alpha_2$ -globulins is almost parallel in the serums of tench males and females. These processes are partly explained by LIEBMANN (1960),



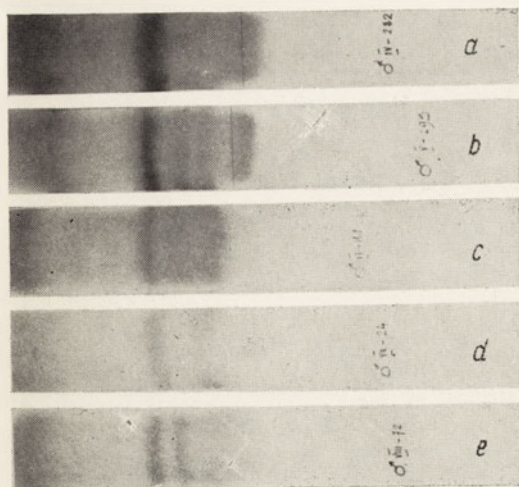


Fig. 9. Comparison of the separation of blood serum of tench males from April till August

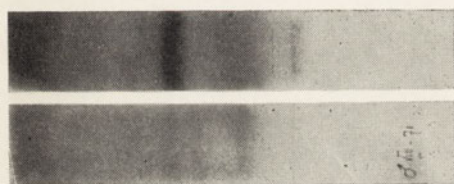


Fig. 10. Differences in the separation of normal blood serum of tench after spawning

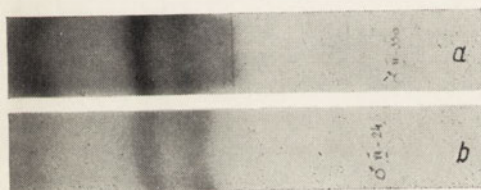


Fig. 11. Typical separation of globulins of males during the spawning in two successive years. a — spawning in June; b — delayed spawning in July

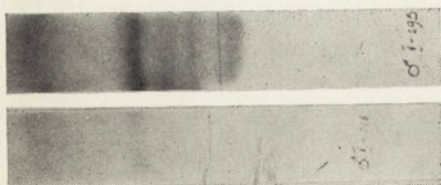


Fig. 12. Differences in the dyeing intensity of protein fractions in blood serum of tenches in spring



who says that  $\alpha$  and  $\beta$ -globulins are responsible for the transport of hormones, phospholipids, bile pigments, cholesterol and some vitamins.

$\beta$ -globulins of freshwater fish are not a subject to seasonal variations according to KIRSIPUU (1964). Also the properties of  $\beta$ -fraction of tench serum are similar, but these proteins increase during the spawning of males and females.

The last fraction from the group of globulins denoted as  $\gamma$ , is distinguished by a considerably lower level in blood serum and especially in early spring and during spawning. At high concentrations 2 subfractions differentiated and smaller amounts did not separate well from  $\beta$ -fraction.

KIRSIPUU (1964a) observed the same among *Abramis brama* (L.) and *Perca fluviatilis* L. Whereas RIEDMÜLLER (1965) observed at least three groups of carp having a different amount of  $\gamma$ -globulins. Fish with a maximum amount of these proteins visibly separated into two  $\gamma$ -fractions.  $\gamma$ -globulins were also estimated in the blood of perch-pike, bream, roach, rudd, perch, white bream (KIRSIPUU 1964a, b, KIRSIPUU and PIHU 1965), horse mackerel (GOLOVKO 1964) and other.

All these data justify the presence of  $\gamma$ -globulins in Teleostei fishes. But the opinions on the subject are not sufficiently proved. Other scientific workers such as DEUTSCH and MCSHAN (1949), ENGLE et al. (1958) and DRILHON (1959) suppose that Teleostei fishes do not contain  $\gamma$ -globulins. ENGLE et al. (1958) suggest that the proteins of fish containing antibodies may have a different mobility than human  $\gamma$ -globulins. Explanation of this and several other problems requires further investigations.

## 5. SUMMARY

The level of total protein in the blood serum of tench changes during the annual cycle. 320 fish were examined, and these variations were from 4.5 to 9.0 g<sup>o</sup>/. Essential changes in the amount of total protein are especially visible in spring (spawning-season) and in winter. The character of protein changes is different in tench males and females. During that period the increase of total protein in the serum of tench males is short lasting and mainly depends on the synthesis of  $\alpha$ -fraction and  $\gamma$ -globulins. The transport of albumins during spawning is limited; the lowest annual value of these proteins is about 1.0 g<sup>o</sup>/. Opposite than in tench females the considerable accumulation of protein in serum in the pre-spawning period remains as such more than two months till the spawning moment. In that time an increased synthesis of albumins takes place. The level of these proteins as compared with that in April or May increases almost twice to 46<sup>o</sup>/o i.e. 3.1 g<sup>o</sup>/. This shows the specific significance of albumins in the process of spawning preparations.

From August till October, at a relative stability of reproduction processes, there is the period of intensive feeding. Increased albumin synthesis is expressed by an increased participation of these proteins in blood serum at a simultaneous equalizing of the level of total protein. In autumn, the level of total protein generally remains within the average annual value, which is about 6 g<sup>o</sup>/, and increases again in March and then decreases in early spring.

## 6. STRESZCZENIE

Poziom białka całkowitego w surowicy krwi linów zmienia się w cyklu rocznym. Wahania te, wyznaczone na podstawie oznaczeń dla 320 ryb, wynoszą od 4,5 do 9,0 g<sup>o</sup>/. Istotne zmiany ilości białka całkowitego występują szczególnie w okre-

sie wiosennym (tarłowym) i zimowym. W czasie tarła charakter zmian białkowych różni się u samców i samic lina. Wzrost ilości białka całkowitego w surowicy samców lina jest w tym okresie krótkotrwały i uzależniony głównie od syntezy frakcji  $\alpha$  i  $\beta$ -globulin. Transport albumin w czasie tarła jest natomiast ograniczony, wyznacza to najniższą wartość roczną tych białek około 1.0 g<sup>0</sup>/. Odwrotnie u samic lina, znaczne nagromadzenie białka w surowicy w okresie przedtarłowym utrzymuje się ponad dwa miesiące, aż do momentu złożenia ikry. Występuje w tym czasie wzmoczona synteza albumin; poziom tych białek w stosunku do kwietnia lub maja wzrasta prawie dwukrotnie (do 46<sup>0</sup>/), tzn. 3.1 g<sup>0</sup>/. Sugeruje to, że albuminy odgrywają szczególną rolę w czasie przygotowań tarłowych.

Od sierpnia do listopada przy względnej stabilizacji procesów rozrodczych, następuje okres intensywnego żerowania. Wzmoczona synteza albumin wyraża się zwiększeniem udziału tych białek w surowicy krwi, przy równoczesnym wyrównaniu poziomu białka całkowitego. W okresie jesiennym poziom białka całkowitego utrzymuje się na ogół w granicach przeciętnej rocznej wartości, tj. około 6 g<sup>0</sup>/. Zwiększa się on ponownie w marcu, a następnie obniża wczesną wiosną.

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QUANTITATIVE CHANGES IN THE CIRCULATING  
BLOOD OF TENCHES (*TINCA TINCA* (L.)) INFESTED  
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ABSTRACT

In order to show the effect of parasites *Ergasilus sieboldi* Nordm. on tenches 143 fish of this species were examined during the annual cycle. A relatively slight infestation (from 100 to 1000 ind. in one fish) causes the anaemia, i.e. decrease of the number of erythrocytes in the circulating blood. The hemoglobin level and hematocrit's index also partly decrease as well as the level of total protein. Changes are noticed in the electrophoresis pattern of proteins in blood serum of infested fish. Generally, but with the exception of September, the albumin amount decreases, while the proteins belonging to  $\alpha$ -group or  $\gamma$ -globulins increase within the globulin fraction.

CONTENTS

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1. INTRODUCTION

*Ergasilus sieboldi* Nordm. parasitizing on gill of tenches cause considerable changes of a necrobiotic character. They damage the structure of gill filaments with their mouth parts, swimming appendages and 2nd antennae and feed on mucus and epithelial cells and erythrocytes (EINSZPORN 1965a, b). As a result of long-lasting feeding disintegration, hyperaemia and frequently even extravasation take place in the gill epithelium. These changes result in permanent disturbances in the functioning of gill, which at the mass invasion of *Ergasilus sieboldi* is one of the reasons of the death of fishes.

This paper aims at an explanation of the effect of parasites by determining the changes in the circulating blood and the differences in the composition of proteins in blood serum.

## 2. MATERIAL AND METHODS

4-6 year old tenches caught in Drwęckie and Łańskie Lakes (Olsztyn province) were examined. The number of blood constituents, hemoglobin content, level of total protein and fractionating of serum proteins were determined according to the standard methods described in the previous papers (EINSZPORN-ORECKA 1970a, b).

The main part of investigations was carried out on tenches infested with a relatively small number of parasites from 100 to 1000 individuals in one fish. On the average ten specimens were examined each month. The presence of 200 parasites resulted in significant changes in the number of blood constituents of the circulating blood, while 100 *Ergasilus sieboldi* already affected the separation of proteins in blood serum. The diagrams (Fig. 1-6) contain numerical data for groups infested with 200 to 1000 parasites and data on protein fractions (Fig. 9-16) for males infested with 100 to 1000 *Ergasilus sieboldi*.

In order to verify the results single fish specimens with a higher invasion extensiveness above 1000 parasites were investigated (Fig. 17-20).

On the whole, apart from 320 control fishes (162 males and 158 females), 143 tenches infested by *Ergasilus sieboldi* were examined, and in that 71 males and 72 females. These fish underwent a detail parasitological dissection in order to eliminate fish with an excessive amount of other parasites and those displaying anatomopathological changes as a result of diseases.

## 3. RESULTS

The number of erythrocytes in infested tenches during the year is on the average smaller, about 100,000-500,000 in 1 mm<sup>3</sup> of circulating blood (Fig. 1, 2), but is different in males and females and depends on the period of the annual cycle. The greatest decrease of the number of erythrocytes in the blood of males is in the pre-spawning period (Fig. 1). When comparing with control fishes the average difference is 850,000/mm<sup>3</sup>, and some of the infested fish have only e.g. 1,120,000 instead of 2,129,000/mm<sup>3</sup>. The influence of parasites is much smaller in autumn (October, November), but increases again in winter and early spring (January-May).

In females the changes connected with the formation and maturation of spawn and the spawning itself basically change the blood pattern of control fishes. At a relatively low infestation it is difficult to observe the effect of parasites in that period (Fig. 2), but even then in the blood of some females the number of erythrocytes decreases maximally to 1,040,000/mm<sup>3</sup> (the mean for control fishes — 1,406,000/mm<sup>3</sup>). Only from October to March, similarly as in the instance of males, the variations increase and amount on the average from 250,000 to 400,000 in 1 mm<sup>3</sup>.

Essential differences in the numbers of leucocytes between the control and infested group take place during spawning. The spawning leucocytosis, which precisely determines the period of changes connected with the reproduction processes, takes place for about one month and disappears immediately after spawning. Fish infested (Fig. 3, 4) during spawning preparations have a smaller number of leucocytes, and the level of erythrocytes does not



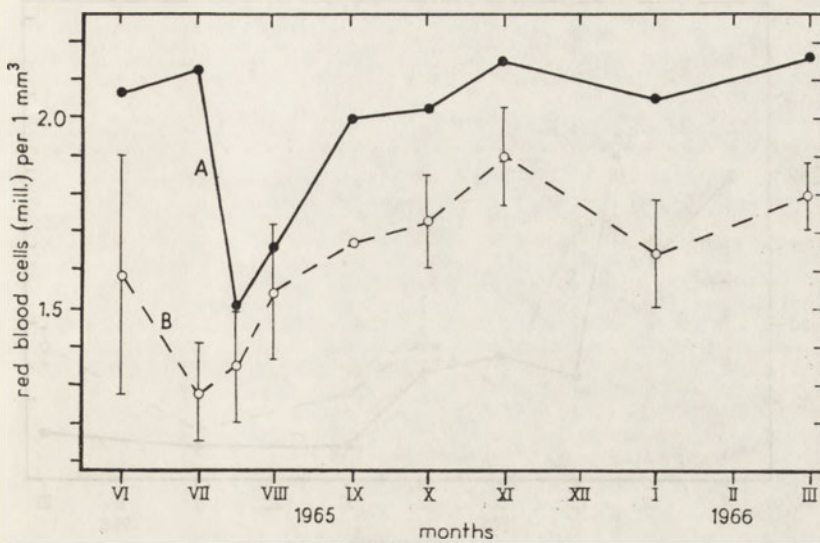


Fig. 1. Differences in the number of erythrocytes in 1 mm<sup>3</sup> of circulating blood of control males (A) and those infested by *Ergasilus sieboldi* (B) (vertical lines in the Figures 1-8 show the standard deviation).

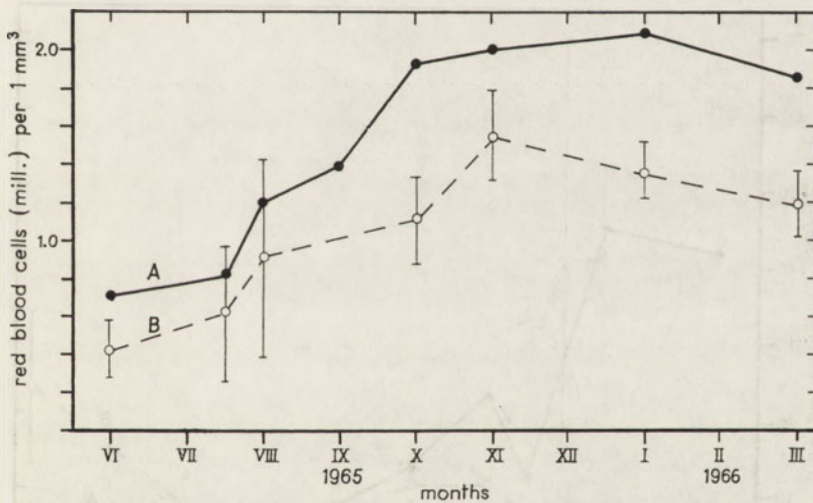


Fig. 2. Differences in the number of erythrocytes in 1 mm<sup>3</sup> of circulating blood of control females (A) and those infested by *Ergasilus sieboldi* (B)

reach the level typical for control tenches. Increased number of leucocytes remains in the blood of females and males slightly longer (on the average 15 days), and that is why the number of leucocytes in August is still 32,686 ( $t = 2.7$ ), and even 40,800 in 1 mm<sup>3</sup> ( $t = 3.83$ ).

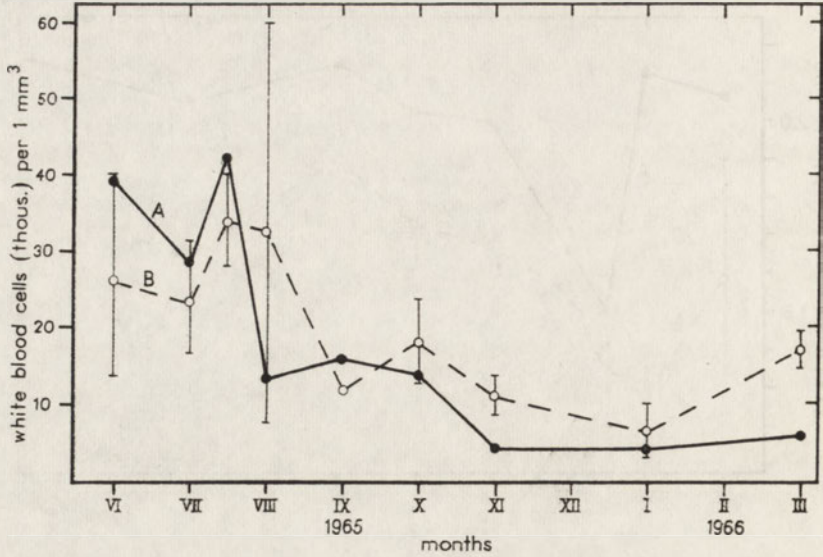


Fig. 3. Number of leucocytes in 1 mm<sup>3</sup> of circulating blood of control males (A) and those infested by *Ergasilus sieboldi* (B)

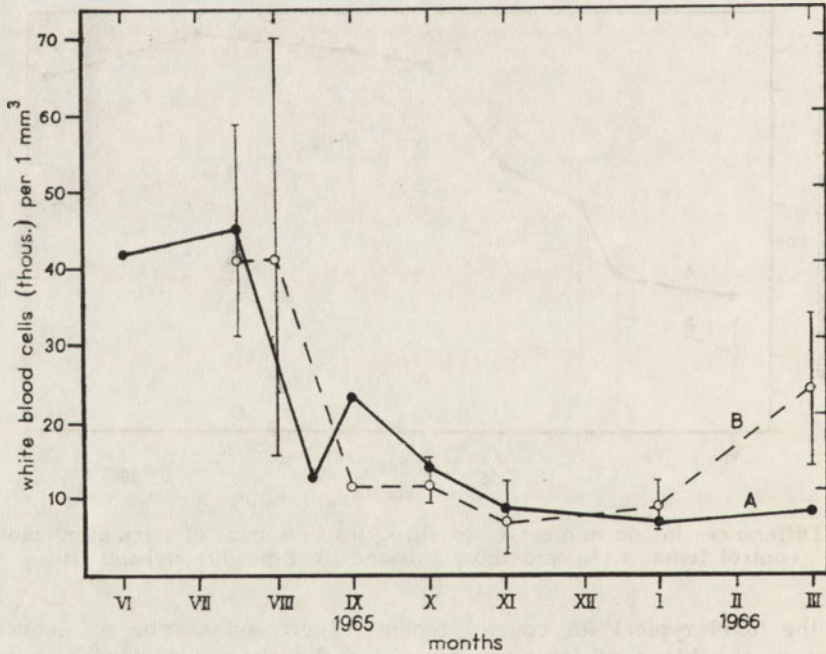


Fig. 4. Number of leucocytes in 1 mm<sup>3</sup> of circulating blood of control females (A) and those infested by *Ergasilus sieboldi* (B)

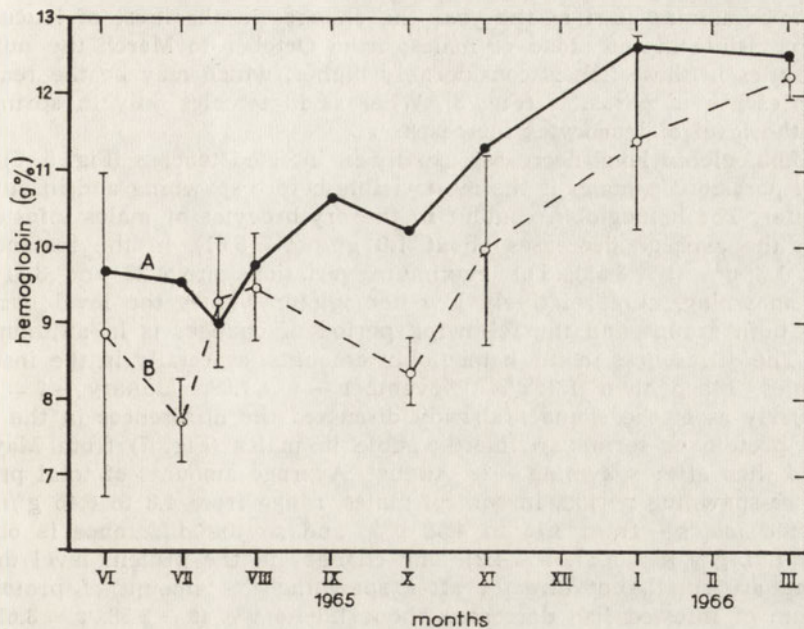


Fig. 5. Fluctuations of hemoglobin level of control males (A) and those infested by *Ergasilus sieboldi* (B)

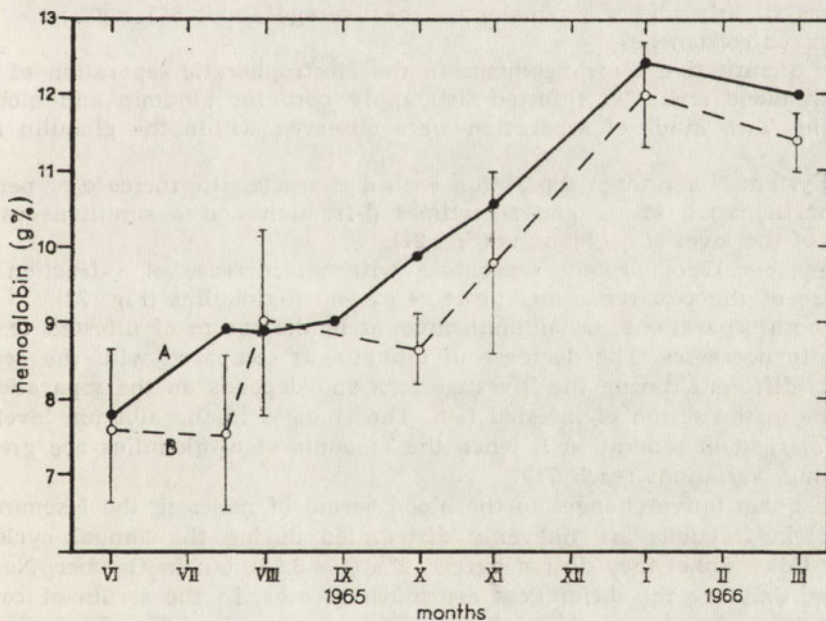


Fig. 6. Fluctuations of hemoglobin level of control females (A) and those infested by *Ergasilus sieboldi* (B)

In the remaining part of the year the changes in the level of leucocytes are more visible in the blood of males; from October to March the number of leucocytes in these fish is considerably higher, which may be the reaction to the presence of parasites (Fig. 3). Whereas in females only in spring, in March, the level of leucocytes increases.

The hemoglobin level decreases usually in infested tenches (Fig. 5, 6). The effect of parasites in males is the most visible before spawning and in autumn and winter. The hemoglobin content in the erythrocytes of males infested in July on the average decreases about 1.8 g% ( $t = 5.61$ ), in the instance of females 1.3 g% ( $t = 3.63$ ). The maximum variations are 4.26 and 3.31 g%. During spawning, at a relatively low hemoglobin values the level becomes even in both groups and the following period of changes is in autumn and winter. The differences in the hemoglobin amounts, especially in the instance of females, reach then 1.3 g% (November —  $t = 1.89$ , January —  $t = 2.97$ ).

Similarly as in the changes already discussed the differences in the level of total protein of serum are more visible in males (Fig. 7) from May till July and then after spawning — in August. Average amounts of total protein in the pre-spawning period, in control males, range from 4.8 to 6.45 g%, and in infested males — from 3.16 to 4.58 g%, and so the difference is on the average 1.81 g% ( $t = 5.67$ ,  $t = 4.2$ ). The changes in the protein level during spawning are small, but directly after spawning the amount of protein in the serum of infested fish decreases about 1.0–1.4 g% ( $t = 3.58$ ,  $t = 3.61$ ). In autumn the difference in the protein level between control and infested fishes again decreases to about 500 mg%. In winter (e.g. in January), both in the instance of males and females (Fig. 8) a decrease of protein was not observed, but just the opposite — the protein level in the serum of infested fish was slightly higher (in males on the average about 547 mg%;  $t = 2.54$ ) than in control tenches.

The quantitative rearrangements in the electrophoretic separation of proteins in blood serum of infested fish apply both for albumin and globulin fractions. Two kinds of separation were observed within the globulin fractions:

1. System I of protein separation with a characteristic increase of percentage participation of  $\alpha_2$ , and sometimes  $\beta$ -fraction and a simultaneous decrease of the level of  $\gamma$ -globulins (Fig. 21).

2. System II of protein separation with an increase of  $\gamma$ -fraction and decrease of the two remaining proteins  $\alpha_2$  and  $\beta$ -globulins (Fig. 22).

In both separations the albumin amount in the serum of infested tenches generally decreases. The decrease of albumin as compared with the control level is different during the investigations and depends on the separation of proteins in the serum of infested fish. The changes in the albumin level are more marked in separation I, when the amounts of  $\alpha_2$ -globulins are greater. Maximum variations reach 7%.

The quantitative changes in the blood serum of males in the I separation of protein fractions are unevenly distributed during the annual cycle. In spring (May, June) they do not exceed 2% ( $t = 3.65$ ), but in October, November and January the differences are much greater. In the serum of control tenches the albumins reach relatively high concentrations 27.7, 27.08, 25.92%. The protein values of males infested during that period are accordingly lower — 24.5, 22.63%, and single specimens even 18.03 or 19.88% (Fig. 9, curve

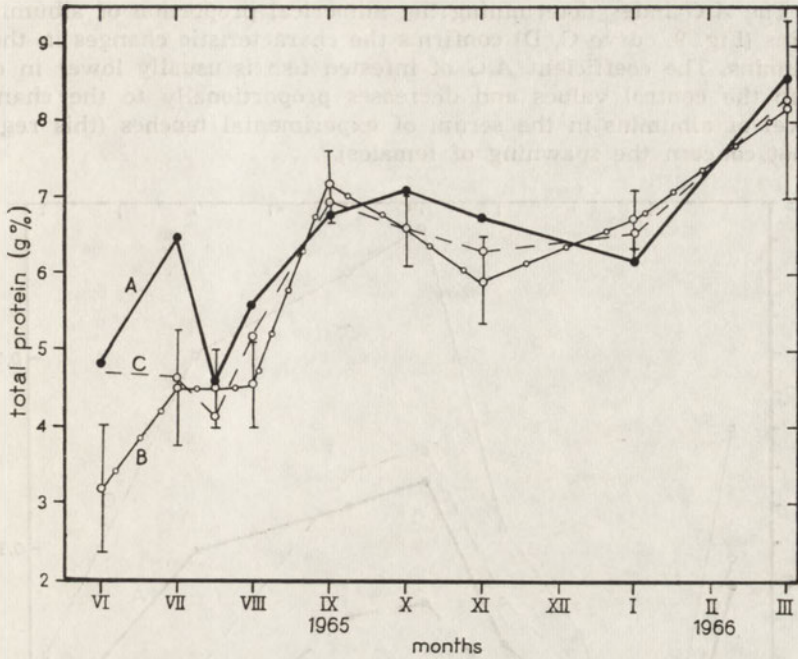


Fig. 7. Amount of total protein in blood serum of control males (A) and those infested by *Ergasilus sieboldi* (infestation degree: 200-1000 parasites — B; 100-1000 parasites — C)

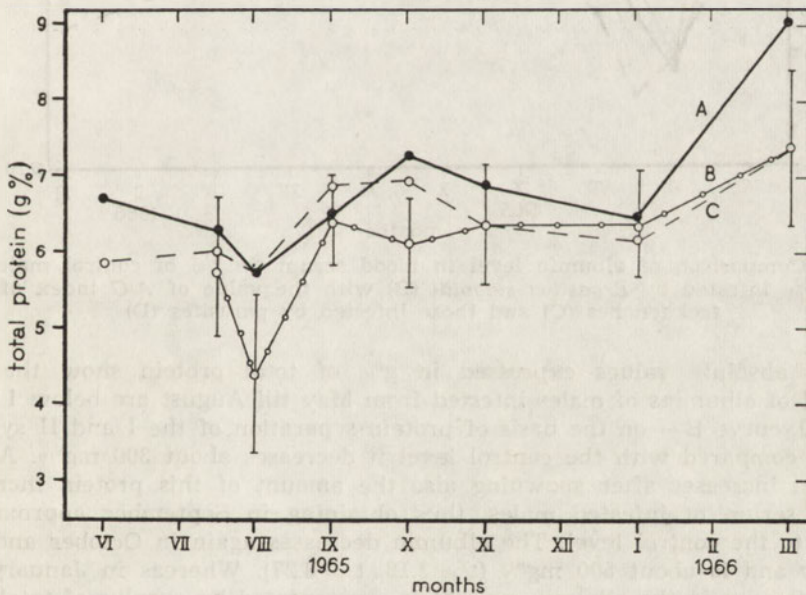


Fig. 8. Amount of total protein in blood serum of control females (A) and those infested by *Ergasilus sieboldi* (infestation degree: 200-1000 parasites — B; 100-1000 parasites — C)

A, B). The A/G index determining the numerical proportion of albumins and globulins (Fig. 9, curve C, D) confirms the characteristic changes in the level of albumins. The coefficient A/G of infested fish is usually lower in comparison to the control values and decreases proportionally to the changes in the level of albumins in the serum of experimental tenches (this regularity does not concern the spawning of females).

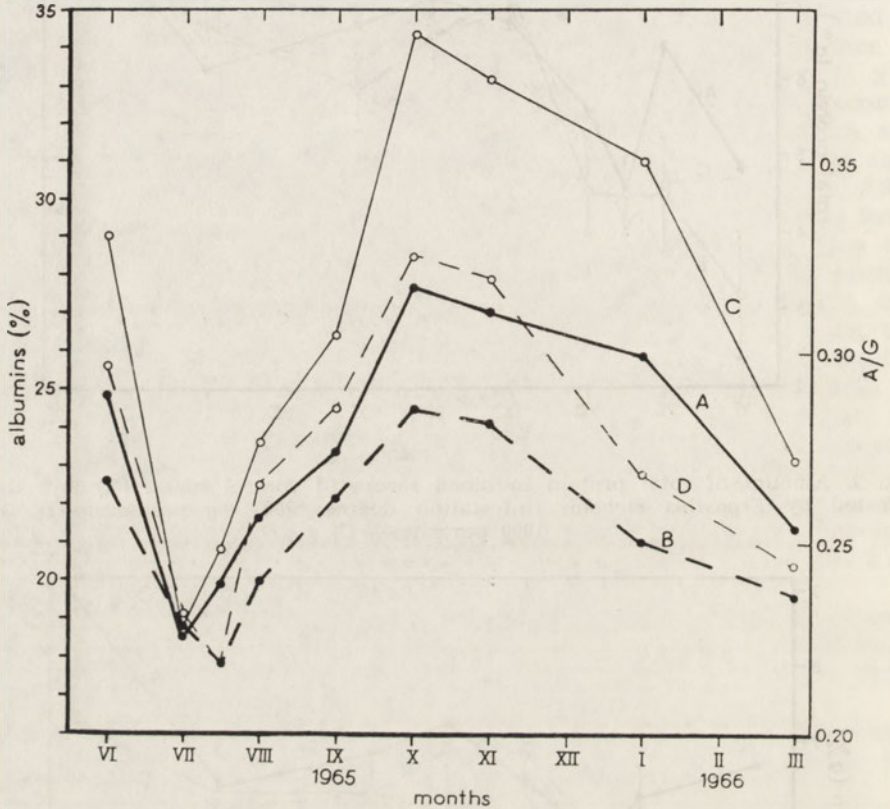


Fig. 9. Comparison of albumin level in blood serum (in %) of control males (A) and those infested by *Ergasilus sieboldi* (B) with the value of A/G index of control tenches (C) and those infested by parasites (D)

The absolute values expressed in g% of total protein show that the amount of albumins of males infested from May till August are below 1.0 g% (Fig. 10, curve B — on the basis of protein separation of the I and II system) and as compared with the control level it decreases about 300 mg%. As the albumin increases after spawning also the amount of this protein increases in the serum of infested males, thus obtaining in September approximate values to the control level. The albumin decreases again in October and November and is about 500 mg% ( $t = 3.18$ ,  $t = 3.27$ ). Whereas in January the 3% difference in the albumin amount is compensated by surplus of total protein, which increases in that period in the serum of infested males about 510 mg% ( $t = 2.30$ ).

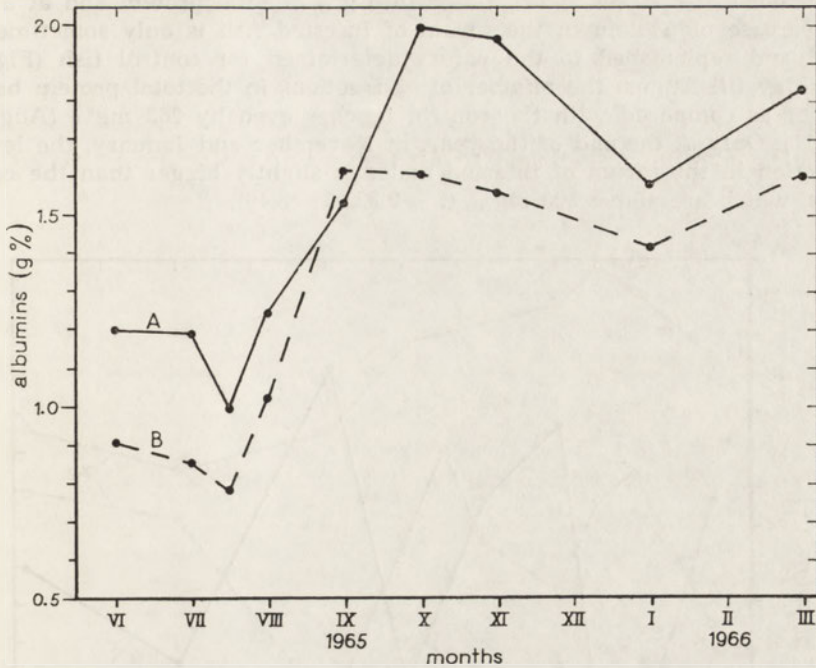


Fig. 10. Differences in the albumin amount (in g%) in blood serum of control males (A) and those infested by *Ergasilus sieboldi* (B)

In the II separation of serum proteins and an increased level of  $\gamma$ -globulins the changes in the albumin amount are much smaller than in the previous one. The essential differences are observed only in winter, the albumin decrease in January is 6.57% i.e. 300 mg%.

The temporary period of accumulation of albumin in the serum of females when the spawn matures and at this degree of infestation is independent from the parasites. Their effect is visible after the spawning is finished. Some of the infested females (6 out of 14 examined ones) have still a high concentration of albumins in the blood serum (34.9%) in August, which proves about the irregular or delayed spawning. The albumin level of the remaining group of females is about 300 mg% ( $t = 3.83$ ) lower. After the moderate autumn the decrease of albumin amount in January is more visible and reaches 5-7%, i.e. 400-500 mg% after taking into account the variable level of total protein.

Typical for this parasitic disease is the separation of serum proteins (system I), which is characterized by a considerable decrease of the percentage albumin participation, increase of  $\alpha_2$  and partly of  $\beta$ -fraction and decrease of  $\gamma$ -globulins (Fig. 23). The level of particular fractions in this system depends on the annual cycle and to a lesser extent on the sex of tenches.

Percentage increase of  $\alpha_2$ -fraction in the serum of males infested in some months (October, January, March) does not exceed 1.2-1.8%, while in other months the much greater differences reach even 4.68 and sometimes 7.52%. The maximum increase during July and August is about 10% (Fig. 11).

The level of  $\alpha_2$ -fraction calculated into g% of total protein and at a marked decrease of protein in the serum of infested fish is only sometimes balanced and replenished to the values determined for control fish (Fig. 12). From May till August the number of  $\alpha_2$ -fractions in the total protein balance is lower as compared with the control tenches even by 263 mg% (August —  $t = 2.17$ ). Only at the end of the year, in November and January, the level of  $\alpha_2$ -fraction in the serum of infested males is slightly bigger than the control values, which are above 300 mg% ( $t = 2.22$ ,  $t = 3.46$ ).

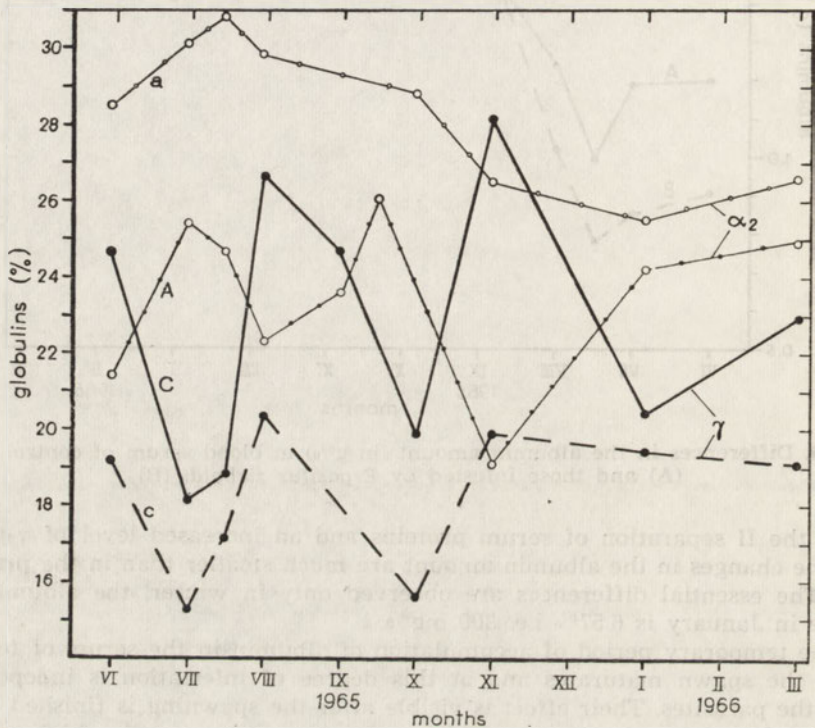


Fig. 11. Differences in the % values of fraction of  $\alpha_2$  and  $\gamma$ -globulins in blood serum of control males (A, C) and those infested by *Ergasilus sieboldi* (a, c)

The values of  $\alpha_2$ -fraction in the serum of females are similar with the exception of the period following the spawning, during which the obtained results are not the same. Increased amounts of  $\alpha_2$ -globulins in the serum of some infested females may point both to the changes after spawning and to the effect of parasites. Definite differences are observed in winter (January) at a 5.23% increase and similarly in the serum of males the fraction level increases by 391 mg%.

The level of  $\beta$ -globulins (Fig. 13, 14) in the separation of proteins (system I) with a dominant  $\alpha_2$ -fraction in the serum of infested males is generally higher than the determined average for control males. Essential differences are observed from August till March and are 1.16–5.6% ( $t = 1.99$ ,  $t = 4.41$ ). Only the numerical relations are different during spawning (Fig. 13). Then



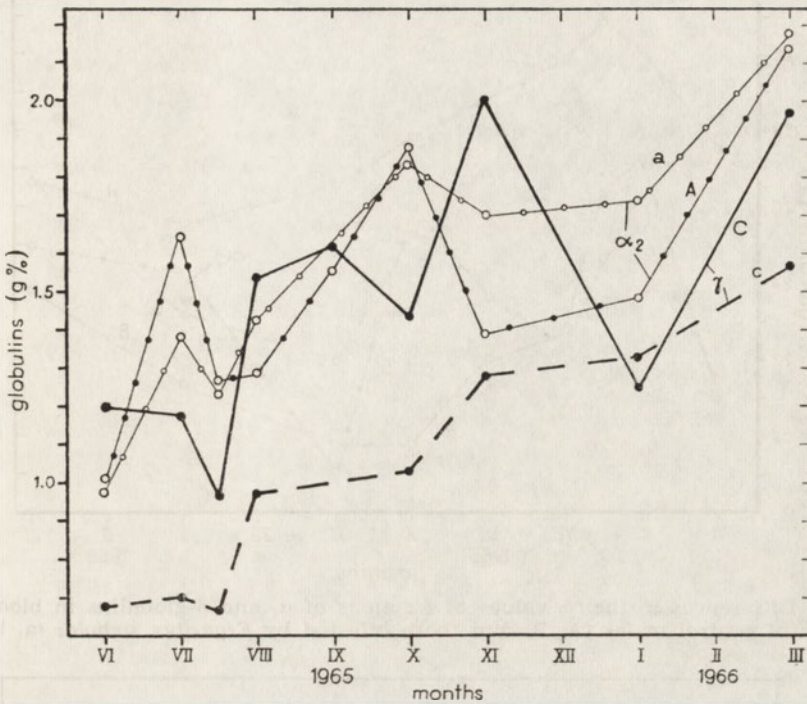


Fig. 12. Differences in the protein level of  $\alpha_2$  and  $\gamma$ -globulins (in g%) of blood serum of control males (A, C) and those infested by *Ergasilus sieboldi* (a, c)

the number of fractions is smaller as compared with the control level about 1.0–1.75%, which is due to protein shortage during the reproduction of these fish. These differences are more visible in the absolute values at a simultaneous decrease of total protein. During spawning of males the  $\beta$ -fraction level decreases about 273 mg% to 556 mg% ( $t = 2.51$ ,  $t = 3.05$ ), and in the instance of females, after spawning this decrease is 7.48% ( $t = 8.25$ ) i.e. 570 mg% ( $t = 3.34$ ).

The percentage increase of  $\beta$ -fraction is more or less constant in the remaining part of the year as compared with the control level. The surpluses, both in the serum of males and females do not exceed 300 mg% (Fig. 14).

As opposed to the two previous fractions the values of  $\gamma$ -globulins (Fig. 11, 12) are uniformly distributed during the annual cycle. The level of this fraction in the group of infested fish falls below 20% and the average differences essential for statistic purposes vary from 2.9 to 8.22%, with the exception of January. The maximum levels reach 10%.

The deviations from the control level (Fig. 12) of absolute values are from 281–768 mg%. Therefore the index  $\alpha_2/\gamma$  is always higher than the coefficient obtained for control fish.

The separation pattern of some serums of infested tenches is in each monthly sample different from the one already described. In the II system of serum proteins  $\gamma$ -globulins are the prevalent fraction (Fig. 22) and also

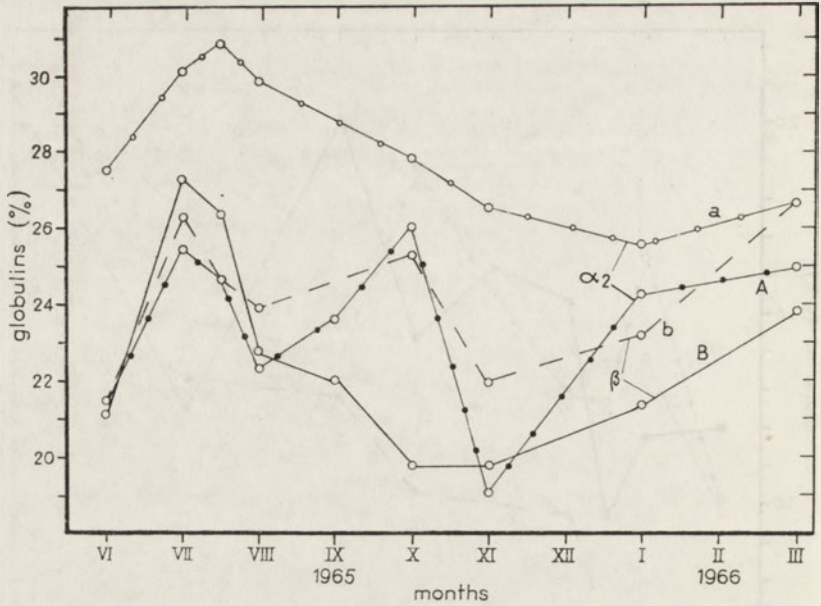


Fig. 13. Differences in the % values of fractions of  $\alpha_2$  and  $\beta$ -globulins in blood serum of control males (A, B) and those infested by *Ergasilus sieboldi* (a, b)

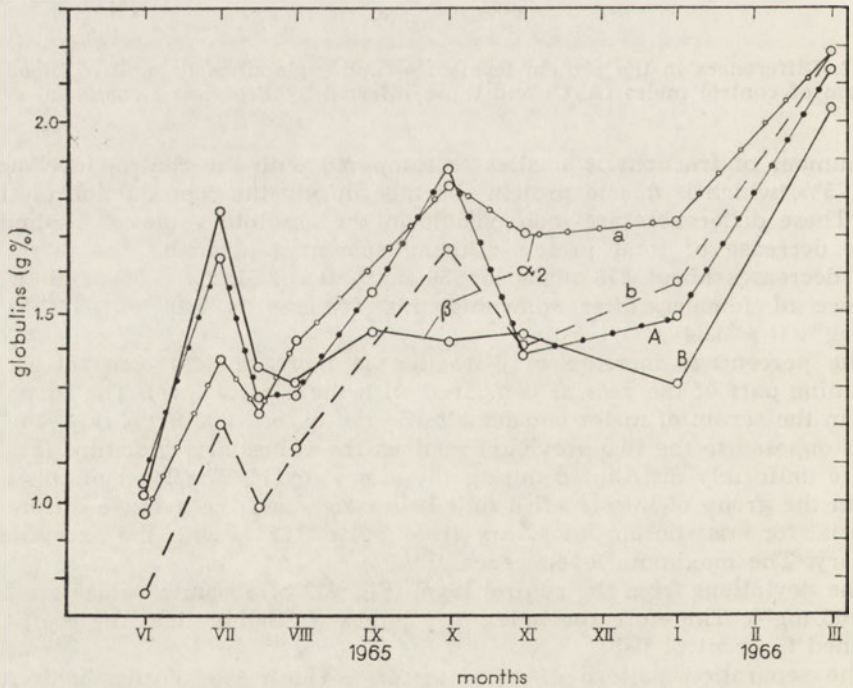


Fig. 14. Differences in the protein level of  $\alpha_2$  and  $\beta$ -globulins (in g%) of blood serum of control males (A, B) and those infested by *Ergasilus sieboldi* (a, b)

the two remaining groups of protein  $\alpha_2$  and  $\beta$ . Sometimes even the albumin values are much lower in comparison to the control level.

The protein amount of the dominant  $\gamma$ -fraction (Fig. 15) increases to 28.7 and even to 32.7%, whereas in control fish it is hardly 18.8–26.7%; the differences are within the range 2.02–11.36%.

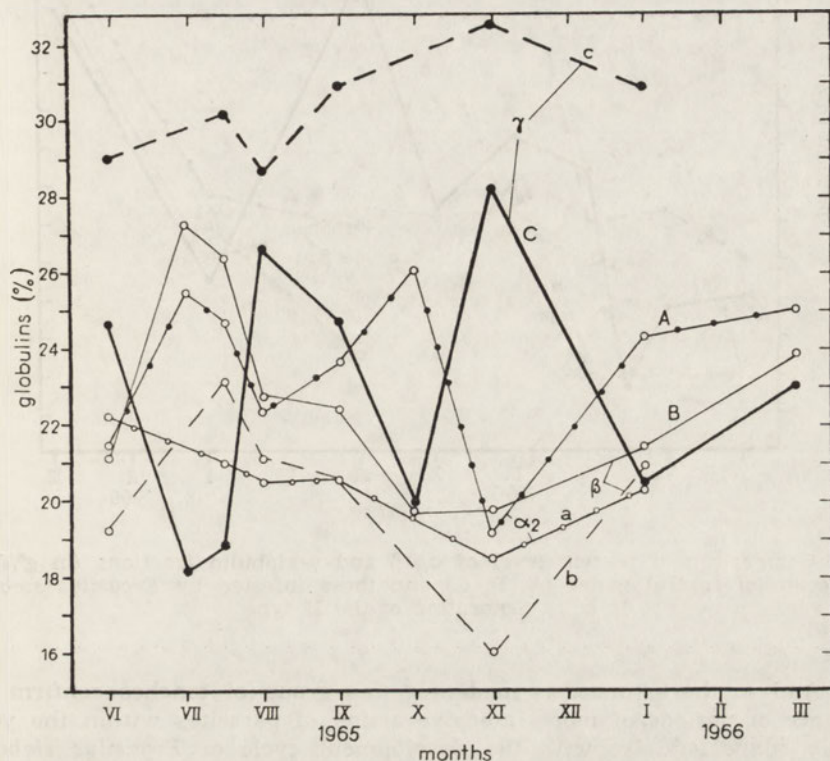


Fig. 15. Comparison of % values of  $\alpha_2$ ,  $\beta$  and  $\gamma$ -globulin fractions of blood serum of control males (A, B, C) and those infested by *Ergasilus sieboldi* (a, b, c). Separation of the II type

The values in the g% system as in the previous globulin fractions are a bit different (Fig. 16). During summer the differences are more visible as independently from the percentage increase the differences in the control level are rather small — within the range 107–455 mg%. Much greater changes in this protein system are observed in autumn; in September, October or January the average deviations increase to 641, 699 mg%, the maximum up to 810 mg%.

The  $\alpha_2$  and  $\beta$  globulin fractions (Fig. 15, 16), apart from September and January when the amount of these two proteins is almost equal, have smaller values as compared with the control level. In the instance of  $\alpha_2$ -fraction the difference amounts to 4%, and in the instance of  $\beta$ -fraction — about 2%. When calculated into g% these variations are within the range of about 300 mg%.

The pattern of changes in the blood of tenches at a greater invasion of parasites (from 1,000 to 2,000) is respectively more marked than in the former

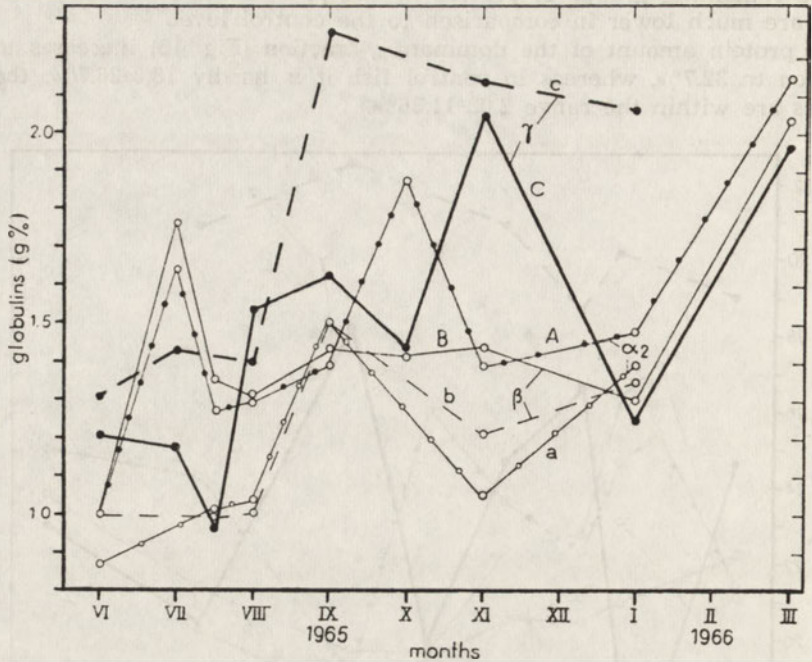


Fig. 16. Comparison of protein level of  $\alpha_2$ ,  $\beta$  and  $\gamma$ -globulin fractions (in g%) of blood serum of control males (A, B, C) and those infested by *Ergasilus sieboldi* (a, b, c). Separation of the II type

group. And so the estimations made for this group of tenches confirm the occurrence of periods of more intensive action of parasites within the year, probably connected also with the development cycle of *Ergasilus sieboldi*. A greater influence is noticed e.g. during the spawning preparations (June), when the differences in the number of erythrocytes in the blood of a male infested by 1,247 parasites are 921,000/mm<sup>3</sup>, at a simultaneous decrease of hemoglobin content (2.4%) and hematocrit's index (12%). Similar or slightly smaller deviations were observed in the two following months during spawning or directly after spawning. However, during these periods the changes resulting from the presence of parasites are more difficult to explain because of the considerable variations of control values.

The survivorship of tenches in conditions of extreme weakness of organism is quite big. It has been found that a fish (male — 24.5 cm, 140 g) having more 28,000 of erythrocytes in 1 mm<sup>3</sup> of circulating blood and 1.09 g% of hemoglobin is still alive with and its movement capacity is not limited. The value of total protein in the serum of the investigated male was also only 1.5 instead of 5.6 g% (Fig. 17).

In autumn the differences determining the effect of parasites are considerably smaller (Fig. 17, 18); in the blood of a female the number of erythrocytes decreases only by 128,000, in the male by 377,000/mm<sup>3</sup>, and also the hemoglobin level decreases by 0.99 and 2.84 g%, respectively.

Periodical action of parasites was observed, similarly as in other parameters, in the fluctuations of the level of total protein. Great differences take place in June and August; the protein value in the serum of males is hardly 0.67 or 1.3 g% at a control level 5.57 g%. Contrariwise, despite the infestation with 2000 parasites, the level of total protein in autumn increases by 732 mg%, and in January at a similar infestation it decreases by 796 mg%.

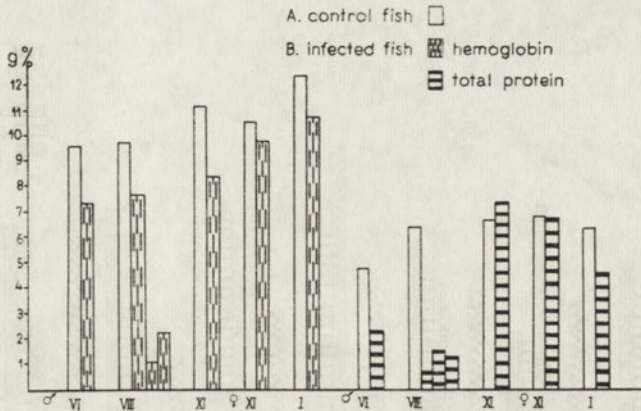


Fig. 17. Differences in the hemoglobin level and value of total protein in blood serum of control males and females (A) and those infested by *Ergasilus sieboldi* with 1100–2000 parasites (B)

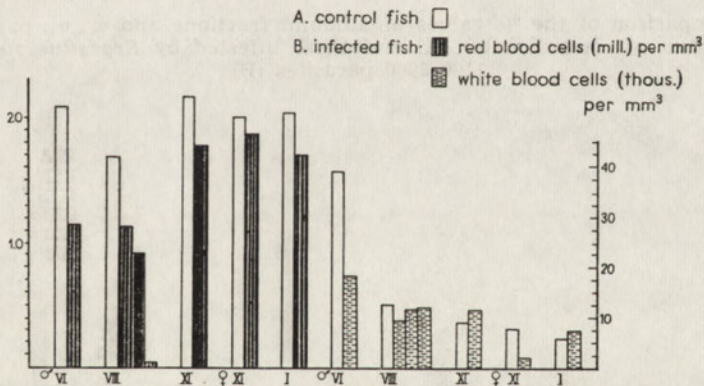


Fig. 18. Number of erythrocytes and leucocytes in the circulating blood of control males and females (A) and those infested by *Ergasilus sieboldi* with 1100–2000 parasites (B)

Analogically as in the first group of fish two types of separation of serum proteins were distinguished (Fig. 19). In both these systems the decrease of albumin level was 4–10%. The increase of  $\alpha_2$ -fraction in the I separation of serum proteins amounts to 35.96%, and simultaneously the  $\beta$ -fraction maximally decreases to 29.27%, whereas the decrease of proteins from the  $\gamma$  group amounts to 6.75%.

In the g<sup>0</sup>/<sub>0</sub> system (Fig. 20), at a minimal level of total protein the value of respective fractions is accordingly low and the right proportions between successive proteins are observed (Fig. 24). This character of changes is most precisely reflected by the fractions of male in November as exceptionally there the protein level is approximate to the control one. An increase of  $\alpha_2$ -fraction is then 926 mg<sup>0</sup>/<sub>0</sub>, of proteins  $\beta$  — 643 mg<sup>0</sup>/<sub>0</sub>, and the decrease of  $\gamma$ -globulins — 439 mg<sup>0</sup>/<sub>0</sub>.

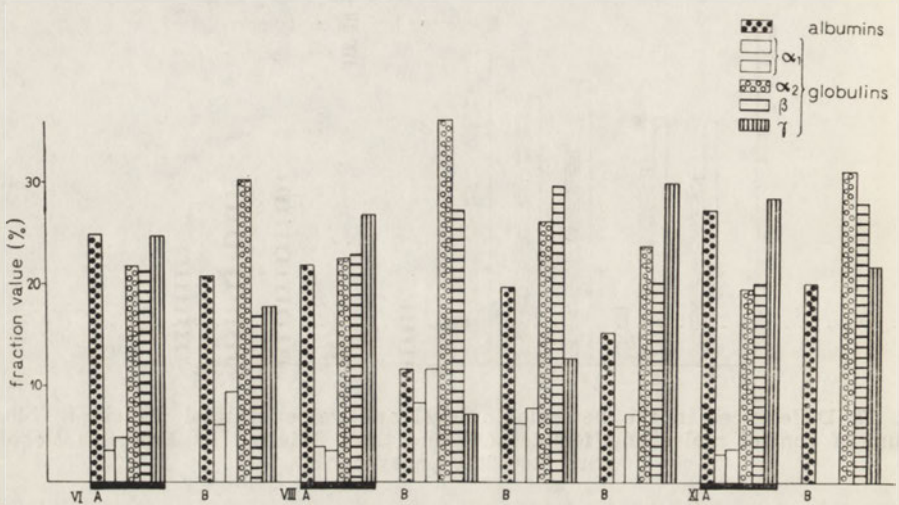


Fig. 19. Comparison of the % values of albumin fractions and  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ ,  $\gamma$ -globulins in blood serum of control males (A) and those infested by *Ergasilus sieboldi* with 1100–2000 parasites (B)

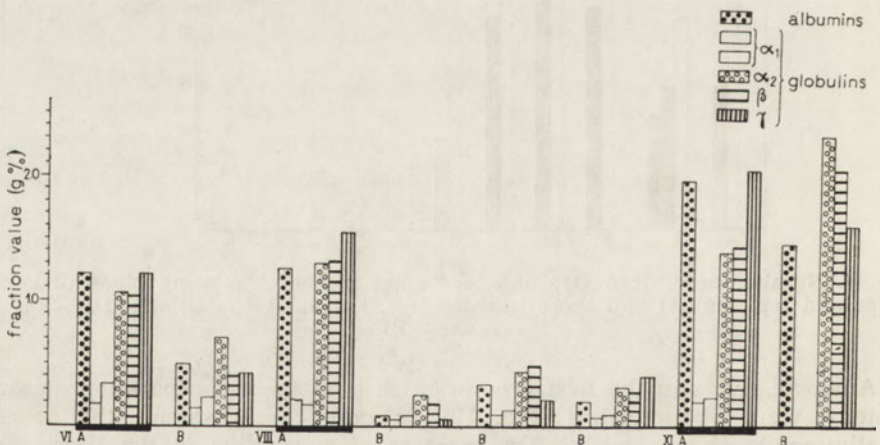


Fig. 20. Comparison of albumin level and  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ ,  $\gamma$ -globulins (in g<sup>0</sup>/<sub>0</sub>) in blood serum of control males (A) and those infested by *Ergasilus sieboldi* with 1100–2000 parasites (B)

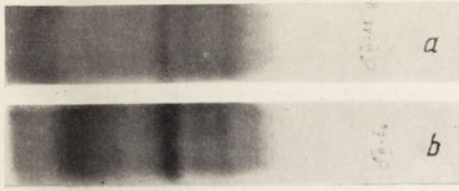


Fig. 21. Normal electrophorogram (a) with a changed pattern of protein separation (b) of a male infested by *Ergasilus sieboldi*

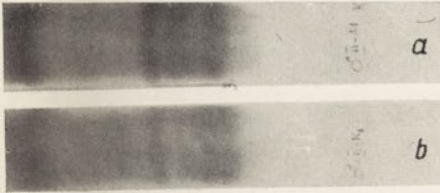


Fig. 22. Differences in the dyeing intensity of  $\gamma$ -globulin fraction in the electrophorogram of a male infested by *Ergasilus sieboldi*. a — separation of normal serum; b — separation of changed serum

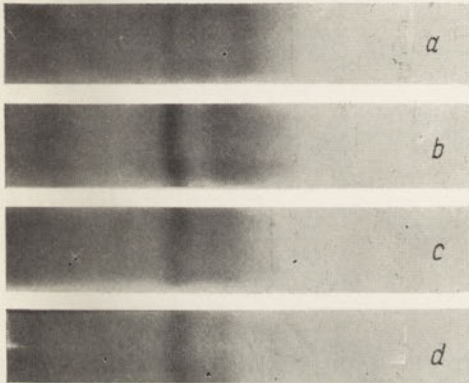


Fig. 23. Increase of proteins of  $\alpha_2$ -globulins and decrease of  $\beta$  and  $\gamma$ -fractions in blood serum of males infested by *Ergasilus sieboldi*. a — normal separation; b, c, d — changed separation

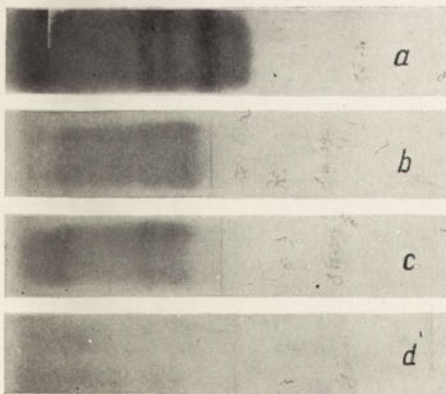


Fig. 24. Changes in the separation and dyeing of electrophorograms of proteins of males infested by *Ergasilus sieboldi* with 1100–2000 parasites. a — control male; b, c, d — infested males





## 4. DISCUSSION

Essential changes in the annual cycle in the circulating blood of tenches infested with *Ergasilus sieboldi* are observed in the number of erythrocytes, partly of leucocytes and hemoglobin. Apart from that the level of total protein of blood serum of these fish decreases and changes in the electrophoresis pattern of proteins connected with the rearrangement of albumin and globulin fractions are observed. Relatively slight infestation from 200 to 1000 of parasites results in anaemia, which is expressed by a decrease of the number of erythrocytes, and frequently of hemoglobin and hematocrit's index.

The histopathological analysis showed (EINSZPORN 1965a, b) that the parasites damage the respiratory epithelium of gill filaments mainly with their swimming appendages, mouth parts and 2nd antennae. The damages caused by the movements, feeding and attachment of parasites result in the development of necrobiotic changes. The deadened and the scattered epithelium fragments are usually washed out from the gill surface forming disintegrations, which uncover the capillary vessels. The destruction of capillaries or deeper placed gill arteries by females causes a constant hyperaemia, which results in a decrease of the number of erythrocytes in the circulating blood of tenches. Frequent hyperaemia lead to a chronic anaemia. Repetitive extravasation of blood result in an iron deficiency. The analysis of the hemoglobin amount in infested tenches shows that there not always are disturbances in the level of this dye. These changes even at a constant number of parasites depend on the length of the feeding period and the extent of damage of gill filaments. Longer action of "pathogenic factor" results in worse haematopoietic activity. These disturbances always lead to an oxygen deficit in the tissues, which while becoming more intensive results in further disturbances in metabolism making worse all the activities of organism.

Among other things it is visible in the insufficient preparation to spawning of infested tenches. Smaller numbers of blood constituents, and especially of leucocytes (Fig. 3, 4), and the irregularities of other factors such as: total protein (Fig. 7) fractions of  $\alpha_2$  and  $\beta$ -globulins of males (Fig. 14) or albumins of females in the period directly preceding and during the spawning show that during the necessary time the fish are not sufficiently prepared for spawning. Spawning leucocytosis, closely connected with the reproduction period is about 10 to 14 days longer in the blood of infested fish. Perhaps some of the fish are later for spawning, but these observations require separate investigations of the fertility of females infested by *Ergasilus sieboldi*.

The anaemia after the haemorrhage is connected with the decrease of the level of total protein in blood. These changes are more visible in the separation of serum proteins with the dominant  $\alpha_2$ -fraction, in the I system. Apart from that, similarly as in other parameters, the action of parasites is especially visible in males during spawning preparations and in females after spawning. Greater protein demand of infested fish may be partly balanced in autumn after a period of longer feeding. Also, it seems that the effect of parasites is much smaller in late autumn than in spring. In winter the protein level in blood serum, mainly in males, is higher than in control fishes. This proves that greater supplies are being used and that the winter reserves are used much quicker.

In the I separation especially, together with a decrease of the level of total protein the amount of albumins in the serum of infested tenches also decreases. Essential differences in males are observed in spring, late autumn and in winter. Whereas in females the influence of parasites is observed after spawning. However the albumin amount, in the instance of males, can be replenished in autumn. Therefore it should be assumed that hypoalbuminemia is mainly connected with the deficiency of nutritious proteins at a simultaneous quickening of the catabolism of albumins.

In pathological conditions the changes in the protein content in serum are not only with regard to albumins but are observed to a greater extent within the globulins, which are more unstable proteins. Increased globulin amounts in blood serum take place usually in the initial phases of various diseases and frequently in severe cases or in chronic inflammation.

Separation of protein of globulin fractions is not typical for the discussed parasitic disease, because apart from the effect of the crustaceans themselves there are also secondary bacterial processes connected with the development of mould (NEUHAUS 1929). And this is probably why two types of separation were observed in the separation of globulin fractions in the serum of infested tenches. In the I system there is an increased amount of  $\alpha_2$ -fractions, in some periods of  $\beta$ , and a decrease of the level of  $\gamma$ -globulins. But in the II system an increase of  $\gamma$ -fraction is observed and also a decrease of the other two proteins. In the instance of examined fish the separation of the I type prevails as a more characteristic one for severe inflammatory states. However, a more precise interpretation of quantitative rearrangements is very difficult because of a lack of thorough investigations on the properties of particular fish proteins. On the basis of the observations presented here it can be only said that the increase of the fraction of  $\alpha$ -globulins is undoubtedly connected with pathogenic changes in gill accompanied by necrocytosis of tissues and their intensive disintegration. Probably the increased synthesis of  $\alpha_2$ -globulins is also the result of a quicker destruction of erythrocytes than normally.

Using the accepted criteria of the estimation of changes in the electrophoretic pattern of serum proteins for the course of inflammations it can be said (this is also in agreement with the histopathological analysis) that in the instance of this parasitic disease the inflammation is in gill. At a severe or almost severe inflammation mainly  $\alpha$ -globulin fractions increase, while chronic inflammation is characterized by a moderate level of  $\alpha$  and  $\beta$ -fractions at a simultaneous increase of  $\gamma$ -globulins. Probably the increase of  $\gamma$ -globulins in the chronic phase connected with the inflammatory state of the gill of fish infested is as in other animals due to the production of antibodies in secondary bacterial processes.

## 5. SUMMARY

Parasites *Ergasilus sieboldi* Nordm., even in a number 200-1000 individuals cause the anaemia in tenches, which is expressed by a decrease of the number of erythrocytes in the circulating blood, and partly of hemoglobin and hematocrit's index. They also cause a deficiency of total protein in blood serum (hypoalbuminemia) and rearrangements within the globulin fractions. The pattern of these changes is more visible in tench males, as they are more sensitive to the action of parasites than the females.

Greater protein demand of infested tenches at a relatively small invasion may be replenished after a longer feeding period in autumn. Simultaneously it seems that the influence of parasites is much smaller in late autumn than in other seasons of the year e.g. spring and summer. And in the same conditions the albumin amount may be balanced with the control level, which shows that hypoalbuminemia is mainly connected with a shortage of nutritious proteins at a simultaneous quickening of the catabolism of albumins.

Rearrangements of serum proteins within globulin fractions are regarding to: increase of  $\alpha_2$ -globulins, and partly of  $\beta$  at a simultaneous decrease of  $\gamma$ -globulins or increase of  $\gamma$ -fraction and a decrease of proteins from the  $\alpha_2$ -group and  $\beta$ -globulins. Increase of  $\alpha_2$ -fraction is connected with the inflammatory state and increased destruction of erythrocytes by constant extravasation of blood in the gill. More frequently occurring separation of proteins with a characteristic increase of  $\alpha_2$ -fraction proves about the severe state of inflammation of gill caused by *Ergasilus sieboldi*. This disease may also take the form of chronic gill inflammation: then it may be characterized by an increased synthesis of  $\gamma$ -globulins, which is probably due to the formation of antibodies in the organism of infested tenches.

## 6. STRESZCZENIE

Pasożyty *Ergasilus sieboldi* Nordm., nawet przy stosunkowo nieznacznym zarażeniu (200–1000 sztuk), wywołują u linów niedokrwistość, która wyraża się zmniejszeniem liczby erytrocytów we krwi obwodowej, częściowo hemoglobiny i obniżeniem wskaźnika hematokrytu. Powodują ponadto niedobór białka całkowitego w surowicy krwi (hypoalbuminemię) oraz przegrupowania w obrębie frakcji globulinowych. Obraz tych zmian jest wyraźniejszy u samców lina, ponieważ są one wrażliwsze na oddziaływanie pasożytów niż samice.

Zwiększone zapotrzebowanie na białko u linów zarażonych, przy stosunkowo niewielkiej inwazji, może być uzupełnione po okresie dłuższego żerowania w jesieni. Równocześnie wydaje się, że wpływ pasożytów jest późną jesienią znacznie mniejszy niż w innych porach roku np. wiosną lub latem. Podobnie w tych samych warunkach, ilość albumin może być wyrównana do poziomu kontrolnego, co sugeruje, że hypoalbuminemia wiąże się głównie z niedoborem białek pokarmowych przy równoczesnym przyspieszeniu katabolizmu albumin.

Przegrupowania białek surowicy w obrębie frakcji globulinowych dotyczą: wzrostu  $\alpha_2$ -globulin, częściowo  $\beta$  przy równoczesnym obniżeniu  $\gamma$ -globulin lub zwiększenia frakcji  $\gamma$  i spadku białek z grupy  $\alpha_2$  i  $\beta$ -globulin. Wzrost frakcji  $\alpha_2$  związany jest ze stanem zapalnym oraz wzmocnionym niszczeniem erytrocytów na skutek stałego wykrwienia w skrzelach. Częściej występujący rozdział białek z charakterystycznym zwiększeniem frakcji  $\alpha_2$  świadczy o nasileniu ostrej formy stanu zapalnego skrzeli w wyniku oddziaływania pasożytów *Ergasilus sieboldi*. Schorzenie to przybiera również formę przewlekłego zapalenia skrzeli: cechuje go, być może, wzmoczona synteza  $\gamma$ -globulin, związana między innymi prawdopodobnie z tworzeniem przeciwciał w organizmie linów zarażonych.

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## THE INFLUENCE OF STRONG ULTRAVIOLET RADIATION ON HATCHABILITY OF *TRIOPS CANCRIFORMIS* (BOSC) EGGS

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### ABSTRACT

The eggs of *Triops cancriformis* show a considerable resistance to ultraviolet radiation of a maximum length of waves 2537 Å and 3660 Å. The applied dosages up to 6,804,000 erg/mm<sup>2</sup> had a weak stimulating effect on the development.

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### 1. INTRODUCTION

Investigations on the effect of ultraviolet radiation on the development of eggs have been carried out already for a long time on the material of various invertebrates — both aquatic and terrestrial. Depending on the wavelength, radiation intensity and specific properties of the investigated material the ultraviolet radiation has either a stimulating or inhibiting effect on the development. This may take the form of activation of unfecundated eggs to parthenogenetic development — e.g. in the instance of sea-urchins (HOLLAENDER 1938, HARVEY and HOLLAENDER 1938), or in inhibition of the course of cleavage (CHASE 1938, GIESE 1938a, b, WELLS and GIESE 1950), degeneration of cells (GIESE 1946, BIZJULJAVIĆJUS 1966) and decrease of the hatching ability (AMY 1964, 1965).

Biological effects of ultraviolet radiation also show a close dependence on the development stage, in which the exposure takes place, which is connected with the course of morphogenetic changes within the embryo (AMY 1964).

The eggs of *Triops cancriformis* show a great capability of adaptation to the changes of medium conditions such as temperature (HEMPEL and DUTRIEU 1965), water salinity (KLEKOWSKI and HEMPEL-ZAWITKOWSKA 1968, HEMPEL-ZAWITKOWSKA 1969) and drying up (HEMPEL-ZAWITKOWSKA and KLEKOWSKI 1968). In their normal development cycle they are subject to solar radiation staying during one year or several years on shallow or dry bottoms of seasonal water bodies. Investigations of the resistance of *Triops cancriformis* eggs to ultraviolet radiation and the possible effects of this radiation on the hatchability are the subject of this paper.

## 2. MATERIAL AND METHODS

The material for investigations were *Triops cancriformis* eggs taken out of egg-pouches (the method of HEMPEL 1963) of females of the parthogenetic population, caught in fish ponds in Jaktorów near Warsaw, in June, 1968. The eggs were kept in pond water till the experiment.

Two lamps were used as sources of ultraviolet radiation:

1. Hanovia "Chromatolit", England, low-pressure. The lamp is stopped by a filter OX-7 acc. to the Chance-Pilkington catalogue, transmitting radiation within the range 2400–4500 Å. The range of emitted waves, their percentage participation and energy were as following:

UV 2537 Å — 55% = 40.70 erg/mm<sup>2</sup> sec,

UV 2537 — 2700 Å — 18% = 12.85 erg/mm<sup>2</sup> sec,

UV above 2700 Å — 27% = 13.90 erg/mm<sup>2</sup> sec.

As the mean radiation energy of the lamp — 67.5 erg/mm<sup>2</sup> sec was assumed. The distance of the object from the lamp was 8.5 cm.

2. Q400 lamp, Famed 1, Poland, mean-pressure, with a filter, chiefly transmitting 3660 Å radiation. The energy of the lamp = 52.5 erg/mm<sup>2</sup> sec. The distance of the object from the lamp was 16 cm.

Dosimetry of both lamps prepared for the applied distances from the objects was made<sup>1</sup> acc. to the HATCHARD and PARKER method (1956).

Out of the eggs chosen for the experiment two groups were distinguished: those developing in diffused day-light in a closed compartment, and those developing in the darkness and brought into light only during the exposure. The eggs were divided into series differing as to the development conditions, which differed among themselves by the time of exposure. The exposure of eggs was made according to two patterns: 1. everyday, beginning from the second day of egg development to the hatching moment in the given series and 2. only during three successive days, starting after 4 and 7 days from the beginning of development.

In both patterns the radiation dosages once in a day were identical for each sample. During the whole time of experiment each sample was placed in a glass crystallizer and covered with settled tap water, the column of which was 1 cm high. The exposures and the development took place in the same vessels. The experiments were carried out in the temp. 20°C.

In order to find out the effect of radiation on dry eggs the part of eggs dried in the open after a previous 6-day development in water were exposed.

The series of experiments compared in 5 Tables were distinguished (Tables I–V).

At the exposure of eggs with Q400 lamp it was noticed that in the instance of longer exposures the samples were warmed up to the temperature 40°C. In order to check whether the increased temperature does not change the time of egg hatching few samples were placed in a thermostat at this temperature for a time equal to the longest exposure under Q400 lamp. The time of development and per cent of hatched eggs did not differ basically from the control hatch.

<sup>1</sup> I am grateful to Dr. Z. TRAMER, from the Institute of Biophysics and Biochemistry, Polish Academy of Sciences, for carrying out the dosimetry.

Table I. Series I: eggs developing in the diffused day-light, exposed with Hanovia lamp. The hatch began on the 9th day

Sample	The beginning of exposure at the day of development	The number of days of the exposure	Daily exposure in min	Total time of exposure in hr and min	Total radiation dosage in erg/mm <sup>2</sup>
1	2	8	10	1 hr 20'	324,000
2	2	8	30	4 hr	972,000
3	2	8	60	8 hr	1944,000
4	2	8	90	12 hr	2916,000
5	4	3	90	4 hr 30'	1093,500
6	7	3	90	4 hr 30'	1093,500

Table II. Series II: eggs developing in the diffused day-light, exposed with lamp Q400. The hatch began on the 9th day

Sample	The beginning of exposure at the day of development	The number of days of the exposure	Daily exposure in min	Total time of exposure in hr and min	Total radiation dosage in erg/mm <sup>2</sup>
1	2	8	20	2 hr 40'	504,000
2	2	8	60	8 hr	1512,000
3	2	8	120	16 hr	3024,000
4	2	8	180	24 hr	4536,000
5	4	3	180	9 hr	1701,000
6	7	3	180	9 hr	1701,000

Table III. Series III: eggs developing in the darkness exposed with Hanovia lamp. The hatch began on the 15th day

Sample	The beginning of exposure at the day of development	The number of days of the exposure	Daily exposure in min	Total time of exposure in hr and min	Total radiation dosage in erg/mm <sup>2</sup>
1	2	13	10	2 hr 10'	526,500
2	2	13	30	6 hr 30'	1579,500
3	2	13	60	13 hr	3159,000
4	2	13	90	19 hr 30'	4738,500
5	4	3	90	4 hr 30'	1093,500
6	7	3	90	4 hr 30'	1093,500

Table IV. Series IV: eggs developing in the darkness exposed with lamp Q400. The hatch began on the 13th day

Sample	The beginning of exposure at the day of development	The number of days of the exposure	Daily exposure in min	Total time of exposure in hr and min	Total radiation dosage in erg/mm <sup>2</sup>
1	2	12	20	4 hr	756,000
2	2	12	60	12 hr	2 268,000
3	2	12	120	24 hr	4 536,000
4	2	12	180	36 hr	6 804,000
5	4	3	180	9 hr	1 701,000
6	7	3	180	9 hr	1 701,000

Table V. Series V: eggs exposed in dry state after a previous 6-day development in water, in the light. After the exposure the eggs were once again covered with water

Sample	Exposure with the lamp	The number of days of the exposure	Daily exposure in min	Total time of exposure in hr and min	Total radiation dosage in erg/mm <sup>2</sup>
1	Hanovia	3	90	4 hr 30'	1093,000
2	Q400	3	180	9 hr	1701,000

In all the mentioned series for 50 days the hatches were noted and when they finished the hatching per cent was calculated. The obtained results were compared with the results of hatches of non-exposed eggs treated as control ones, which was carried out separately for eggs developing in the light, in the darkness and those slightly dried up.

### 3. RESULTS

As it is shown from a comparison of the hatchability and the rate of egg development in the light (series I and II) and those exposed everyday by two lamps (Fig. 1A, B) there are no basic differences between the hatch of experimental and control eggs. The final per cent of the hatch of exposed eggs was slightly lower than that of control ones: eggs under the exposure of Hanovia lamp attained from 84.3 to 92.4% of hatch, the eggs under the exposure of Q400 lamp — from 82.9 to 90.6% of hatch, while the control eggs attained 90.8% of hatch. The first hatches took place simultaneously in all samples, but respective samples differed among themselves as to the course of the further hatch in time and as the radiation dosages increased the hatches were quicker. The hatch of eggs exposed with Q400 lamp as compared with the control hatch was exceptionally fast.

The hatchability of eggs developing in the darkness and those exposed by UV rays was compared with the hatchability of control eggs developing in diffused day-light and control eggs developed in the darkness (Fig. 2A, B). From the comparison of both control samples it can be seen that the lack of day-light lowers the final per cent of hatch (control in the light 90.8% of the hatch, control in the darkness 73.5% of hatch) and delays the beginning of hatch some 19 days. But in both cases the hatch ends almost simultaneously, because the eggs developing in the darkness hatch in a very short period of time.

Exposure of eggs developing in the darkness with UV rays results in a hatch, which is much higher than that of control eggs (88.4 to 94.1% — the hatch of eggs exposed by Hanovia lamp, and 90.4 to 95.5% — the hatch of eggs exposed by Q400 lamp as compared with the control hatch — 73.5%). The exposed eggs begin to hatch 13 days earlier than the control ones at the exposure with Hanovia lamp (eggs exposed in the 14th day and non-exposed in the 27th day) and 13–15 days quicker at the exposure with Q400 lamp (eggs exposed in the 12–14th day). As the radiation dosages increase at the exposure with both lamps the rate of hatches increases (counting from the first day of



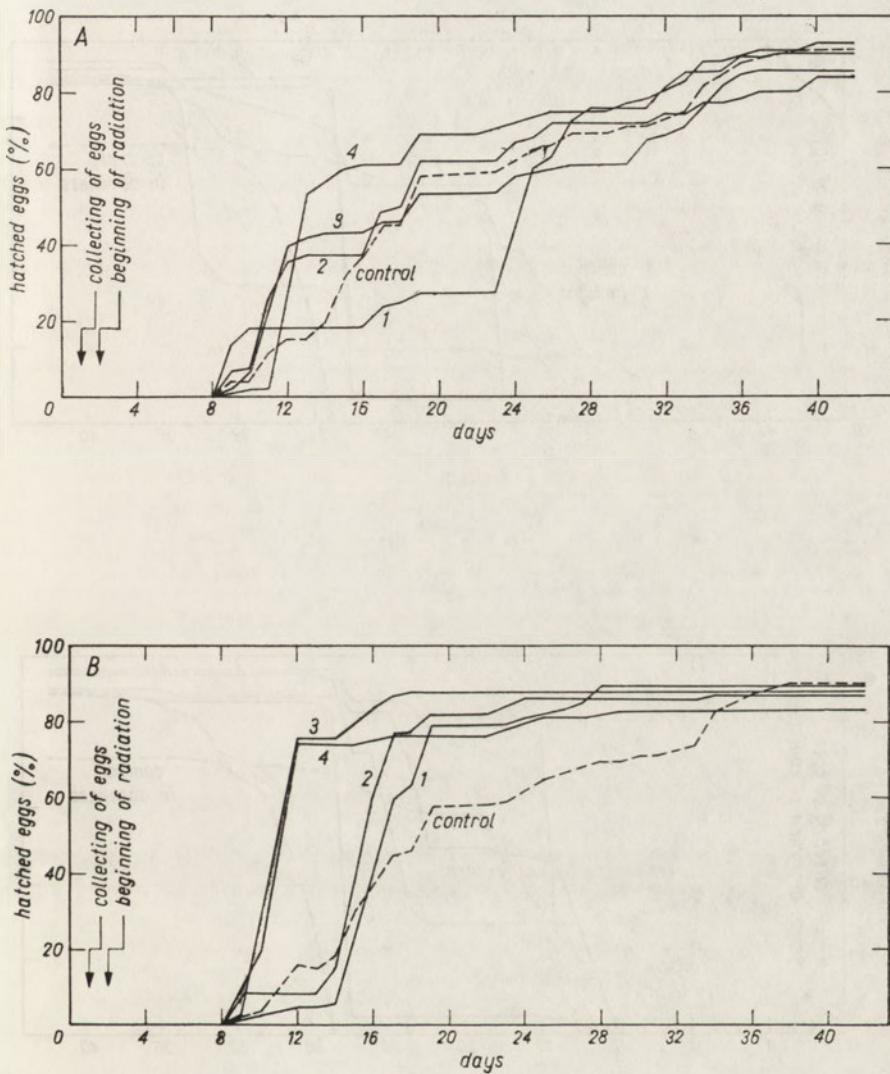


Fig. 1. Hatchability of eggs developing in the light. A—exposed with Hanovia lamp, 1-4 samples of series I; B—exposed with Q400 lamp, 1-4 samples of series II

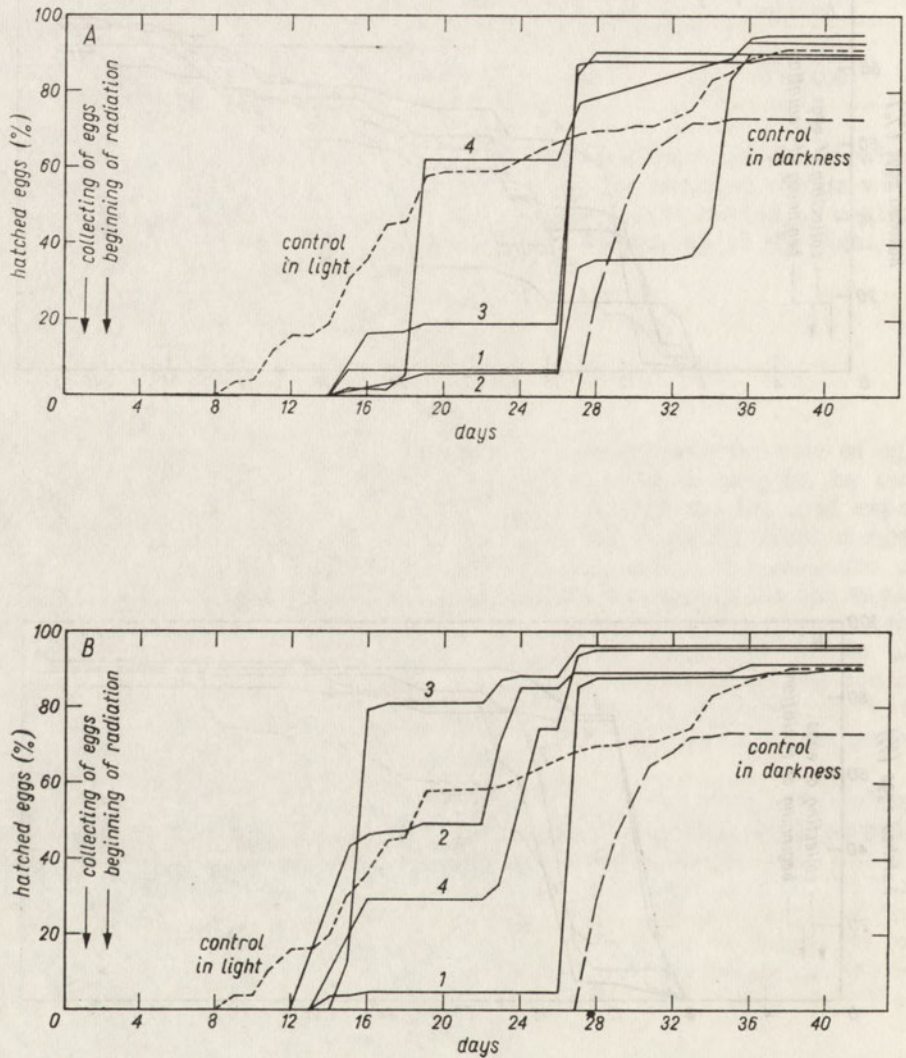


Fig. 2. Hatchability of eggs developing in the darkness. A—exposed with Hanovia lamp, 1-4 samples of series III, B—exposed with Q400 lamp, 1-4 samples of series IV

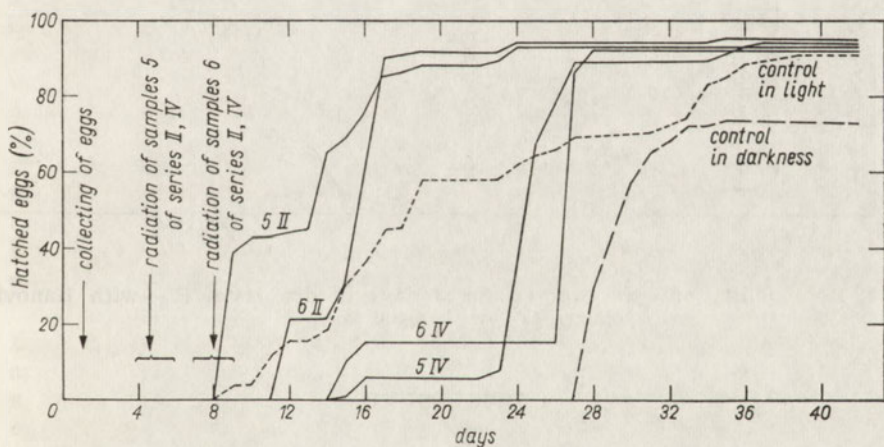
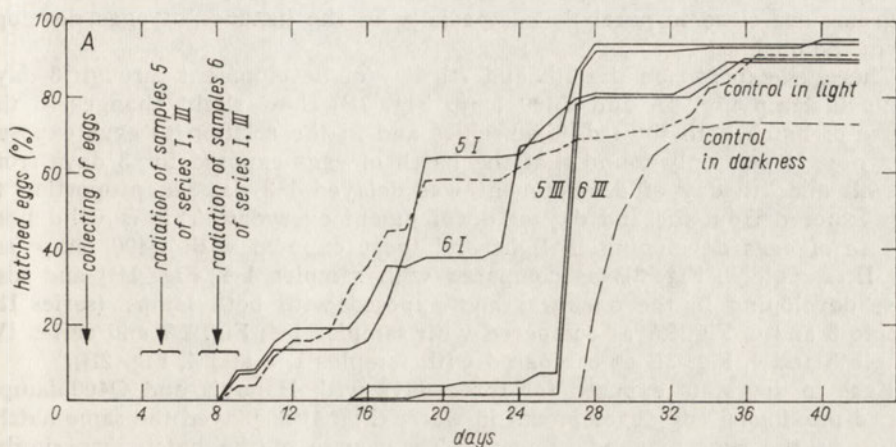


Fig. 3. Hatchability of eggs exposed for 3 days, starting from the fourth and seventh day of development (Arabic numerals designate the number of sample, Roman numerals—the number of series). A—exposed with Hanovia lamp 5 I, 6 I—development in the light, 5 III, 6 III—development in the darkness; B—exposed with Q400 lamp 5 II, 6 II—development in the light, 5 IV, 6 IV—development in the darkness

hatch) — an exception is sample 4 from the IVth series, which has a slower hatch than samples 2 and 3.

On the whole it can be said that the course of the hatch of exposed eggs is stepwise, while the control hatches have a more even course and their diagrams are close to parabola — especially in the instance of eggs developing in the darkness.

The eggs exposed on the 4th and 7th day of development through 3 days (Hanovia lamp Fig. 3A and Q400 lamp Fig. 3B) show slight changes in the course of hatch both towards themselves and in the relation to eggs exposed everyday. It was only found that the hatch of eggs exposed for 3 days from the 4th and 7th day of development was delayed 1–3 days in proportion to eggs exposed from the 2nd day of development everyday. This is valid both in case of eggs developing in light and those exposed with Q400 lamp (series II, sample 6, Fig. 3B as compared with samples 1–4, Fig. 1B) and also those developing in the darkness and exposed with both lamps (series III, sample 5 and 6, Fig. 3A as compared with samples 1–4, Fig. 2A and series IV, sample 5 and 6, Fig. 3B as compared with samples 1, 2 and 4, Fig. 2B).

Eggs in dry state exposed for three days with Hanovia and Q400 lamps, after a previous 6-day development in water (Fig. 4) displayed the same hatchability as the eggs exposed in water. The course of the hatch was similar to that of the control series.

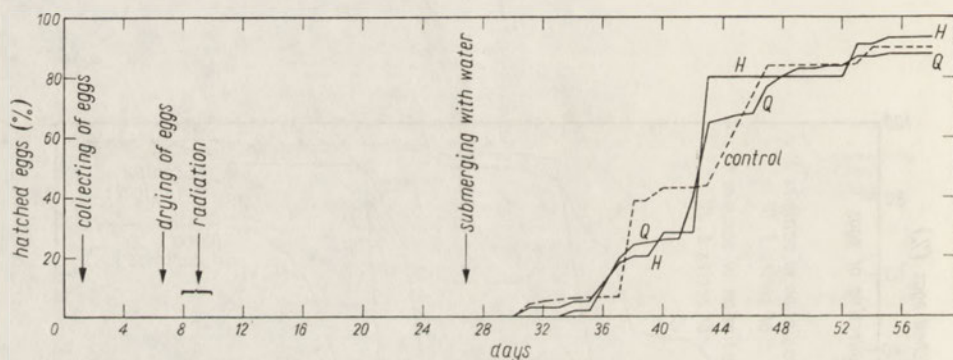


Fig. 4. Hatchability of eggs exposed for 3 days in dry state. H — with Hanovia lamp, Q — with Q400 lamp

#### 4. DISCUSSION

The results that have been obtained hitherto about the ultraviolet radiation on eggs or the embryos of invertebrates show that depending on the length of waves their results have a double character: 1. radiation of the area C (2000–2800 Å) and area B (2800–3200 Å) depending on the dosage may have a stimulating or an inhibiting influence on the development (BIZJULJAVIČJUS 1966), 2. radiation of the area A (3200–3800 Å) has a positive effect and is used sometimes as a reactivator after the harmful action of UV rays having shorter waves (WELLS and GIESE 1950, AMY 1965).

The applied here two sources of UV radiation having the maximum emiss-

ions 2537 Å and 3660 Å should have a completely different action according to the above presented data. However, the presented results of hatches show that the action of both lamps differed only slightly and also in both instances there were no distinct differences observed in the hatch of exposed and control eggs. The reason for it, as it seems, is the choice of applied here dosages. Therefore for the purposes of comparison I shall present the dosages applied in investigations on the eggs of other invertebrates, which have visible biological effects. GIESE (1946) while investigating the effect of UV radiation of a wavelength 2537 Å on the development of eggs of sea invertebrates applied dosages (depending on the species of animal) from 100 to 8000 erg/mm<sup>2</sup>, and exceptionally to 25,000 erg/mm<sup>2</sup> attaining at the maximum dosages the effect of the embryo's degeneration or their death. Within these limits are the dosages applied by WELLS and GIESE (1950) for the *Strongylocentrotus purpuratus* eggs and also applied by AMY (1965) for the *Habrobracon* sp. eggs.

The dosages, which I have applied at the maximum wavelength 2537 Å are from 324,000 erg/mm<sup>2</sup> to 4,738,000 erg/mm<sup>2</sup>, thus being on the average 1000 times bigger than the dosages in quoted works. Despite this these dosages did not cause any visible biological effects in the hatch of *Triops cancriformis* eggs. This is probably due to the great resistance of the eggs of this species to the UV radiation, which is probably the result of their two egg covers: cuticular and chitin (HEMPEL 1965), and is a general ecological adaptation to settle in astatic water bodies. The outside cover of a brown colour contains the pigment — derivative of hemoglobin (HEMPEL-ZAWITKOWSKA 1967) characterized by great absorption among others in this spectrum band. Therefore, possibly only a small part of radiation gets inside the egg. However, on the other hand solar radiation (within the range of the length of waves getting through the earth atmosphere) is necessary for normal development and penetrates the egg covers because the eggs developing in the darkness have a lower hatchability than those developing in the light. And apart from that their development is much slower. It should be pointed out that the eggs named in the discussed experiments as the eggs developing in the darkness were not completely deprived of light as they were taken out of the egg pouches in light, and the control of their hatch, lasting also few minutes was also in the light. Therefore it is possible that in a complete darkness there would be no development at all.

A comparison of the influence of radiation of a length 2537 Å (Hanovia lamp) and 3660 Å (Q400 lamp) on eggs developing both in the light (Fig. 1 and 2) and in the darkness (Fig. 3 and 4) shows that the waves 3660 Å have a better effect because they quicken the hatch of a considerable amount of eggs. This is consistent with the results of experiments carried out by AMY (1965) on the embryos of *Bracon* sp., in the instance of which the exposure with rays from 3200 Å to 4400 Å with the peak 3600 Å caused 93.6% of hatch, and the control eggs were hatched in 92.9%. The same author found that the above mentioned radiation causes a photoreactivation (withdrawal of negative effects caused by UV radiation of shorter waves), which takes place in a short time, and the longer exposures are without effect on further development. Possibly the UV radiation of the area A — like visible radiation — is a development stimulus, which acts in threshold quantities after which further effects do not take place. This could explain the fact that activation of the

hatch of *Triops cancriformis* eggs is not proportional to the increased radiation dosages of a wave length 3660 Å.

The stage of embryonic development is a factor changing the resistance of various eggs to the UV radiation. As it is seen in the investigations of AMY (1964) this dependence is not proportional, but the sensitivity increases and decreases during the development several times. BIZJULJAVIČJUS (1966), while investigating the influence of UV radiation on maturation and vitality of *Ascaris lumbricoides* and *Trichocephalus trichiurus* eggs found that the eggs in the stage of first division stria have the smallest resistance, while the larvae just before the hatch — the greatest.

A dependence of the radiation effects on the development was not found in this paper, because the eggs exposed in the second, fourth and seventh day of development do not display any basic differences in the hatch. Only a slight delay of hatches of eggs exposed in the later development stages may be noticed, but this requires repeated investigation using radiation sources of a greater emission intensity. As it seems, the applied dosages (perhaps as a result of little penetrable egg covers for UV radiation) are within the range of minimum stimulating stimulus, and only their considerable increase might show the total changes caused by this factor in the developing *Triops cancriformis* eggs.

## 5. CONCLUSIONS

1. *Triops cancriformis* eggs display a great resistance to UV radiation of a maximum wavelength 2537 and 3660 Å. This is connected with the presence of two egg covers, out of which the outside one — cuticular — is impregnated with pigment similar to hemoglobin (HEMPEL-ZAWITKOWSKA 1967).

2. Despite the application of almost 1000 times greater dosages of UV radiation (up to 6,804,000 erg/mm<sup>2</sup>) than in the papers quoted a decrease of hatchability of eggs was not noticed. The applied radiation had a rather stimulating effect, as it resulted in activation of the hatch of a considerable part of the eggs (especially of those exposed with rays 3660 Å) in relation to control eggs.

3. It was found that the diffused day-light is a factor mobilizing the hatch of eggs, as the hatch of eggs developing in the darkness was 17% lower and took place 19 days later than the hatch of eggs developing in the light.

## 6. SUMMARY

*Triops cancriformis* (Bosc) eggs from fish ponds from Warsaw surroundings were displayed to the action of UV radiation of a maximum wavelength 2537 Å and 3660 Å. For respective samples radiation dosages were applied from 324,000 erg/mm<sup>2</sup> to 6,804,000 erg/mm<sup>2</sup>. The eggs developing in the light and in the darkness (taken into the light only for the time of exposure) were exposed using two patterns of exposure: everyday till the beginning of hatch in the given sample and during for 3 days, starting from the fourth or seventh day of development. In respective samples the time of the hatch was noted, and then its per cent was calculated. *Triops cancriformis* eggs display a considerable resistance to UV rays and the applied dosages have a weak stimulating action.

## 7. STRESZCZENIE

Jaja *Triops cancriformis* (Bosc) pochodzące ze stawów rybnych z okolic Warszawy poddano działaniu promieniowania UV o maksimum długości fali 2537 Å i 3660 Å. Dla poszczególnych prób stosowano dawki promieniowania od 324 000 erg/mm<sup>2</sup> do 6 804 000 erg/mm<sup>2</sup>. Naświetlano jaja rozwijające się na świetle oraz w ciemności (wydobywane na światło jedynie na czas ekspozycji), stosując dwa schematy naświetlań: codzienne, aż do rozpoczęcia wylęgu w danej próbie, i okresowe — przez trzy dni, począwszy od czwartego lub siódmego dnia rozwoju. W poszczególnych próbach notowano czas wylęgu, a następnie obliczono jego procent. Stwierdzono, że jaja *Triops cancriformis* wykazują znaczną odporność na działanie promieni UV, a stosowane dawki mają słabe działanie stymulujące.

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## NUMBER DYNAMICS OF BENTHIC CHIRONOMIDAE (DIPTERA) IN RIVER SUPRAŚL

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### ABSTRACT

The species composition and the number of occurrence of benthic Chironomidae species were investigated on two sections of River Supraśl. 44 Chironomidae species were found. In autumn the numbers were several times greater than in the other seasons; the larvae number was greatest in the place where the organic sewage waters flow in. Species domination as well as maximal numbers depending on the kind of substrate were investigated.

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### 1. INTRODUCTION

*Chironomidae* larvae are quite important in production of benthos biomass. However, the dynamics and by the same biomass production varies in different types of water bodies.

KONSTANTINOV (1953), KAJAK (1958, 1959, 1960) and others discussed the Chironomidae of rivers and river-side water bodies. The increase of benthos biomass together with the increasing trophy of water body seems to be beyond discussion. A considerably greater number and richer development of benthos was noticed in water bodies enriched with organic substances of plant origin (MARKOSJAN 1959, LOFFE 1954), from mineral and organic fertilization (ZADIN 1957, 1959, PANKRATOVA 1957, WŁODEK 1962 and others), by leading organic sewage waters (JOHNSON and MUNGER 1930). But RYBAK (1962), while investigating the Chironomidae of River Wkra found that the number of larvae decreased in water polluted with sugar-factory sewage waters.

This research work has been carried out in order to determine the specific composition, observe the dynamics of more numerous Chironomidae species of River Supraśl taking into consideration some ecological factors. Systematic investigations were carried out from April 1967 to February 1968, and fragmentary samples were taken in 1965. This paper is an introduction to investigations, which are planned for the following years.

## 2. DESCRIPTION OF THE FIELD, MATERIAL AND METHODS

River Supraśl is the right-sided tributary of River Narew and forms numerous meanders. Its width ranges from few to several dozens of metres. The part of the river, on which the investigations were carried, was meliorated in the years 1965-1966. The mean depth during summer of the investigated part of river was about 1 m, and during the autumn and spring tides from 1.5-2.5 m. Table I and II present the temperatures of water, chemical composition of water and deposits. The municipal sewage waters are led into the River Supraśl some 300 m before the stations No. 2.

Table I. Chemical composition of the waters of River Supraśl in the period of investigations from the 11th of April to the 4th of February 1967

Specification	Range	Specification	Range
Temp. °C	2.5 -15.5	SO <sub>4</sub> mg/l	23.0 -27.0
pH	7.8 - 8.2	PO <sub>4</sub> mg/l	0.07- 0.09
O <sub>2</sub> mg/l	12.2 -15.2	suspension mg/l	20.0 -30.0
Fe mg/l	0.5 - 0.8	solid residue mg/l	40.0 -60.0
Ca mg/l	60.0 -68.12	organic substances mg/l	20.0 -30.0
NH <sub>3</sub> mg/l	0.12- 0.15	mineral substances mg/l	20.0 -30.0

Table II. Chemical composition of deposits on investigated stations

Station	Date from-to	Organic substances (%)	SiO <sub>2</sub> (%)	Fe <sub>2</sub> O <sub>3</sub> (%)	CaCO <sub>3</sub> (%)
1A	11.IV.67-4.XI.67	5.2 - 8.11	75.76-79.2	0.34-0.52	1.6-2.5
1B	15.V.67-4.XI.67	3.52-11.54	75.62-78.0	0.28-0.36	5.1-1.3
1C	15.V.67-4.XI.67	0.29- 0.46	78.26-86.4	0.2-0.013	0.8-1.3
2A	4.IX.67-4.XI.67	1.9 - 1.93	71.56-79.5	0.5	2.6-1.7
2B	4.IX.68-4.XI.68	0.91- 5.3	79.84-81.14	0.2-0.25	2.8-1.5
2C	4.XI.67	21.09-20.85	56.04-61.9	5.3-5.5	5.1-4.6
2D	4.IX.67	63.0-34.3	20.14-55.4	4-4.5	8.3-1.6

The material was sampled on the average twice during the month from six stations in two cross-sections of the river width. The stations were chosen by means of organoleptic estimation of substratum. On section No. 1 the series of samples were taken at the bank (1A), 1 m from the bank (1B), in the middle of the river (1C), and on the section No. 2 at the bank (2A), 1 m from the bank (2B), 5 m from the bank (2C) and 10-15 m from the bank (2D). The deposit on stations 1A, 1B, 2C was slimy, while on 1C the bottom was covered with fine yellow sand. In substratum of stations 2A and 2B gravel with pebbles of a diameter several cm, and on 2D great amounts of organic substances brought in by water. The material was sampled with a loaded Ekman dredge (on a line, the surface 225 cm<sup>2</sup>)—3 samples from determined

places of each station. Samples with a small amount of deposit were not taken into account. The deposit was sieved through sieves having the mesh surface  $0.5 \times 0.5$  mm. When selecting the material a simple microscope was used (Jena  $2 \times$ , made in Germany). In order to designate the growth classes the body length was measured with the help of millimetre scale exact to a millimetre and the length of cranium with eyepiece micrometer. The composition of species was determined acc. to the keys of GOETGHEBNER and LENZ (1937, 1939), ČERNOVSKIJ (1949), ROMANISZYN (1958) and FITTKAU et al. (1967). While analysing the chemical composition of deposit, the organic substances, silica, iron and calcium were determined.

### 3. RESULTS

In the basin of River Supraśl 20 Chironomidae species were found in station 1A, 18 — 1B, 26 — 2A, 32 — 2B, 23 — 2C, and 27 — 2D (Table III). The dominant species of the investigated water body are *Microtendipes ex. gr. chloris* (Meig.), *Endochironomus ex gr. dispar* (Meig.), *Cryptochironomus ex. gr. conjugens* (Kieff.) and *Procladius Skuse*.

Rarely and in small quantities occurred: *Potthastia longimanus* (Kieff.), *Psectrotanytus varius* (Fabr.), *Polypedilum ex gr. convictum* (Walk) and *Cryptochironomus ex gr. viridulus* (Fabr.) and others.

The average density of Chironomidae in section No. 1 (1A, 1B) in the investigated season was 927, and in the section No. 2 (2A, 2B, 2C, 2D) — 1485 larvae per  $1 \text{ m}^2$ .

However, in respective series and stations (Table IV) the species composition and number can considerably change. On section No. 1 an increase in number was observed in the middle of June, at the beginning of September and November, and a decrease — in the middle of June and end of September. In autumn a maximum increase of the number took place, i.e. from 2620 (1A — bank) to 4751 (1B — 1 m from the bank) larvae per  $1 \text{ m}^2$  (Table IV). During the period of investigations on station 1C the larvae were found only sporadically. More numerous both in species and number were the samples from section No. 2 (Table IV). On all stations after the fluctuations in the spring-summer period there is a rapid increase in number during late autumn, and a decrease in winter (with the exception of 2D).

The greatest number was found on station No. 2A — first days of November (4741 larvae per  $1 \text{ m}^2$ ) and No. 2B — in the middle of November (gravel — 10,123 larvae per  $1 \text{ m}^2$ ). At the increase in number the greatest number of species (16) was found in samples taken from the section 2B (thick gravel) at the beginning of September. At the beginning of February the number decreases in the majority of stations (Table IV). These great differences in the total number were conditioned by the dynamics of dominant species and those less numerous, but frequently found, and so:

1. *Microtendipes ex gr. chloris* (Meig.) — is a dominant species on station 2B (Fig. 1), in autumn, on other stations occurred rarely and in small amounts. After domination in April (from 46.4% — 1A to 68.7% — 2B) the number of this species rapidly decreases. During spring its number increases twice but very slightly. This species attained its maximum on station 2B at the end of September (8148 ind. per  $\text{m}^2$ , i.e. 87% of all Chironomidae). On the

Table III. Species composition and frequency of occurrence of Chironomidae on the investigated stations of River Supraśl (total number of samples — 270 in 90 series)

Species	Station						1	2	3
	1A	1B	2A	2B	2C	2D			
<i>Cryptochironomus ex gr. defectus</i> (Kieff.)	+	+	+	+	+	+	20	48.0	4.0
<i>Cryptochironomus ex gr. vulneratus</i> (Zett.)	+	+	+	+	+	+	17	44.0	2.9
<i>Cryptochironomus ex gr. viridulus</i> (Fabr.)			+				1	88.0	5.2
<i>Cryptochironomus borysthenicus</i> Tshern.				+	+	+	3	317.0	2.7
<i>Harnischia fuscimana</i> Kieff.	+	+	+	+	+	+	24	87.0	9.0
<i>Cryptochironomus ex gr. conjugens</i> Kieff.	+	+	+	+	+	+	14	119.0	24.4
<i>Paracladopelma campolabis</i> Kieff.	+		+	+	+	+	11	66.0	7.6
<i>Paralauterborniella brachylabis</i> (Edw.)		+				+	2	88.0	9.8
<i>Endochironomus ex gr. dispar</i> (Meig.)	+	+	+	+	+	+	43	626.6	33.3
<i>Endochironomus ex gr. tendens</i> (Fabr.)	+	+	+	+	+	+	14	545.5	4.2
<i>Microtendipes ex gr. chloris</i> (Meig.)	+		+	+	+	+	27	941.6	35.6
<i>Polypedilum ex gr. convictum</i> (Walk.)			+				1	36.5	6.1
<i>Polypedilum ex gr. nubeculosum</i> (Meig.)	+		+		+		4	36.5	3.6
<i>Polypedilum ex gr. scalaenum</i> (Schr.)			+	+	+	+	46	112.6	6.9
<i>Pentapedilum exsectum</i> Kieff.			+	+	+	+	4	117.0	6.3
<i>Chironomus f.l. salinarius</i> Kieff.				+	+		4	48.0	1.3
<i>Chironomus f.l. thummi</i> Kieff.	+	+	+	+			8	119.6	6.7
<i>Paratendipes ex gr. albianus</i> (Meig.)					+	+	2	33.0	5.5
<i>Tanytarsus ex gr. mancus</i> (Walk.)	+		+	+	+	+	24	305.8	15.5
<i>Tanytarsus ex gr. lauterborni</i> Kieff.	+					+	11	135.5	5.7
<i>Tanytarsus ex gr. gregarius</i> Kieff.	+	+	+	+	+	+	60	161.7	18.4
<i>Micropsectra praecox</i> Meig.				+	+	+	3	26.4	1.1
<i>Cricotopus ex gr. algarum</i> Kieff.		+	+	+	+	+	9	61.6	3.3
<i>Cricotopus ex gr. silvestris</i> F.	+		+				3	77.0	6.5
<i>Epoicocladus ephemeræ</i> (Kieff.)				+		+	9	44.0	1.6
<i>Orthoclaadiinae gen? l. triquetra</i> Tshern. sp.n.				+		+	9	44.0	1.2
<i>Synorthocladus semivirens</i> (Kieff.)		+	+		+	+	5	79.2	8.5
<i>Cricotopus inaequalis</i> Kieff.		+	+	+	+	+	15	66.9	2.7
<i>Eukiefferiella hospita</i> Edw.?			+	+			2	17.6	1.2
<i>Diamesa ex gr. insignipes</i> Kieff.			+	+			2	220.0	6.2
<i>Potthastia longimanus</i> (Kieff.)				+			2	36.5	1.2
<i>Prodiamesa ex gr. bathyphila</i> (Kieff.)	+	+	+	+		+	17	74.8	6.0
<i>Prodiamesa flabellata</i> Kieff.	+	+	+	+			9	47.5	1.8
<i>Thienemanniella flaviforceps</i> Kieff.		+		+	+		5	40.9	3.3
<i>Corynoneura celeripes</i> Winn.				+	+		6	22.0	3.0
<i>Ablabesmyia ex gr. monilis</i> (L.)				+		+	2	22.0	10.0
<i>Psectrotanypus varius</i> (Fabr.)					+	+	1	44.0	2.0
<i>Anatopynia plumipes</i> (Fries.)	+			+			2	6.6	1.4
<i>Apsectrotanypus trifascipennis</i> (Zett.)	+	+	+	+			8	44.0	6.3
<i>Clinotanypus nervosus</i> (Meig.)				+	+		4	41.8	5.1
<i>Procladius Skuse</i>	+	+	+	+	+	+	41	374.0	26.6
<i>Ablabesmyia ex gr. lentiginosa</i> (Fries.)	+		+	+	+	+	30	211.0	12.7
	20	18	26	32	23	27			

1 — Number of series containing the given species.

2 — Average density per 1 m<sup>2</sup> (in samples containing the given species).

3 — Mean per cent of individuals in samples containing the given species (%).

Table IV. The number of Chironomidae on the investigated stations of River Suprasl taking into consideration the species numbers

Date of sampling	1 A		1 B		2 A		2 B		2 C		2 D	
	No. sp.	No. ind./m <sup>2</sup>	No. sp.	No. ind./m <sup>2</sup>	No. sp.	No. ind./m <sup>2</sup>	No. sp.	No. ind./m <sup>2</sup>	No. sp.	No. ind./m <sup>2</sup>	No. sp.	No. ind./m <sup>2</sup>
12.IV.1967	5	556	6	178	4	238	11	1244	8	1232	6	510
26.VI.	3	440	5	133	5	133	10	1200	13	1056	8	510
15.V.	4	266	5	155	10	682	12	1820	6	660	7	511
1.VI.	5	244	2	66	12	1144	9	1243	14	1056	7	244
15.VI.	4	400	9	755	10	44	8	2156	13	1221	10	1110
2.VII.	1	133	5	488	8	1232	8	755	6	444	6	488
17.VII.	2	344	3	89	1	22	1	89	4	355	4	577
29.VII.	3	133	3	533	2	264	3	400	3	488	4	355
13.VIII.	4	189	5	440	3	308	7	1021	4	533	1	44
1.IX.	10	1598	10	1354	7	1276	16	5372	5	1243	8	444
4.IX.	10	821	7	488	6	572	11	2464	11	511	8	311
15.IX.	6	577	4	777	8	1584	9	10034	4	710	5	1287
16.IX.	5	2176	2	2975	2	844	11	3286	6	1421	8	2575
30.IX.	6	2620	5	4751	10	4741	12	5280	7	1332	8	2353
14.X.	6	2200	6	4600	3	2353	8	10123	7	1954	7	2220
4.XI.	5	622	5	666	3	488	8	2309	7	622	7	1998
18.XI.	5	622	5	666	3	488	8	2309	7	622	7	1998
2.II.1968	5	622	5	666	3	488	8	2309	7	622	7	1998
Av. No. ind./m <sup>2</sup>		826		1028		992		3051		928		971

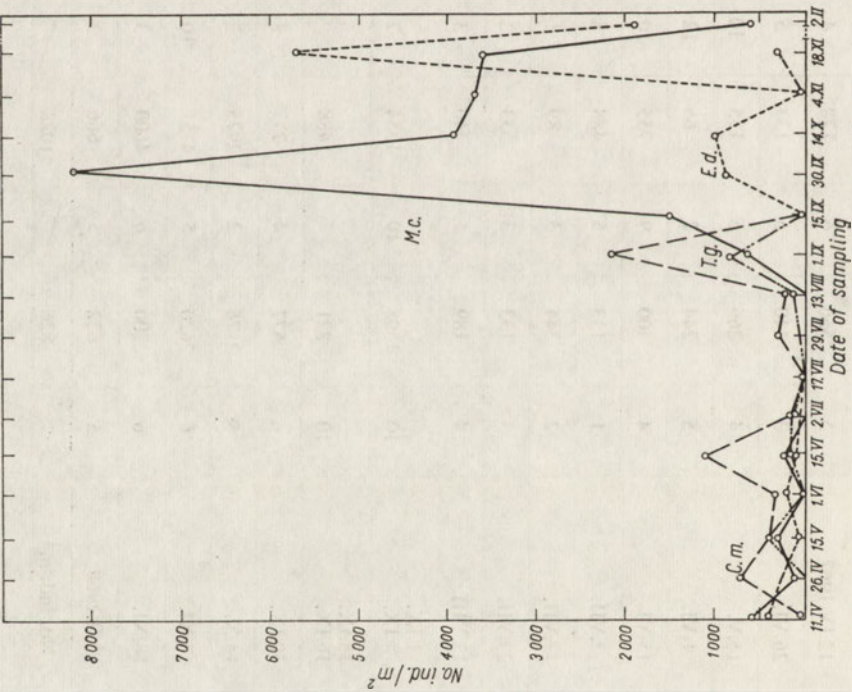


Fig. 1. Number dynamics of dominant species on station 2B (thick gravel). M.c. — *Microtendipes* ex gr. *chloris* (Meig.); T. g. — *Tanytarsus* ex gr. *gregarius* Kieff.; E.d. — *Endochironomus* ex gr. *dispar* (Meig.); C.m. — *Tanytarsus* ex gr. *manomus* (Walk.)

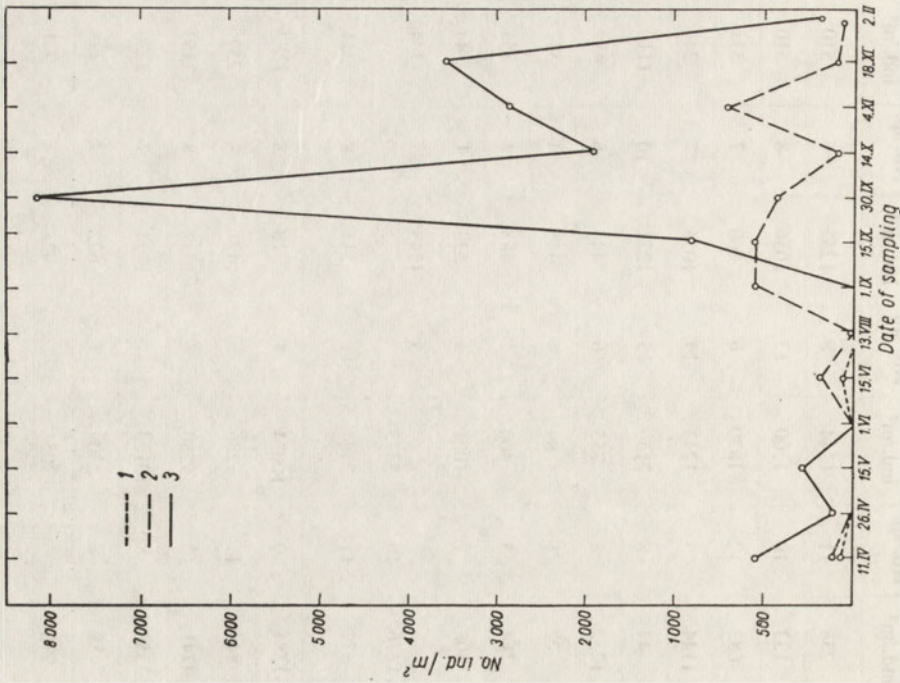


Fig. 2. Number of *Microtendipes* ex gr. *chloris* (Meig.) in the period of investigations on station 2B (gravel). 1 — class 1; 2 — class 2; 3 — class 3

basis of the cranium measurements three classes of growth were distinguished: the first to 200  $\mu$ , the second 225–350  $\mu$  and the third 375–550  $\mu$  (Fig. 2).

2. *Endochironomus ex gr. dispar* (Meig.) — in spring and summer a slight, several times increase in number of this species was observed on stations 1A and 1B, and a greater increase on 2A and 2B (Fig. 3). The maximum increase in number ranged (in autumn) from 2110 (1A) to 5632 (2B) larvae per 1 m<sup>2</sup>. On the basis of cranium measurements three growth classes were distinguished: the first to 150  $\mu$ , the second from 175–300  $\mu$ , and the third from 325–450  $\mu$ . In the examined samples the two last classes were usually found with the dominance of the third one (Fig. 3).

3. *Procladius Skuse* — found on all stations, most numerous on 2C and 2D. During autumn its number rapidly increases attaining even 60% (i.e. 1320 larvae per 1 m<sup>2</sup> — 2D) of all Chironomidae (Fig. 4, 5, 6, 7, 8).

4. *Cryptochironomus ex gr. conjugens* Kieff. — found almost on all stations, but most numerous on station 2B during summer months (July, August — up to 480 larvae/m<sup>2</sup>).

5. *Ablabesmyia ex gr. lentiginosa* (Fries) — this species was only found in greater amounts on stations 2C and 2D — with a large content of organic substances. The greatest amount of this species was found at the beginning of February (1480 larvae per 1 m<sup>2</sup> — 2D) (Fig. 5 and 6).

6. *Tanytarsus ex gr. gregarius* Kieff. — most numerous occurred on station 2C. An increase in number was observed at the beginning of September and in the middle of November. During winter the increase in number is very slight only on station 2D (Fig. 1, 5, 6, 8).

7. *Tanytarsus ex gr. manicus* (Walk) — occurs more numerous only on 2 stations of section No. 2 (2A and 2B). During the investigated period its number increased several times (Fig. 1 and 4). In samples taken in late autumn only few individuals of this species were found.

8. *Polypedilum ex gr. scalaenum* (Schr.) — frequently found in the examined samples, but in small quantities. Its greatest number was found on station 2A (beginning of June) — 600 larvae per 1 m<sup>2</sup> (Fig. 4, 7 and 8).

9. *Harnischia fuscimana* Kieff — larvae of this species occurred in small amounts on all stations. During the spring-summer period its number increased 3 times (Fig. 4, 5, 6, 8).

10. *Paracladopelma camptolabis* Kieff. — found on 5 stations of River Supraśl. Altogether several hundred of individuals of this species were found (Fig. 7).

#### 4. DISCUSSION

On all stations considerable differences were observed in the dynamics and number of Chironomidae. In the second part of summer the number (on majority of stations) is greater than that in the early spring. The greatest numbers were observed in autumn, which is consistent with data from literature. MCTHES (1966), when investigating Chironomidae of Stechlinsees Lake observed a maximum increase in number of *Microtendipes ex gr. chloris* (Meig.) in the early spring. During winter the number decreases (with the exception of 2D). Great differences were observed both in the species com-

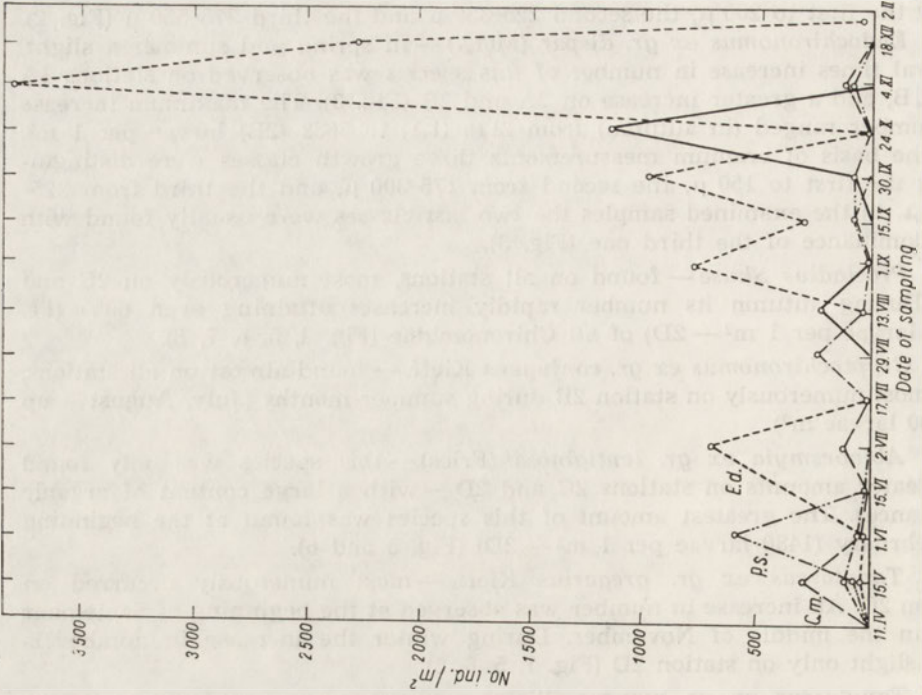


Fig. 4. Number dynamics of dominant species on station 2A (bank). C.m. — *Tanytarsus ex gr. mancus* (Walk.); P.s. — *Polypedilum ex gr. scalaenum* (Schr.); E.d. — *Endochironomus ex gr. dispar* (Meig.)

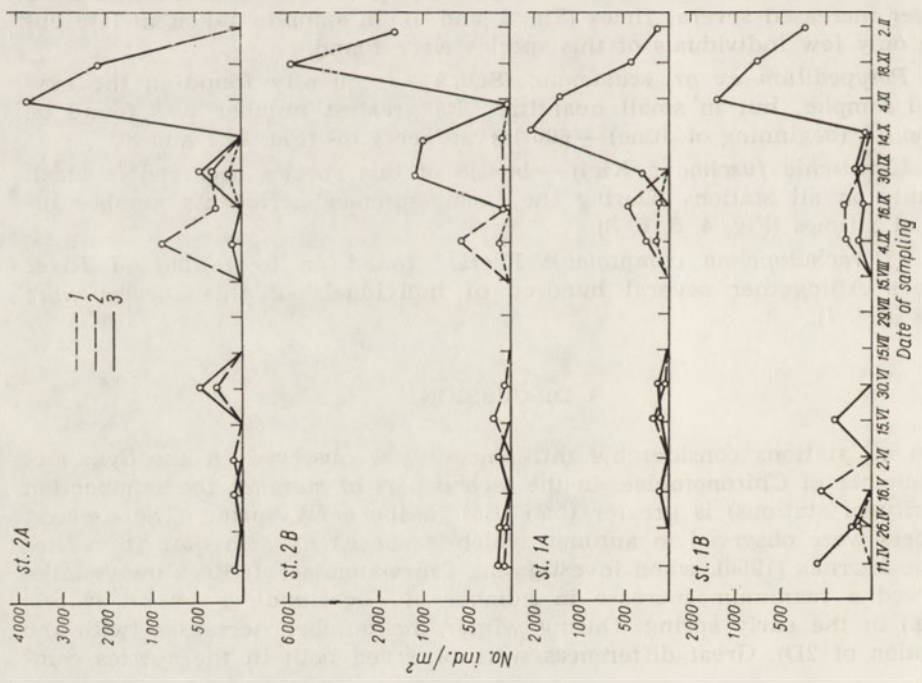


Fig. 3. Number of *Endochironomus ex gr. dispar* (Meig.) on respective stations. 1 — class 1; 2 — class 2; 3 — class 3



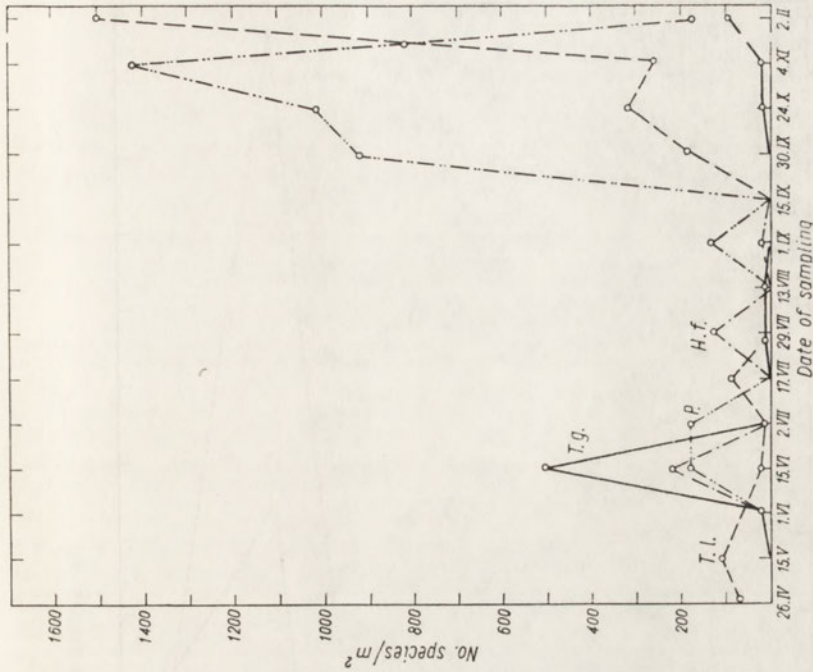


Fig. 6. Number dynamics of dominant species on station 2D. T.l. — *Ablabesmyia* ex *gr. lentiginosa* (Fries.); T.g. — *Tanytarsus* ex *gr. gregarius* K.; P. — *Procladius* Skuse; H.f. — *Harnischia fuscimana* Kieff.

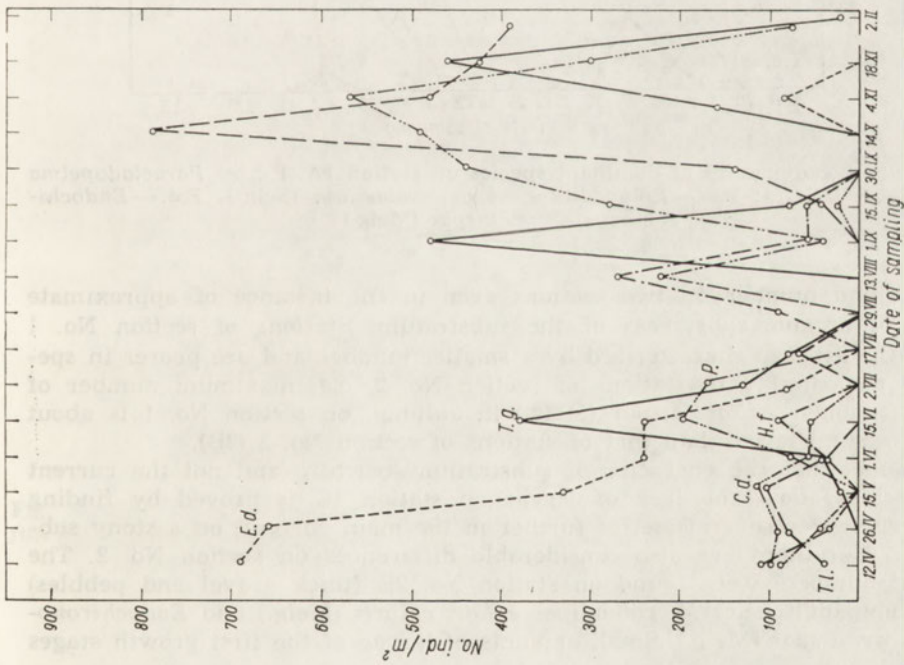


Fig. 5. Number dynamics of dominant species on station 2C. C.d. — *Cryptochironomus* ex *gr. defectus* (Kieff.); T.l. — *Ablabesmyia* ex *gr. lentiginosa* (Fries.); H.f. — *Harnischia fuscimana* Kieff.; T.g. — *Tanytarsus* ex *gr. gregarius* K.; P. — *Procladius* Skuse; E.d. — *Endochironomus* ex *gr. dispar* (Meig.)

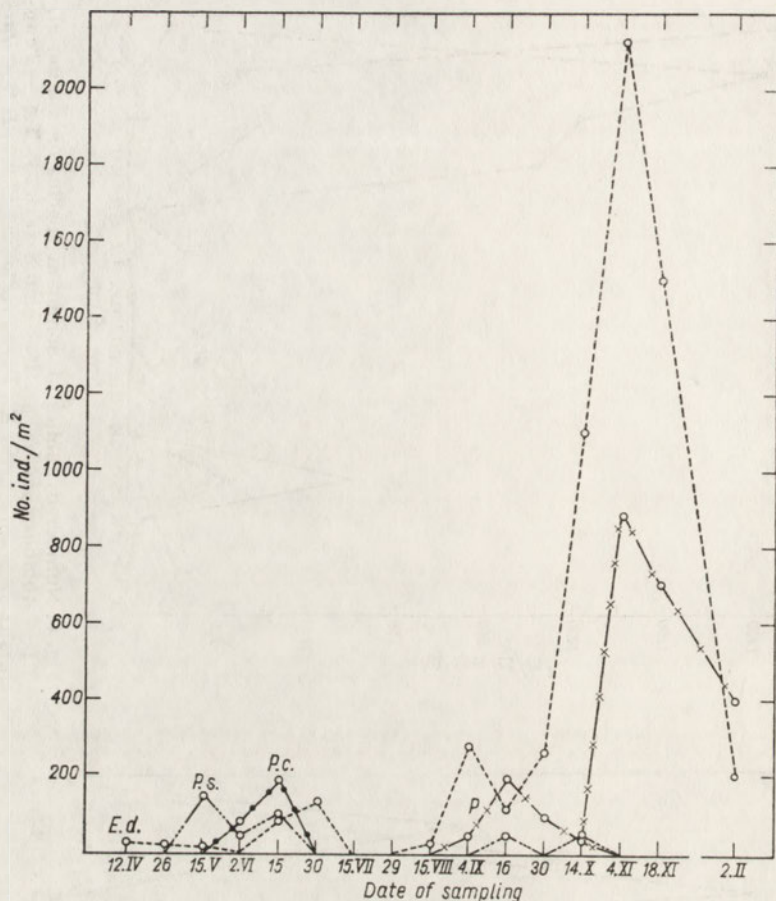


Fig. 7. Number dynamics of dominant species on station 1A. P.c. — *Paracladopelma camptolabis* (Kieff.); P.s. — *Polypedilum ex gr. scalaenum* (Schr.); E.d. — *Endochironomus ex gr. dispar* (Meig.)

position and number on two sections even in the instance of approximate values of organic substances of the substratum. Stations of section No. 1 are on the average characterized by a smaller number and are poorer in species as compared with stations of section No. 2, e.g. maximum number of *Endochironomus ex gr. dispar* (Meig.) in autumn, on section No. 1 is about 3500 larvae/m<sup>2</sup> lower than that of stations of section No. 2 (2B).

The fact that the character of substratum structure and not the current rate decides about the lack of larvae on station 1C is proved by finding numerous larvae several metres further in the main current, on a stony substratum. But there are also considerable differences on section No. 2. The greatest numbers were found on station No. 2B (thick gravel and pebbles) with dominant species *Microtendipes ex gr. chloris* (Meig.) and *Endochironomus ex gr. dispar* (Meig.). Small amounts of larvae of the first growth stages

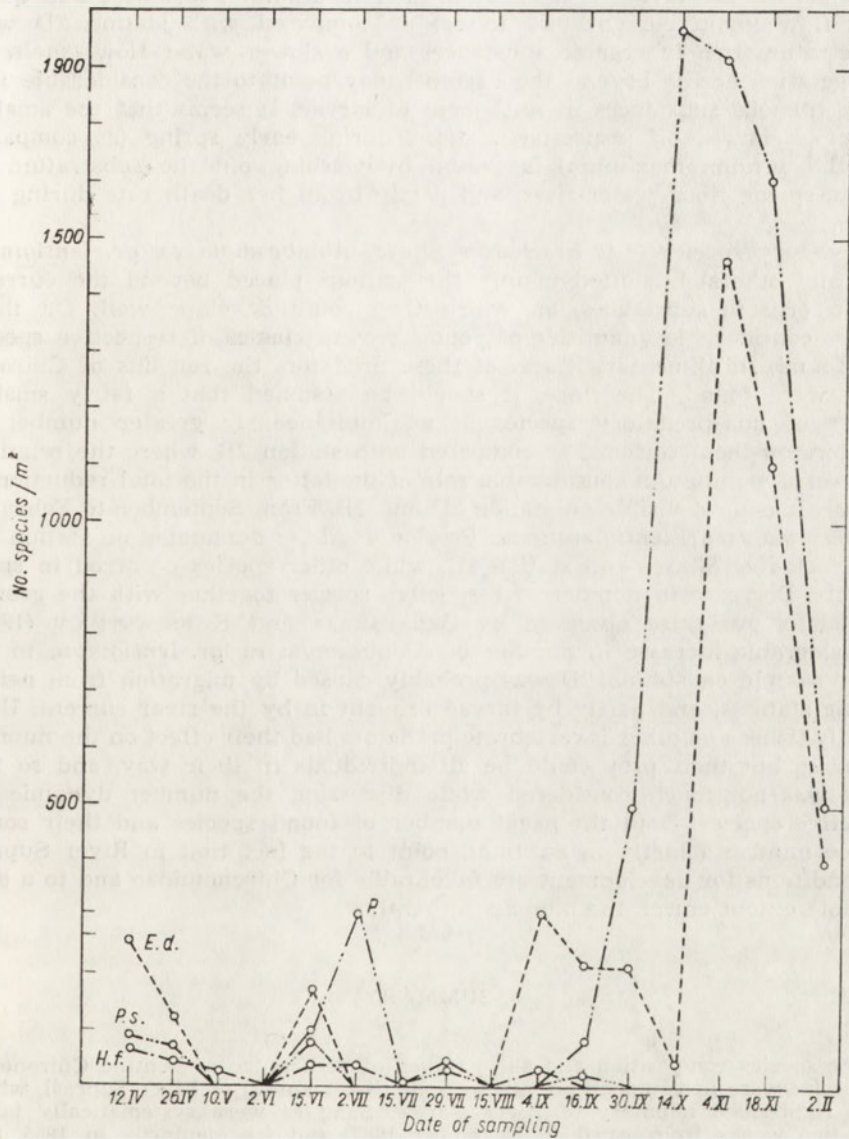


Fig. 8. Number dynamics of dominant species on station 1B. H.f.—*Harnischia fuscimana* Kieff.; P.s.—*Polypedilum ex gr. scalaenum* (Schr.); E.d.—*Endochironomus ex gr. dispar* (Meig.); P.—*Procladius* Skuse

of these species in samples should be explained by the possibility of getting through the mesh of sieves by larvae of this size.

Both the differences in the species composition and the total higher number of station 2B having a substratum poor in organic substances and quick water flow (main current 0.50 m/sec) as compared with station 2D with a substratum rich in organic substances and a slower water flow (sheltered by vegetation and is beyond the current) may point to the considerable role of allochthonous substances in settlement of larvae. It seems that the smaller number of larvae on respective stations during early spring (as compared with the autumn maximum) is caused by washing out the substratum by autumn-spring flood water-rises, and partly by higher death rate during the winter.

Predatory species (e.g. *Procladius Skuse*, *Ablabesmyia ex gr. lentiginosa* Fries and others) inhabited mainly the stations placed beyond the current, rich in organic substances, on which they could develop well. On these stations considerable quantities of young growth classes of respective species were found. In alimentary tracts of these predators the remains of Chironomidae were found. Therefore, it should be assumed that a fairly smaller number of non-predatory species at a simultaneously greater number of predators on these stations, as compared with station 2B, where the relations are reverse, points to a considerable role of the latter in the total reduction of number. It is most visible on station 2D and 1B. From September to February *Ablabesmyia ex gr. lentiginosa* and *Procladius Skuse* dominated on station 2D, and *Procladius Skuse* — on station 1B, while other species occurred in small amounts. Decrease in number of respective species together with the growth of predator was also observed by BELAVSKAJA and KONSTANTINOV (1956). A considerable increase in number of *Ablabesmyia ex gr. lentiginosa* in the winter sample on station 2D was probably caused by migration from neighbouring stations, and partly by larvae brought in by the river current. Undoubtedly fishes and other invertebrate predators had their effect on the number reduction, but their prey could be all individuals in their way, and so this factor was not much considered while discussing the number dynamics of respective species. Both the great number of found species and their considerable number (chiefly in autumn), point to the fact that in River Supraśl the conditions for development are favourable for Chironomidae and to a considerable extent enrich the biomass of benthos.

## 5. SUMMARY

The species composition and the number of occurrence of benthic Chironomidae species were investigated on two sections (No. 1 and 2) of River Supraśl, which is the right-sided tributary of River Narew. Samples were systematically taken every two weeks from April to November 1967, and fragmentarily in 1965, and at the beginning of 1968.

44 Chironomidae species were found in River Supraśl. In the place, where the organic sewage waters flow in, a greater number of larvae was found as compared to the place without sewage waters.

In autumn the numbers were several times greater than in the other seasons. The maximum number — in autumn on section No. 1 was 4751, and on No. 2 — 10123 larvae/m<sup>2</sup>. The average number of Chironomidae on section No. 1 was 927, and on the section No. 2 — 1485 larvae per 1 m<sup>2</sup>.

On section No. 1 the smallest number was found on sandy substratum with a small amount of organic substances, and the greatest — on sandy-loamy.

On section No. 2 the smallest number occurred in substratum with a large amount of decomposed organic substance, and the greatest among the thick gravel and pebbles.

The following species dominated in River Supraśl: 1. *Microtendipes ex gr. chloris* (Meig.), 2. *Endochironomus ex gr. dispar* (Meig.), 3. *Procladius Skuse*, 4. *Cryptochironomus ex gr. conjugens* Kieff.

On the basis of cranium measurements three growth classes were distinguished for the two first species. Predatory species dominated on stations with substratum having a large content of organic substances.

## 6. STRESZCZENIE

Badano skład gatunkowy oraz liczebność występowania bentosowych gatunków Chironomidae na dwóch przekrojach (nr 1 i 2) rzeki Supraśl, która jest prawostronnym dopływem rzeki Narew. Próby pobierano systematycznie co dwa tygodnie, od kwietnia do listopada 1967 r., zaś wycinkowo w 1965 oraz na początku 1968 r.

W rzece Supraśl stwierdzono występowanie 44 gatunków Chironomidae. W miejscu, gdzie dopływają ścieki organiczne stwierdzono kilkakrotnie większą liczebność larw w porównaniu do miejsca niepodlegającego wpływowi ścieków.

Jesienią stwierdzono liczebności kilkakrotnie większe niż w pozostałych sezonach. Maksymalna liczebność — jesienią na przekroju nr 1 wynosiła 4751, zaś nr 2 — 10123 larw/m<sup>2</sup>. Średnia liczebność Chironomidae na przekroju nr 1 wynosiła 927, a na przekroju nr 2 — 1485 larw na 1 m<sup>2</sup>.

Na przekroju nr 1 najmniejszą liczebność stwierdzono na podłożu piaszczystym, z niewielką zawartością substancji organicznych, zaś największą — w piaszczysto-ilałym.

W przekroju nr 2 najmniejsza liczebność występowała w podłożu z dużą ilością częściowo rozłożonej substancji organicznej, a największa wśród grubego żwiru i otoczków.

W rzece Supraśl dominowały następujące gatunki: 1. *Microtendipes ex gr. chloris* (Meig.), 2. *Endochironomus ex gr. dispar* (Meig.), 3. *Procladius Skuse*, 4. *Cryptochironomus ex gr. conjugens* Kieff.

W oparciu o wymiary długości puszki głowowej wyróżniono trzy klasy wzrostowe dla dwóch pierwszych gatunków. Gatunki drapieżne dominowały na stanowiskach o podłożu z dużą zawartością substancji organicznych.

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SEASONAL CHANGES OF NITROGEN-FIXING AND  
NITRIFYING AND DENITRIFYING BACTERIA IN  
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## ABSTRACT

The distribution of nitrogen-fixing bacteria (*Azotobacter* and *Clostridium pasteurianum*) in the bottom sediments of the Iława lakes depends mainly on the bottom type. These microorganisms occur more numerous in bottom deposits of the muddy type and much less in sandy bottom deposits. Seasonal fluctuations in the development of these bacteria display maximum numbers in the vegetative period and minimum numbers in winter. The temperature of bottom sediments and their character, to a lesser extent, are of great significance in the development of nitrifying bacteria. This is the same also for the nitrification intensity. The number of denitrifying bacteria is higher in the muddy sediments at a limited oxygen access. Their activity increases in the vegetative period.

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## 1. INTRODUCTION

Nitrogen is one of the more important biogenic components and the productivity of waters depends on its amount and character of its compounds. Several authors (BIRGE and JUDAY 1911, NAUMANN 1932, YOSHIMURA 1932 and others) consider nitrogen as one of the main indicators of the degree of trophy of lakes. This element is found in the reservoir mainly by the process of bonding out of the atmosphere by microorganisms and some algae, and also as a result of mineralization of plant and animal remains.

The spread of nitrogen-fixing bacteria in water bodies is uneven. *Azotobacter*, which undoubtedly is the most significant in the nitrogen-fixation process, occurs in greater amounts only in the littoral zones of lakes (KOSOVIC 1896, ISACENKO (1914) 1951, SALIMOVSKAJA-RODINA 1939) and in bottom deposits (NEČAEVA and

SALIMOVSKAJA-RODINA 1935, SALIMOVSKAJA-RODINA 1939, KUZNECOV 1952, SEN 1955, KRASHENNIKOVA and NOVOZILOVA 1959, SALMANOV 1959, CORBERI and SOLAINI 1960, VARGA and TAKÁTS 1960 and others), frequently together with cellulose bacteria using the metabolism products of the latter (IMSENECKIJ 1949, OSTERTAG 1952). Also considerable numbers of anaerobic bacteria of *Clostridium* type which can fix nitrogen are found in bottom deposits of lakes (ŠTURM and SIMAKOVA 1929, ŠTURM et al. 1941, ŠTURM and KANUNNIKOVA 1945, GAMBARIAN 1957, 1958, KUZNECOV 1952, RODINA 1958) and also bacteria mineralizing the plant and animal remains. Among the latter, the nitrifying bacteria are of special significance for the productivity of water bodies. According to ULJANOVA (1961) these microorganisms occur in greater numbers only in bottom deposits containing some oxygen reserve. But even at a lack of oxygen the reduction processes which are the result of development and activity of denitrifying bacteria, mainly of *Pseudomonas* type, are also possible (SALMANOV 1966).

Although the occurrence of bacteria taking part in the nitrogen cycle in lakes has been thoroughly studied, the information about their distribution and activity in bottom deposits is barely sufficient. In Poland such investigations were not carried on a larger scale. The scarce data on the subject are only with regard to bottom deposits of small water bodies of Warsaw surroundings, which are not typical for the majority of our lakes. Therefore, in the years 1960–1963 an attempt was made to characterize the development dynamics of this microflora in bottom deposits of the Ilawa lakes (Jeziorak Mały and Jeziorak) on the Mazurian Lakeland. Because of their situation and various degree of pollution (NIEWOLAK 1966a, b, 1968) these lakes are an interesting object for hydrobiological investigations.

## 2. MATERIAL AND METHODS

Samples of the bottom deposits were taken more or less once a month by means of the Ekmann sampler into sterile Petri dishes on 15 various sites of the Ilawa lakes (Fig. 1). The choice of sites was done taking into account their distribution and type of bottom (sandy, muddy, sapropel). A separate paper (NIEWOLAK 1968) contains a description of investigated lakes and chosen sites. Then in the laboratory 10 g of wet weight of the deposit was dissolved in 90 ml of 0.85% NaCl solution and shaken for 15 min on a shaker and plated on suitable media.

The following determinations were made in the samples of bottom deposits under investigation:

1. Bacteria of *Azotobacter* type on a breeding-ground (MALISZEWSKA 1954) at 25°C during 6 days.
2. Anaerobic nitrogen-fixing bacteria (titre *Clostridium pasteurianum*) in Winogradski's medium at 25°C during 14 days (RODINA 1956).
3. Nitrifying bacteria of the I and II phase (titre *Nitrosomonas*, *Nitrosococcus* and *Nitrobacter*) in liquid mediums (FICDOROW 1952) at 25°C during 28 days.
4. Denitrifying bacteria (titre) in Giltay's medium at 25°C during 2 days (RODINA 1956).

All the determinations were accomplished in three parallel platings and the results obtained calculated per 1 g of dry or wet weight of the deposits. When determining with the titre method in liquid media (anaerobic bacteria of *Clostridium* type, nitrifying and denitrifying bacteria) the positive titre was the solution, in which at least 2 out of three parallel platings displayed the presence of a determined group of bacteria.



In years 1962-1963 simultaneously with the determination of particular physiological groups of bacteria in sandy and sapropel deposits of the Iława lakes, the oxidation intensity of ammonium salts and nitrites by nitrifying bacteria was investigated as well as the reduction of nitrates by denitrifying bacteria.

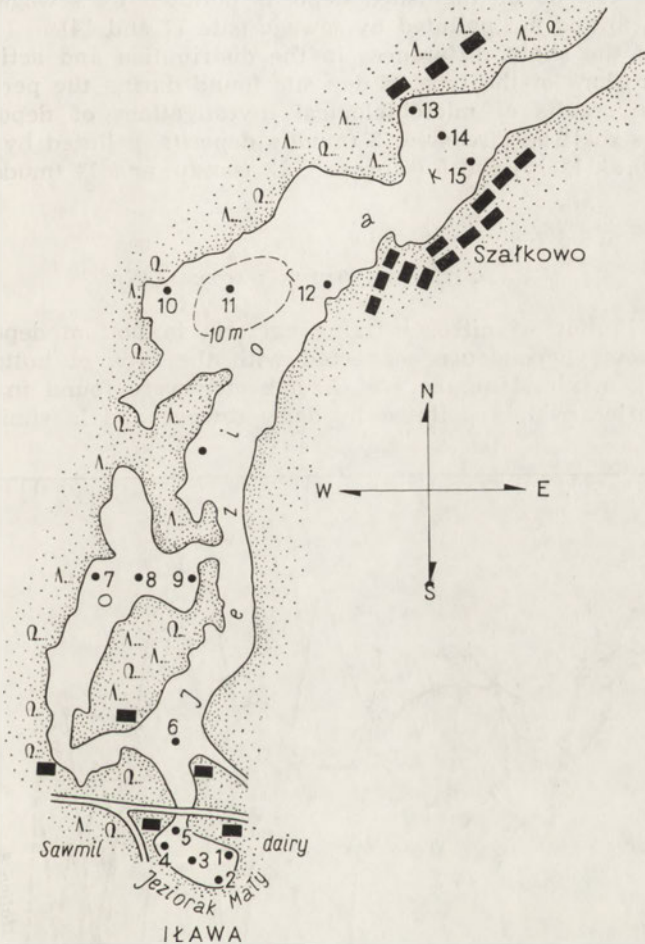


Fig. 1. Localization of the investigated Iława lakes (Jeziorak Mały and Jeziorak). Numbers denote sampling stations

In the studies on the intensity of nitrogen conversions the applied method was the one used by RHEINHEIMER (1959) in his investigations of similar processes in the waters of River Elba.  $N-NO_2$  was determined according to ALLPORT (1956) using the photoelectric colorimeter of KF2 type (dark-green filter No. 4 VG6 Schott).

## 3. RESULTS

From the point of abundance in organic matter of the Ilawa lakes three main types of deposits can be recognized: sandy type containing 1-2% of organic substances (sites 7, 9, 10, 12, 13 and 15), muddy deposits containing about 40% of organic substances (sites 1-6, 11 and 14), and sapropel deposits in the region of Moty (site 8), on the depth from 2 to 6 m. Among the muddy deposits there can be distinguished deposits polluted by sewage of organic origin (sites 1-6) and not polluted by sewage (site 11 and 14).

Because of the slight differences in the distribution and activity of biochemical microflora of the type of deposits found during the period of investigations, the results of microbiological investigations of deposits on the following sites shall be discussed: 3 (muddy deposits, polluted by sewage deposits of Jeziorak Mały) and 8 (sapropel), 13 (sandy) and 14 (muddy in Jeziorak).

## A. NITROGEN-FIXING BACTERIA

In the distribution of nitrogen-fixing bacteria in bottom deposits of the Ilawa lakes some dependence connected with the type of bottom may be observed. E.g., maximal numbers of *Azotobacter* were found in the muddy grounds of Jeziorak Mały polluted by dairy sewage and in similar deposits

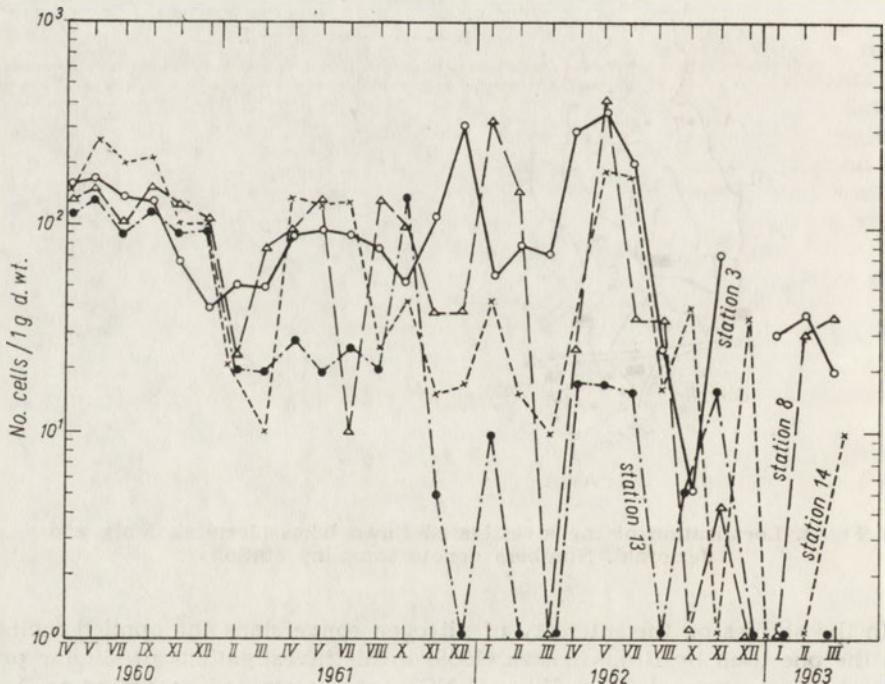


Fig. 2. Number of *Azotobacter* cells in the bottom deposits of Ilawa lakes in the years 1960-1963 (mean values out of 3 parallels)

of Lake Jeziorak. Approximate numbers of these bacteria were also found in the sapropel deposits of Lake Jeziorak in the region of Moty (Fig. 2). In sandy parts, poor in organic substances, *Azotobacter* were found in small numbers rarely exceeding 100 cells/g dry weight of deposits. In all types of bottom deposits the *Azotobacter* occurred in the spring-autumn period. In autumn their number begins to decrease to tens of cells/g dry weight, attaining its lowest value in winter.

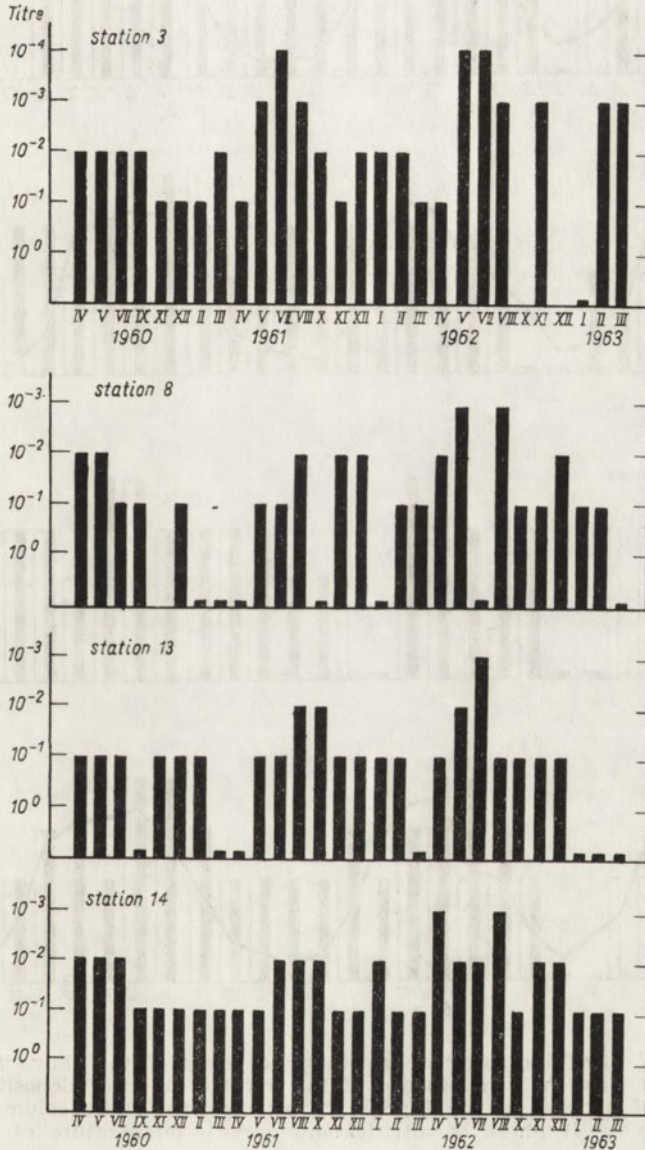


Fig. 3. Titre of sporeforming anaerobes (*Clostridium pasteurianum*) fixing nitrogen in bottom deposits of the Ilawa Lakes in the years 1960-1963

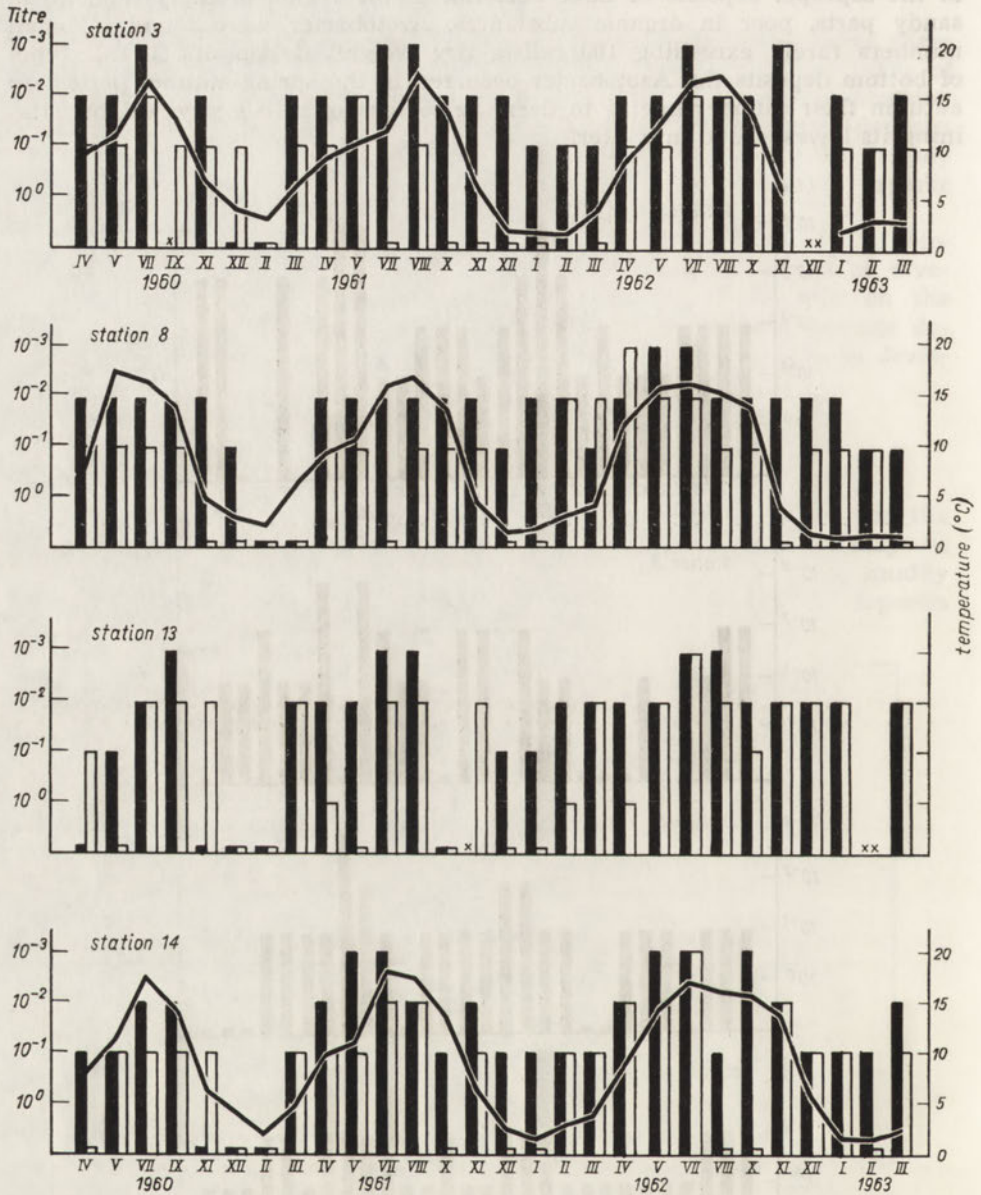


Fig. 4. Titre of nitrifying bacteria in bottom deposits of the Ilawa lakes in the years 1960-1963, and the temperature of the surface layer of deposits. Dark columns — titre of bacteria of the I phase of nitrification; white columns — titre of bacteria of the II phase of nitrification; curve — temperature of deposits

The titre of *Clostridium pasteurianum* in bottom deposits of the Ilawa lakes (Fig. 3) ranged from 0 to 0.001, exceptionally 0.0001 as on site 3 in the deposits of Jeziorak Mały polluted by dairy sewage. In general greater numbers of these bacteria were found in spring and summer, and smaller — in the autumn-winter period at a lowered temperature of the medium. Differences in the numbers of anaerobic nitrogen-fixing bacteria in the bottom deposits of various type were slight, however smaller numbers of these bacteria were more frequently in sandy deposits.

#### B. NITRIFYING BACTERIA

Figure 4 presents the distribution of nitrifying bacteria in the bottom deposits of the Ilawa lakes. The maximum development of nitrifying bacteria in the I phase most frequently takes place in summer (titre 0.001), more rarely in winter as e.g. in the muddy deposits of Jeziorak Mały (site 3). In winter the numbers of these bacteria were minimal or even non-existent at the plating of 1 ml of deposits. Also the differences in the quantitative occurrence of nitrifying bacteria of the I phase were not observed depending on the type of bottom deposits.

Nitrifying bacteria of the II phase usually occurred in smaller numbers than the bacteria of the I phase of nitrification. Their maximum (titre 0.01) occurred more frequently in summer, although in some instances their similar numbers were also observed in spring, summer and even in winter, which proves that in the development of this group of microorganisms the temperature is probably of lesser importance than in the instance of bacteria from the I nitrification phase.

Simultaneously with the intensification or inhibition of the development of nitrifying bacteria an increase or weakening of the nitrification strength was observed in the bottom deposits of the Ilawa lakes. The maximum intensity of oxidation of ammonium to nitrites took place in the period of maximal temperatures at the greatest number of nitrifying bacteria, i.e. from July to August, attaining 48–51 mg N-NO<sub>2</sub>/l (Fig. 5). As the temperature of the medium and the number of nitrifying bacteria of the I phase decrease, the intensity of the process decreases to 0.5–3 mg N-NO<sub>2</sub>/l in winter. The maximum intensity of this process is observed in the muddy deposits of Jeziorak in summer, and in the sapropel deposits of this reservoir in winter.

In the instance of the II nitrification phase an increase of the nitrification strength is observed starting May to the end of August within the limits 180–200 mg oxidized N-NO<sub>2</sub>/l and a considerable decrease in autumn and winter. In this last period the amount of oxidized N-NO<sub>2</sub> was 54–85 mg/l. With the exception of winter the differences in the intensity of the process in particular types of deposits were relatively small (of the order of several mg oxidized N-NO<sub>2</sub>/l). Sapropel deposits of Lake Jeziorak displayed in winter a greater nitrification strength.

#### C. DENITRIFYING BACTERIA

Denitrifying bacteria display a relatively high sensibility to temperature. These microorganisms were found in bottom deposits of the Ilawa lakes quite numerously, and their titre ranged from 0.1 to 0.0001 depending on the de-

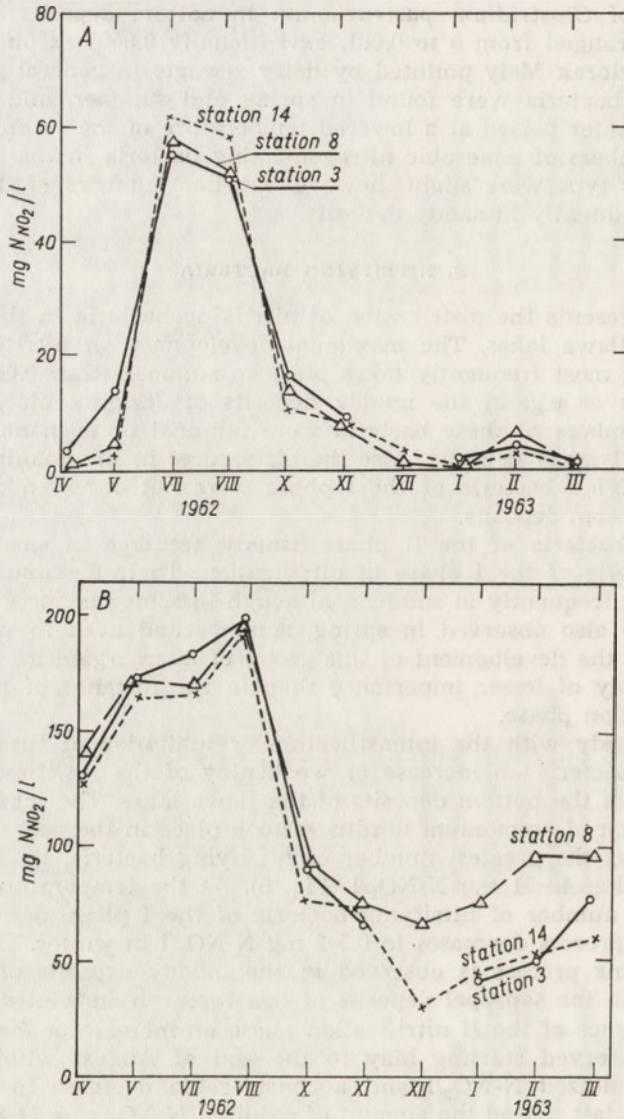


Fig. 5. Course of nitrification in bottom deposits of the Ilawa lakes on stations 3, 8, 14 in the years 1962/1963 (mg of N-NO<sub>2</sub> consumption/l after 21 days of incubation at 25°C). A—I phase; B—II phase

posit type and season of the year (Fig. 6). In muddy deposits of the polluted by sewage Jeziorak Mały and in sapropel deposits in Lake Jeziorak the denitrifying bacteria occurred in maximal amounts almost during the entire period of investigations. The seasonal differences were small and were of 1 order of magnitude. The medium temperature is significant in the distribution of denitrifying bacteria in sandy grounds and muddy bottom deposits of Lake

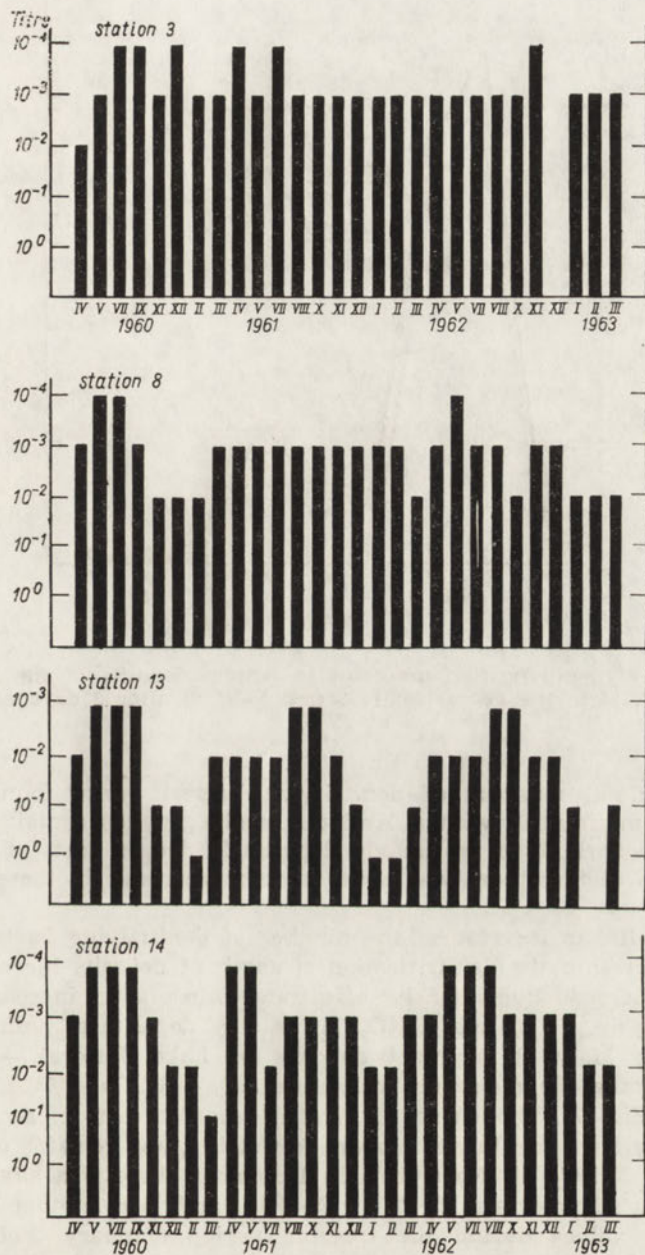


Fig. 6. Titre of denitrifying bacteria in bottom deposits of Ilawa lakes (mean values out of 3 parallels)

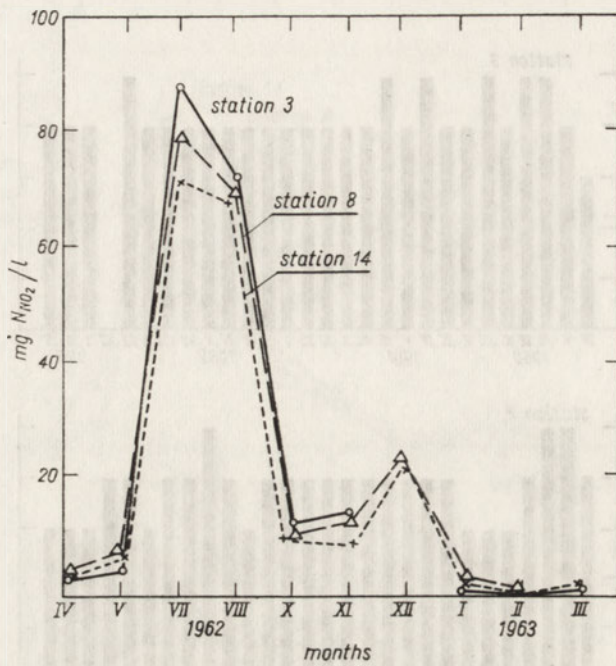


Fig. 7. Course of denitrification processes in bottom deposits of the Iiawa lakes on stations 3, 8, 14 in the years 1962/1963 (mg N-NO<sub>2</sub>/l after 2 days of incubation at 25°C)

Jeziorak. Maximal numbers of denitrifying bacteria were found there in summer, and minimal in winter. And the sandy grounds contain much less denitrifying bacteria than the muddy deposits in deeper parts of this reservoir. It is typical that there was never a negative result in these investigations.

Together with an increase of the number of denitrifying bacteria and of medium temperature the denitrification strength of deposits increases. In all experiments the reduction intensity of nitrates into nitrites increased already early in the spring to 2.5 mg N-NO<sub>2</sub>/l in muddy deposits of polluted by sewage Jeziorak Mały, in sapropel deposits of Lake Jeziorak—to 4.2 mg N-NO<sub>2</sub>/l, attaining the maximum values in July—88.0 and 79.0 mg N-NO<sub>2</sub> respectively (Fig. 7). The curve of denitrification in bottom sediments decreases rapidly from the July maximum to October, and remains on the level of several mg N-NO<sub>2</sub> till November. In December there is a slight increase of the intensity of process to 23-24 mg N-NO<sub>2</sub>/l and then another decrease to the minimum values during the freezing period (January, February and March). In July and August the muddy deposits of sewage Lake Jeziorak Mały and sapropel sediments of Jeziorak display a maximal denitrification. In the remaining period the differences in the denitrification density in particular types of deposits were small and only in November they exceeded 5 mg N-NO<sub>2</sub>/l.



## 4. DISCUSSION

Nitrogen-fixing bacteria, and especially *Azotobacter* are a relatively small group of microflora of bottom deposits of the Hława lakes. The bottom type is decisive in their distribution. In the muddy deposits, where the content of organic substances is quite big and in poorly mineralized sapropel deposits *Azotobacter* occurred sometimes in several times greater numbers than in sandy deposits. In that respect the results of investigations carried out on the Hława lakes are equal to the data of KUZNECOV (1952) and GAMBARIAN (1958) obtained for bottom deposits of lake reservoirs. The first quoted author found in the bottom deposits of some eutrophic lakes of the U.S.S.R. (the lakes Białe near Pokrowo, Czernoe in Kosino and other) less than 300 cells of *Azotobacter* in 1 g dry weight, while the second observed in the bottom deposits of Lake Sewan smaller numbers of these bacteria in the inshore sandy deposits, and higher in the central part of the reservoir with a muddy bottom. Quantitative differences in the occurrence of *Azotobacter* cells in the bottom deposits of the Hława lakes are conditioned by a varying content of mineral compounds and organic substances, which are the source of carbon and energy for these microorganisms. More frequent occurrence of *Azotobacter* in the muddy deposits of Jeziorak Mały is undoubtedly in relation with the dairy sewage containing a large amount of carbohydrates and possibly the accumulation of organic acids during the decomposition of mill sawdust which are found on a quite big surface of lake bottom.

Anaerobic nitrogen-fixing bacteria of *Clostridium* type were found in the bottom deposits of the Hława lakes in slightly greater numbers than *Azotobacter*. They were frequently observed in platings of 0.001 and even 0.0001 g wet weight of deposits. This seems to correspond approximately with the number of these bacteria in bottom deposits of eutrophic Lake Czernoe in Kosino. KUZNECOV (1952) sometimes found there up to 1000 *Clostridium pasteurianum* cells in 1 g deposits. Higher numbers of anaerobic nitrogen-fixing bacteria especially in bottom deposits of Jeziorak Mały are obviously the result of oxygen-free conditions frequently occurring there and of the content of carbohydrates supplied by the dairy sewage (NIEWOLAK 1966a).

The seasonal fluctuations of the number of nitrogen-fixing bacteria in bottom deposits of the Hława lakes observed during the investigations can be connected with the reproduction period of bottom invertebrates (ŻUKOWSKA 1960) and also with the dying of the planktonic and benthic plant and animal organisms, which enriches the bottom deposits with nourishing substances for these bacteria. For example, RODINA (1958) noticed that fertilization of ponds with plant mass increased 100 times the number of *Azotobacter* and *Clostridium pasteurianum* in the bottom deposits. As the nourishing substances disappeared, consumption of the cells of these bacteria by zooplankton considerably decreased the number of nitrogen-fixing bacteria.

Lack of data on the intensity of nitrogen-fixation by *Azotobacter* and *Clostridium pasteurianum* does not allow to draw final conclusions about the significance of these microorganisms in the investigated water bodies. But the number of these bacteria allows to assume that the nitrogen-fixation in bottom deposits of the Hława lakes is due to the participation of both types of bacteria as opposed to the water habitat of these reservoirs, where only *Azotobacter* are abundant (NIEWOLAK 1966a).

Also, nitrification bacteria are a small group of microorganisms in the bottom deposits of the Hawa lakes. The titre of nitrification bacteria of the I phase generally corresponded with the values obtained by RODINA (1958) for bottom deposits of some Lithuanian ponds, where they occurred from 1 to 100 cells/ml deposits. These numbers are higher than those given by KUZNECOV (1952) for bottom deposits of lake reservoirs of the European part of the U.S.S.R. In the latter the nitrification bacteria occurred in numbers from 1 to 10 cells/ml deposits. However, there is no indication whether these data were obtained in summer or in some other season of the year. But it is known that the summer maximum of nitrifying bacteria is clearly marked. This is indicated by the observations of many authors and results of investigation of this group of bacteria in the Hawa lakes (NIEWOLAK 1966b). Intensification of development of nitrifying bacteria of the I phase in summer is also reflected by an increase of nitrification strength of bottom deposits during that period. In that respect the investigations described here are equivalent to the data by RHEINHEIMER (1959, 1964) on the seasonal changes in the activity of the discussed process in the water bodies. This dependence was also observed by FISCHER (1960) in small water bodies in the surroundings of Warsaw.

In the development of nitrifying bacteria of the II phase (Nitrobacter) the temperature is probably of lesser significance and therefore it frequently happened that in spring, autumn and even in winter they occurred in numbers found in summer. However, their biochemical activity was considerably higher in summer than in winter.

The occurrence of nitrifying bacteria — outstanding anaerobes in the bottom deposits of the Hawa lakes — is very interesting because of the frequently taking place there oxygen-free conditions (NIEWOLAK 1966a). But these observations are not a novelty. There are reports proving the existence of nitrifying bacteria in bottom deposits of Barents Sea (NIKITINA 1955) and in the hydrogen sulphide zone of Black Sea having oxygen-free conditions (KRIS 1959). Perhaps as ULJANOVA (1961) says, in various types of waters, soils and bottom deposits there are ecological *Nitrosomonas* strains having specific morphological and physiological properties adapted to life in conditions of limited oxygen access.

The denitrifying bacteria are a relatively widespread group of microorganisms in bottom deposits of the Hawa lakes, as they have favourable development conditions especially in summer, when there is sometimes a considerable oxygen deficit and great amount of easily accessible carbon resources. The greatest numbers of these bacteria in bottom deposits of the Hawa lakes (titre 0.0001) were approximate to the data of KUZNECOV (1952) obtained for similar eutrophic habitats of eutrophic lakes of the U.S.S.R. The great number of denitrifying bacteria usually corresponds with a great number of proteolytic bacteria (NIEWOLAK 1968). Among the latter there are many species displaying reduction abilities. It is even considered that the summer maximum of the development of proteolytic bacteria is caused in the majority by species partially denitrifying (NIKITINA 1955). Together with the decrease of temperature of the medium the number of denitrifying bacteria decreases to such an extent that the autumn maximum of proteolytic bacteria is not accompanied any more by stronger development of species that can partially reduce the nitrates.

The decrease of the number of denitrifying bacteria in the bottom deposits of lakes investigated in winter 1962/1963 was also reflected in the experiments on the intensity of the course of nitrate reduction. While the number of denitrifying bacteria decreases the chemical analyses of experimental samples display a slight increment of nitrites. This is in total agreement with the data of RHEINHEIMER (1964), who said that the activity of denitrifying bacteria is imperceptible at low temperatures and practically equals 0.

The differences in the number of denitrifying bacteria and the reduction intensity of nitrates in various types of bottom deposits can be explained by a various content of organic substances and by oxygen conditions.

## 5. SUMMARY

The type of bottom and the temperature of medium are of the decisive significance in the distribution of nitrogen-fixing bacteria (*Azotobacter* and *Clostridium*). The maximum development of these bacteria is observed in bottom deposits rich in organic substances, especially in the spring-summer period. In the development of nitrifying bacteria, and especially of *Nitrosomonas* and *Nitrosococcus*, first of all the dependence on the temperature of surroundings can be observed. The maximum development of these bacteria and the intensity of processes they cause are observed in summer at higher temperatures, independently from the type of deposits, while in winter it is minimal. Smaller numbers of nitrifying bacteria of the II phase (*Nitrobacter*) are found in bottom deposits of the Iława lakes, and temperature is not so important for their development. The development of denitrifying bacteria is connected with the bottom type and temperature of bottom deposits. The latter affects also the intensity of reduction processes.

## 6. STRESZCZENIE

W rozmieszczeniu bakterii wiążących azot atmosferyczny (*Azotobacter* i *Clostridium*) w osadach dennych jezior iławskich zasadnicze znaczenie posiada charakter dna oraz temperatura środowiska. Maksymalny rozwój tych bakterii obserwuje się w osadach dennych bogatych w substancje organiczne zwłaszcza w okresie wiosennó-letnim. W rozwoju bakterii nityfikacyjnych, zwłaszcza *Nitrosomonas* i *Nitrosococcus*, zauważyć można przede wszystkim zależność związaną z temperaturą otoczenia. Maksymalny rozwój tych bakterii oraz intensywność wywołanych przez nie procesów obserwuje się latem przy wyższej temperaturze, niezależnie od typu osadów, minimalny zimą. Bakterie nityfikacyjne II fazy (*Nitrobacter*) występują w osadach dennych jezior iławskich w ilościach mniejszych, przy czym w ich rozwoju temperatura zdaje się odgrywać mniejszą rolę. W rozwoju bakterii denityfikacyjnych obserwuje się zależność związaną z typem dna oraz z temperaturą osadów dennych. Ta ostatnia wpływa również na intensywność procesów redukcyjnych.

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H. WYSOCKA-BUJALSKA

This is the last paper by the late Dr. Hanna WYSOCKA-BUJALSKA. She spent the last years of her scientific activity in Department of Experimental Hydrobiology and she left the best memory as a scientist, fellow-worker and Human Being. May this posthumous publication be an homage paid to her by the Editorial Board and her fellow-workers.

THE ALGAE FROM A MODEL POND  
OF THE M. NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY<sup>1</sup>

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ABSTRACT

A list of algae occurring in the model pond was made up basing upon the material left by the late Authoress. The number of six species of algae not noticed up to date in Poland was found, as well as one new species, denominated in memory of the late Authoress — *Clathrochloris hanna*e.

After unexpected death of Dr. Hanna WYSCCKA-BUJALSKA, member of the research staff of the Nencki Institute, notices and preparations of algae last worked out by her were handed me by her daughter Dr. Gabriela GRIM-BUJALSKA. The materials concerned plankton or seston collected in the small experimental pond situated on the grounds of the Institute.

The basin was excavated of the ground in a shape of a truncate inverse pyramid, its surface was nearly 6.5 m<sup>2</sup> at the ground level and 1 m<sup>2</sup> at the bottom; it was isolated from the ground with a layer of igelite foil supported by wooden slats. The 30 cm layer of mud at the bottom was formed of bottom sediment brought from a natural pool in Puszcza Kampinoska forest, where from also originated the water used for filling the experimental pool. Plants were not included deliberately.

The pool was investigated first in 3 years after filling. During the period of observation from May 1966 to May 1967 the pool was periodically overgrown by abundant vascular autochthonous vegetation and covered with duck-weed (*Lemna minor*). The occurrence in water of Cladocera, Copepoda, Protozoa, Rotatoria as well as of insect larvae (Ephemeroptera, Diptera etc.) was noticed. The density of bacteria in water oscillated within a wide scope from 4.3·10<sup>9</sup> to 120.6·10<sup>9</sup> cells per litre.

The water temperature (measured with thermistor thermometers with continuous recording) was relatively stabilized due to the duck-weed cover. Diurnal temperature changes did not exceed 2.5°C; over the year the temperature oscillated from 0.5 to 21°C. The water level depended solely upon natural factors, i.e. evaporation and refilling by atmospheric precipitations. The water pH over the period of observation was 6.6–7.5.

Chemical analysis of water was performed every 7 days. The concentrations of particular constituents were as follows: sulphate 15.15–99.15 mg/l, orthophosphate 0.02–0.72 mg/l, nitrate nitrogen 0–0.15 mg/l, nitrite nitrogen 0–0.17 mg/l, ammonium nitrogen 0.04–2.72 mg/l, oxygen 0–12.4 mg/l, carbon dioxide (total) 12.5–233.2 mg/l.

These materials consisted of samples of seston obtained by centrifugation or by filtration through membrane filters Coli 5, and of several glass plates with algae settled on them. The Authoress left also detailed notices and drawings of the identified algae; I saw moreover a part of this material in the course of its working out during her two visits in my Laboratory in Cracow.

<sup>1</sup> The material was put together and elaborated by Prof. Dr. K. STARMACH, Department of Hydrobiology, Jagiellonian University, Cracow, Oleandry 2a. Poland.



The material although rather scanty comprises several species found in Poland first, as well as one new species not described till now. For this reason I have undertaken willingly the task of composing the notices and preparing them for publication. They deserve it undoubtedly.

The new species of the genus *Clathrochloris* was characterized according to the preparation and notices of the late Authoress and in her memory denominated *Clathrochloris hannaë*.

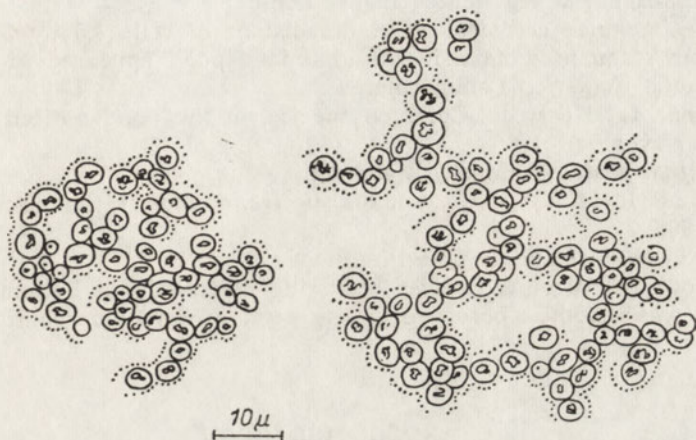


Fig. 1. Typical colonies of *Clathrochloris hannaë*

The following species were till now not reported from Poland: *Microcystis testacea*, *Tetrachloris merismopedioides*, *T. diplococcus*, *Achroonema macromeres*, *A. simplex*, *Pelonema subtilissimum*, *P. aphane*.

The species *Gloeoskene turfosa* is identified only with partial certitude; it could not be found in dry preparations available, whereas the drawing and notices were too scanty, insufficiently emphasizing specific features of the species.

#### CYANOPHYTA

##### 1. *Synechocystis aquatilis* Sauvageau

Cells about  $5.8 \mu$  in diameter (in diagnosis of the species  $4.5-6.0 \mu$ ), singular or double. Common species, occurring usually in bottom mud of stagnant water bodies.

##### 2. *Synechococcus cedrorum* Sauvageau

Eliptical cells,  $6-8 \mu$  long,  $3-4 \mu$  wide. Common species, known from stagnant waters. In the investigated pond it appeared in May, 1966.

##### 3. *Rhabdoderma lineare* Schmidke et Lauterborn

Irregular small colonies, cells  $4.7-8.2 \mu$  long,  $0.9-2.3 \mu$  wide (diagnostic dimensions:  $6-8 \mu$  long,  $0.8-2.5 \mu$  wide). Species frequently found in pond plankton. In the investigated pond found first in July, 1966.

##### 4. *Microcystis testacea* (Nägeli) Elenkin

Colonies nearly spherical, cells  $7.5-9.5 \mu$  in diameter. Species common in mud and in damp places. In the pond it was found in July, 1966, first time in Poland.

5. *Aphanothece saxicola* Nägeli

Irregular diffused colonies, cells 2–3  $\mu$  long, 1.5  $\mu$  wide. Common species, in the investigated pond found in June, 1966.

6. *Gomphosphaeria compacta* (Lemm.) Strom.

Irregular spherical colonies, cells 4–6  $\mu$  long, 1.5–3.0  $\mu$  wide. Species occurring in small water bodies, in the pond under investigation found in July, 1966.

7. *Gomphosphaeria năgeliana* (Unger) Lemm.

Oval or irregular colonies, mean dimensions of cells 4.5 by 2  $\mu$ . Species known from stagnant waters, in the examined pond appeared in July, 1966.

8. *Lyngbya limnetica* Lemmermann

Trichomae 1–1.5  $\mu$  wide. Common species, in the pond noticed from September to November, 1966.

9. *Lyngbya aestuarii* (Mert.) Liebmann

Trichomae 10–12  $\mu$  wide. Common species, found in the discussed pond in June, 1966.

10. *Oscillatoria splendida* Greville

Trichomae 2–3  $\mu$  wide. Species occurring commonly in bottom sediments of various water bodies, observed in the pond in September and November, 1966.

## CYANOCHLORIDACEAE

11. *Tetrachloris merismopedioides* Skuja

Fine tablet like colonies of pale blue-green cells, 0.7–0.9  $\mu$  long and about 0.6  $\mu$  wide. Species described in 1948 as found in the hypolimnion of some Swedish lakes, where it occurs together with sulphur bacteria. In the pond under investigation it was found in July, 1966, first in Poland.

12. *Tetrachloris diplococcus* Pringsheim

Most cells 1.4  $\mu$  in diameter, usually grouped in pairs, but also singular. Observed in July, 1966. The species was described by PRINGSHEIM in 1953 as found in polluted ditch water in Germany. In Poland was not found till now.

13. *Clathrochloris hannaе* Wysocka-Bujalska et Starmach

Floating colonies, irregular, discontinuous, forming however no regular web. Groups or individual rows of cells are surrounded by loose jelly, scarcely visible, becoming more accentuated after staining with methylene blue. Colonies of various size, 30–90  $\mu$  in diameter. Cells round or short-elliptical, 1.9–5.8  $\mu$  in diameter, most frequent diameter being 4.7  $\mu$ : 1.9—3 cells, 2.3—4, 2.5—4, 3.5—10, 4.3—20, 4.7—60, 5.0—8, 5.8—11.

In cells the gas vacuoles are visible. The colour is yellowgreen, usually rather pale.

The species was found in the artificial small pond situated on the grounds of the Nencki Institute in May and June, 1966, among patches of duckweed.

This *Clathrochloris* species, not described up to date, was measured and drawn by Dr. WYSCCKA-BUJALSKA. Its description was completed basing upon the preparations and notices of the late Authoress.

14. *Pelodictyon clathriforme* (Szafer) Geitler

Irregular net like colonies, composed of cells about 0.8  $\mu$  in diameter.

Species common in hydrogen sulphide containing waters, described primarily by SZAFER in sulphur springs at Lubień Wielki near Lwów, found later in springs at Swoszowice near Cracow. In the examined pond found in July, 1966.

15. *Achroonema macromeres* Skuja

Trichomae 3.3–4.6  $\mu$  wide, cells about 8  $\mu$  long. The width is then slightly smaller than the diagnostic one (according to SKUJA trichomae are 5–6.8  $\mu$  wide). The species was known till now only in Swedish lakes, appearing chiefly in the hypolimnion. In Poland reported first.

16. *Achroonema simplex* Skuja

Trichomae 1.7–1.85  $\mu$  wide (diagnostic values after SKUJA are 1.8–2.0  $\mu$ ). The species known from Swedish lakes where it occurs chiefly in the hypolimnion. In Poland found first time.

17. *Pelonema subtilissimum* Skuja

Trichomae about 0.5  $\mu$  wide and 59  $\mu$  long. Appeared in July and August, 1966. New species for Poland, known from Swedish lakes.

18. *Pelonema aphane* Skuja

Trichomae 0.6–1.4  $\mu$  wide, cells 4.7–6  $\mu$  long (in diagnosis according to SKUJA the width is 1–1.7  $\mu$ ). New species for Poland, found in June, 1966. Described first in benthos and plankton of Swedish lakes.

#### HETEROKONTAE

19. *Gloeobotrys chlorinus* Pascher

Colonies of an elliptical contour, cells 4.2–4.8  $\mu$  in diameter. Species found on microscopic slides placed in the pond. Reported second time from Poland.

20. *Gloeoskene turfosa* Fott (?)

Fine shapeless colonies, oval cells 4.7–9.4  $\mu$  in diameter. The identification is dubious since the notices of the Authoress were not enough accurate. No specimens could be found in the material available.

21. *Ilsteria lobata* Pascher

Two or four cell colonies, cells 9  $\mu$  wide. Occurred frequently in June, 1966.

#### BACILLARIOPHYCEAE

22. *Navicula cryptocephala* var. *veneta* (Kütz.) Grun. Occurred in typical form in July, 1966.

23. *Nitzschia paleacea* Grun.

Common species, in the pond under investigation observed particularly in July, 1966.

24. *Nitzschia Clausi* Hatzsch.

Species rather common, found in July, 1966.

25. *Siderocapsa* cfr. *maior* Molisch

An iron bacterium, belonging probably to the species *Siderocapsa maior*, settles on microscopic slides immersed for a certain time in the pond. Frequently observed in all preparations.

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1. REYNOLDS, T. B., YOUNG, J. O., TAYLOR, M. C. 1965. The effect of temperature on the life-cycle of four species of lake dwelling triclads. *J. anim. Ecol.*, 34, 23-43.
2. [ИМСЕНЕЦКИЙ, А. А.] ИМШЕНЕЦКИЙ, А. А. 1949. ОПТИМАЛЬНЫЕ ПИТАТЕЛЬНЫЕ СРЕДЫ ДЛЯ АНАЭРОБНЫХ ЦЕЛЛЮЛОЗНЫХ БАКТЕРИЙ. [Optimal nutritional environments for anaerobic cellulose bacteria]. *Микробиология*, 18, 215-223.
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4. ЕКМАН, S. 1953. *Zoogeography of the sea*. London. Sidgwick and Jackson.
5. БЕТОН, А. М., ЧАНДЛЕР, D. C. 1963. The St. Lawrence Great Lakes. In: FREY, D. C. [Ed.] *Limnology in North America*, 535-558, Madison, The University of Wisconsin Press.

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