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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	15 (28)	2	79—86	1968

L. Szlauer

INVESTIGATIONS UPON ABILITY IN PLANKTON CRUSTACEA TO ESCAPE THE NET

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ABSTRACT

The escape ability in lake plankton animals was estimated on the basis of comparisons between catchings into two nets which, because of their construction, were giving decidedly different chances of escaping to the animals. The greatest ability to escape the net was found in *Cyclops vicinus*, the least one — in *Daphnia cucullata, Chydorus sphaericus*, and *Bosmina coregoni crassicornis*. Among *Cladocera*, the greatest escape abilities were found in *Leptodora kindtii* and *Diaphanosoma brachyurum*. Diurnal and seasonal changes in escape ability of Crustacea were confirmed. Crustacea were proved to show the least escape abilities in dusk and in winter. Moreover, the influence of the speed at which the net was drawn on the escape ability in Crustacea was ascertained.

CONTENTS

1. INTRODUCTION

Flight of plankton animals from catching objects is always a problem arising on occasion of quantitative investigations on zooplankton. In general, these animals are thought to reveal a great escape ability and investigators supposed them to differ in this respect in particular species (Fleminger, Clutter 1965). Large plankton animals are thought to have greater possibility to escape and this may explain their scarce occurrence in the nets (Bridger 1956). Increased in the day-time escape ability of these animals may be an explanation of a generally observed, smaller numbers caught during the day as compared with those caught at night (Southern, Gardiner 1926, Colton, Honey, Temple 1961, Patalas 1954, Bridger 1956, Fleminger, Clutter 1965). Unproportionally low catches when using smaller nets in comparison with those obtained with larger ones, can be also explained by escaping of animals from the catching nets (Bridger 1956, Fleminger, Clutter 1965).

Opinions on the escape ability of plankton animals, often expressed on occasions of interpretation of results based on catches with nets, are nothing but hypotheses. Papers describing some well-evidenced studies on this phenomenon in natural conditions are lacking in the literature. Under laboratory conditions, studies on this subject have been carried out by Szlauer (1964, 1965).

2. METHODS

The technique used in this work allowed to estimate the escape ability of plankton animals on the basis of comparison between catchings done with different nets, A and B, which gave the animals decidedly different chances of escaping (Fig. 1). Net A had a rectangular inlet, 20 cm by 1 cm, its surface being 20 cm². The dimensions of square-shaped inlet of net B were 6.4 cm by 6.4 cm, with the surface of 40 cm². A surface of 25 cm² of stilon gause (with mesh 0.2 mm wide) fell on each 1 cm² of the inlet of both the nets.

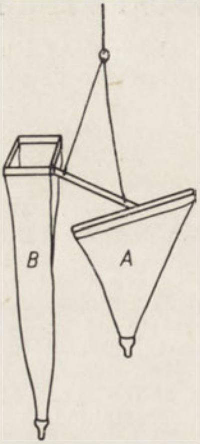

Fig. 1. Nets A and B used to observe plankton Crustacea escape ability

Net A rendered the animals exceptional possibility for successful flight. Some of the animals, found on the way of net A drawn upwards were caught, however the rest of them avoided catching. The animals caught in the nets were then caunted. In order to find out the number of escaping individuals, the number of individuals caught in net A was subtracted from the general quantity of animals present on the way of this net. This general quantity of individuals present on the way of net A was determined on the basis of the number of individuals caught into larger net B (Fig. 1), which was of construction making the escape of Crustacea difficult. The number of caught individuals falling on the surface of inlet of net B, equal to that of net A, was related to the number of individuals present on the way of net A. It equaled to half the catches in net B as this net had twice as big inlet surface as that of net A. Thus estimated number of individuals escaping net A was expressed as per cent of the number of Crustacea present on the way of this net. This value was accepted as a measure of escape ability of Crustacea plankton. Preciseness of the described method depends on conducting catches with both nets along the same distance, with the same speed, in the same place, and at the same time. All these conditions were met by joining both the nets with a crossbar, 18 cm long (Fig. 1).

The method applied in experiments had some inaccuracy resulting from the fact that net B was catching not all of Crustacea because of their partial escape. Hence, the obtained data represent only relative values.

By using the method described above, the escape ability of different species was defined as well as the influence of the day-time, season, and the speed with which the net was drawn.

Catches were carried out with the speed of 0.08 m/sec, except for the experiments on the influence of varying speed on the flight, when the catches were conducted at speeds of 0.08 m/sec., 0.33 m/sec., and 0.66 m/sec.

The nets were drawn upwards to the surface from the depth of 10 m. When lowered, the nets were not catching because their inlets were then directed to the surface.

The catches were carried out usually between 11:00 and 13:00, and in addition, during diurnal observations, at 19:00 and 22:00.

Each single test consisted of 9—10 droughts whose catchings were mixed together and placed for each of the nets in separate bottles. Each of the tests was twice repeated. Averaged results from both the repetitions are shown in tables. Stress was laid on the exact counting of the collected animals. In the case of Cladocera adults, females were counted together with grown individuals. In Copepoda, *Cyclops vicinus* and *Eudiaptomus graciloides*, adult individuals and III, IV, and Vth copepodits were treated together. The same holds for *Mesocyclops leucarti* and *Thermocyclops oithonoides*. Significance of differences between results was verified by HILL's method (1961).

Experiments were carried out on an eutrophic lake, Kortowo, in Olsztyn. The majority of the material was collected in September 1966, some small part of it in winter 1965 and 1967.

3. RESULTS

Samples taken in September 1966 allowed to determine the escape ability in various species. The results, as shown in Table I, were based on a total amount over 40,000 individuals. They describe the escape ability of Crustacea in the day-time from the net drawn with a speed of 0.08 m/sec. From Table I, it is evident that large *Cyclops vicinus* had the greatest possibility for successful flight, whereas water fleas, *Daphnia cucullata, Chydorus sphaericus*, and *Bosmina coregoni* revealed, on the contrary, the lowest ability to escape. Among Cladocera, *Leptodora kindtii* and *Diaphanosoma brachyurum* were the most successful in running away.

Diurnal observations upon the escape ability of Crustacea were conducted on 7-th and 8-th of September. During the catches carried out on these days at 13:00 the wind was blowing hard but it was rather sunny. Catches at 19:00 were conducted after the sunset, at the twilight. The least series of catchings were taken at 22:00, by moonlight, almost without wind. During the observations the temperature of upper water layer was 16°C. To a depth of 11 m the capacity of oxygen saturation was from 8.6 to 8.0 mg O_2/l. Close to the bottom, at a depth of 15 m, there was no oxygen and the temperature was 9.7°C. The results shown in Table II were based on catches amounting to 300,000 individuals.

The escape ability of all species, except for *Eudiaptomus graciloides*, were higher in the day-time than in dusk and at night. It is difficult to define the escape ability of *Chaoborus* larvae in the day-time because they were not caught in the nets at this time of day. Other results concerning these larvae are not clear because of scarcity (150) of individuals caught. These results show that *Chaoborus* has a great ability for escaping which permits to compare it with the most successful species of Crustacea, *Cyclops vicinus*. Differences in the escape ability of *Chaoborus* larvae between the dusk and the night were statistically insignificant.

Table I

Escape ablitlity of different species expressed as % of individuals escaping before the net drawn with a speed of 0.08 m/sec (September 1966, 11 : 00—13 : 00)

1) *Cyclops vicinus* Uljanin	— 75.7
2) *Leptodora kindtii* Focke	— 53.7
3) *Eudiaptomus graciloides* Lilljeborg	— 50.6
4) *Diaphanosoma brachyurum* Liéven	— 45.8
5) *Mesocyclops leuckarti* Claus+ *Thermocyclops oithonoides* Sars	— 41.9
6) *Bosmina coregoni crassicornis* Lilljeborg	— 36.6
7) *Chydorus sphaericus* O. F. Müller	— 34.7
8) *Daphnia cucullata* Sars	— 34.1

The majority of examined Crustacea were more successful in the flight from the net, both in the day-time and at night than in dusk. Only *Cyclops vicinus* and *Leptodora* varied in their behaviour. In general, very distinct limitations of the escape ability of *Crustacea* were observed in dusk.

Table II

The percentage of Crustacea escaping from the net drawn with a speed of 0.08 m/sec in different times of the day (September 1966)

Species	13 : 00	19 : 00	22 : 00
Diaphanosoma brachyurum	52.9	33.7	46.5
Daphnia cucullata	50.1	24.0	47.3
Bosmina coregoni crassicornis	44.7	30.3	40.7
Chydorus sphaericus	44.5	21.0	39.6
Leptodora kindtii	61.7	42.9	35.8
Eudiaptomus graciloides	58.7	37.1	66.7
Mesocyclops leuckarti+ *Thermocyclops oithonoides*	53.1	45.0	48.4
Cyclops vicinus	84.0	72.3	56.0
Chaoborus sp.	—	74.1	71.5
Crustacea total	50.7	34.9	46.5

Appearance of the majority of species only at given seasons have limited to a high degree the seasonal observations on the escape ability of plankton animals. Only *Eudiaptomus* because of its full-year's appearance, could be taken into account as an object of such observations. This species showed considerably greater ability of escaping in September 1966 with 51% of individuals fled than in January 1967, when only 19.7% of individuals escaped the nets. Analysis of catches involved about 11,000 individuals. Environmental conditions differed considerably in months when observations were made. In September, the visibility amounted to 2.2 m and temperature of epilimnion was about 16°C, whereas in January, the visibility reached 3.5 m, but the temperature decreased to 0.0—5.0°C.

Experiments concerned with the influence of speed of net on the escape ability of plankton animals, conducted in September 1966, did not give any satisfactory results. It was only found that when the net was drawn at a very low speed (0.08 m/sec.) much more Crustacea fled away than when it was drawn with higher speeds. The next regularity was that of relatively higher ability of escape at a higher speed of the movement of the net in these species which fled most effectively from the net drawn at the lowest speed. It concerns *Eudiaptomus, Leptodora,* and *Cyclops vicinus.*

Table III

The percentage of plankton animals escaping from the net drawn with different speeds (January 1965, 11 : 00—13 : 00.)

Species	Speeds of the net mevement			
	0.08 m/sec	0.16 m/sec	0.33 m/sec	0.66 m/sec
Daphnia cucullata	37.7	0.0	0.0	0.0
Eudiaptomus graciloides	59.2	23.2	14.6	0.0
Keratella cochlearis	0.0	0.0	0.0	0.0
Keratella quadrata	0.0	0.0	0.0	0.0

More satisfactory picture of the influence of various speeds on the escape ability in plankton animals was obtained during the winter of 1965. These observations were carried out, using the similar method, only the nets were made of fine mill gause (No. 16). Because of winter scantiness of plankton, these observations were conducted only on 10,200 Crustacea and 4,700 Rotatoria. The results shown in Table III are so evident that more detailed explanations are unnecessary. To avoid misunderstandings, it should be said that the results shown in this table are not comparable with other data given in this work, since they were obtained with somewhat different method.

4. DISCUSSION

The obtained data, in spite of successful going through the statistical tests and basing on the material amounting to thousands of individuals, did not always give a satisfactorily clear evidence of a depedence. It can be partially explained by the fact that in quantitative observations of plankton, results of a very high precision are never obtained. Correctness of the applied method as well as of the obtained results, is affirmed by accordance with the previous results (Szlauer 1965) concerning the escape ability of Crustacea. They were obtained by using a somewhat different method and under laboratory conditions. The results of present investigations (Tab. I) demonstrate that Cladocera, *Daphnia cucullata, Bosmina coregoni,* and *Chydorus sphaericus,* were characterized by the lowest escape ability and were in this regard preceded by *Diaphanosoma brachyurum.* In both the experiments, a considerably greater escape ability of *Eudiaptomus graciloides* than those of *Thermocyclops oithonoides* and *Mesocyclops leucarti,* was affirmed.

The results of investigations, concerning the escape ability in different species, explained and affirmed to a certain degree some observations of other authors. For instance, low catchings of *Leptodora kindtii,* reported by PATALAS (1954) resulted from high escape abilities, is partialy confirmed by the results of the present experiments. This species surpassed all Cladocera and the majority of Copepoda in the escape ability (Tab. I). A relatively great escape abilities in *Eudiaptomus* and *Diaphanosoma* (Tab. I) may also elucidate the almost complete lack of these species in the bowles of ablens in spite of their contemporary frequent appearance in plankton, as reported by MARCIAK (1962). It seems that these species are able to escape the plankton-feeding fish.

Often observed, smaller catches of plankton animals in the day-time than at night (FLEMINGER, CLUTTER 1965, PATALAS 1954, BRIDGER 1956) and explanation of this phenomenon by increased escape ability during the day-time, finds its partial affirmation in the results of the present investigations. It was observed that plankton Crustacea showed a greater escape ability in the day-time than at night (Tab. II). Previous observations (SZLAUER 1965) proved also seasonal differences in the escape ability of *Eudiaptomus graciloides* and made the present results more evident. These results, however, do not coincide, what is certainly due to another method and other experimental conditions in which previous investigations were conducted.

Separate species show a great differentiation in the escape ability (Tab. I). This is probably conditioned by the speed with which Crustacea move, related with size of these animals. Last supposition is affirmed by the fact of demonstrated greater escape ability in the largest species, *Leptodora kindtii, Cyclops vicinus,* and *Chaoborus* larvae. Other factor which can decide upon escape ability, is the type of animals' movement. Copepoda owe their high escape abilities to typical, quick and long jumps.

It is interesting to discuss the observations showing that some Crustacea had evidently lower escape ability in the dusk than in the day-time or at night (Tab. II). Incongruence of results concerning *Cyclops vicinus* and *Leptodora kindtii* certainly need not show that these animals behave differently. Let us suppose the results to be casual because of less numerous appearance of these animals in plankton community. Coincidence of the periodical, minimum escape ability in Crustacea, with the fact of an intense upward migration in dusk permits to bind these two phenomena. Probably an intensive upward movement decreases escape ability in Crustacea. It is difficult to explain the periodical increase of Crustacea escape ability at night, in comparison with that in dusk.

Striking differences in escape ability of *Eudiaptomus* found in September and January (Tab. III) allow to suppose that they may cause also changes in reaction of this species and of other Crustacea to plankton-feeding fish. The great difference in lake temperature between summer and winter as well as a known fact of a high influence of the temperature upon physiological processes in poikilotherm animals show that seasonal differences in escape ability were, in general, caused by this factor.

Investigations upon the influence of speed at which the net was drawn on escape ability in zooplankton gave, in general, expected results. An increase of the speed caused gradual decrease of escape ability in *Eudiaptomus graciloides* and an abrupt one in *Daphnia cucullata* (Tab. III). Speeds at which these species were losing their escape abilities, were different and to a greater degree, dependent on the speed of movements of the animals. *Eudiaptomus*, moving in quick jumps, was losing its escape ability only at a speed of 0.66 m/sec, but Daphnia — already at a speed of 0.16 m/sec. Remarks given above pertain to the winter time. In summer, both species and also other Crustacea show some abilities to escape even at the speed of 0.66 m/sec of the movement of the net. It follows that for the purpose to decrease the influence of escape ability on the catches of Crustacea, it is necessary to draw the net with possibly high speed. This conclusion should be considered as a practical recomendation concerning plankton catches in general.

Rotatoria do not escape from the net, even when caught at lower speed; it proves their minimum escape ability in comparison with Crustacea. Results of previous observations (Szlauer 1965) confirm this finding.

Experiments described above show several factors influencing escape ability in Crustacea. It is doubtless that these factors can influence the quantitative results of catches conducted with the use of standard plankton nets. The flight phenomenon, however, is rather of a little importance in this case. With affirmed in all examined species, relatively low percentages of flight from the experimental net which favours the animals' escape, let us suppose that this phenomenon does not affect, to a greater extent, the results obtained with regular, big and fastly drawn plankton nets. This conclusion refers to the lake plankton only.

5. SUMMARY

1. Among Crustacea, *Cyclops vicinus* had the greatest escape ability; *Daphnia cucullata*, *Chydorus sphaericus*, and *Bosmina coregoni* — the lowest one. *Leptodora kindtii* and *Diaphanosoma brachyurum* showed larger escape abilities when compared with other Cladocera.
2. Differences in Crustacea escape ability during 24-hour cycle were ascertained. The lowest escape ability was found in dusk, when compared with those in the day-time or at night. The highest escape ability was found during the day-time.
3. Escape ability in *Eudiaptomus graciloides* was more than 30% lower in winter than in summer.
4. Speeds of the movement of the net evidently influenced escape ability in plankton animals. Crustacea were losing possibilities of successful flight when caught with higher speeds. Species with slow movements were losing effective escape ability at lower catching speeds than fast moving Crustacea. Rotatoria were not able to escape from the net when the catching speed was within the range of 0.08—0.66 m/sec.
5. During summer, Crustacea were able to escape at higher catching speeds than during winter.
6. Degree of the flight of lake Crustacea from the net used in experiments, exceptionally facilitating the escape, permits to suppose that these animals had low possibilities to escape from commonly used, standard plankton nets.

6. STRESZCZENIE

Na podstawie porównywania połowów do dwu siatek planktonowych, które z powodu różnic wielkości i kształtu wlotu (rys. 1) dawały łowionym skorupiakom jeziorowym zdecydowanie różne możliwości ucieczki, określano ich zdolność ucieczki przed siatką. Za miernik zdolności ucieczki przyjęto % osobników uciekających przed siatką A (rys. 1) o długości prostokątnego wlotu 20 cm i szerokości 1 cm. Wartości takie przedstawiają tabele I—III.

Stwierdzono, że wśród badanych skorupiaków *Cyclops vicinus* posiadał największą zdolność ucieczki, najmniejszą natomiast wioślarki — *Daphnia cucullata, Chydorus sphaericus* i *Bosmina coregoni. Leptodora kindtii* i *Diaphanosoma brachyurum* wyróżniały się największymi zdolnościami ucieczki wśród wioślarek.

O zmierzchu skorupiaki wykazywały najmniejszą zdolność ucieczki w porównaniu z dniem i nocą. Zdolność ucieczki *Eudiaptomus graciloides* w okresie zimowym była o 30% niższa w porównaniu z latem.

Ponadto stwierdzono wpływ prędkości ciągnięcia siatki na zdolność ucieczki zwierząt planktonowych.

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A. KOSMAL

ON THE DISTRIBUTION OF OSTRACODA IN THE LITTORAL OF LAKE KISAJNO (MASURIAN LAKE DISTRICT)

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ABSTRACT

Studies were made of the occurrence of Ostracoda in the littoral of lake Kisajno during a year cycle. It was found that these animals formed several ecological groups which occurred depending on zonation of the littoral. A total of 27 species were identified, 7 of which were new to Poland. Time of reproduction and the number of generations have been also studied and the dominance patterns were analyzed.

CONTENTS

1. INTRODUCTION AND METHODS

Collection and elaboration of ostracods from the littoral of lake Kisajno aimed at learning about occurrence of these animals in different zones of the littoral as well as gathering the material concerning the life cycles of ostracod species. Samples have been collected during one year (July, 1958 — July, 1959): in summer — at fortnight intervals, in autumn and spring — once a month. During the winter, sampling was carried out in January before the lake freezed and in February from under the ice. The material was gathered from so-called "High reeds" profile, located on the western shore of lake Kisajno, the latter being a part of the Mamry lake complex.

The following zones have been distinguished along the profile in the littoral: shallow zone, intermediate zone, and zone of submerged meadows, all of them depending on the depth, type of vegetation, and the occurrence of ostracods.

I. The shallow zone was characterized by a prodigal, floating and immerged vegetation, muddy bottom, and a small depth (to 1 m). In this zone, samples were collected from 3 different habitats:

1. shore habitat, at a distance of 2 m from the shore, depth up to 50 cm, muddy bottom, plants: *Equisetum plumosum, Carex sp. Hydrocharis morsus ranae, Lemna trisulca, L. minor, L. gibba.* The site was plenty of rotting plant debris and fallen leaves. The water just above the bottom was often deficient in oxygen.

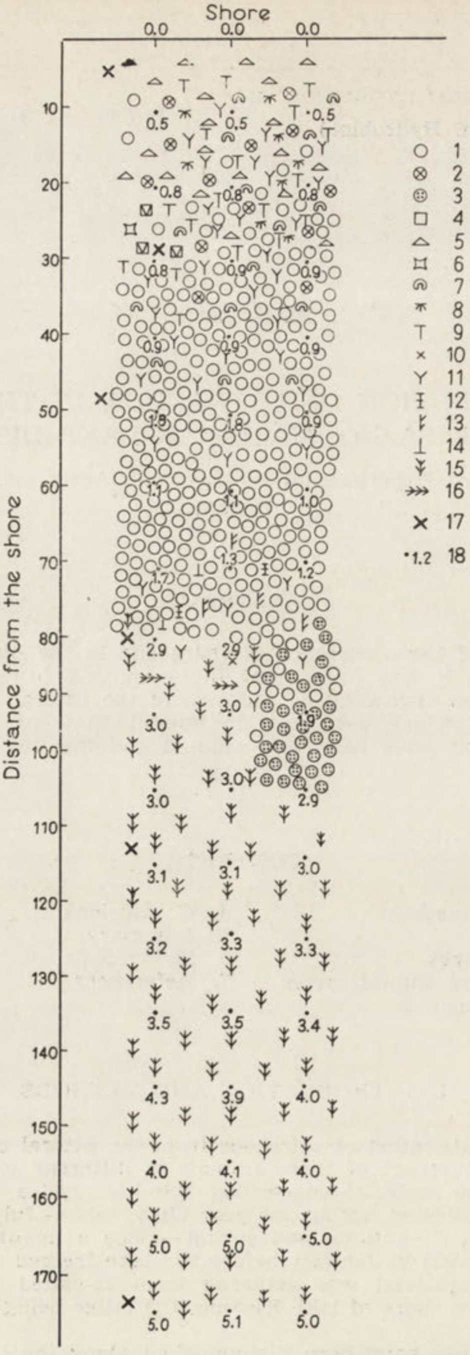

Fig. 1. Lake Kisajno, Floristic profile of "High reeds" August 23, 1957. The map was drawn by the staff of the Department for Lake Management, directed by doc. Dr. St. Bernatowicz.

1 — Phragmites communis, 2 — Equisetum palustre, 3 — Scirpus lacustris, 4 — Stratiotes aloides, 5 — Carex, 6 — Sparganium neglectum, 7 — Hydrocharis morsus ranae, 8 — Spirodella polyrrhiza, 9 — Lemna minor, 10 — Ranunculus circinatus, 11 — Lemna trisulca, 12 — Potamogeton densus, 13 — Ceratophyllum demersum, 14 — Potamogeton perfoliatus, 15 — Nitella, 16 — Elodea conadensis, 17 — Sampling sites, 18 — Depths,

2. habitat of Stratiotes, at a distance of 8 m from the shore, depth about 80 cm, muddy bottom, vegetation: mainly Stratiotes aloides, also *Lemna minor, L. trisulca, Hydrocharis morsus ranae, Sparganium ramosum,* and *Phragnithes communis* growing sparsely;

3. habitat of compact reeds, 50 m from the shore, depth of about 100 cm, hard bottom, vegetation: *Phragmithes communis, Hydrocharis morsus ranae, Lemna trisulca.*

II. Intermediate zone was characterized by scarce immerged vegetation, depth of about 1.5 m. Samples were taken from 2 habitats:

1. boundary of reeds, i.e., the boundary between the immerged and submerged vegetation, 90 m from the shore line, depth to 1.5 m, soft bottom, plants: *Cerathophyllum demersum, Potamogeton densis, P. perfoliatum;*

2. habitat of Scirpus, the site located aside of the main profile, but included here on account of its specificity: its vegetation consisted exclusively of *Scirpus lacustris;* 80 m from the shore, depth of 60 cm, sandy bottom.

III. Zone of submerged meadows comprised patches of submerged vegetation, reaching a depth of 6 m. Samples were taken from 2 sites at different depths:

1. submerged meadows I, 120 m from the shore, depth of 3—4 m, soft bottom, plants: *Elodea canadensis, Nitella sp.*

2. submerged meadows II, 180 m from the shore, depth of 5—6 m, soft bottom, plants: *Nitella sp.*

Casually, samples were taken from the deep zone of the lake. The description of the "High reeds" profile is supplemented with a floristic map * on which the sampling sites are marked.

In the shallow and intermediate zones of the littoral, a net sampler was used, and a dradge in the submerged meadows. The latter was dragged along the bottom of the lake for a distance of about 20 m, parallel to the shore line. In the winter, the apparatus was dragged under the ice through the ice-holes. Few bottom samples were collected with Eckman dradge from the deep zone.

In order to obtain ostracods with open shells, the samples, preserved with formalin not later than two hours after their collection, were then transferred to 70 per cent alcohol solution.

2. RESULTS

A. FAUNISTIC REMARKS

The following species of Ostracoda were found in the littoral and profundal of lake Kisajno:
Ilyocypris decipiens Masi *, *Notodromas monacha* (O. F. Müller), *Dolerocypris fasciata* (O. F. Müller), *Dolerocypris pellucida* Klie, *Cypridopsis newtoni* Brady et Robertson, *Cypridopsis vidua* (O. F. Müller), *Cypridopsis helvetica* Kaufmann *, *Cyclocypris ovum* (Jurine), *Cyclocypris laevis* (O. F. Müller), *Cyclocypris globosa* (G. O. Sars), *Cypria exculpta* (Fischer), *Cypria ophthalmica* (Jurine), *Cypria curvifurcata* Klie *, *Physocypria fadeevi* Dubowsky *, *Pseudocandona insculpta* (G. W. Müller), *Candona marchica* Hartwig, *Candona candida* (O. F. Müller), *Candona weltneri* Hartwig, *Candona crispata* Klie *, *Candona fabaeformis* (Fischer), *Candona holzkampfi* Hartwig, *Candona hyalina* Brady et Robertson *, *Candona acuminata* (Fischer), *Candona pratensis* Hartwig, *Candona parallela* G. W. Müller, *Metacypris cordata* Brady et Robertson *.

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* Species new to Poland, found in the littoral and profundal of lake Kisajno.

Till now, 48 species of Ostracoda were recorded for Poland. Out of 27 species listed here for lake Kisajno, 7 are new to Poland.

Such a large list of species unknown for Poland can result, among other factors, from the lack of extensive studies of lake environments throughout the year cycle. Most of the data on Polish ostracods refers to small-pools, ponds, etc. Below, remarks are given on the occurrence of ostracod species new to Poland, or of rare species which were found in lake Kisajno.

Ilyocypris decipiens Masi 1905, found twicely: in a sample of 9 August, 1958 from submerged meadows, at a depth of about 6 m (4 specimens ♀) and in a sample of 6 July, 1959 in the profundal zone (1 specimen ♂).

Species belonging to genus Candona, *C. acuminata, C. hyalina, C. crispata,* and *C. holzkampfi*, and also *Pseudocandona insculpta,* are usually very rarely found. They were reported only for Germany, Poland, and the Soviet Union (*C. hyalina* was also found in Great Britain). Thus, they seem to have, according to our present knowledge a very restricted range of geographical distribution, covering only Europe (mainly East Europe).

Similar was found for *Phyrocypria fadeevi* (Dubowsky), the species which was reported only from the Soviet Union and Roumania. Three species of this genus were reported from Europe, but two of them, acc. to BRONSTEIN (1947), turned to be synonymous to *Phyrocypria fadeevi* (Dubowsky).

It is not certain whether the species above mentioned have such a narrow occurrence range, or, due to their scarcity, they were omitted by investigators in some regions.

B. DISTRIBUTION AND ANNUAL CYCLES

a. Occurrence in various zones of the littoral

Ostracoda formed several ecological groups which were distinguished on the basis of their occurrence in different zones of the littoral (Tab. I).

Group I, species occurring in the shallow littoral zone:

Dolerocypris fasciata (Masi)

Mentioned by ALM (1915), BRONSTEIN (1947), and KLIE (1938) as a species of small ponds, but in larger reservoirs it occurs close to the shore, at a depth of 20—30 cm, in the mass of decomposing leaves which had fallen from the trees growing ashore.

Candona parallela (G. W. MÜLLER, var. albicans)

In July 1958, two females were found in a sample from the shore habitat. This species was mentioned in the literature to occur in small ponds and in shallow littoral of lakes. It was reported from Poland by GROCHMALICKI (1912).

Cyclocypris globosa (G. O. Sars)

Described in the literature as typical for ephemeral ponds, found also in permanent waters. In the littoral of lake Kisajno the species was found to occur in close-to-shore waters and in the habitat of Stratiotes.

Table I

The occurrence of Ostracoda in the littoral zones of lake Kisajno

Species	Shallow littoral zone			Intermediate littoral zone		Zone of submerged meadows		
	Shore	Stratiotes	Compact reeds	Boundary of reeds	Scirpus	Submerged meadows	Submerged mead ows II	Profundal
Dolerocypris fasciata Candona parallela	×							
Cyclocypris globosa	×	×						
Candona holzkampfi		×						
Candona fabaeformis	×		×					
Dolerocypris pellucida Candona acuminata			+					
Candona weltneri	×	×	×	×				
Notodromas manacha	×	×	×	×	×			
Cyclocypris ovum, C. laevis Cypria opthalmica, Cypridopis vidua	+	+	+	+	+	+	+	
Cypria exculpta	×	×	×		×	×	×	
Pseudocandona sp.		×	×	×	×	×		
Cypridopsis helvetica Candona hyalina			+			+		
Candona candida				×		×		
Pseudocandona insculpta			×				×	
Cypridopsis newtoni, Cypria curvi furcata Physocypria fadeevi, Metacypris cord.							+	
Ilyocypris decipiens							×	×
Candona pratensis, C. marchica								+
Candona crispata	×	×	×	×	×			×

× = Occurrence

+ = Co-occurrence wish several species

Candona holzkampfi (Hartwig)

Reported from various water bodies. Usually, it occurs together with C. fabaeformis. In lake Kisajno, a single specimen, ♂, was captured in the habitat of Stratiotes.

Candona fabaeformis (Fischer)

Rare, occurs in various waters. Hartwig (1901) reported on C. fabaeformis occurring in drying ponds located in meadows. Alm (1915) observed this species in large, permanent water bodies. In Poland, the species was found by Grochmalicki (1912) in small ponds. In the shallow zone of the littoral of lake Kisajno, 3 specimens (including 1 ♂) were found at the sites located in the shore habitat and in the habitat of compact reeds.

Doloeocypris pellucida (Klie)

Reported twicely: by Klie (1926) from small ponds in Sumatra and by Bronstein (1947) from rice fields in the vicinity of Samarkand, Uzbek S. S. R. Two specimens of this species were found in the habitat of compact reeds of the shallow zone.

Candona acuminata (Fischer)

Very rarely encountered, described as a form occurring in small ponds and in the littoral of lakes /Klie (1926)/. Two females of this species were found in the habitat of compact reeds.

Group II. Only two species can be listed in this group, both occurring in the shallow zone and in the intermediate zone down to the border of submerged meadows (cf. Tab I).

Candona weltneri (Hartwig)

It lives in various types of waters. In lakes, it occurs in the littoral, but can also descend into deeper strata (Klie 1926). For Poland, the species has been described by Grochmalicki (1912) as occurring in small ponds. In the littoral of lake Kisajno, it occurred only at small depths of the shallow and intermediate zones in all the habitats examined, except for the habitat of Scirpus. The highest number of specimens was found in the habitat of compact reeds.

Notodromas monacha (O. F. Müller)

The form is typical for various, non-drying water bodies. In lake Kisajno, it was observed to occur rather abundantly in the shallow zone and accidentally in the intermediate zone. The highest number of specimens was gathered from the shore samples (up to 40), less from Stratiotes (up to 28), and much les from compact reeds (up to 18). In the intermediate zone, only a few specimens were found.

Group III. It includes species which occurred in all three zones of the littoral. They were rather abundant.

Cyclocypris ovum (Jurine)

A very common species, found in a variety of environments: small ponds, lakes (littoral and profundal), brackish waters (in the Baltic Sea, at salinity of 6.4‰). C. ovum appeared in great numbers in all the habitats distinguished of the littoral of lake Kisajno. It was most abundant in the shallow zone. The numbers were clearly lower in the intermediate zone, especially at the border of reeds, and the increased again in submerged meadows I.

Cyclocypris laevis (O. F. Müller)

This species is as common as the previous one to which it is closely related. It occurs in various types of water bodies, usually together with *C. ovum*. The range of its occurrence in lake Kisajno was roughly similar to that of *C. ovum,* i.e., it was found in all the habitats explored of the three littoral zones. Its occurrence differed from that of *C. ovum* in the highest numbers of the latter found in the shallow zone, where *C. leavis* was scarcely represented. In the intermediate zone, the number of specimens was found to increase reaching its peak in submerged meadows.

Cypria ophthalmica (Jurine)

A very common, wide-spread species (not reported from Australia only). It occurs in various environments: small ponds, lakes (in the littoral and a very deep profundal), subterraneous waters (Wolff 1919), waters with a high iron content (Wolff 1919, Kaufmann 1900), brackish waters (in the Baltic Sea, at salinity of $6.4^{0}/_{00}$). The species was found in all three zones of the littoral of lake Kisajno with its abundance differing from zone to zone. It was highest in the shallow zone, and especially in the habitata of compact reeds. Only a few specimens were found in the intermediate zone, but the species was abundant again in submerged meadows.

Cypridopsis vidua (O. F. Müller)

A common species, reported from various water bodies: marshes, small ponds, lakes (down to 70 m of depth, acc. to Klie (1926). In the Alps, it ascends to 2,000 m a.s. l., in the Fatra Mts — to 1,800 m a.s.l. It is eurythermic. In lake Kisajno, this species was encountered in all three littoral zones, occurring most numerously in the habitats of compact reeds and submerged meadows.

Cypria exculpta (Fischer)

Mentioned in the literature as a form typical for small ponds, lakes and rivers. It was found in all three littoral zones of lake Kisajno, except for the border of reeds. Most numerous in submerged meadows.

Pseudocandona sp. (Fig. 2)

Rare specimens were caught in all littoral habitats except for the shore habitat of the Kisajno littoral.

Group IV. This group includes species habitually appearing in moderate numbers mainly in two habitats: of compact reeds and of submerged meadows.

Cypridopsis helvetica (Kaufmann)

The species closely related to *C. vidua,* was described by Alm (1915 and Müller (1912) as its subspecies. It usually occurs together with the latter. The maximum occurrence of this species, found in the habitat of compact reeds and of submerged meadows I, overlapped that of *C. vidua.*

Candona hyalina (Brady et Rob.)

A rare species found in small ponds and in lakes. It is little known about its biology. In the littoral of lake Kisajno, 3 specimens of this species were found in the habitats of compact reeds and submerged meadows.

Pseudocandona insculpta (G. W. Müller)

Reported from Germany and the Soviet Union; occurs in small ponds and in rivers. In lake Kisajno, a small number of specimens was found in the habitats of compact reeds and submerged meadows II.

Candona candida (O. F. Müller)

Cosmopolitan species, reported for cold waters. In the littoral of lake Kisajno, separate specimens were found at the border of reeds and in submerged meadows II.

Group V. This group, comprising 4 species *Cypriodopsis newtoni* (Brady and Robertson), *Cypria curvifurcata* (Klie), *Physocypria fadeevi* (Dubowsky), and *Metacypris cordata* (Brady and Robertson), is represented by their single specimens found in submerged meadows II.

Ilyocypris decipiens (Masi)

Reported from Italy, Sweden, and Germany, for river environments. In lake Kisajno, single specimens of this species were encountered twicely in sumberged meadows and in profundal.

Moreover, *Candona pratensis* (Hartwig) and *Candona marchica* (Hartwig) both were found to occur in the profundal of lake Kisajno with no specimens found in the littoral. *Candona crispa* (Klie) was excluded from the above list since it occurred both in the littoral and profundal. Up to now, *Candona crispata* was reported from Germany, the Soviet Union, and Poland. Its biology is unknown. In the littoral of lake Kisajno, it occurred in all the environments explored, save submerged meadows II. It was most numerous (7 specimens) in the samples from the shore habitat. In profundal sampled, only one specimen was found.

b. Species characteristic for the littoral of lake Kisajno

In the shallow littoral, 19 species occurred, 7 of which exclusively in this zone (Tab. I, 1—5). The habitat of a shallow water is typical for these species. In the majority, they were summer forms and their development was conditioned by a fairly high temperature (except for *Candona acuminata*). For example, *Dolerocypris fasciata* (O. F. Müller) and *D. pellucida* (Klie) are exceptionally fond of high temperature, which is affirmed by the occurrence of these species in Sumatra. Some of these species, e.g. *Dolerocypris fasciata, Candona parallela, Cyclocypris globosa* easily survive dessication, although it is not necessary for their development. It is little known about the biology of *Candona fabaeformis, C. holzkampfi,* and *C. acuminata* and it is difficult to explain why they occur exclusively in shallow waters. Besides the species above mentioned, the shallow litoral zone was also inhabited by several species which occurred also in deeper waters.

The intermediate littoral zone with the two environments distinguished, the boundary of reeds and the habitat of Scirpus, is poorest both in species and in numbers of Ostracoda. A total of 10 species were found in this zone, 3 of which were more numerous, *Cyclocypris ovum, C. laevis,* and *Cypridopsis vidua*. They were occurring abundantly within the whole littoral. A good swimming ability of these species can explain more abundant occurrence of these ostracods than of others in the intermediate zone. However, their numbers were somewhat lower in this zone. The remaining 7 species (cf. Tab. I) occurred in much lesser numbers. There is no one species which would occur exclusively in this part of the littoral. It could be explained by the fact that this environment was deficient in food, without a definite character, and transient between immerged and submerged vegetations.

Submerged meadows were inhabited at least by 16 species of Ostracoda. Four of them, *Cypridopsis newtoni, Cypria curvifurcata, Physocypria fadeevi,* and *Metacypris cordata,* were found exclusively in submerged meadows. These were only single specimens encountered only once, in spite of continuous catching carried out throughout the year. Among species occurring in more than one zone, a certain regularity was observed, namely, an aggregation of their individuals in the two habitats: of compact reeds and of submerged meadows. This aggregational tendency was most evident in *Cypridopsis vidua* (cf. Tab. III). *Cypridopsis helvetica, Candona hyalina,* and *Pseudocandona insculpta* occurred exclusively in these two habitats, in spite of a clear difference between these environments, which differed in depth, vascular vegetation, bottom, etc.

c. *Cypria cuevifurcata* (Klie) — a species unknown in the lake littoral

This species has been described by Klie (1926) and Dubowsky (1927). Reported only from USSR for rivers and their old basins (the rivers: Kama, Kostroma, Don, and Wołchow). This ostracod has hard, heavy shell, antennae II void of swimming bristles, the furca is of a heavy built. Such anatomy points to the fact that this species is well adjusted for creeping and digging movements. In lake Kisajno it was found in submerged meadows II.

d. Phenology

The occurrence of Ostracoda within the year cycle is hown in Table II. As it can be seen from this table, only 6 species were found to occur throughout the year. These were: *Cypria exculpta, Cypria Opthalmica, Cypridopsis ovum,* and *Pseudocandona sp.* The highest number of species (23) appeared in July and August, the smallest one (4) in November. In the autumnal period (September, October and November) 9 species were found altogether. This is the smallest number of species recorded within the whole year of sampling. In the winter and spring (from December to mid June) 10 species were usually found during each sampling. *Candona holzkampfi* was encountered solely in the spring. However it is not certain whether this species is exclusively vernal one since on account of being closely related to *C. holzkamfi* its females are difficult to discern them from those of *C. fabaeformis.* In April one specimen of *C. holzkampfi,* ♂, was found. There were 14 species occurring exclusively in summer. Candona crispata was found to be a vernal-summer species: it appeared in the second half of April and vanished in the beginning of August. *Notodroma monacha* appeared in late spring (the beginning of June) and disappeared in autumn (mid November). Cypridopsis helvetica was a summer-autumnal species occurring from the end of June to mid November. *Candona weltneri* appeared in autumn (mid November) and vanished in the spring (second half of April). *Candona hyalina* occurred in the winter (February) and disappeared in spring (April). Two species, *Candona candida* and *Candona acuminata,* were found to occur in samples which were taken in winter months (January, February).

e. Life cycles of some species of Ostracoda

Sampling throughout the year allowed to observe the reproduction periods and the number of generations per year in some ostracod species.

Notodromas monacha (O. F. Müller)

In samples of 1958, it appeared in the second half of July, with males and females occurring simultaneously. In the second half of August and the first one of September, the numbers were decreasing and vanished by the end of September. In 1959, separate individuals of females and males were found in the beginning of June. Klie (1938) reported on two generations per year with a very short periods of development (6 weeks). In lake Kisajno, N. monacha had only one generation per year.

Doloeocypris fasciata (O. F. Müller)

A typical summer species. Its females were encountered twicely, in July and August, 1958. According to Alm (1916), D. fasciata has two generations per year, the first one in April, and the second in June. Klie (1926) also mentioned two generations in this species. Wolf (1919) reported on one summer generation fithin an annual cycle. In the littoral of lake Kisajno, only one generation was observed, with the maximum of abundance in the first half of August.

Cypridopsis vidua (O. F. Müller)

This species occurred in the littoral of lake Kisajno throughout the year. Individuals with matured eggs were observed to occur in July. In August, numerous larvae, which probably belonged to this species, were encountered. It can be inferred from this that C. vidua had only one generation per year in the lake with reproduction period following in a full summer (second half of July).

Cyclocypris ovum (Jurine)

It appeared in the habitats of lake Kisajno during the whole year with maximum numbers found during summer months. According to Alm (1915), the species has one generation per year with a very long period of development. Females and males could be found throughout the year, but their numbers were decreasing in the winter.

Cypria exculpta (Fischer)

Low numbers of this species can be found within the whole year. Acc. to Bronstein (1947), the development of *C. exculpta* lasts around 5—6 months, from April to August or September. In the littoral of lake Kisajno, matured females and one male were found in early summer (June-July). In July,

Table II

The occurrence of Ostracoda in the littoral of lake Kisajno within an annual cycle

Species	17.IV. 59	1.VI. 59	19.VI. 59	6.VII. 59	9.VII. 59	19.VII. 59	9.VIII. 59	21.VIII. 59	15.IX. 59	13.X. 58	8.XI. 58	3.I. 59	20.II. 59			Spring	Summer	Autumn	Winter
Candona holzkampfi	×															×			
Dolerocypris pellucida, Cand. pratens. Cand marchica, Physocypria fadeevi				+													+		
Candona parallela Candona fabaeformis					+												+		
Ilyocypris decipiens				×			×										×		
Cyclocypris globosa					×	×	×										×		
Dolerocypris fasciata					×		×										×		
Cypria curvifurcata Psudocandona insculpta							+										+		
Metacypris cordata Cypridopsis newtoni								+									+		
Candona crispata	×	×		×	×	×	×									×	×		
Notodromas monacha		×				×	×	×	×								×	×	
Cypridopsis helvetica			×						×								×	×	
Cypriaexculpta	×	×	×	×	×			×	×	×			×			×	×	×	×
Cypria ophthalm, Cypridopsis vidua Cyclocypris laevis, C. ovum	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+
Pseudocandona sp.	×	×						×	×	×		×	×			×	×	×	×
Candona weltneri	×								×				×			×		×	×
Candona hyalina	×								×				×			×		×	×
Candona candida												×	×					×	
Candona acuminata													×					×	

× = period of occurrence
\+ = period of co-occurrence with several species

the larvae of last stage of this species were encountered (with a clear carving pattern on the shell surface). The highest numbers were caught in September. In a sample of February females with matured eggs and a male were found. This would suggest an early spring reproduction period (March). The development ended in July, lasting around 5 months. The males predominated quantitatively over the females. The species had one generation within the year.

Cypria ophthalmica (Jurine)

This ostracod occurred throughout the year, except for autumnal period when its numbers were clearly at a decline. The highest numbers of specimens were found at the beginning of June and then in the second half of August. The females with matured eggs were encountered twicely, in the first half of July and in February. This suggested that the species had two generations per year.

Candona weltneri Hartwig

Matured individuals, females and males, were observed in the samples collected in September, February, and April. In April, only females with matured eggs were found. In lake Kisajno, this species was found to lay eggs in April and its development lasted for about 5 months, till September, when matured females and males appeared again. The lack of males in the samples collected in April could be explained by the fact that they had died out earlier. This finding corroborates Klie's (1926) opinion. Thus C. weltneri has one generation per year.

Candona crispata Klie

The biology of this species is unknown. Only Klie (1936) reported that a few matured individuals were observed to occur in May (with males prepondering) and in July (when females prepondered). In the littoral of lake Kisajno, a few individuals of *C. crispata* were observed from April to August. Thus, this is a spring-summer species. In the vernal period (April — first half of June) some females were caught in the shallow littoral. The maximum of abundance was recorded in the full summer (first half of July). In this period males appeared for a short time only and vanished later so that by the first half of August only females were noted. No females were carrying eggs, therefore it is not possible to define the reproduction period in this species. However, it probably followed in the end of July and in August.

Candona fabaeformis (Fischer)

Little is known about this species. Alm (1915) supposed that this species had a very long period of development, from May to October. Hartwig (1901) considered it to be exclusively vernal form. Müller's (1912) observations showed this species to occur all the year round with two generations per year. In the littoral of lake Kisajno, 3 adult specimens (including 1 male) were found in the samples taken close to the shore and among compact reeds in July, 1958.

Candona hyalina Brady et Robertson

The biology of this species was little studied. According to Klie (1926), it develops in early spring, with males prepondering at the beginning.

In lake Kisajno, specimens of this species were encountered 3 times, in February 1958 and 1959, and in April 1959. Probably there is only one generation of this species per year. Matured individuals can occur in the winter months. The reproduction would follow in the winter period what has been suggested by discovering the females with matured eggs in February.

Candona acuminata (Fischer)

A rare species, its biology unknown. According to KLIE (1926), this ostracod matures probably in the late autumn. In the littoral of lake Kisajno, matured individuals were encountered in the winter samples, collected on 20 February 1959 from the habitat of compact reeds (two females); also in other samples (not included in this characteristics), collected on 7 December 1957 (both females and males) and on 24 February 1958 (one female with matured eggs). This species has probably one generation with adults occurring axclusively in winter, when the reproduction takes place.

3. DOMINANCE PATTERNS

For characteristics of the dominance patterns in ostracods, only these species were taken into acount which showed a higher abundance and lasted in the environment for at least 4 months. These were: *Cyclocypris ovum, C. laevis, Cypria ophthalmica, Notodromas monacha,* and *Cypridopsis vidua.* The species which showed the highest numbers in a given sample were accepted as dominants. The dominance of ostracod species related to the habitats examined and to the time of their occurrence is illustrated in Figs 1 and 2. Two reasons can be mentioned as responsible for the observed dominance. One is coincidence of sampling with the maximum abundance of a species and the second is the maximum occurrence in a specially favourable habitat for this species, e.g., in the habitat of reeds, with its simultaneous non-dominant position in other environments. *Cypria ophthalmica* predominated consistently in the three habitats of the shallow littoral zone (shore, Stratiotes, and compact reeds) during the spring. In the habitat of compact reeds, its dominance was prolonged till the beginning of summer (second half of June and the first one of July) with not so clear domination in the second half of June, when *Cypridopsis vidua* showed but little lower percentage of occurrence (cf. Fig. 1). In the shore and Stratiotes habitats, *C. ophthalmica* was losing its dominance in the summer period in favour of *Cyclocypris ovum*. In the habitat of compact reeds, the latter predominated in July, but in the first half of August *Cypridopsis vidua* dominated there. Later on, in the second half of August, the numbers of *Cypria ophthalmica* were increasing again, so that this species re-established then its dominant position. In mid September, *Notodromas monacha* predominated in the shore habitat, and *Cypria ophthalmica* in the habitat of compact reeds. In general it can be said that in the autumn and winter the abundance of species decreased considerably and the dominance patterns were losing their clarity, except for the habitat of compact reeds, where *Cypria ophthalmica* predominated in February. In the intermediate zone, at the boundary of reeds and in the habitat of Scirpus, where both the numbers of species and numbers of individuals were low, a clear dominance was sporadically observed of only one species, *Cyclocypris laevis* (cf. Fig. 1).

Within submerged meadows environment, in submerged meadows I, *Cyclocypris laevis* showed a clear dominance by the second half of June. Similarly this species predominated in submerged meadows II in the samples of April and June. Later on, the abundance of this ostracod decreased with a simultaneous increase in numbers of *Cypridopsis vidua*. Thus, in submerged meadows I, in the sample of 6 July, two species, namely *Cyclocypris laevis* (49%) and *Cypridopsis vidua* (44%) predominated numerically over the others (cf. Fig. 1). In submerged meadows II, *Cypridopsis vidua* showed a clear dominance (94%) whereas numbers of C. laevis were found to decrease forming only 4% but in the following month they increased to the dominance level. During the second half of August in submerged meadows I and II *Cyclocypris ovum* developed greatly, its dominance becoming conspicuous, and later on, in September, its numbers dropped down.

In September and October, *Cyclocypris laevis* predominated in submerged meadows I and *Cypridopsis vidua* in submerged meadows II. In the winter, *Cyclocypris ovum, C. laevis,* and *Cypria ophthalmica*, all were fairly abundant in submerged meadows I whereas in submerged meadows II — *Cyclocypris laevis, Cypridopsis vidua,* and *Cypria ophthalmica* occurred numerously but without clear dominance of one species over the others.

By and large, of a total of 27 species, only 5 were observed to be dominants.

The dominance is clearly expressed during the summer months (July, August and September) and in the zone of submerged meadows also in the autumn (September, October, November).

Not all of the littoral habitats are favourable for appearance of dominance. The dominance can be most often observed in the habitat of compact reeds and in the zone of submerged reeds. The preponderence of *Cypria ophthalmica* in the shallow littoral during the spring and early summer as well as in the second half of August and September, coincided clearly with the maximum occurrence of this species. Similarly, the dominance of *Cypridopsis vidua* in the compact reeds in August and in submerged meadows in September coincided with its maximum numbers.

Cyclocypris ovum developed later than *Cypria ophthalmica;* its maximum of occurrence was noted in the full summer and that is why it outnumbered *Cypria ophthalmica*, becoming the dominant species of the shallow littoral.

4. CONCLUSIONS

In the material collected from the littoral of lake Kisajno, 27 species of ostracods were identified. They formed several ecological groups, discerned on the basis of their place of occurrence in different habitats.

In the shallow littoral zone, species reported usually as forms of small ponds were encountered: *Dolerocypris fasciata* — reported by Alm (1915), Bronstein (1947) and Klie (1938), *Candona prallela* — reported by Bronstein (1947), *Cyclocypris globosa, Doloeocypris pellucida* — reported by Klie (1926), and Bronstein (1947). All these species are characterized by a short life cycle, they are fond of high temperature and occur in shallow waters. Similarly the remaining species of the shallow littoral are forms whose typical environment is that of shallow waters and their development is conditioned by rather a high temperature. *Cypria curvifurcata* (Klie), till now reported twicely: by Klie (1923) and Dubowsky (1927) for rivers, has been found in the lake Kisajno at a depth of 6 m, in the habitat of submerged meadows. Likewise *Ilyocypris decipiens* Masi (1905) described as typical for running waters and for lakes, has been found in this zone. This species was reported by Alm (1915) from Italy and Sweden, by Müller (1912) and Klie (1926) from the Soviet Union where it occurred in rivers. Besides, it has been reported by Bronstein (1947) as a species occurring in lakes: Caca, Barmanca, and Pieščanoje in USSR.

Reproduction seasons and the number of generations found for some of the ostracod species in lake Kisajno differed from those given in the literature.

Most abundant were wide-spread, well-swimming species occurring in the summer months, when the number of species was highest. The smallest number of species (4) was observed in November. There were 14 species which occurred exclusively in summer and 2 — exclusively in winter.

AKNOWLEDGEMENTS

This paper is devoted to memory of the late Professor Marian Gieysztor who was initiator and guide of this work.

I wish to thank Doc. Dr. R. Z. Klekowski and Doc. Dr. St. Bernatowicz for their help and invaluable assistance in performing this work.

6. SUMMARY

Ostracods were collected during a year cycle from the littoral of lake Kisajno. Three environmental zones were distinguished: Shallow littoral zone, intermediate zone and zone of submerged meadows. Type of vegetation, depth, and the character of ostracod occurrence formed the basis for this classification. A total of 27 species were identified, 7 of which were new for the Polish fauna. Several ecological groups were distinguished basing on their occurrence in various littoral zones. In a number of cases, species were found which up to now were thought to be typical for rivers and small ponds. Sampling throughout a year allowed to define the precise time of appearance of various species and the number of their generations per year. The dominance patterns have been analyzed for ostracods occurring in various zones of the littoral of lake Kisajno.

6. STRESZCZENIE

Ostracoda zbierano w cyklu rocznym z litoralu jeziora Kisajno. Wyróżniono 3 strefy: litoralu płytkiego, przejściowego i łąk podwodnych. Podstawą podziału był typ roślinności, głębokość i charakter występowania Ostracoda. W zebranym materiale Ostracoda stwierdzono i oznaczono 27 gatunków, w tym 7 nowych dla fauny Polski. Stwierdzono istnienie kilku grup ekologicznych, wyróżnionych na podstawie ich występowania w poszczególnych strefach litoralu. W szeregu przypadkach stwierdzono występowanie gatunków, które dotychczas znajdowane były jedynie w rzekach i drobnych zbiornikach. Pobieranie prób w cyklu rocznym pozwoliło określić pory pojawiania się poszczególnych gatunków, oraz ilość ich pokoleń. Zanalizowano zjawisko dominacji gatunków w poszczególnych strefach litoralu jeziora Kisajno.

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DEVELOPMENT AND REPRODUCTION OF ROACH (*RUTILUS RUTILUS* L.) IN THE SZCZECIN FIRTH

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ABSTRACT

The roach under study, *Rutilus rutilus*, formed a population occurring in brackish waters, with a part of the population migrating for food to inshore waters of the Baltic Sea. From the macroscopic survey of the gonads and their relative weight, the Maier's scale of fish maturation has been adopted to study the roach of the Szczecin Firth. The single-cast spawning was observed in the females and many-cast spawning in the males. The period of development is very long. The sexual maturity is attained by this species after 2—6 years at the body length of 9—23 cm; the males mature earlier than the females.

Studies were made of the seasonal changes in relative weight of gonads of *Rutilus rutilus*. This feature was found to depend on the fish dimensions. The sex ratio of males to females, was 9:16 during the year cycle and was found to change with aging of the fish. In the spawning population this ratio was 8:9. The absolute fecundity of roach was increasing with age from 3,000 to 27,000 eggs per female. The relative fecundity was found to be constant within the individual life period.

A description has been made of the spawning places as well as of the spawning behaviour in this species. It was found that the roach started spawning at the end of April or in early May depending on the weather conditions and continued this process for 3 to 4 weeks with some intervals in it.

CONTENTS

1. INTRODUCTION

The roach of the Szczecin Firth has not been yet studied except the investigations by Neuhaus (1936). The roach population under study differs from the others occurring in freshwater environments and is similar to those of the Vistula

Firth and the Pucka Bay (Romański 1963, 1965) in terms of the growth rate, survival, reproduction, and behaviour. A part of the population migrates to the inshore waters of the Pomeranian Bay to feed there. Probably on account of the latter peculiarity Staff (1956) in his commentary to the Polish translation of the Detailed Ichthyology has considered it as a semi-migratory form, similar in this respect to *Rutilus rutilus caspicus* (Jak.) and *Rutilus rutilus aralensis* (Berg).

The roach takes first or second place in commercial catches at the Szczecin Firth, alternately with the bream. The roach yield showed a very great variation, in the years 1900—1937 it amounted to 3.2—17.3 kg/ha (the average for the whole Firth), in 1948—1966 — to 5.0—35.7 kg/ha (the average for the Polish part of the Firth).

The present paper includes a partial elaboration of the materials collected in the years 1956—1966 and it aims at learning about some problems on the reproductive behaviour with a certain attention given to a possible effect of the brackish waters.

2. MATERIAL AND METHOD

The material for this paper has been collected (together with biological analyses) in the years 1956—1958 in monthly intervals. In the years 1959—1966 the material was gathered twice a year, in the spring and autumn, basing on both the commercial and experimental catches. From small catches the whole material was analyzed; from larger ones biological analyses were made proportionally to the length composition of the fish. The material for analysis can most often of the catches made at Wolin since in this fishing base the fishermen were catching the roach in relatively large numbers and the catches extended almost all over the entire Polish part of the Firth. The experimental catches were made in the inshore zone of the Firth and in its adjacent waters where spawning and fry feeding occurred as well as in the central part of the Great Firth. Observations on the spawning process and sites were done by the authoress, supplemented with information supplied by fishermen and inspectors of the Department of Fishery Protection, the Szczecin Marine Office.

The biological analysis included the following determinations: total length (longitudo totalis) — accuracy of 0.5 cm, body weight — accuracy of 5 g, sex and maturity of the gonads according to a 7-degree scale *. A total of 6.636 young and mature individuals were examined. In order to obtain more precise information on the sex ratio in Rutilus, additional 4.255 fish were surveyed, their total length and sex recorded. In 1.219 fish (maturation degree II—VII) the gonads were weighed and fecundity of 122 females with gonads at degree IV and V, was determined. The sex of young fish was identified with the use of a lens. The gonads after the fat tissue had been removed were weighed to an accuracy of 0.1 g. When determining fecundity, external membranes were removed from the ovaries and the ova were weighed to an accuracy of 0.1 g. Fecundity was estimated by weighing fresh, unpreserved roe acc. to weighing method (Załachowski 1959).

Since the macro-characteristics of subsequent stages of maturation described by Maier (1906) did not correspond thoroughly to those of gonads neither of the roach nor of the bream of the Szczecin Firth (Pęczalska 1963a),

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* The Maier's scale adopted by the authoress for *Rutilus rutilus* L.

a trial was made to describe macro-characteristics of the gonads of the roach population within an annual cycle. From the survey of gonads of 1.541 young and mature fish a scale of sexual maturation for roach has been elaborated.

3. RESULTS

A. THE SCALE OF SEXUAL MATURATION OF GONADS IN ROACH OF THE SZCZECIN FIRTH

(Includes only these macro-characteristics which are typical for the roach gonads, characteristics which agree with those described by MAIER are omitted).

Degree I (acc. to MAIER — juvenile stage)

The gonads are in shape of very thin cords expandend somewhat in their middle sections. They spread up to the posterior chamber of the swimming bladder. Glassy in appearance. Centrally, along the gonads rather bulky blood vessels can be seen with branching visible only through the lens. The reproductive glands are often covered with the fat tissue.

Degree II (acc. to MAIER — resting stage)

The glands are essentially in the shape of laminae expanding in their middle parts. In alive or freshly dead fish, sex differences can be easily recognized; the testes form flat laminae, the cross-section of the ovaries forms a triangle with open arms and round angles. In the middle of the triangle base, there is an oblong, shallow furrow. At transparent light, the lobular structure of the ovary can be seen with translucent ova. Under magnification, they are similar in shape to those of degree I, but the cells are larger. After some training it is possible to distinguish the sex without using a lens, but not in all individuals of this class. The gonads are almost white or slightly rose and somewhat lustreless. Lateral branching of the blood vessels can be seen without magnification.

Degree III (acc. to MAIER — preparatory stage)

Large ovaries extend for one third of the abdominal cavity length. Inside the ovaries, ova of various size can be easily seen. They are still transparent and difficult to separate from the thick and hard membrane of the ovary. The lobular structure of the ovaries is very clear. They acquire coloration typical for further developmental stages.

Testes are much bigger than in degree II. Their surfaces fold in and the first, shallow furrows can be seen. The fat which surrounded the gonads disappears. The blood vessels form a dense network.

Degree IV (acc. to MAIER — hardening stage)

The ovaries fill in almost the entire space of the abdominal cavity; by the end of winter they attain maximum dimensions. Cross-sected, are still triangular with the edges more concave than in degrees II and III. There is a deep, oblong furrow along the wrinkled base of the triangle. The ovaries are narrow at the ends, the one directed towards the head is more dull. The ovaries acquire rather vivid colours, grey, yellowish, rosy, or crimson. The round, intransparent eggs of even size covered with hard shells are distributed sparsely and grow rather considerably during the winter. It is still difficult to separate them from the inner connective tissue and the outer membrane of the ovary.

Large testes but twice or three times as small as the ovaries in the roach of a similar body length. They are slightly wrinkled in the middle parts. Their coss-section forms equilateral triangle, its base being shorter than the laterals. The testes are springy, of body-milky colour. In early spring the testes when pressed strongly release highly concentrated sperm.

Degree V (acc. to Maier — lengthening stage)

The whole ovaries are glassy, their coloration faded. Strong venation mostly in the oblong furrow of the ovaries. The latter are strongly wrinked, their membranes becoming thinner. Inside the ovaries, the roe is interspaced with the ovarian liquid. The females pressed strongly on the both sides release the roe aggregated in clusters. After fractioning the ovarian membrane, the eggs stay compact and do not disperse. They are transparent as though slightly glittering. The egg shell is very thin; touched slightly with a needle disintegrates and the whole egg content pours out.

The testes are dull-white, strongly wrinkled; when pressed, the drops of averagingly concentrated sperm appear along the dorsal part of the testis, on its upper edge. The fish may not reveal as yet the sexual coloration. In some years, the display coloration appears in this stage of maturation. Maturation degree V lasts in the roach for a very short period, about 2—3 weeks.

Degree VI (acc. to Maier — maturity stage)

The ovaries lose their turgidity, become dull and filled with much liquid. The genital papilla is swallen and reddish. The spawning females spray the roe at the slightest stimulation, e.g. after a gentle taking the fish in the hands, when the fish jump on the boat bottom, etc. At spawning, the females of the roach population examined eject the roe only once and within a relatively short time (for about 10 hrs). It happens that in this period some females reveal a slight pearl eruption and all have silver body colour and vivid coloration of the fins.

The testes are ready for reproduction probably for a longer time (over 10 days) than are ovaries, but much shorter than the testes of the bream in the same environment (Pęczalska 1963a). During the reproduction readiness, the turgidity of the testes decreases in considerably. Milky-white coloration is maintained, only the posterior parts of the testes become softer and overflown with blood. In these parts the sperm is less concentrated than in the anterior ones. When the fish sides are pressed, the sperm comes out, often it even sprays out. The display coloration occurs during the entire period of sexual readiness, its intensity increasing with the age of fish.

Degree VII (acc. to Maier — half-spawned and spawned stage)

The ovaries diminished by several times as compared with those at degree VI. They contain more or less numerous eggs which undergo adsorption. The eggs are surrounded by a dilute, transparent substance — developing cells of the following generation. They are round and can be seen only when magnified. The genital papilla is smaller and paler.

The testes are also smaller, acquiring the form of thin cords with almost equal diameter throughout their length; overflowned with blood. The display coloration disappears changing into normal.

B. ANNUAL CYCLE OF MATURATION

After retrogressive changes (degree VII), the gonads of roach were found to return to degree II in June and July for a relatively short period. In August all mature individuals had the gonads at degree III, which also lasted short time. The gonads reached degree IV in September and October, earlier in females than in males. The males at degree III could be still encountered in January. Degree IV in the roach of the population examined lasted long, almost until the spawning period. During this period the gonads were gaining weight only. According to Meien (1940) this stage is characterized by a fast growth. The duration of degree IV was longest of all the developmental stages except for the first one. On the other hand, degree V was the shortest one, especially in males. The fish of this stage were found only for several weeks prior to the spawning (before attaining degree VI). Readiness for reproduction, i.e., degree VI of maturation in the roach females lasted probably from several hours to several days in its utmost. The females were probably discharging almost the entire storage of the roe and shortly thereafter they left the spawning place, their gonads empty being at degree VII. Very similar-sized eggs, visible at the first look at the ovaries of a female more advanced in the development, can suggest an one-cast spawning in this sex. Similar-sized eggs were reported for bream, and the opposite was found in *Blicca bjoernca* L. and *Scardinius erythrophthalmus* (L.) of the Szczecin Firth (Pęczalska 1963a). Disscecting the roach females for ascerting the fecundity also proved the eveness of roe in this species.

The males stayed probably longer at the spawning places than did the females and they were participating in the spawning repeatedly. This has been suggested by the state of their gonads examined at different stages of the spawning process. Sexual coloration was found to appear essentially during the phase of sexual readiness, but it can be sometimes observed as early as at degree V. The top of the head and the dorsal part of the body were covered with an intense eruption which was less conspicuous on the sides of the head and the body. Pelvic fins and the anal fin were bright-flame-orange. The caudal fin, pectoral fins and the aterior dorsal fin were red with black edging. The eyes were ruby-red, the body — bright silver.

The retrogressive changes in the gonads after the spawning occurred for a short period of about 6 weeks. At the end of this period, both the ovaries and the testes returned to the shape, size, and coloration typical for degree II.

The phenomenon of "pausing", i.e., spawning not every year, has been observed only in several individuals of the length over 20 cm whose gonads had been at degree II during the winter. No infertile fish were found even among the oldest ones.

The annual cycle of maturation described above for the roach of the Szczecin Firth did not occur uniformly for the whole population. The phenology of attaining by the gonads more advanced stages of maturation was found to depend on sex, body size, and probably on other factors (climatic conditions of the year, for instance). All these factors were causing that in particular years the spawning was started either by small, or average, or large fish. The duration of spawning was rather long, depending on hydrological conditions of a given year. Some differences were observed in the appearance of subsequent maturation degrees from year to year, depending on the prevailing temperature conditions.

C. GONAD DEVELOPMENT IN ROACH BEFORE THE FIRST SPAWNING

The duration of initial stages of maturation in juvenile roach prior to the first spawning was different from that in the adults which were repeating the reproduction process.

The sex of roach below 7 cm in length was almost indiscernible even with a lens. The reason for this was a later maturation of females than of males. Most of the fish at degree I were 8—9 cm long, although some were as long as 15 cm.

Juvenile individuals with gonads at degrees I and II could be found in the population throughout the year (Fig. 1). These two first degrees lasted probably from several months in individual specimens (in males spawning at the age of 2 years) to several years in the majority of females and in some males reaching their sexual maturity at the length of about 15 cm.

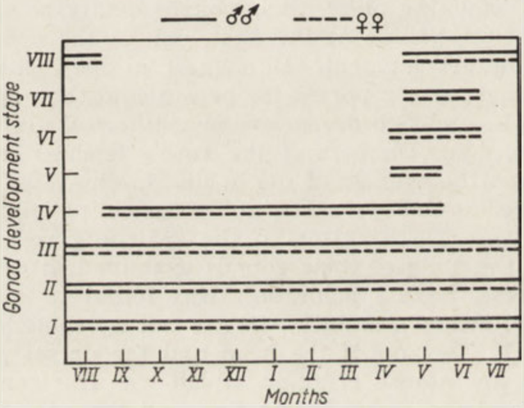

Fig. 1. Frequency of maturation degrees of the gonads within the annual cycle in *Rutilus rutilus* (1956—1964)

Degree II of maturation was observed to occur in males over 7 cm in length (age group I) and became more frequent in bigger fish. Throughout the year except for summer, when all the adult fish were at this stage, the length of males at degree II ranged from 7 to 18 cm, that of females — from 8 to 22 cm.

Degree III of reproductive maturation was also frequently found throughout the year. It can be assumed that the majority of juvenile, growing up fish had their gonads at degree III for a considerably longer time than the adults, for this degree occurred in old fish at a definite time of the year (in females through August, in males from the mid summer to late autumn).

Attaining degree IV and further maturation of the gonads followed rather concurrently both in juvenile and adult fish.

The minimum length of males that spawned for the first time was 9—10 cm, the minimum age being 2 years; in females these minima were 10—11 cm and also 2 years. By the length of about 12 cm, 50% of males were already matured (degree III including); in females this percentage was attained not earlier than at the length of 15 cm. Immature males of the length up to 18 cm and immature females up to 23 cm were rare exceptions (Fig. 2). This would indicate that the first sexual maturation followed very irregularly; the duration of degree I and that of further maturation stages (degree II to the first spawning) varied with individuals. This second period of maturation, lasting from one to several years, corresponded to the linear growth of the fish from 7 to 23 cm and age of 2 to 6 years.

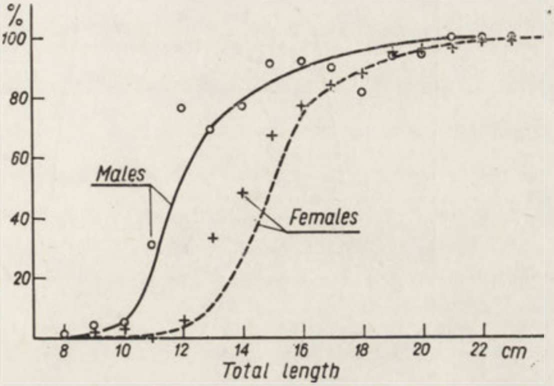

Fig. 2. Length data for the fish reproducing for the first time in *R. rutilus*

D. RELATIVE WEIGHT OF GONADS

The relative weight of gonads (percentage ratio of the gonad weight to the body weight) is a good index (besides the histological picture and macro-characteristics) for determining the degree of sexual maturation of fish. This index was variable even for the fish of the same age, same developmental stage, and of the same sampling. In general, the bigger was the fish the higher was the relative weight of its gonads. For example, the relative weight of ovaries of the roach which in springtime were at maturation degree IV and 15.5—17.4 cm long was 11.14%, whereas for the individuals of 31.0—33.4 cm length-class it amounted to 17.52%. In all the degrees of maturation the individual differences in relative weight were considerable.

The changes in the relative weight of the gonads during a year cycle are presented in Figures 3 and 4. The causes of these changes were the following: (1) an increase in the gonad weight during the subsequent stages of maturation, followed by a decrease on account of the spawning; (2) earlier attaining the further degrees of maturation by larger individuals *, there-

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* Early degrees of maturation (up to IV or perhaps V, inclusive) were, as a rule, attained earlier by larger fish.

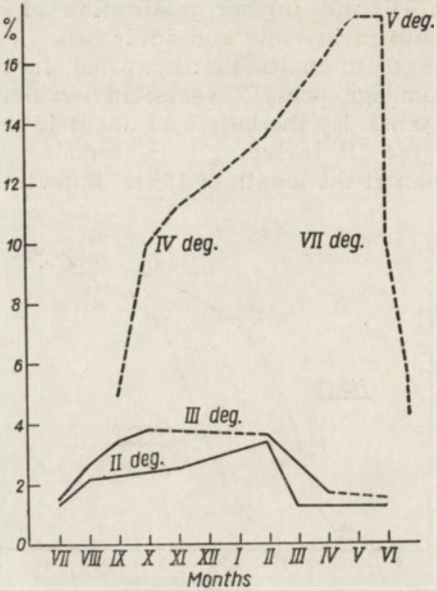

Fig. 3. Relative weight of ovaries at subsequent degrees of maturation within the annual cycle in *R. rutilus*

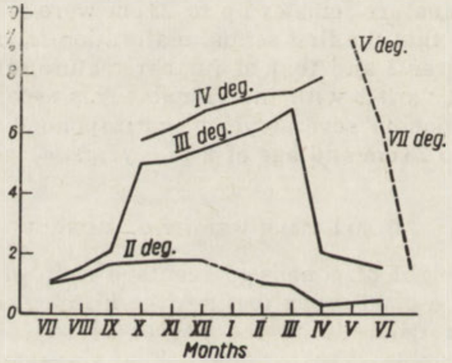

Fig. 4. Relative weight of testes at subsequent degrees of maturation within the annual cycle in *R. rutilus*

fore those with heavier gonads, e.g., bigger females were reaching degree IV in the autumn; (3) attaining a given degree of maturation by young fish with small and light gonads, e.g., attaining degree II by the fish of both sexes that up to then had the reproductive glands at the juvenile stage of development with a minimum weight.

The average relative weight of the ovaries at degree II was low throughout the year, ranging from 1.20 to 3.35%. This weight was still low at degree III, 1.54—3.81%. At degree IV, on the contrary, the average relative weight of the ovaries was much higher. It was increasing intensely in the autumn, slowly in the winter, and rapidly again in the spring. Extreme values of the relative weight of the ovaries at degree IV amounted to 28%.

The ovaries at degree V were of the same relative weight as those of the maximum weight at degree IV, which would suggest that during this period the ovaries did not grow but ripened only. It was not possible to ascertain the relative weight at degree VI since the captured females were discharging the roe already during the fishing. After the spawning, a rapid decrease in the relative weight of ovaries was observed until July.

During the whole annual cycle of maturation, the relative weight of the testes was considerably lower than that of the ovaries. At degree II it ranged from 0.29 to 1.74%, at degree III — from 1.13 to 6.77%. Degree IV appeared in October; the testes grew then not very intensely until the following spring. Their relative weight ranged then from 5.86 to 7.91%. The corresponding weight at degree V was 8.97% with no increase observed during this degree of maturation. The testes were losing their weight slowly at degree VI, and more rapidly at degree VII, i.e. during the retrogressive changes.

The changes in relative weight of gonads described above permit to conclude on the phenology of these degrees of maturation which can be found throughout the year (degrees I—III).

Sex ratio in the roach population.

Of a total of 8.477 individuals examined, ranging in length from 8 to 42 cm, 3.046 (36%) were males and 5.421 (64%) — females. This ratio was rather constant in subsequent years of the study although it varied with the body length. The males under 12 cm in length were more numerous, within 13—15 cm class this ratio was almost 1:1. With a further growth of the fish, the numerical preponderence of females was increasing although it showed high variations. Unaccountable was for example an increase in the incidence of males within 23—30 cm class. That increase cannot be explained by different sex ratios of various generations of the roach.

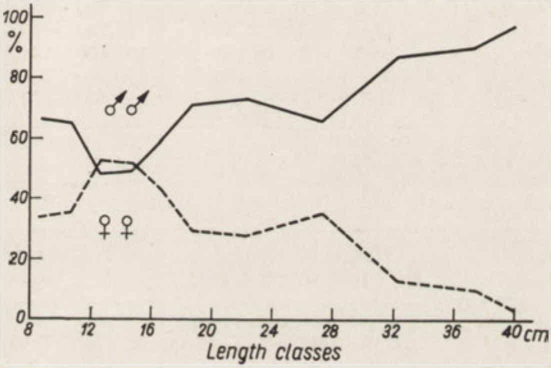

Fig. 5. Sex ratio in relation to body length in *R. rutilus* (per cent)

E. FECUNDITY

The absolute fecundity of Rutilus rutilus ranged from 3,400 to 272,800 eggs per female. Individual variation was very high. Depending on the fish dimensions, the fecundity ranged from several to a score of thousands of eggs within the same length-class. There were observed some individuals with high numbers of eggs even over the average value for a given length-class, although the weight of their gonads was low as for this class. On the other hand, there were other females whose ovaries were small, containing low numbers of minute eggs. Usually the roe of smaller females was finer; as the fish were growing from 16 to 37 cm in length, the average weight of 100 eggs increased from 64 to 105 mg, this increase being unproportional (Tabl. I). In general, the dimensional differences of eggs of one female were inconsiderable.

Table I

The absolute fecundity — body length dependence in *R. rutilus*

Length class, cm:	Average body weight g	No. observations	Weight of gonads, IV-V maturation degree,		No. eggs in ovaries, thousands		Average g
			from-to	average	from-to	average	
13.5—15.0	28	8	1.30—4.03	2.92	3.094—6.790	4.353	60
15.5—17.0	35	10	2.70—5.36	3.90	4.774—8.576	6.358	60
17.5—19.0	52	10	4.38—7.90	6.0	6.072—11.591	8.908	67
19.5—21.0	89	11	5.84—21.0	13.8	10.278—26.520	18.906	72
21.5—23.0	125,5	10	12.0—28.0	19.3	14.640—34.020	24.646	78
23.5—25.0	179	10	20.0—34.0	26.4	16.000—43.833	34.319	76
25.5—27.0	239	15	20.0—68.0	36.3	29.240—66.640	47.185	77
27.5—29.0	296	11	32.0—66.0	53.0	43.650—92.400	62.920	84
29.5—31.0	350	11	33.0—87.0	71.0	43.560—87.000	66.846	101
31.5—33.0	485	10	65.0—105.0	85.0	76.700—116.000	95.117	89
33.5—35.0	598	8	80.0—120	103.2	102.400—184.080	128.870	80
35.5—37.0	725	6	100—145.5	126.3	120.000—221.000	165.924	78
41.5—43.0	1140.0	2	220—235	225.5	258.500—272.800	265.650	84,8

Table II

The absolute fecundity-body weight dependence in *R. rutilus*

Weight class, g	Average body length cm	No. observations	Weight of gonads, IV-V maturation degree		No. eggs in ovaries, thousands,		Weight of 100 eggs, mg, average
			from-to	average	from-to	average	
25—74	16.3	15	1.42—7.90	4.18	3.408—9.164	6.455	64
75—124	20.8	15	5.84—26.0	14.9	10.278—29.680	20.199	73
125—174	23.1	10	14.0 —34.5	21.8	16.000—34.020	25.218	86
175—224	25.2	10	20.0 —47.0	29.5	28.160—52.400	40.720	72
225—274	26.6	11	24.5 —68.0	35.8	32.881—66.640	47.353	75
275—324	28.1	11	30.0 —66.0	51.4	47.839—92.400	61.755	83
325—374	29.6	6	52.0 —87.0	68.0	64.000—79.040	71.025	95
375—424	30.8	4	43.0 —77.0	60.0	54.180—87.010	76.631	78
425—474	32.6	3	65.0 —82.0	75.6	76.700—100.860	89.320	84
475—524	32.6	6	70.0 —105.0	87.6	88.200—110.000	97.100	90
525—574	34.0	1		83.0	103.750		80
575—624	34.6	4	100.0 —115.0	105.0	112.700—126.000	118.675	88
625—674	33.6	1	—	116.8	—	109.792	106
675—724	35.5	4	88.0 —130.0	117.0	135.600—221.000	165.360	70
825—874	37.0	1		145.4		137.548	105
1140.0	42.0	2	220.0 —235.0	225.0	258.500—272.800	265.650	84

The absolute fecundity was found to depend on the size of the female and was increasing with the growth of the fish (Tab. I and II, Figs 6 and 7). Especially high increase in absolute fecundity was observed in the biggest (the longest) specimens, though not in the expense of the eggs weight, since, as it has been already said, bigger females had bigger eggs as compared with those of smaller ones.

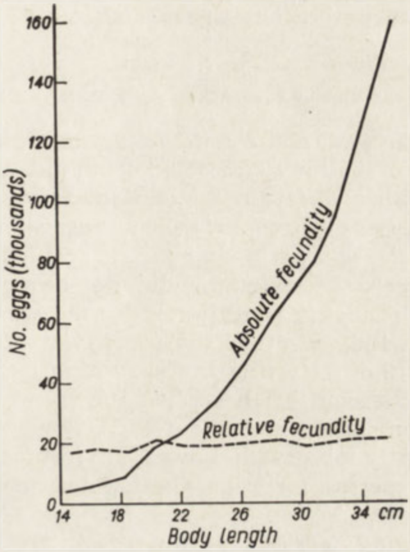

Fig. 6. Absolute and relative fecundities related to the fish growth (length) in *R. rutilus*

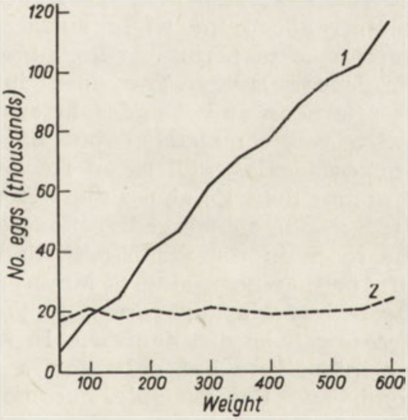

Fig. 7. Absolute and relative fecundities related to the fish growth (weight) in *R. rutilus*

The dependence of absolute fecundity from the female body weight was not so regular as that from the body length. This pertains mostly to the heaviest individuals. It is still possible that there was inadequate number of sampled individuals among these weight-classes.

The relative fecundity, i.e., the number of eggs per female related to 100 g of female's body, was found to be independent from the female size (Figs 6 and 7). It varied within length classes from 17.000 to 23.000.

F. SPAWNING PLACES AND BEHAVIOUR

The Szczecin Firth and mainly its adjacent waters, plentiful of calm small bays, canals, and shallows protected from the wind, their shores and bottoms overgrown with proliferous vegetation, formed suitable habitats for spawning of the roach, the species eurytypic as regards the choice of reproduction sites. The roach can spawn in any place, provided there is calm water and vegetation. However, the spawning did not occur in all of the suitable sites, the choice being probably conditioned by the weather factor of a given year. Nevertheless at windy weather the roach was often observed to linger in the belt of reeds without starting the spawning.

The roach of the Szczecin Firth started the spawning as the first of the ciprinid species at a temperature of about 14°C, however, at 16°C the spawning was more frequently observed. Lower environmental and temperature requirements in this species for reproduction led usually to a successful course of the spawning.

The largest spawning terrains of the roach are those of the northen part of the Firth (so-called "Kamionka", "Wucyk", the upper course of the Swina River with its numerous canals, and the Dziwna River). In these habitats the roach spawned at the shallowest places overgrown with high reeds or among off-shore reed clumps, where the plants immerged not more than for several centimeters above the water surface. The spawning was even observed in the water void of plants. At the sunset or sunrise the fish came to these sites from deeper layers. The most intense spawning was observed to occur in the afternoon and it ended before the dusk.

The roach at spawning was completely void of fear. The courtship depended on persuits and escapes as well as on frequent jumps (2 to 3) of the whole groups of fish (more than 10) above the water surface.

The roe was deposited on the submerged sections of plants.

The spawning of the roach in the Szczecin Firth lasted for about 3 to 4 weeks, from the end of April, or beginning of May (the second half of May was an exception) to the end of May or, rarely, to the beginning of June. Three phases of this process can be distinguished. In some years, the roach spawned in masses very intensively but only for a short period under extreme overcrowded conditions. For example, enormous abundance of the roach and bream was recorded in the Dziwna River on 24th of May, 1958, when the water temperature was 16.5°C. The bream was spawning near the surface, the roach in deeper waters under the bream in any place where a tiny spot of vegetation was present. This strong spawning followed there till midnight. On 25th of May (temp. 18°C) the swarms of fish disappeared. A smaller surge of the spawning population of roach was observed on the following day, 26th of May; the fish spawned in the evening. On 27th of May, the spawning was less intense on account of lower temperature (15°C). A further drop in temperature to 14.5°C has stopped the spawning in this region on 28th of May. A similar intense spawning took place in the Dziwna River in 1964. In other years, the spawnings were rather dispersed without such strong aggregation of the fish in the spawning places.

The roach was observed to spawn together with the bream as well as with *Scardinius erythrophthalmus* (L.) and silver bream, *Blicca bjoernka* L. For example the fishing in the spawning grounds of the north-western part of the Wicko Lake, which had a broad connection with the Szczecin Firth, carried out by night of 15th of May, 1956, yielded 41 roach, 39 bream and 32 silver bream, all spawning.

In general, smaller roach was found to spawn earlier than the larger one. Some exceptions were noted, when, for example in 1959 (a very early and warm spring) these were large fish and in 1960 — the medium-sized fish, which started the spawnings. The males, maturing earlier than females, came to the spawning places earlier and stayed there, or in the vicinity, longer than did the females. The females, after having discharged the whole roe stayed only for a while in the spawning place and later on they were returning promptly to the feeding population abode. The fishings during the roach reproduction period yielded mostly one sex. At the feeding places, juvenile fish with the gonads at degree I or II was well as the females (very often) at maturation degree V and VII have been usually caught. At the vicinity of the spawning place or in the spot of spawning the yields contained almost exclusively either males or females, rarely equal proportions of the both sexes. The roach was found to feed during the reproduction, but less actively.

G. COMPOSITION OF THE SPAWNING POPULATION

The sex ration in the spawning population captured at the spawning places or in their vicinity can differ. Among the fish of more or less equal length, one sex prevails or counterbalances the other, irrespective to the size of the fish. In the total material collected from the spawning places within 10 years, the ratio of males to females was 8:9, i.e., almost 1:1. This ratio was found to depend to a greater degree on the phase of the spawning. For example, in the catch of 8th of May, 1958, the sex ratio was 13:1; on 9th of May — 1:1; and on 27th of May (the end of spawning) — 1:36.

The spawning population of the roach under study consisted of the fish of total length 11—44 cm, with males measuring from 11 to 38 cm and females from 12 to 44 cm. Most numerous were the males 16—30 cm long and the females 20—34 cm long.

4. DISCUSSION

The detailed studies of the maturation cycles of various species of fish showed that the commonly used maturation scale worked out by Maier is hardly applicable to most of them (Backiel 1952, Brylińska 1963, Chełkowski 1965, Pęczalska 1963 a, and 1966). The roach of the Szczecin Firth also renders such difficulty.

In describing the macro-characteristics of gonads in the roach population examined an ust was made of the Maien's (1940) paper on the maturation of *Rutilus rutilus caspicus* (Jak.) of the Caspian Sea. The maturation cycles of these two forms were found to be similar in their macro-characteristics, the relative weight of gonads, the duration of subsequent degrees of maturation, and in an one-cast of the roe at spawning. Taking into account the histological studies, macro-characteristics, and relative weight of gonads of *Rutilus rutilus caspicus*, Maien (1940) has adopted a 6-degree scale for this form. In his scale, stage V of Maier's scale has been pooled together with stages VI, VII, and VIII. However, the authoress of the present paper is inclined to consider a 7-degree scale as most suitable for the roach.

The results of the present observations on the annual cycle of the roach corroborate those reported by Romański (1963) on the roach of the Vistula Firth, but they do not agree somewhat with those given by Załachowski (1959). According to him the ovaries of the roach in the period from March to early May can indicate the transfer stage from V to VI of the Maier's scale. This difference could probably result from different environmental conditions.

One-cast spawning in the females of R. rutilus was reported by Pliszka (1964) and indirectly by Załachowski (1959) who wrote about the uniform size of the roach roe. One cast of roe and a short period that the females stay at the spawning place were reported by Maien (1940) as typical for *R. rutilus caspicus* of the northern part of the Caspian Sea.

The results of the present paper indicate that both the range of the body length and of maturation age in the roach are broader as compared with those reported by Neuhaus (1936) and by Skóra (1964 a i b). However, they confirm the findings given by Stangenberg (1953) and Załachowski (1953).

The relative weight of gonads in the roach under study was similar to that reported by Skóra (1964 a and b) from the water bodies in Kozłowa Góra and in Goczałkowice, but the obtained values were much higher than those reported by Pliszka (1964).

The sex ration in a fish population can vary greatly. The reasons of this variation can be different and numerous: the species differences, the body length of the fish, the seasonal aspect, the feeding and reproductive periods, different growth rates of males and females, earlier maturation of one of the sexes, overall mortality and accidental (due to the fishing technique) mortality differen for each sex, and perhaps the environmental conditions. The sex ratio in fish was also found to change with time factor (Beverton 1964, Brylińska 1963, Chrzan 1959, Gorbacz 1962, Gundrizer and Titow 1962, Wescłowicz and Wiktor 1959, Filuk 1962 and 1963, Nikitińskaja 1962, Pęczalska 1963, Romański 1962 and 1964, Sclewski 1961 and 1962, Starmach 1963, Strzeżewska 1959, Starmach and Rosół 1961, Suworow 1954, Żarnecki 1963). Most of the authors mentioned have reported on the numerical preponderence of females over the males. This finding is in accord with a known ecological fact that the survival of females is higher than that of males (Park 1958).

The fecundity of the roach from the Szczecin Firth was much lower than that of the roach collected from the Goczałkowice dam reservoir (Skóra 1964b), but it was much higher than the values observed for the roach of some of the Masurian Lakes (Załachowski 1959). The latter author has also reported on a high individual variation of this feature. Although the data presented by him on the fecundity of roach in USSR are related to the body length (l. corporis), they allow to conclude indirectly that the only population of roach which is more proliferous than the roach of the Szczecin Firth is that of the mid-course of the Volga River.

Załachowski (1959) has reported on the increasing relative fecundity of the roach in certain Masurian Lakes with the growth of the fish, whereas the fecundity of the roach of the Szczecin Firth remained almost constant during the entire life cycle. It is possible that the relative fecundity in fish, depending on the fish growth, can follow different patterns in various species (Kaj and Wołoszyński 1957, Filuk 1962, Sołewski 1960 a, 1962, 1964), but it can be also affected by environmental factors. The relative fecundity can vary with years (Wołodin 1963).

The earlier (by 1—2 years) maturation of males than females, thus males maturing as smaller, is rather common in fish. The roach of the Szczecin Firth follows this rule.

The dependence of the spawning time (within several weeks' change) from the size of the fish differs from species to species (Filuk 1962, Wiktor and Żukowski 1962). The lack of this dependence in the roach of the Masurian Lake District has been reported by Wilkońska and Żuromska (1967). The roach of the Szczecin Firth spawned at a higher temperature and for a shorter period than that of the Vistula Firth or the Pucka Bay (Romański 1963 and 1965).

According to Pliszka (1953), the diel peak of spawning of the roach from lake Harsz was observed to occur in the noon and not in the evening, as it was the case for the roach of the Szczecin Firth.

The findings of the present paper corroborate those reported by Swärdson (1962) who dealt in detail with the roach spawning under natural conditions.

The percentage incidence of males in the spawning population of roach of the Vistula Firth was lower (Romański 1963) than the corresponding percentage for the roach of the Szczecin Firth.

An increased salenity of water in the Szczecin Firth as compared with that of freshwater environments had no perceptible effect on the reproductive behaviour of the roach.

5. SUMMARY

The roach of the Szczecin Firth can be defined as a semi-migratory form, similar in this respect to *Rutilus rutilus caspicus* (Jak.). The material, gathered in the years 1956—1966, involved biological analyses of 6.636 specimens; in 1.219 fish the gonads were weighed, and the fecundity has been ascertained for 122 females by means of the weighing method. From the macroscopic description of the gonads and their relative weight, the Maier's scale of sexual maturation has been adopted to study the roach of the Szczecin Firth. Studies on the cycle of sexual maturation involved the phenology (time and duration) of subsequent developmental stages in the gonads (Fig. 1). It was found that (1) after the spawning the gonads were at maturation degree II in June and July, and at degree III in August. The females attained degree IV in autumn (September-October), the males at the beginning of winter. Degree V appeared several weeks prior to the spawning. Degree VI, readiness for reproduction, lasted over 10 hours in females and around 10—20 days in males. Recurring casting of the reproductive products was observed to occur in males and only one cast of the roe in females. The retrogressive changes in the reproductive glands lasted for about 6 weeks following the spawning; (2) the cycle of the first maturation in the roach showed a high variation. The fish at degree I were encountered throughout the year among the individuals of the total length up to 15 cm. Those at degree II were also found through out the year among males 7—18 cm in length and among females 8—22 cm in length. Degree III lasted longer

in the maturing individuals than in the older fish which have been reproducing for several times. Further developmental stages were similar in these two age groups; (3) the roach was found to mature at the age of 2—6 years with a total length (l.c.) of 9—23 cm (Fig. 2): (4) annual changes in the relative weight of gonads followed the patterns presented in Figs 3 and 4; this weight was found to increase with the growth of the fish; (5) the sex ratio of males to females was 9:16 in the commercial population and 8:9 in the spawning population. The ratio was found to change with the age of roach; (6) the absolute fecundity amounted to 270.000 eggs per female (Tab. I and II, Fig. 6 and 7) and it showed rather high individual variation.

The numerous spawning places in the Szczecin Firth did not differ much in their character from those of the freshwater environments. The spawning was found to begin in the end of April or early May and lasted 3 to 4 weeks with some intervals. The fish of the spawning population had a total length from 11 to 44 cm.

6. STRESZCZENIE

Płoć Zalewu Szczecińskiego można uważać za formę półwędrowną, podobną w tym do wobły.

Materiał do pracy zebrano w latach 1956—1966, wykonując analizy biologiczne (6 636 płoci). Zważono gonady 1 219 ryb, obliczono płodność 122 samic metodą wagową.

Na podstawie opisu makroskopowego i względnego ciężaru gonad płoci młodocianej i dorosłej przystosowano skalę Maiera do badań płoci, zbadano cykl dojrzewania, porę przechodzenia gonad w poszczególne stopnie rozwojowe i czas ich trwania (rys. 1). Stwierdzono, że: 1. Gonady płoci po tarle są w czerwcu i lipcu w II stopniu dojrzałości, w sierpniu — w III. Samice IV stopień osiągają jesienią (wrzesień-październik), samce na początku zimy. V stopień pojawia się dopiero na kilka tygodni przed tarłem. Stopień VI — gotowość do rozrodu — trwa u samic około kilkunastu godzin, u samców — do kilkunastu dni. Samce mają tarło wielomiotowe, samice wycierają się jednorazowo. Zmiany wsteczne zachodzą w gruczołach płciowych w ciągu około 6 tygodni. 2. Pierwszy cykl dojrzewania płciowego przebiega w badanej populacji bardzo nierównomiernie: I stopień spotyka się w ciągu całego roku u płoci do 15 cm długości całkowitej. Stopień II spotyka się w ciągu całego roku u samców o długości 7—18 cm, u samic — 8—22 cm. III stopień u osobników dorastających trwa dłużej niż u ryb powtarzających cykl rozwoju. Dalsze stadia rozwoju przebiegają w obu grupach ryb podobnie. 3. Płoć ta dojrzewa w wieku 2—6 lat, przy długości całkowitej 9—23 cm (rys. 2). 4. Roczne zmiany w ciężarze względnym gonad przedstawiono na rys. 3 i 4. Zwiększa się on w miarę wzrostu płoci. 5. W stadzie przemysłowym znaleziono ogólnie 36 samców na 66 samic i ten stosunek zmienia się z wiekiem płoci (rys. 5). W stadzie tarłowym ogólnie przypada 9 samic na 8 samców. 6. Płodność absolutna dochodzi do 270 000 jaj (tabl. I i II, rys. 6 i 7). Wahania osobnicze wysokie.

Liczne w Zalewie tarliska nie odbiegają charakterem od tychże w wodach słodkich. Tarło zaczyna się w końcu kwietnia lub pierwszej połowie maja i trwa z przerwami 3—4 tygodnie. Stado tarłowe składa się z ryb o długości całkowitej 11 do 44 cm.

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FLOWING-WATER POLAROGRAPHIC RESPIROMETER FOR AQUATIC ANIMALS

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ABSTRACT

Construction and technique of using flowing-water polarographic respirometer designed for the research on aquatic invertebrates are described. The apparatus assures continuous, required conditions of both temperature and oxygen concentration, and guarantees a stable water flow.

CONTENTS

1. INTRODUCTION

Studying the oxygen consumed by the aquatic animals in flowing-water respirometers one escapes certain undesirable phenomena that take place when confined respirometers are used. In the flowing-water respirometers neither oxygen concentration decreases, nor accumulations of metabolites appears, and conditions are stable and controlled. Due to this, long-term measurements are possible, and the period of acclimatization to the respirometer can be rejected (Mann, 1965). Here, the concentration gradients of oxygen and of metabolites at the body surface connected with the boundary layers (Ambühl, 1959) are shortened (Philipson, 1954). Furthermore, in the flowing-water respirometers one can perform studies on the role of current speed, and no problems exist as concerns the exchange of gases between the water and gas phases, as it can be observed in manometric respirometers.

In the case of a low oxygen consumption by the organisms here examined, thus, during a feeble water flow through the respirometer, when a continuous recording is necessary, and/or when the oxygen cannot be estimated chemically, the oxygen concentration should be determined using the polarographic method. Hence, the flowing-water polarographic respirometers are more and more applied at present (Spoor 1948, Jaag 1955 and Ambühl 1959, Mann 1958, 1965, Edwards, Learner 1960, Bielawski 1959, 1961, Berg, Jonasson 1965, Harrison 1966, Eriksen, Feldmeth 1967).

The present paper deals with the description of the flowing-water polarographic respirometer, describes the procedure, as well as gives some results of measurements. The apparatus discussed in the paper is designed for the measurements of respiration of the individual aquatic invertebrates, particularly of the Plecoptera, Ephemeroptera and Odonata larvae, and guarantees the controlled conditions of oxygen contents and of temperature, as well as a constant current speed.

The prototype of this apparatus was constructed by one of us (R. Z. Klekowski) in the Freshwater Biological Laboratory of the University Copenhagen, Hilerød (Denmark) under the direction of Prof. Dr. K. Berg. We wish to thank him for many helpful suggestions. The prototype was done from the glass. It was discussed in a congresspaper (Klekowski, 1964).

The content of this paper was presented as a laboratory manual in Klekowski, Kamler (1967).

2. CONSTRUCTION

The apparatus is placed in a large water thermostat (1_1) *. The thermostat characterized by the capacity amounting to 180 l has its glass walls insulated at the bottom and at the sides with styrene foam mats, 5 cm in thickness. The front wall insulation can be taken off to facilitate visual observations. Temperature can be controlled precisely with an accuracy of $\pm 0.015^\circ C$ from a point slightly above $0^\circ C$, using a cooling device (7_{81}) or a heater, and toluen thermostat. The temperature was continuously recorded (7_{80}).

Water prepared in a way described below was poured into a reservoir-bottle. Two kinds of bottles were applied alternately:

I. The outlet of water is assured mainly due to a stable gas overpressure above the water level in the bottle. The required amount of O_2 in water can be obtained by ventilating the water in the bottle with gas mixtures of required composition.

II. The outlet of water is assured mainly due to the differences in levels between the water in the bottle and final outlet. The required amount of O_2 in water can be obtained by ventilating the water with homogeneous gas (N_2 or O_2) during a defined time span.

Bottle I (1_2) characterized by a capacity amounting to 10 l is fastened firmly to the edge of the thermostat. The rubber stopper (1_3, 2_3) of the reservoir-bottle I is protected against the gas overpressure that tends to push it out. Three glass tube pass through the rubber stopper. The first glass tube (1_4, 2_4) serves for filling in the reservoir-bottle with water. The second glass tube (1_5, 2_5) leads the ventilating gas that flows out through a gas diffuser Schott G3, 20 mm in diameter (1_6, 2_6). The last glass tube (1_7, 2_7) leads off, during the ventilation, the excess gas through the other glass tube (1_8, 2_8) into a container with water, approximately 50 cm in depth (1_9), (Fig. 2A), or feeds gas that maintains the constant pressure in the reservoir-bottle after the ventilation (Fig. 2B). The excess of gas escapes then through the glass tube (1_{10}, 2_{10}) into a glass cylinder, 45 mm in diameter, 200 cm in height, filled in with water (1_{11}). The level of the gas outlet in water (consequently also the gas pressure onto the water level in the reservoir-bottle) is controlled. The water from the reservoir-bottle is led off to the respirometer through a glass tube (1_{12}).

Reservoir-bottle II (1_{13}) characterized by a capacity amounting to about 10 l is fastened firmly to the thermostat bottom. The neck of the reservoir-bottle, plugged with a rubber stopper, is directed downward; the bottom of the bottle is cut off, its upper edges emerging from water in the thermostat. Three glass tubes pass through the stopper (1_{14}). The first tube (1_{15}) serves for filling in the reservoir-bottle with water. The second tube (1_{16}) is for ven-

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*) Left cipher above means the number of the figure; right cipher below — the number of the element considered.

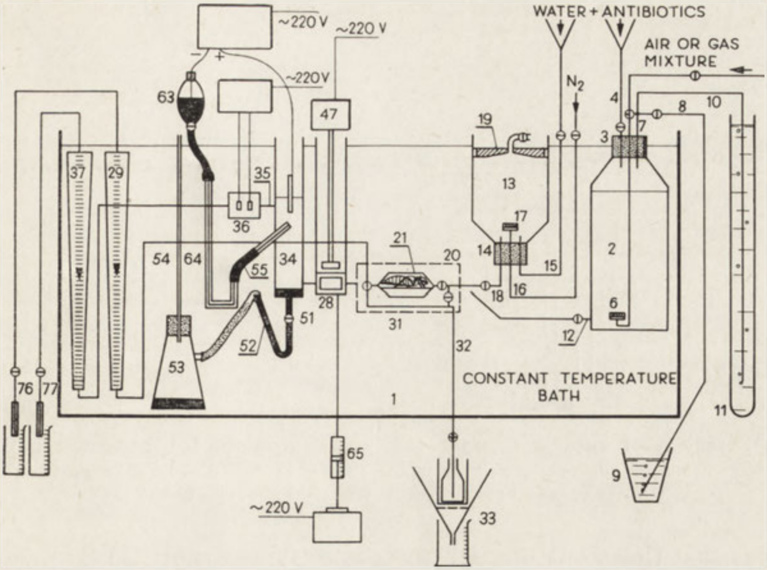

Fig. 1. General diagram of polarographic respirometer (for explanation of numbering see denotation)

1 — constant temperature bath, 2 — reservoir-bottle I, 3 — rubber stopper of the reservoir-bottle I, 4 — glass tube for filling up the reservoir-bottle I with the water, 5 — glass tube ventilating gas inlet to the reservoir-bottle I, 6 — gas diffuser Schott G3 for reservoir-bottle I, 7 — glass tube, gas outlet from the reservoir-bottle I during ventilation, or gas inlet during regulation of gas pressure onto the water in the reservoir-bottle I, 8 — gas tube, final outlet for the gas from the reservoir-bottle I during ventilation, 9 — container with water, 10 — glass tube, final outlet for the gas from the bottle during regulation of the gas pressure onto the water in the reservoir-bottle I, 11 — glass cylinder filled with water, 12 — glass tube, water outlet from reservoir-bottle I, 13 — reservoir bottle II, 14 — rubber stopper of the reservoir-bottle II, 15 — glass tube for filling up with water the reservoir-bottle II, 16 — glass tube, ventilating gas inlet into the reservoir-bottle II, 17 — gas diffuser Schott G3 for reservoir-bottle II, 18 — glass tube, water outlet from the reservoir-bottle II, 19 — polyethylene disk, 20 — blick of plexiglass, 21 — respiration chamber, 22 — supporting brass rods, 23 — water inlet to the respirometer, 24 — direct route, 25 — tap, 26 — braking capillary for inflowing water, 27 — three-way tap, 28 — electrolyte-mixer, 29 — flow meter, 30 — fine-control tap, 31 — control route, 32 — glass tube for water sampling, 33 — water sampling for the analysis of oxygen concentration by Winkler method, 34 — polarograph electrode chamber, 35 — electrode chamber outlet, 36 — conductometer chamber, 37 — flow meter, 38 — nylon fibre filter, 39 — inlet part of the respiration chamber, 40 — middle part of the respiration chamber, 41 — animal chamber, 42 — insert, 43 — outlet part of the respiration chamber, 44 — electrolyte inlet to the electrolyte-mixer, 45 — magnetic stirrer, 46 — magnet, 47 — electric motor, 48 — water-proof plexiglass cylinder, 49 — water inlet to the polarograph electrode chamber, 50 — mercury pool, 51 — waste mercury, 54 — decompression glass tube, 55 — wide-bore dropping-mercury electrode, 56 — screw plug, 57 — rubber washer, 58 — silver (silver chloride) reference electrode, 59 — seal of the Mendeleev (or Dekhotinsky) sealing wax, 60 — silver rod coated with silver chloride, 61 — cable, 62 — paraffin wax, 63 — mercury reservoir, 64 — braking capillary for mercury, 65 — electrolyte supply device, 66 — glass syringe, 67 — electrolyte reservoir, 68 — three-way tap, 69 — kymograph axis, 70 — claw coupling, 71 — hollow metal cylinder, 72 — gap in the metal cylinder, 73 — pilot bar of feel screw, 74 — feed screw, 75 — quick-change clasp nut, 76—77 — braking capillaries for outflowing water, 78 — polarograph, 79 — conductometer, 80 — temperature recorder, 81 — cooling device.

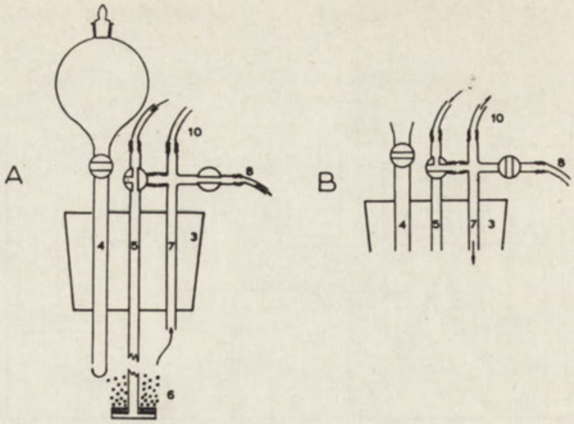

Fig. 2. Diagram of rubber stopper and its equipment for reservoir-bottle I
A — Taps in position of gas-ventilation of water in the bottle. B — Taps in position of regulation of gas pressure onto the water in the bottle

tilating gas that flows out through the gas diffuser Schott G3 (1_{17}), 20 mm in diameter. Through the third tube (1_{18}) water is led off to the respirometer. The upper surface of water in the reservoir-bottle II is closed with a 20 mm thick polyethylene disk (1_{19}) that floats on it. The lower surface of the disk is funnel-shaped, and in its centre there is an opening with a tube and a tap for the gas that flows out during the ventilation of the water. A 1—2 mm gap between the disk and bottle walls is filled in with fluid paraffin oil.

The respiration chamber (1_{21}, 3_{21}) is found in a cut of a 30 mm thick plexiglass block (1_{20}, 3_{20}, 4). Canals for water flow ($\varnothing$ 3 mm) are pierced in the block of plexiglass. The block is fastened to the thermostat frame with the aid of supporting brass rods (3_{22}). The reservoir-bottle is connected with the water inlet to the respirometer (3_{23}) using glass tubes. The connection between the glass and plexiglass is safeguarded with a short rubber pipe. The water flowing into the block can flow in two ways:

I. In a direct route (3_{24}) through the tap (3_{25}), or through a braking capillary for the inflowing water (3_{26}), into the respiration chamber (1_{21}, 3_{21}), and then towards the three-way tap (3_{27}), and from here to the electrolyte-mixer (1_{28}, 3_{28}), or immediately to the flow meter (1_{29}) and outlet.

II. Through the fine-control tap (3_{30}) to the control route (1_{31}, 3_{31}), three-way tap (3_{27}), and electrolyte-mixer (1_{28}, 3_{28}), or to the glass tube for water sampling (1_{32}), where water samples are taken for Winkler determination (1_{33}).

Water that flows into the electrolyte-mixer (3_{28}) from the respiration chamber (1_{21}, 3_{21}), or from the control route (3_{31}), passes then to the polarograph electrode chamber (1_{34}, 3_{34}, 5), and through its outlet (3_{35}) into the conductometer electrode chamber (1_{36}), to reach the outlet through the flow meter (1_{37}).

The braking capillary (3_{26}) is made of glass. In the inlet branch flaring there is a nylon fibre filter (3_{38}). The connection between the capillary branches and plexiglass block is sealed with the Silicon rubber mouding paste "Escorubber" SR 300, ESCO (Rubber) Ltd, London E 8, and with a rubber hose.

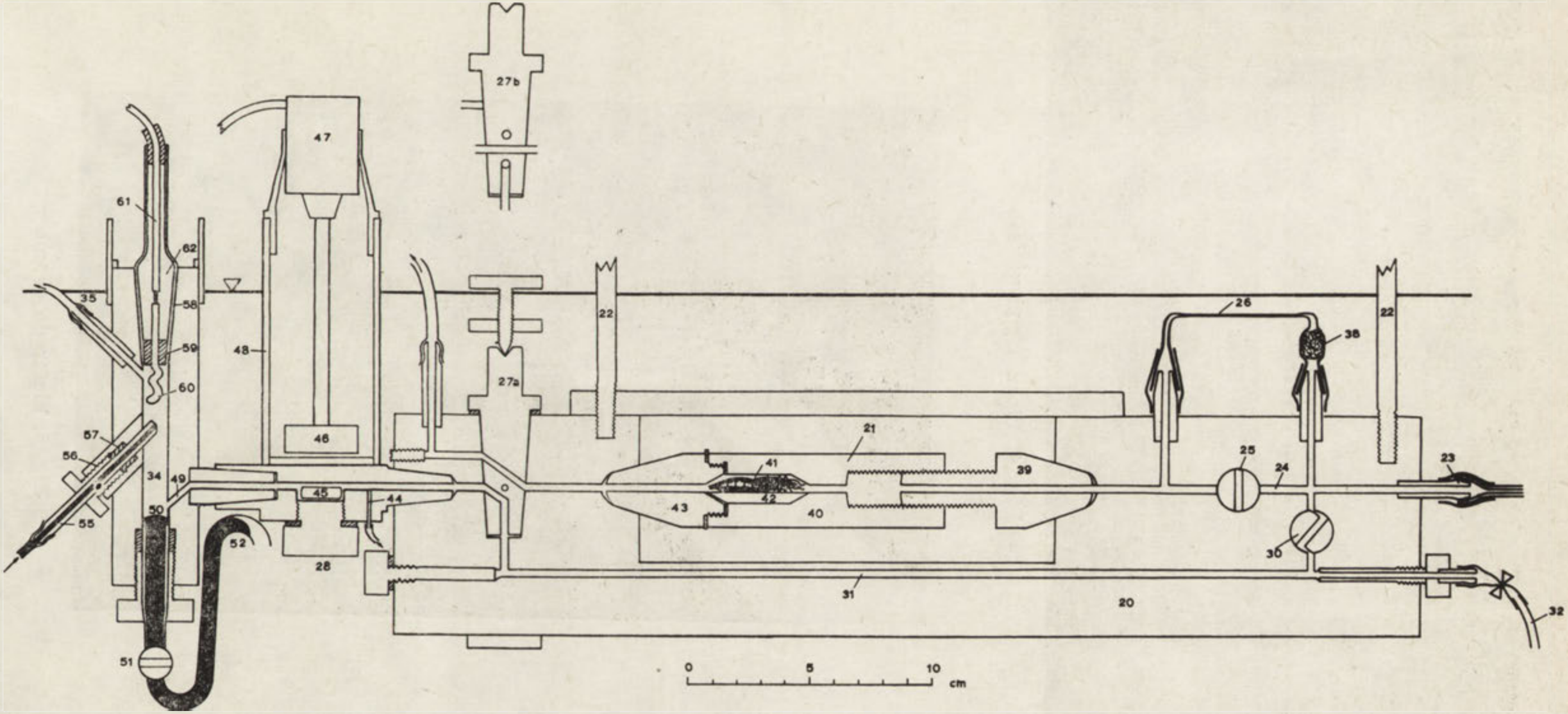

Fig. 3. Diagram of respiration chamber, control route, electrolyte-mixer and electrode chamber

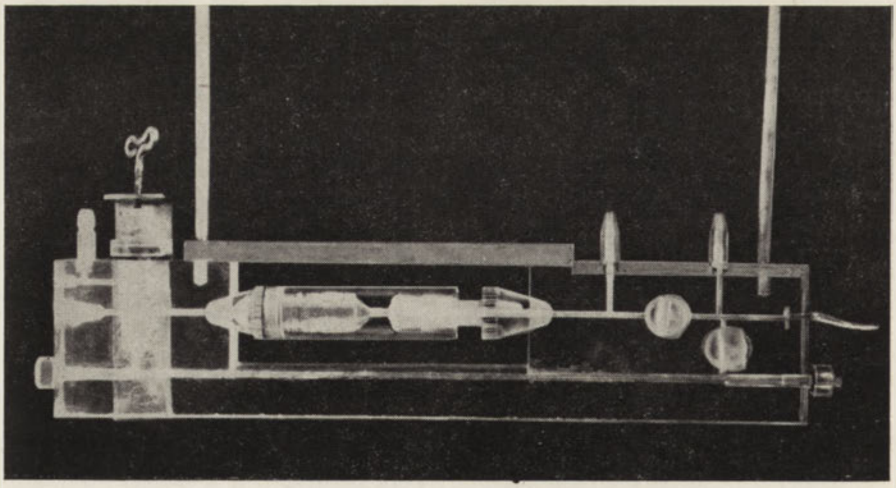

Fig. 4. Block of plexiglass

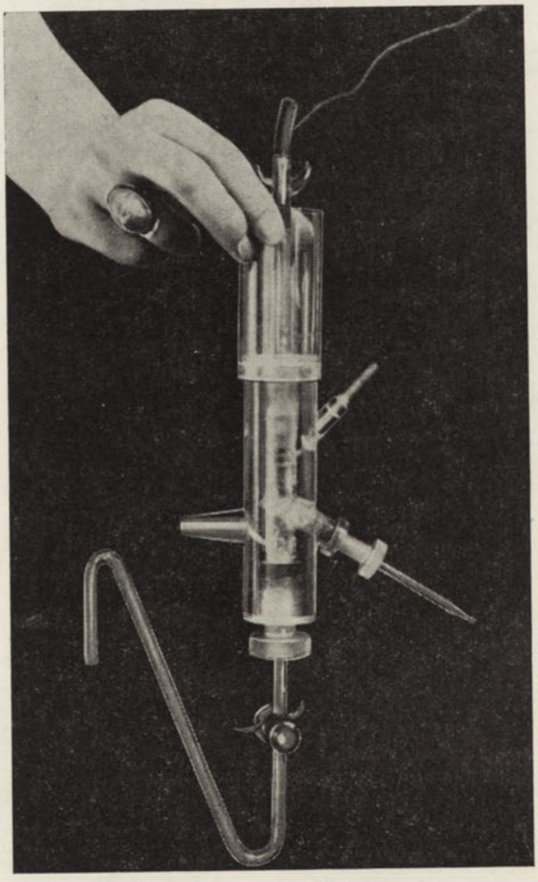

Fig. 5. Electrode chamber

The respiration chamber is made of a plexiglass shaft and consists of three parts. The cone-shaped inlet part (3_{39}), sealed with vaseline, sticks in the hollow of the block. Another threaded end of the inlet part is screwed to the middle part of the respiration chamber (3_{40}). Such a screw-like connection, sealed with vaseline, makes it possible to change the lenght of the chamber, or to take the chamber away, or even to put it into the block. In the middle part there is found animal chamber (3_{41}). The chamber is semicircular in section and an insert (3_{42}) constitutes its flat bottom. Behind, the chamber is closed up with the outlet part of the respiration chamber (3_{43}) placed on a screw thread and sealed with rubber rings. The cone-shaped outlet part sticks in the hollow of the block. The middle part of the respiration chamber (3_{40}) is replaceable. This allows one to adapt the size of the animal chamber (3_{41}) to that of the animals under examination.

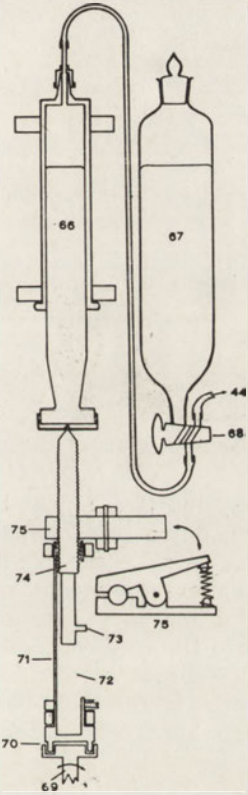

Fig. 6. Electrolyte supply device

The electrolyte-mixer (1_{28}, 3_{28}) made of plexiglass sticks with its cone-shaped inlet part in the block. The concentrated solution of electrolytes flows through the lateral inlet (3_{44}) and, in the mixer chamber, undergoes mixing with the aid of a magnetic stirrer (3_{45}). The stirrer is coated with a polyethylene tube and can be rotated by a magnet (3_{46}) fastened to the axis of an electric motor (1_{47}, 3_{47}). The upper edges of the water-proof plexiglass cylinder (3_{48}) emerge from the water in the thermostat.

The polarograph electrode chamber (1_{34}, 3_{34}, 5) is made of plexiglass as well, and resembles a vertical tube with thick walls. The inlet of the chamber is placed in the electrolyte-mixer. Both the electrolyte-mixer and the electrolyte chamber are fastened to the block with the aid of proper catches and rubber rings, invisible in the figure. The water inlet to the polarograph electrode chamber (3_{49}) is obliquely directed downwards to avoid the penetration of mercury to the electrolyte-mixer. From below, the electrode chamber is closed by a mercury pool (3_{50}), whose level can be regulated by changing the position of the waste mercury tap (1_{51}, 3_{51}) and mercury outlet (1_{52}, 3_{52}). The excess of mercury flows then down to a conical flask (1_{53}) without touching the water of the thermostat. Air in the conical flask above the mercury is connected with the atmosphere by means of a decompression glass tube (1_{54}). The wide-bore dropping-mercury electrode (1_{55}, 3_{55}) (pyrex-glass capillary, $\varnothing$ 0.8 mm) passes into the electrode chamber under an angle of 45°. Its position can be regulated and protected by means of a screw plug (3_{56}) and a rubber washer (3_{57}). The outlet of the wide-bore dropping-mercury electrode is situated close to the centre of the chamber, the lower edge of the inlet being slightly truncated.

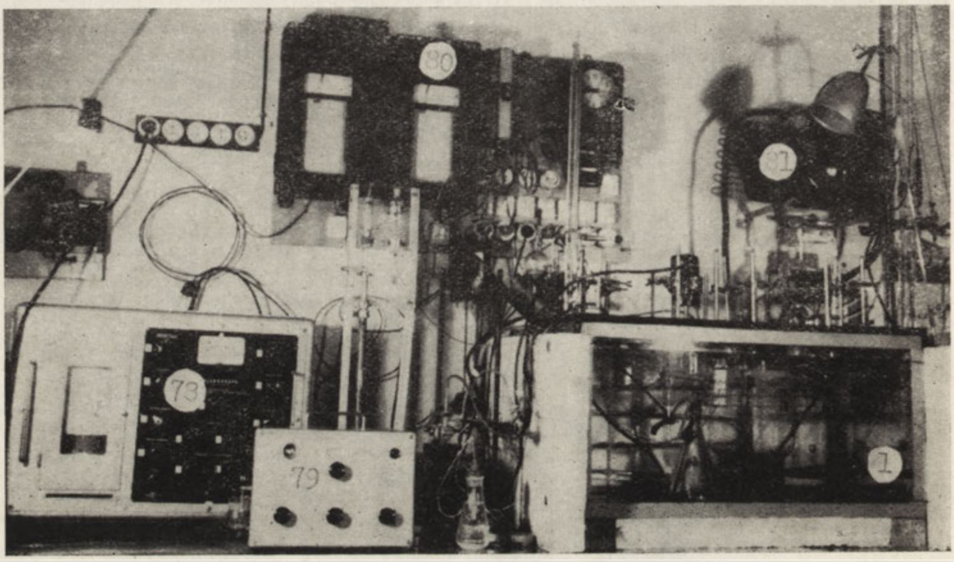

Fig. 7. General view of the apparatus

From above, the electrode chamber is closed with the Ag/AgCl reference electrode (3_{58}). The external part is made of glass or of ground plexiglass. At the bottom, there is a silver rod (3_{60}) coated with silver chloride, sticking firmly in a seal of Mendeleev (or Dekhotinsky) sealing wax. The rod plays here the part of an anode. A cable is soldered to the silver rod (3_{61}). The electrode is filled in with hard paraffin wax (3_{62}). Immediately under the lower edge, there is an electrode chamber outlet (3_{35}).

The mercury reservoir (1_{63}), with height control, fastened on a stand is connected with the braking capillary for mercury (1_{64}), about 68 cm in length, and 0.2 mm in diameter, and then with the wide-bore dropping-mercury electrode (1_{55}). Here, a polarograph of the type 7-77-4/b "Orion" produced in Budapest, Hungary (7_{78}), was used. Polarographic curves were recorded using ink pen on a paper tape. Conductometer electrode chamber (1_{36}) consists of a glass tube, 13 mm in diameter, having terminals for hose, with two soldered electrodes (Pt wire, 0,5 mm in diameter, length of the free part of the electrode about 8 mm). A conductometer of GP-2 Solomierz "Energopomiar" type, Gliwice, Poland (7_{79}) was applied.

The length of the scale of the flow meters ($1_{29, 37}$) amounts to 33 cm and is characterized by a range of flow measurement from 20 to 200 ml/hour. Floats to measure such low flows must be made of a relatively light material. Here, the floats used in the research works were made of milk perspex, or of PVC.

The electrolyte supply device (1_{65}) consists of a glass syringe (6_{66}), an electrolyte reservoir (6_{67}) with a three-way tap (6_{68}), and of a device moving the piston of the syringe. In the case here considered, the kymograph produced by C. F. Palmor (London) was the drive of the device applied during the examinations. At its upper end, the kymograph axis (6_{69}) is equipped with a claw coupling (6_{70}). The coupling rotates a hollow metal cylinder (6_{71}) with a gap (6_{72}) on one side. In such a cut there is a pilot bar (6_{73}) of the feed screw (6_{74}) that moves within the cylinder hollow. During rotation, the screw enters between the threaded jaws of the quick-change clasp nut (6_{75}). The thread length on the screw is so chosen that the screw stops and does not move the piston after approaching to the syringe bottom.

The infilling of the syringe with the electrolyte solution takes place, after connecting with the electrolyte reservoir (6_{67}), through the three-way tap (6_{68}), due to the release of the jaws of the quick-change clasp nut (6_{75}), and owing to the lowering of both piston and screw. Then, the three-way tap (6_{68}) is placed in a position shown in Fig. 6 to connect the syringe with a thin polyethylene hose leading to the electrolyte inlet in the electrolyte mixer (3_{44}).

The whole device is illustrated in Fig. 7.

3. PREPARATIONS

Reference electrode (3_{38})

Attempts at using a calomel electrode with saturated KCl solution did not give any adequate results; the current diminished. After immersing the electrode in the saturated KCl and after repeated placing in the examined water for several seconds, the current flowed normally, to diminish, however, again. Most probably, the salt concentration across the bridge changed. This phenomenon was stressed already by Spoor (1948). The difficulty was overcome when a silver/silver chloride reference electrode was used. During the measurements, the electrode was immediately immersed in the water under examination.

The reference electrode is prepared in the following way (according to Briggs, Knowles 1961, and Briggs, Mason 1962): a pure silver rod, 2.5 mm in diameter, and about 25 mm in length, is washed in diluted HNO_3 (1+1) for 1 min.; then it is put, as anode, into 0.1 n HCl, where Pt wire plays the part of a cathode. A current of 5 mA is passed for 5 min. The whole is then washed in 15% aqueous ammonia to obtain pure silver, and then in HNO_3 (1+1). A current of 0.5 mA is passed for 1.5 hours in 0.1 n HCl.

The preliminary coating with silver chloride (5 mA for 5 min.) is here a modification, and guarantees the preparation of the electrode surface, consequently its greater durability, too. The electrode is stored in saturated KCl.

Wide-bore dropping-mercury electrode (1_{55}, 3_{55})

The wide-bore dropping-mercury electrodes were made, according to Briggs, Knowles (1961) of a capillary, 0.8 mm in internal diameter, and 5.5 mm in external diameter, and about 10 cm in length, in the following way: 1. The inlet part is widened and olive-shaped. The outlet opening is in the non-grinded part of the capillary; the lower edge of this part is obliquely truncated. 2. Washed in ethyl methyl ketone. 3. Dried in dustless air for 2 hours. 4. Filled in with diluted (1+9) HCl for 24 hours. 5. Washed with distilled water. 6. Dried in dustless air for 2 hours. 7. Washed with ethyl methyl ketone. 8. Dried in dustless air for 2 hours. 9. Washed with Silicone Fluid MS 200/1000 cs, 2 per cent solution in xylene (Hopkin Williams, Ltd.). 10. Dried in dustless air for 4 hours. 11. Roasted in +110°C for 30 min., and then in 250°C for 3 hours, and chilled. 12. Washed with ethyl methyl ketone. 13. Dried in dustless air for 2 hours. Point 1 is a supplement of the method given by Briggs, Knowles (1961). Furthermore, another kind of silicon was used, as well.

The wide-bore dropping-mercury electrodes described, among others, by Briggs et al. (1957), Briggs et al. (1958), Briggs, Knowles (1961) and Briggs, Mason (1962), probably represent the best solution as concerns the dropping-mercury electrodes for respirometry purposes, similarly as the membrane-covered solid electrodes, described by Clark et al. (1953), Carrit, Kanwisher (1959), Tümmler, Reitnauer (1964) and Carey, Teal (1965), are the best ones among the solid electrodes. The wide-bore dropping-mercury electrode was chosen mainly because it is cheaper and produced of easily available material resistant to contamination. Moreover, it does not require equilibrium time, its response time being shorter.

Braking capillary for mercury (1_{64})

The terminals of the U-shaped capillary, having 0.2 mm in internal diameter and about 68 cm in length, are widened and olive-shaped. The capillary is washed with ethyl methyl ketone and dried in dustless air for about 2 hours. The method was borrowed from the work published by Briggs, Knowles (1961). These authors used, however, a short braking capillary, about 30 cm in length. A longer one (60 cm) was applied by Edwards, Learner (1960) and Briggs, Mason (1962). The application of a longer braking capillary permits to increase the difference between the level of mercury in the mercury reservoir (1_{63}) and the outlet of the wide-bore dropping-mercury electrode. In this case, the changes in the mercury level do not influence so intensely the time of the drop.

Braking capillary for inflowing water (3_{26})

The braking capillary is U-shaped and consists of a capillary middle part and of a widening at the inlet portion, where nylon fibre filter is placed. Both the inlet and outlet of the capillary are funnel-shaped. By means of burner, the glass tube terminals are funnel-shaped, the filter container is made, and the middle part is stretched to form a capillary. At the same time, the funnel-shaped terminals are placed on metal cones, whose shape and distance resemble those of the plexiglass cones, on which the braking capillary is placed.

The capillaries prepared in that way should be calibrated. The capillary with the filter is connected with the bottle by means of a hose, the length of which is somewhat shorter than a half the height of the cylinder filled in with water (1_{11}). The flow rate is measured. Those capillaries are chosen, which assure a flow rate approximate to the required one.

Reagent solution

A description of composition and of preparation of the reagent solution is given in the papers by Briggs, Knowles (1961), Briggs, Mason (1962), Painter, Jones (1963). There is also a commercially available reagent solution for dissolved oxygen meter, Baird and Tatlock Ltd. Chadwell Heath, Essex, characterized by the same composition: sodium hexametaphosphate — 14 g NaCl — 210 g, KCl — 24.5 g, K_2CO_3 — 59.5 g, KNO_3 — 70.0 g, glycine — 42.0 g made up to 1000 ml with distilled water. They increase the conductivity, pH, buffering capacity and guard against deposition of calcium carbonate. The role of maximum suppressor is played by the sodium starch glycollate, 55 g per liter of reagent solution.

Since certain difficulties can be met with by some laboratories in buying the sodium starch glycollate and/or ready-made reagent solution, attempts were made to replace the sodium starch glycolate with another maximum suppressor. Tests were made using air-saturated distilled water. The following art variants applied in the examinations: a) 0.5 ml reagent solution without maximum suppressor per 100 ml of sample; there is a distinct maximum on the first part of the second oxygen plateau and a hump on the first part of the second oxygen plateau of polarograms; b) 0.5 ml of 1.5% aqueous solution of methycellulose (tylose M) (Zagórski, 1956) +0.5 ml of reagent solution without the maximum suppressor per 100 ml of sample. Neither the maximum on the first plateau, nor the hump on the second one was removed; c) 60 mg of thymol (Chodkowski, 1958) per 100 ml of reagent solution without the maximum suppressor. Dosage: 0.5 ml per 100 ml of sample. Neither the maximum, nor the hump was removed (Fig. 8A); d) 0.5 ml of 2% aqueous solution of gelatin (Zagórski, 1956) +0.5 ml of reagent solution without the maximum suppressor per 100 ml of sample. The maximum on the first plateau was removed (Fig. 8B). The hump on the second plateau was not removed. With the increase in gelatin dose (0.25, 0.50, 1.00, 2.00, 2.50, 3.00 ml of 2% gelatin per 100 ml of sample) the hump diminishes (Fig. 8C). When gelatin is used, the current is not stable, although other factors are constant. The stabilization of current was obtained with the aid of opposite potential; after a few minutes, however, the current was not stable again. The gelatin

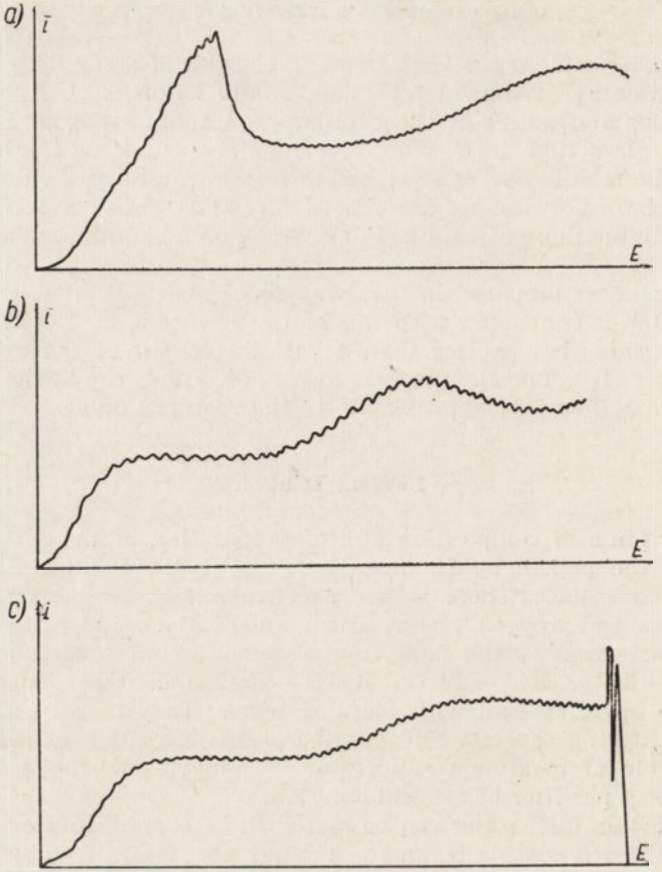

Fig. 8. Polarograms of air-saturated distilled water.
a — with thymol (see text), compensation 4. b — with 0.5 ml of 2% solution of gelatin +0.5 ml of reagent solution without maximum suppressor per 100 ml of sample, compensation 3. c — with 2.5 ml of 2% solution of gelatin + 0.5 ml of reagent solution without maximum suppressor per 100 ml of sample, compensation 3

solution undergoes bacterial decomposition. In the connection with the concentrated electrolytes it precipitates and therefore must be added to the sample separately; e) 50 mg of methyl red (Chodkowski, 1958) per 100 ml of reagent solution without the maximum suppressor. The dosage is 0.5 ml per 100 ml of sample. No difference was recorded in the shapes of the polarograms, as compared with the polarograms made with the aid of original Baird and Tatlock reagent solution. By means of the device described above, the conductivity of water was controlled; the reagent solution with methyl red being added automatically. During addition to 100 ml of tap water:

solution ml	0.000	0.089	0.239	0.458	0.627	1.485,
conductivity was µS·cm⁻¹	758	2477	3886	5521	6220	9743.

The reagent solution with methyl red is coloured, thus an additional, visual control of dosage is possible. The reserves can be stored in a dark bottle for about 4 months.

Antibiotics

The following was the dose of the antibiotics: streptomycin (source Polfa), 32 mg/l+chloromycetin (Polfa), 25 mg/l. When choosing the antibiotics and determining the concentrations, the results of MARSHALL, ORR (1958), and those of ZEISS (1963) were taken into account.

Calculations

1°. Calculations when wentilation with gas mixtures having known contents of O_2 is used (as in the case of reservoir-bottle I).

Polarographic measurements of the water equilibrated with gas mixture are made. Fig. 9 illustrates an example of a calibration curve (relation between the diffusion current and dissolved oxygen concentration). The curve should at least consist of three points (or better of four points) and be controlled before each experiment. On the X axis, ammeter readings are marked, on the Y axis — O_2 percentage in the gas mixture. The points obtained should form a straight line (Fig. 9 — dotted line).

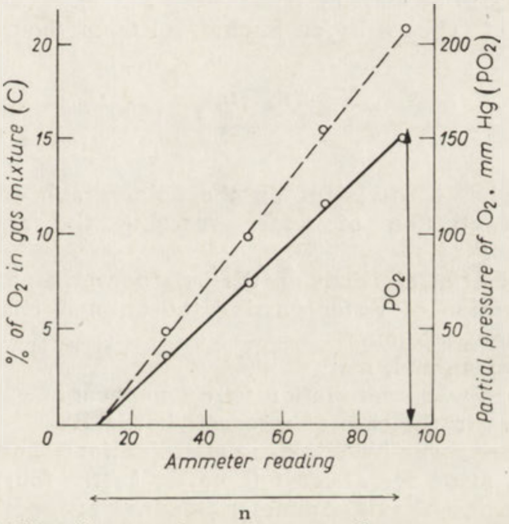

Fig. 9. Calibration graph. — — — — — C, ————— PO_2

Calculations of partial pressure of O_2, mm Hg (Fig. 9 — continuous line):

$$PO_2 = \frac{C(H_A - P_V)}{100},$$

where:

C — % of O_2 in gas mixture (known from gas analyse in cylinder, or from flow meter readings at gas stirrer),

H_A — atmospheric pressure, mm Hg (barometer reading),

P_v — vapour pressure of water at experimental temperature, mm Hg (from physical tables).

Both lines (% and partial pressure of O_2) should come into a contact on the X axis at the same point.

Calculations of ml O_2/l per ammeter scale division, ml O_2/l (referred to STP conditions):

$$K = \frac{\alpha \cdot PO_2}{760 \cdot n},$$

where:

α — absorption coefficient of O_2 at experimental temperature (from physical tables),

n — the number of divisions on the polarograph ammeter scale (n — must be read off, considering the cutting point of calibration curve with the X axis to be starting point — see Fig. 9).

Calculations of O_2 used by an animal, ml O_2/mg/hour:

$$X = (n_a - n_b) \frac{K \cdot D}{C \cdot 1000},$$

where:

n_a — the number of divisions on the polarograph ammeter scale when O_2 concentration of water entering the animals chamber is measured;

n_b — the number of divisions on the polarograph ammeter scale when O_2 concentration of water passing the animals chamber is measured;

D — water flow, ml/hour;

C — weight of animal, mg;

2°. Calculations when ventilation with homogenous gas (N_2 or O_2) is used in defined time (as in the case of reservoir-bottle II).

Calibrations are made by Winkler determinations. The results of Winkler analysis, ml O_2/l, made for at least three (or better four) concentrations of O_2 are marked on the Y axis. Ammeter readings are marked on the X adis.

Calculations of ml O_2/l per 1 ammeter scale division:

$$K = \frac{a}{n_a},$$

where:

a — O_2 concentration, ml O_2/l (from Winkler analysis),

n_a — corresponding of the polarograph ammeter scale divisionsis (read off, considering the cutting point of curve with the axis to be starting point).

Either calculation method is applied alternatively, depending on the laboratory equipment. However, to obtain precise results a verification of the calibration curve is desirable, using method 1° by making 1—2 Winkler analyses. When method 2° is used, a point corresponding to the air-saturation ($C = 20.9\%$) can be controlled on the calibration curve by an additional calculation by means of the method 1°.

4. PROCEDURE

Procedure when reservoir-bottle I is used (1_2)

Tap water (from the river Vistula *) was ventilated for, at least, 24 hours to remove chlorine. After the previous filtration through mill gauze No. 25, water is poured through the glass tube (1_4, 2_4) into the reservoir-bottle (1_2) immersed in the thermostat. When pouring, one adds the antibiotics dissolved in a small amount of water. Here, the water is left for at least 12 hours to egualize the temperature. The taps are placed in a position shown in Fig. 2A, water in the bottle being ventilated through a glass tube (1_5) and gas diffuser Schott G3 (1_6) with air or another gas mixture. The gas excess passes through the glass tube (1_7) and the final outlet to the container with water (1_9). To obtain gas equilibrium in water, ventilation time must be empirically chosen previously. Then the taps are placed in a position shown in Fig. 2B. The amount of gas flowing out of the compressor or reductor is so chosen that single gas bubbles leave the outlet tube immersed in the glass cylinder filled in with water (1_{11}) in the intervals of several seconds. The interruptions between the end of ventilation and the begin of measurement, was not shorter than 10 min.

The following is the procedure applied to determine the contents of oxygen in the water that enters the respirometer; the tap on the outlet glass tube (1_{12}) is open. The water flows through the inlet of the respirometer (3_{23}). By opening the tap (3_{25}) the water can wash up the animal chamber (3_{41}) and flow out through the tap (3_{27}) placed in a position "a" towards the flow meter (1_{29}). After washing the chamber, the tap (3_{25}) is closed, and the water passes to the animal chamber through the braking capillary (3_{26}). The flow rate is controlled by the flow meter (1_{29}) due to the changes of pressure in the bottle by changing the position of the gas outlet in the glass cylinder (1_{11}).

Simultaneously, the remaining portion of water, that enters the perspex block, flows through the tap (3_{30}) that controls the ratt of its flow through the control route (3_{31}), the lower route in the three-way tap (3_{27}), as well as through the electrolyte-mixer (3_{28}), polarograph electrode chamber (3_{34}), conductometer electrode chamber (1_{36}) and flow meter (1_{37}) towards the outlet. The flow meter mentioned above can control the rate of the water flow through the control route. Then, a series of polarograph measurements is made (detailed description is given in the following part of the present paper).

The following is the procedure to determine the oxygen contents in the water that flows out of the animal chamber. The three-way tap (3_{27}) is placed

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* Average monthly chemical composition of tap water for September 1966 (time of experiment) was: turbidity — 0 mg SiO_2/l, colour — 11 mg Pt/l, pH — 7.5, nitrate — 1.20 mg/l, chloride — 38.1 mg/l, iron — 0.02 mg/l, sulphate — 47.3 mg/l, total hardness — 4.1 mval/l, alkalinity — 3.1 mval/l, COD — 4.8 mg O_2/l, calcium — 61.5 mg/l (data obtained from the Service Laboratory Station of Water Works, Warsaw).

in position "b". To change the position of the tap (3_{27}) the screw should be loosened. The screw passing through a threaded opening in double-bent brass plate that clasps the perspex block, presses the tap. After changing the position of the tap, the screw is tightened again. In this way, the flow through the control route is cut off, and the water flowing from the animal chamber passes to the electrolyte-mixer and, through the polarograph and conductometer electrode chamber, reaches both flow meter (1_{37}) and outlet. A series of polarographic measurements is then made in the water which has flowed through the animal chamber with animal.

The oxygen content in water that flows to the animal can be determined by the two methods described above. When oxygen concentration analyses should be made using Winkler method, water is taken from the glass tube (1_{32}, 3_{32}) into Winkler flasks (1_{33}). During water sampling, the flasks are immersed in a vessel, through which the water excess flows, preventing thus from too sharp changes of temperature in the flask, especially when experiment is made in a temperature greatly different from room temperature. To this effect, the glass tube for water sampling (1_{32}) is, also, thermically insulated with a latex sponge layer. Water in the flasks was being changed threefold before the fixation of the sample.

The constructional principles of the reservoir-bottle I affect its positive and negative features (the water outflow from the bottle is assured mainly due to the stable gas overpressure onto the water level; the required amount of O_2 in water is obtained by ventilating the water in the bottle by means of gas mixture of required composition). The application of this bottle allows us to control precisely the water flow rate, and O_2 content in water. The latter can be determined by two methods. However, the application of the reservoir-bottle I requires additional apparatuses to prepare gas mixtures and to control their composition. The construction of the bottle and its application are complicated, and the gas consumption is considerably high.

Procedure when reservoir-bottle II is used (1_{13})

The tap water, prepared as in the case of the reservoir-bottle described previously, is poured through the glass tube (1_{15}), polyethylene disk (1_{19}) being moved upwards. If a measurement of the oxygen consumption is to be made in the water saturated with air, the water in the bottle is ventilated additionally, at least for 1 hour. If, however, the O_2 content in water should be different, homogeneous gas (N_2 or O_2) must be used in a time period empirically chosen. An example of the decrease in oxygen contents in water, depending upon the time of ventilation with N_2 is shown in Fig. 10. Gas flows through the glass tube (1_{16}) and gas diffuser Schott G3 (1_{17}). During the ventilation, the tap in the polyethylene disk is open to remove the excess of gas. After the ventilation, it is closed. The water flows to the perspex block through a glass tube (1_{18}). Both the further route and procedure necessary to make the measurements are, generally speaking, the same as those described in the case of the reservoir-bottle I (1_2).

It should, however, be kept in mind that in the reservoir-bottle II, the water flow through the whole system is assured mainly due to the difference in the inlet and outlet levels. In this case, the regulation of the flow by

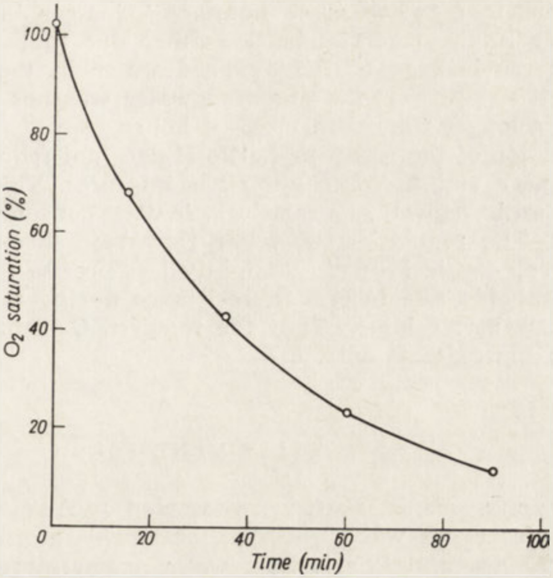

Fig. 10. Changes in oxygen contents in water during ventilation with nitrogen. (Reservoir-bottle II, capacity about 10 l, gas diffuser Schott G3, ϕ 20 mm, temperature +15°C, barometric pressure 745.8 mm Hg, admixture of oxygen in nitrogen — 0.2%)

means of the braking capillary (3_{26}), or with the aid of fine-control tap would cause lovered pressure in the farther parts of the system. This is highly disadvantageous, since it leads to the acceleration of mercury outflow from the wide-bore dropping-mercury electrode, the suction of electrolytes, and the rise of meniscus of the outflowing mercury (3_{50}). Thus, the regulation of flow rate through the animals chamber and the control route is made by means of braking capillaries (1_{76}, 1_{77}) placed before the outlet. Their level is controlled. The tap (3_{25}) is continuously open, the braking capillary (3_{26}) removed, and the openings that lead to the braking capillary closed with the aid of some small rubber plugs. During the Winkler determination of the O_2 content in the water entering to the respirometer, the fine-control tap (3_{30}) is open. For these reasons, the water flow through the respirometer should be stopped during the short time span, when the water is sampled. This, f. ex. can be done by closing the tap (3_{25}) and by placing the three-way tap (3_{27}) in a blind position. Otherwise, suction of water takes place in the farther parts of the respirometer. The mercury level in the mercury reservoir (1_{63}) should be gradually lowered, parallelly to the lowering of the water level in the bottle and to the outlet level. If not, the mercury flow through the wide-bore dropping-mercury electrode will accelerate.

The reservoir-bottle II should be controlled, as concerns gas leakage, before its use. A control of this kind was performed in the case here considered. Water was ventilated with N for 6.5 hours. In 10 min. after the ventilation, two determinations of O_2 were made by Winkler method, showing in each case 0.16 mg/l. After 18 hours, three determinations, and after 42 hours another three ones were made. All of them gave the same results

(0.16 mg/l). Thus, in a period of 42 hours, no changes in O_2 content were noted to appear in the reservoir-bottle, either due to their diffusion from air, or due to the leakage of the oxidized water in the thermostat from below. In practice, however, the prepared water was not held in the reservoir-bottle for a longer time than for 3—4 hours.

The application of the reservoir-bottle II does not require any additional devices to prepare and to control the gas mixtures. This, and the simple service of this bottle, as well as a relatively low gas consumption are no doubt its advantages. The regulation of water flow rate through the apparatus with the reservoir-bottle II used, is slightly less precise than in the case of the bottle I, probably due to certain resistance during the lowering of the polyethylene disk in the bottle. Thus the required O_2 contents in water can be obtained as approximate only.

5. EXPERIMENT

Larvae *Anax imperator* Leach were sampled on Aug. 26, 1966, in a pond in Warsaw. Water depth was 20—50 cm, bottom — argillaceous and slimy, covered with Elodea and Phragmites: water temperature was 18.2°C. The larvae were individually placed in vessels, 8.5 cm in diameter, each with 100 ml of air-saturated tap water. The vessels were put into a water bath at a temperature of +18°C. During 3 hours the temperature was decreased to 15°C and at that temperature (±0.5°C) the animals were held till the experiment time. Each 2 days the water in the vessels was changed into fresh, air-saturated tap water characterized by the temperature of the culture. Tubifex served as feed; the weight of the consumed feed was noted, and the

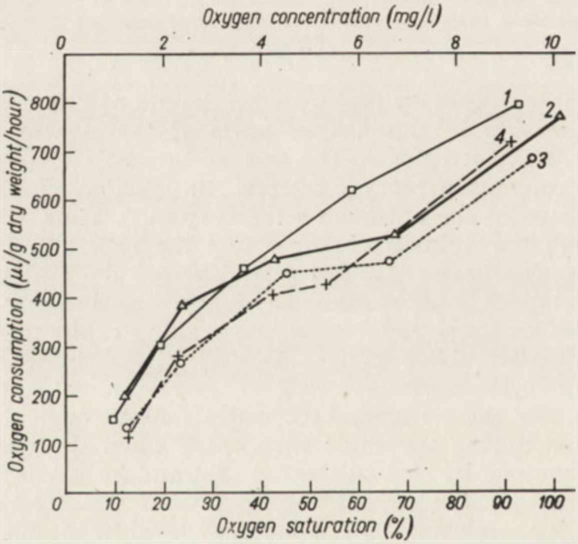

Fig. 11. Oxygen consumption of four *Anax imperator* larvae in relation to the oxygen content in water. Numbers are the same as in Tab. I

Table I

Larvae used in experiment

no.	dry weight of Anax mg	body length of Anax mm	average live weight of Tubifex consumed per day mg	average number of faecal pellets produced per day
1	149.8	42	48.4	0.50
2	146.8	39	36.5	0.50
3	140.2	41	47.0	0.43
4	139.0	40	39.1	0.50

number of faecal pellets was recorded (Tab. I). The experiments were conducted from 1 to 13 Sept., 1966.

The procedure was as follows: 1 — placing of one animals in a animal chamber, 2 — ventilation of wattr with gas, 3 — double sampling by Winkler method, 4 — water flow from reservoir to the measuring part, and water flow from animal chamber out-wards, 5 — rapid washing of control route and measuring part, 6 — establishment of required water flow rate, 7 — conductivity of water is controlled to the moment of obtaining proper proportion between the water and reagent solution, 8 — control of stability of polarographic current, 9 — making the final polarograms of water from the reservoir, 10 — water flow from animal chamber to measuring part; control route is closed, 11 — polarographic control of water exchange in electrode chamber. When polarographic current ceased to decrease, 5 polarograms were taken up to make sure that the exchange was finished. This is the longest procedure here, and lasts, when the flow amounts to 33 ml/hour, about 1.5 hours (comp. Harrison, 1966), 12 — making the final polarograms of water from animal chamber. This procedure, except for point 1, was repeated fivefold, for each oxygen concentration separately. Point 3, i.e. Winkler analysis to control the calibration curve, is not to be executed in each case. After the measurements of respiration, body length, live weight and dry weight were determined (Tab. I).

The experiment was conducted at a water flow from 30.0 to 34.5 ml/hour (linear velocity in electrode chamber was 0.0106—0.0122 cm/sec.). Reservoir-bottle II was applied, and Baird and Tatlock reagent solution was used; conductivity was 3800—4700 $\mu S \cdot cm^{-1}$, mercury dropping rate was exactly 1 drop every 2.5 sec. at the potential — 1.5 V, and temperature +15°C (±0.015°C).

The oxygen consumption by 4 larvae *Anax imperator* was measured in 5 oxygen concentrations, separately. The oxygen consumption μl/g dry weight/hour diminished with the decrease in oxygen concentration in water (Fig. 11).

6. DISCUSSION

The accuracy of the polarographic readings is affected by several factors.

Water flow

As concerns the wide-bore dropping-mercury electrode, Briggs and Mason (1962) state that readings are independent of the flow rate from 0 to 0.25 cm/sec. past the electrode, and increase linearly with flow rates 0.25—

54.00 cm/sec. Similarly, other authors inform that as concerns the dropping-mercury electrodes, readings are independent up to a definite flow rate: SPOOR (1948) — up to 2400 ml/hour, BRIGGS et. al. (1958) — up to 2400 ml/hour, BRIGGS, KNOWLES (1961) — up to 1800 ml/hour; above this value they already increase (the last data can hardly be compared, since the linear velocity past the electrode is unknown). The stronger water currents seem to interfere with the formation of mercury drops (SPOOR, 1948).

According to this, our studies have shown that the polarographic current (-1.5 V) is independent of water flow in a range from 52 to 832 ml/hour (0.0184—0.2945 cm/sec.) and amounts to 23.2 μA. On the other hand, in the case of a very slow flow, amounting to 13 ml/hour (0.0046 cm/sec.), a slight decrease in polarographic current to 22.2 μA was observed. It may be that at such a low speed of flow, the oxygen consumption of the dropping-mercury electrode, begins to play a role there. This problem can distinctly be seen at the solid electrodes, where the polarographic current is proportional to the log speed of water current (Chodkowski, 1958), i.e., with the increase in water current speed the polarographic current increases asymptotically to a certain limit. AMBÜHL (1955) has shown that by choosing the adequate shape of a stable cathode, we can displace this limit towards the lower water current speeds. The extremal changes of the water flow observed during the experiment (30.0—34.5 ml/hour) may have caused a reading error lower than 0.5%.

Conductivity

As it is known (f.ex. ZAGÓRSKI, 1956), the increase of resistance of a sample causes the displacement of waves towards more negative potentials. According to BRIGGS and MASSON (1962) the position of the second plateau remains the same with respect to the voltage, when the conductivity of the sample is 3000 $\mu S \cdot cm^{-1}$, or more. AMBÜHL (1955) has studied the curves of the dependence of the polarographic current upon the conductivity for bare solid electrodes. Below certain critical value of the conductivity polarographic current considerably increases with the increase of conductivity, and above this value the increase is less intense. For various types of cathodes studied by AMBÜLH, the value of the conductivity lies at various points, but generally between 100 and 600 $\mu S \cdot cm^{-1}$.

The examinations of the dependence of the polarographic current upon the conductivity have been made for those values of conductivity, which, on the basis of the results obtained by AMBÜHL (1955), can only slightly affect the polarographic current. Air-saturated tap water was used, with various amounts of reagent solution with methyl red added automatically; temperature was $+5.5 \pm 0.015$°C, the water flow speed — 100 ml/hour, and the mercury drop rate was stable (1 drop every 2.5 sec.). With the increase in conductivity (1000—2000, 3000—4000, 5000—6000, 7000—8000 and 9000—10000 $\mu S \cdot cm^{-1}$) the polarographic current also increased by 2.1%, 1.3% 0.8%, 0.5% and 0.4%, respectively.

In the light of the results presented above, we may assume that the extreme changes in conductivity observed during the experiment (3800—4700 $\mu S \cdot cm^{-1}$) may have produced a negligible reading error only, lesser than 0.9%.

Mercury drop rate

It is generally known that the stability of the mercury drop rate is very important in obtaining stable polarographic readings when other factors are constant. Fig. 12 illustrates the influence of the mercury drop rate on the polarographic current. The influence of the mercury drop rate on the polarographic drop rate is highly distinct. Therefore, the stability of the mercury drop rate, amounting to 1 drop every 2.5 sec., was kept very carefully during the experiments.

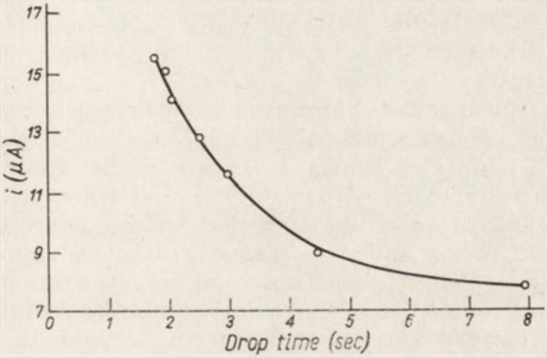

Fig. 12. Influence of mercury drop rate upon polarographic current. Potential — 0.6 V. Air-saturated tap water, flow — 100 ml/hour. Conductivity — 4500 μS·cm^{-1}. Temperature +5.5±0.015°C

Temperature

In polarographic methods temperature changes can be a source of errors. In the experiments described in the present paper, the errors produced due to the temperature changes have been eliminated to considerable extent by introducing a special calibration curve for each series of the experiment, and by the high accuracy of thermoregulation.

Bacterial growth

Bacterial growth can be a source of errors in studies of respiration of aquatic animals mainly because it is responsible for the decrease in oxygen concentration in water. Furthermore, bacterial growth can produce errors owing to the narrowing of tube internal diameters (perturbation in flow rate), overgrowing of flow-meter (errors in measuring the flow rate), as well as due to the clogging of the opening of dropping-mercury electrode (disturbances in mercury drop rate). This problem becomes particularly important when low flow rates are applied to investigate small organisms.

The bacterial growth was suppressed only in a few cases, as can be seen in the papers devoted to the respirometry of aquatic animals. Prior to each experiment, Krüger (1957) sterilized the flowing-water respirometer with hot water steam or with 10% HCl. This allowed him to eliminate the microorganisms that had settled on the apparatus, but did not permit him to counteract the oxygen decrease during the experiment by the microorganisms found in water. Mann (1958) added 1 mg/l chlorine to the water used in his experiment. However, it is not known whether such an amount of chlorine adequately liquidates the bacterial growth, and whether it does not affect badly the animals under examination. Berg, Jonasson (1965) heated water before use up to 70°C. If this really prevents the development of microorganisms in water, it does not liquidate the microorganisms that develop on the walls of the apparatus.

Barnes et al. (1963) added chloromycetin (100 mg/l) to the water in vessels of the manometer respirometers. Marshall, Orr (1958) stated that chloromycetin (50 mg/l) maintains constant oxygen in the water for 11—21 days, at 10 mg/l concentration for 5—7 day only; and streptomycin (50 mg/l) — for 3—7 days. These antibiotics are better fitted for this purpose than the others examined by them. When added to water in a concentration of 50 mg/l of each, they have no significant effect upon the oxygen consumption of Calanus. Before the examination of the oxygen consumption by zooplankton in bottles, Zeiss (1963) also essayed various antibiotics. A couple of antibiotics: dihydrostreptomycin — chloromycetin, at concentrations 25 mg/l, showed no bacterial growth and no apparent effect upon the oxygen consumption of Daphnia. Since during the present experiments antibiotics were added, the errors caused by the bacterial growth were considerably eliminated.

Accuracy of wave height measurement

The errors of the wave height reading from recorder charts does not exceed 0.5 mm. In the case of the wave height, amounting to 105—30 mm, this is less than 0.5—1.7%.

Zagórski (1956) reports that the accuracy of a normal polarographic analysis amounts to about ±2%. As it has been shown above, this is possible in the apparatus here considered.

Berezina (1959) conducted the researches on daily variations of oxygen consumption by larvae *Aeschna grandis* L. about 600 mg in weight, using Drastich's (respirometer, at a temperature of +20°C. Between 13 and 18 hours the oxygen consumption diminished from about 420 to about 270 μl/g live weight/hour. The examinations of oxygen consumption made at the same time during the present experiment, under conditions of a complete oxygenation, at a temperature of +15°C, gave results that amounted to 157.4—130.5 μl/g live weight/hour (mean live weight = 726 mg). Considering the differences in temperature of the experiment, and the weight of the animals, we may state that the result fairly well corresponds to that obtained by Berezina (1959).

7. SUMMARY

1. Construction of flowing-water polarographic respirometer is described (Figs 1—7). Wide-bore dropping-mercury electrode is used. Reagent solution is supplied automatically. Two types of reservoir-bottle are presented. These may be used alternatively, depending upon the required accuracy on the one hand, and the technical possibilities of a laboratory on the other;

2. Preparation of reference electrode, wide-bore dropping-mercury electrode, braking capillary for mercury, braking capillary for inflowing water, reagent solution, antibiotics and calculations are described;
3. The procedure of the experiments is presented;
4. The results of the measurement of oxygen consumption by larvae Anax imperator Leach in various oxygen concentrations are given;
5. Factors affecting the accuracy of measurements in flowing-water polarographic respirometer are discussed.

8. STRESZCZENIE

1. Przedstawiono konstrukcję polarograficznego respirometru przepływowego (Fig. 1—7). Stosowano szerokootworową kroplową elektrodę rtęciową. Elektrolit przewodzący wraz z substancją tłumiącą maksima tlenowe dozowany był automatycznie. Zaproponowano dwa typy butli rezerwuarowych, które mogą być użyte alternatywnie, w zależności od wymaganej dokładności z jednej strony i od możliwości technicznych laboratorium z drugiej strony.
2. Opisano sposób przygotowania elektrody odniesienia, szerokootworowej kroplowej elektrody rtęciowej, kapilary hamulcowej dla rtęci, kapilary hamulcowej dla wpływającej wody, elektrolitu, antybiotyków, oraz wykonania obliczeń.
3. Opisano sposób przeprowadzenia eksperymentu.
4. Przedstawiono wyniki pomiaru zużycia tlenu przez larwy Anax imperator Leach w różnych stężeniach tlenu.
5. Przedyskutowano czynniki wpływające na dokładność pomiarów w polarograficznym respirometrze przepływowym.

9. REFERENCES

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STUDIES ON THE NITROGEN COMPONENTS COMPOSITION OF CRAYFISH (*ASTACUS ASTACUS* L.) MEAT AS RELATED TO ITS NUTRITIVE VALUE

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ABSTRACT

Studies were made of the chemical composition and vield of crayfish (*Astacus astacus* L.) meat with distinguishing males and females. It was found that the meat consists of 83.23% water, 0.60% fat, 1.23% ash and 2.32% total nitrogen, of which 78% is of protein origin. The percentages are averages for males and females. Males contain more total nitrogen, fat, and ash than do females and the water content is lower in the former. In addition, qualitative and quantitative analyses were made of free and total amino acids in the crayfish meat.

CONTENTS

1. INTRODUCTION

The crayfish (*Astacus astacus* L.) is found in the inland waters of Europe from the Pyrenees and Atlantic Coast to the upper course of the Dniepr and Bug Rivers (Z. Gajewski, W. Terlecki, 1956) and is expanding into new areas.

As yet however, no satisfactory analysis of the chemical composition of crayfish meat has been made. The scanty data in the available literature (K. N. Budnikov, F. F. Tretiakov, 1952, K. Smolian 1925) refer only to the contents of the most essential constituents of crayfish meat and are rather confusing.

It was to fill this gap in the literature that the chemical composition of crayfish (*Astacus astacus* L.) meat has been studied for males and females separately. The paper apart from a routine chemical analysis, gives the amino acid composition of crayfish meat (free and combined amino acids), the yield of edible meat, as well as the relation between the total lenght and weight of crayfishes.

2. MATERIAL AND METHODS

The crayfish (*Astacus astacus* L.) were caught in the Długie Lake by Olsztyn, in August 1964. Until being used for the analysis the material has been kept alive in an aerated water tank (about one week) and fed with fish fry. After being removed from the tank the crayfishes were air dried at the temperature of 20°C and then killed by chloroform vapour and the meat from the claws and tails was seccured.

A) MORPHOMETRIC ANALYSIS OF CRAYFISHES

The crayfishes were divided according to the sex, and the total lenght (as the total we agreed to consider the distance from the rostrum to the end of the caudal plates), weight, and the yield of edible meat were measured.

B) PREPARING THE SAMPLES FOR THE ANALYSIS

The meat of crayfish, even very frash one, (immediately post mortem) is characterized by a large amount of tissue drip which makes a uniform sampling for the examination difficult. Therefore meat obtained from 20 specimens (from males and females separately) was first frozen at −30°C and then passed twice through a meat grinder with a sieve with openings mm in diameter. The ground meat was stored in small Petri dishes and kept frozen until needed for the analysis (about 8 hr.).

C) CHEMICAL METHODS

In the crayfish meat there were determined: 1) water — by the method of drying at 105°C, 2) fat — by Soxhlet's method, 3) iodine and acid number of fat according to Krauze et al. (S. Krauze, Z. Bożyk, L. Piekarski, 1962), 4) ash — after carbonizing the samples in crucibles over a burner and combusting them in a muffle furnace at 550—600°C, 5) nonprotein nitrogen — after extracting with 10-per cent CCl_3COOH (A. A. Lazarevskij, 1955) and total nitrogen — by Kjeldahl method (S. Krauze, Z. Bożyk, L. Piekarski, 1962) using the Parnas-Wagner apparatus, 6) protein nitrogen — from the difference between the total and nonprotein nitrogen, 7) amino acid nitrogen — by the Pope-Stevens method (C. G. Pope, M. F. Stevens, 1939).

D) QUALITATIVE AND QUANTITATIVE ANALYSIS OF AMINO ACIDS

Free amino acids were extracted from the tissue by the Jones method (N. R. Jones, 1955) with own modifications (E. Kołakowski, T. Dąbrowski, 1967); separated and identified as described by Awapara (J. Awapara, 1948). Total amino acids (free and combined) were determined after an acid hydrolysis of the tissue in a mixture of hydrochloric and formic acids after Block (R. J. Block, 1960).

Amino acids separation was performed by the combined methods of chromatography and electrophoresis (E. L. Durrum, 1951) using in the first run ascending chromatography in the n-butanol: formic acid: H_2O (75:15:10 respectively) for about 32hr (2×16hr) and then perpendiculary to the first run, the low-voltage electrophoresis (Z. Podeszewski, 1962) was employed in an acetate buffer of pH 2 (300 V, 0.8—1.5 ma/cm of Whatman No. 3 filter paper) for about 2.5 hr.

Quantitative amino acid determination was made by the Fischer and Dörfel method (F. G. Fischer, H. Dörfel, 1953), using calibration curves made on the English standard ("Shandon" — Standard Solution for Paper Chromatography).

3. RESULTS

The preliminary studies comprised a determination of the morphometric qualities of crayfish (*Astacus astacus* L.) — for males and females separately. The results are shown in Table I. Generally it may be assumed that with an increase in the crayfish weight there is an increase in the percentage meat yield. Due to bulkier claws the males yield considerably more meat. The meat yield of crayfish in the lenght class 10.1 to 16 cm amounts to 23.0 per cent for females and 23.9 per cent for males, on the average.

Further, the chemical composition of the crayfish meat was examined taking into account such parameters as water, fat, ash and various forms of nitrogen. The results obtained are shown in Table II.

Table I

Relation between total lenght of crayfisch (*Astacus astacus* l.) and its weight and edible meat yield

Sex	Total lenght (cm)	Total weight (g)	Meat yield	
			(g)	(%)
Male ♂	10.0—11.0 11.1—12.0 12.1—13.0	31.5—33.0° 33.1—34.2 34.3—40.6	7.1—7.5 7.6—9.2 9.3—11.6	22.7 24.8 27.7
Mean;				25.0
Female ♀	10.0—11.0 11.1—12.0 12.1—13.0	26.5—31.7 31.8—33.0 33.1—37.5	5.6—7.1 7.2—8.0 8.1—10.4	21.8 23.4 26.0
Mean;				23.7

Table II

Contents of water, fat, ash, and various forms of nitrogen in the fresh meat eat of crayfish (*Astacus astacus* L.)

Sex	Water %	Fat %	Ash %	Nitrogenous substances g pre 100 g raw meat			
				Total Nitrogen	Protein Nitrogen	Nonprotein Nitrogen	Aminoacid Nitrogen
♂	83.11	0.64	1.25	2.329	1.811	0.517	0.180
♀	83.35	0.57	1.21	2.311	1.814	0.496	0.176

Crayfish fresh meat contains on the average 83,2 per cent of water, 0.60 per cent of fat, 1.23 per cent of ash, and 2.32 per cent of total nitrogen, the latter being in 78 per cent of protein origin. Male individuals contain more total nitrogen, fat, and ashes than do females, but less water. The fat obtained in the quantitative analysis was used to determine the iodine and acid number of fat of crayfish meat. The iodine number of fat for females was 92.5, that for males 91.7, the respective figures for the acid number being 5.54 and 4.49.

The last investigation phase covered the qualitative and quantitative analysis of free and total amino acid (free + combined) of the crayfish muscles. The results obtained are shown in Table III and Fig. 1, 2 and 3.

Table III

Amino acid composition of crayfish (*Astacus astacus* L.) fresh meat

Amino acids	Amino acids %			
	Free amino acids (extr. alcohol.)		Combined amino acids (acid hydrolyzate)	
	♀	♂	♀	♂
1. Cystine	1.57	1.08	1.02	1.03
2. Lysine	2.68	2.70	4.35	4.44
3. Arginine	1.49	3.00	5.13	3.60
4. Histidine	17.59	18.30	3.06	3.08
5. Glycine	5.36	5.74	3.45	4.20
6. Serine	2.30	3.07	3.90	3.74
7. Asparaginic acid	8.69	8.66	10.12	9.93
8. Glutaminic acid	4.29	4.20	12.57	11.44
9. Treonine	4.42	4.48	7.40	7.42
10. Alanine	10.54	10.30	5.46	4.42
11. Proline	3.49	3.85	5.20	6.26
12. Tyrosine	2.79	2.80	5.76	4.13
13. Tryptophane	2.73	—	—	—
14. Methionine	11.66	11.55	5.91	5.78
15. Valine	2.52	2.69	3.27	3.91
16. Phenylalanine	2.45	2.60	6.35	6.87
17. Leucyne+isoleucyne	10.65	10.85	13.66	14.65
18. Taurine	4.78	4.54	5.39	5.10

A preliminary electrophoretic separation of free amino acids showed a high contents of the basic fraction averaging for males and females 47.2 per cent of the total amount of free aminoacids. The fraction consists mainly of histidine and partly of arginine and lysine (Fig. 1). In the group of acid amino acids there were found glutamini and asparaginic acids, as well as an unidentified amino acid distinguished by the highest electrophoretic velocity in this group of amino acids.

Out of 19 identified free amino acids 18 are common to both sexes, whereas one of them, tryptophane, was found only in female individuals and in trace quantities (Fig. 1.). The free amino acids in the muscle tissue of crayfish comprise: histidine, alanine, arginine, lysine, leucine, isoleucine, serine, methionine, treonine, glycine, proline, asparaginic and glutaminic acids, tyrosine, cystine, taurine, and phenylalanine.

Apart from the above, in all the chromatoelectrophorograms there were found additional six spots of unidentified compounds which were marked X_1 to X_6 (Fig. 2).

After hydrolyzing the tissue in a mixture of hydrochloric and formic acids total amino acid composition did not change, however, the additional spots (X_1—X_6) disappeared and there was an increase in the contents of glutaminic acid, phenylalanine, lysine, serine, treonine, proline, arginine, leucine, and isoleucine.

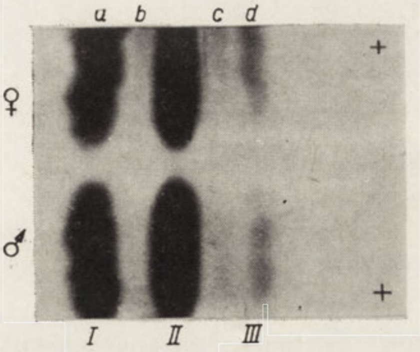

Fig. 1. An elektrophoregram of free amino acids in the muscle of crayfish (*Astacus astacus* L.) (separation in a pyridine buffer pH 6.7; separation time 2 hrs, 300 V, 1.6—3.3 mA/cm of width of Whatman's No. 3 paper strip)

I — basic fraction (a — histidine, b — arginine), II — neutral fraction (a — complex of fourteen amino acids), III — acid fraction (c — glutaminic acid, d — asparaginic acid).

Fig. 2. Chromatoelectrophoregrams of free amino acids of crayfish (*Astacus astacus* L.)

1 — cystine, 2 — lysine, 3 — arginine, 4 — histidine, 5 — glycine, 6 — serine, 7 — asparaginic acid, 8 — glutaminic acid, 9 — treonine, 10 — alanine, 11 — proline, 12 — tyrosine, 13 — tryptophane, 14 — methionine, 15 — waline, 16 — phenylalanine, 17 — leucyne + isoleucyne, 18 — taurine. x_1 — x_2 — unidentified compounds.

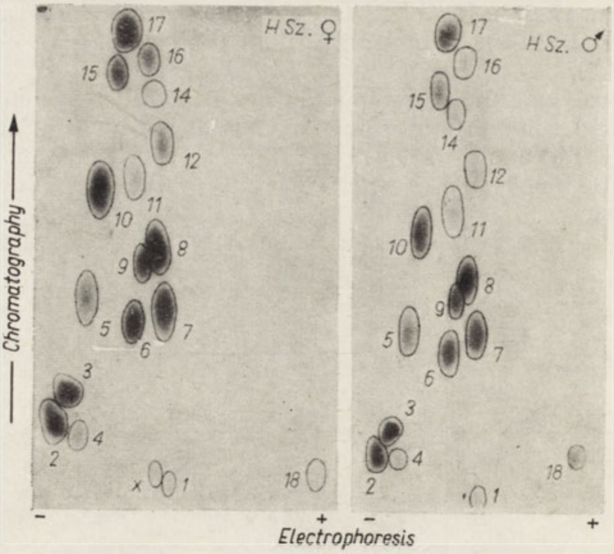

Fig. 3. Chromatoelectrophoregram of an acid hydrolyzate of crayfish muscle. (sec. Fig. 3)

The quantitative composition of free and total amino acids (free + combined) in the muscle tissue of crayfish is shown in Table III. Male individuals showed higher contents of glycine, proline, tyrosine, phenylalanine, and lysine than females but smaller amounts of asparaginic and glutaminic acids, alanine, and taurine.

4. DISCUSSION

It follows from the analysis of the chemical composition of crayfish (*Astacus astacus* L.) meat that it is a food low in fat (average 0.60% fat) containing considerable quantities of water (83.23%) and relatively small amounts of proteins (11.41%). As regards its nutritive value the meat of crayfish cannot compete with that of fish, however, in taste it surpases fish on account of its delicacy.

As concerns the amino acid composition it is a valuable food containing all the essential amino acids including large amounts of such exogenic amino acids as methionine, leucine, isoleucine, phenylalanine, treonine, and lysine.

A characteristics feature of crayfish meat, however, is its high contents of amino acids present in a free state, and especially of their basic fraction. Amino acid nitrogen in females accounts for 35.5% and in males for 34.8% of the total sum of nonprotein nitrogen determined in a trichloroacetic acid extract from the tissue. Among the amino acids present in a free state there is a quantitative preponderance of histidine which amounts in females an males to 17.9 per cent of the total contents of amino acids. Such high amounts of histidine are probably responsible for the characteristic flavour and aroma of crayfish meat (F. Bramstedt, 1957) as well as for some of its nixious effects in case it comes from dead individuals. Histidine is easily decarboxylated by the tissue and bacterial (E. Geiger, G. Courtney, G. Schnakenberg, 1945, H. Igarashi, 1939, M. Kimata, A. Kawai, 1953, R. Legroux, J. C. Levanditi, 1947) enzymes to histamine which, as already shown (A. Strøm, W. Lindberg, 1954, van A. G. Veen, H. E. Latuasan, 1950), caused many cases of poisoning.

A marked increase in the glutaminic acid contents after hydrolyzing the tissue points to the presence of considerable amounts of glutamine in the muscle proteins.

The disappearance of tryptophane and the drop in the contents of histidine and methionine in the tissue hydrolyzates is due, among others, to the decomposition of these amino acids under hydrolytic conditions (A. Niewiarowicz, 1955).

5. SUMMARY

Studies were made of the chemical composition of crayfish (*Astacus astacus* L.) meat for females and males separately, as well as of the relation between the yield of edible meat and the total lenght of crayfish.

Crayfish fresh meat contains 83.23 per cent of water, 0.60 per cent of fat, and 2.32 per cent of total nitrogen, of which 78 per cent is of protein origin. Male individuals contain more total nitrogen, fat, and ashes than females, but less water. The studies comprised also a quantitative and qualitative analysis of free and total amino acids in the meat of crayfish.

6. STRESZCZENIE

Przeprowadzono badania nad składem chemicznym mięsa raka szlachetnego (*Astacus astacus* L.) z podziałem na samce i samice, ze szczególnym uwzględnieniem składu substancji azotowych.

Wykazano, że mięso raka szlachetnego zawiera średnio dla obu płci 83.23% wody, 0.60% tłuszczu, 1.23% popiołu i 2.32% azotu ogólnego, który w 78% jest pochodzenia białkowego. Samce zawierają więcej azotu ogólnego, tłuszczu i popiołu niż samice, a mniej wody. Poza tym oznaczono skład jakościowo-ilościowy aminokwasów wolnych i ogólnych (wolne+związane) mięsa raka szlachetnego oraz wydajność mięsa jadalnego samców i samic w zależności od długości raka.

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HYDROCHEMICAL STUDY OF A SPRINGWATER LAKE ON THE EXAMPLE OF THE LAKE JASIEŃ

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ABSTRACT

On the basis of physico-chemical investigations on the water, conducted in the period from April 1960 to October 1961, a hydrochemical characteristic of the springwater Lake Jasień has been elaborated. Supplied by generous bottom springs the Lake Jasień belongs to the West Pomeranian Lake District and is the river Łupawa's outflow reservoir. The dynamics of thermal and oxygen conditions was distinct, owing to a number of specifities connected with the springwater-flow character of the lake. The ion balance of the water was clearly formed under the influence of ground waters (large content of calcium and magnesium), and had an interesting system of microelements. Also the bottom deposits in the Lake Jasień, among which lime, organic, silica and mixed ones could be distinguished, had individual character, connected with the structure and dynamics of water masses.

CONTENT

1. INTRODUCTION

Limnological investigations conducted in the northern regions of Poland concern almost entirely the Mazurian Lake District and include only a small part, i.e. single lakes of the Pomeranian Lake District. The aim of our work is acquiring knowledge of the physico-chemical elements in the environment of the Lake Jasień,

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supplied by the springs, which is one of the largest and most interesting bodies of the Pomeranian District. For springwater lakes are hitherto only slightly known in world literature and in Polish literature they are indeed quite unknown.

The Lake Jasień belongs to the drainage area of the river Łupawa, which covers a surface of 4045.8 ha. The percentage of lakes is large and amounts to 4049. The biggest lake of the basin is Gardno, with a surface of 2500 ha, it covers 61.7% of all the basin's lake surface. The group of three lakes: Jasień, Potęgowskie and Kamienieckie, with respective surfaces: 587 ha, 146 ha and 128 ha (Majdanowski and Kondracki 1953) is second in the scale of dimensions. The joint surface of these three lakes is 861 ha and represents 21.2% of the basin's lake surface.

Table 1

Limnometric characteristic of the Lake Jasień
(after Waluga, IRS in Olsztyn)

The lake's characteristics	Data concerning	
	southern basin	northern basin
Surface (*S*)		
the water mirror ha	336.7	240.5
the islands ha	10.2	1.1
Depth (*D*)		
maximum m	22.6	32.2
relative $\frac{D \text{ max}}{S}$	0.012	0.020
diameter $\frac{V}{S}$ m	7.75	9.1
index of depth $\frac{\text{mean } D}{\text{max. } D}$	0.34	0.28
Volume (*V* in thous.) m^3	26052.4	21996.4
Dimensions (*L* and *W*)		
maximum lenght (L) m	3100	4690
maximum width (*W*) m	1465	900
mean width m	1086.1	532.0
Coast line (*CL*)		
the lake's depression m	10025.0	13400.0
the island m	3165.0	750.0
total m	13190.0	14150.0
Geogr. latitude	54°16.7′	54°18.5′
Geograph. longitude	17°35.8′	17°37.3′
Altitute above sea level m	112.6	112.6

The Pomeranian Lakeland is limited from the north by a long space of the Baltic Sea coast, has a much differentiated territory, situated along lines marked by barometric depressions, and is distinguished from the surrounding regions by a somewhat harsher climate; its winters are colder and its short vegetation period has not more than about 200 days. More important meteorological events, noted throughout the research period, i.e. in the years 1960 and 1961 are shown in Fig. 1. The climate of this period did not essentially differ from that of average years. An exception was only a far milder winter occurring in 1960—1961.

The first slight frost occurred only on October 21, 1960 and disappeared on April 12, 1961, (it usually lasted longer, from Oct. 5 to May 15), but the temperature of that winter dropped only once below −10° (showing −12,2° on January 25, 1961). The lake was covered with ice only for a short time and this mild winter caused

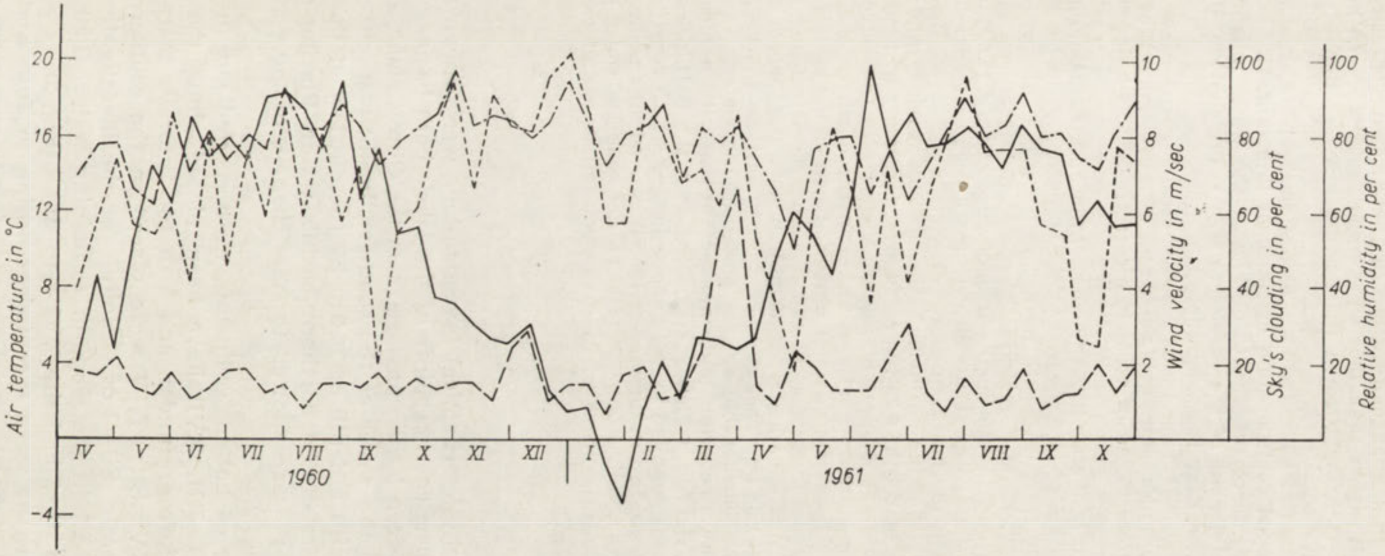

Fig. 1. Diagram of meteorological phenomena for Lake Jasień between April 1960 and October 1961

a rather high medium annual temperature reaching 8.6° (registered from April 1960 to April 1961), much higher than that of average years (6.5°—7.0°).

The Lake Jasień is composed in the north-south direction of two basins: the south and the north one.

The southern basin of Lake Jasień is shaped similarly to an elongated rectangle with weakly developed configuration of its coast line (Fig. 2).

38.7 m of coast line correspond to one ha of the water mirror. The relatively high index of development of the coast line (2.0) is undoubtedly due to a number of six islands situated near the west shore.

The northern basin of the Lake Jasień has the shape of a much elongated gutter, with many gulfs and a high index of development of its coast line, reaching 2.7. Therefore 60.4 m of coast line correspond to 1 ha of the water mirror (tab. I).

The mean depth of the southern basin is 7.7 m and its maximum is 22.6 m. The index of depth 0.34 suggests a mean variety of bottom. The basin's south part is in general shallower than the north part. The peripheral disposition of the lake's deep places is characteristic, namely in the southern extremities we observe depths of 15.3 and 13.7 m, and in the northern ones respectively 22.6 m and 21.9 m. The banks of the lake slope very softly down into the water along the southern part and are very steep in the northern part.

Mean water depth of the northern basin is 8.5 m, reaching 32.0 m in its deepest part, but the index of depth: 0.28 indicates a large variety of bottom. Similarly as in the southern basin the deepest places are disposed in the extremities, reaching a depth 32 m in the southern part and 26.3 m in the northern one.

The Lake Jasień is the springwater outflow basin of the river Łupawa. According to data of the Polish Hydrological and Meteorological Institute the river carries in the summer months an amount of 48 m^3/min of water, which gives an annual outflow of about 25 mln m^3. The ratio of annual outflow to the lake capacity is 0.5 and represents a considerable mixing power of the water.

The river Łupawa covering a distance of 91 km with a mean decline 1,2‰, falls into the Lake Gardno, which is connected with the Baltic Sea.

The Lake Jasień is supplied by only one tributary which constitutes the passage from the Lake Obrowo. In the summer of 1961 it carried an amount of 6—10 m^3 per min. As this lake has no other large tributaries, not counting water supplied directly by rainfall, it is evidently provided by many bottom springs of rich yield.

2. METHODS

Samples for physico-chemical investigations were drawn from four sites placed in the deepest counterpoints of the lake's both basins.

Site 1, 15.3 m deep by the passage from the Obrowo lake, and site 2, 21.9 m deep, characterize the water of the southern basin; respectively site 3, 32 m deep and site 4 with a depth of 26.3 m by the outflow of the river Łupawa, show characteristics of the northern basin's water. Placing sites along the axis of the water passage allowed to state changes of the water composition in both basins (Fig. 2).

Investigations of bottom sediments were carried out in 16 sites, 11 of which (I—V, VIII—XII, XVI) have been placed along the axis of water passage in the lake's profundal, and 5 in the littoral's characteristic points (VI—VII, XIII—XV). Sites I—V were placed in the southern, and VI—XV in the northern basin. Site I of sampling for the investigation of sediments corresponds to site 1 in the scope of examining water, further site IV — to site 2, site X — to site 3 and site XVI — to site 4 (Fig. 2).

All determinations were executed according to Just and Hermanowicz (1959), only selenium has been determined after Struszyński (1952).

Water samples were drawn from a rowing boat by a Patalas sampler in quantities: 3 litres to a general analysis, whereas water samples for O_2, CO_2 and H_2S were placed in separate bottles. Samples for oxygen and hydrogen sulphide were preserved and samples for CO_2 were immediately examined. Basic analyses were carried out every month, drawing samples from depths every 5 m, but temperature and oxygen were investigated in vertical section every 2 m in all sites. The remaining analyses were done only twice — in summer and autumn of 1960.

Samples were drawn at 3—6 weeks' intervals, but in May and June, during the forming of the thermocline, the frequency of sampling grew to 3—4 times per month. The characteristic of bottom sediments has been worked out on the basis of analyses carried out only once in autumn of 1961. Samples were drawn by means of an Ekman bottom sampler.

The analysis of sediments included estimation of organic substance, organic carbon, ammonia nitrogen, organic nitrogen, carbonates, silica, iron and aluminium, phosphates, chlorides and nitrates, carried out after Piper (1957).

Organic substance was determined on the basis of loss on ignition of sediment samples at a temperature of dark red glow (about 500°) and was for comparison calculated after the method of Tjurin (1936), consisting in the multiplication of organic carbon determined by Walkley and Black's titration method (Pifer 1957), by a coefficient 1,724.

3. RESULTS

A. THERMAL AND OXYGEN CONDITIONS

The dynamics of thermal and oxygen conditions in the lake's water have been presented in the diagram of thermo-isoplates at temperature course (Fig. 3), and characteristic thermic profiles in Fig. 4. Accordingly, diagrams presented by Figures 5 and 6, illustrate the dynamics of oxygen conditions. To the computation of percentage of water saturation with oxygen, a value of 761.2 mm Hg was accepted.

The thermal year in Lake Jasień falls into four, well distinguished periods. These are:

1) spring homothermal situation — lasting from April to mid-May
2) summer stagnation — from mid-May to mid-October
3) autumn homothermal situation — from mid-October to December
4) winter stagnation — lasting from January till March.

The run of thermic and oxygen changes was in general typical for holomictic lakes in this area. Some differences will probably be the result of quite specific properties of the springwater Lake Jasień, such as: a rather intense springtime circulation which takes place when the lake's water is not fully saturated with oxygen, the late occurrence and stabilization of summer stratification, very low thermocline gradients (0.88—0.9°/m, Fig. 3), an average heating of hypolimnion, a strong and longlasting autumn circulation, considerable cooling, late freezing followed by short duration of ice cover and exceptionally high oxygen content in the hypolimnion. This proves an appurtenance of the Jasień Lake to the group of eumictic lakes, leaning rather more to tachymixis.

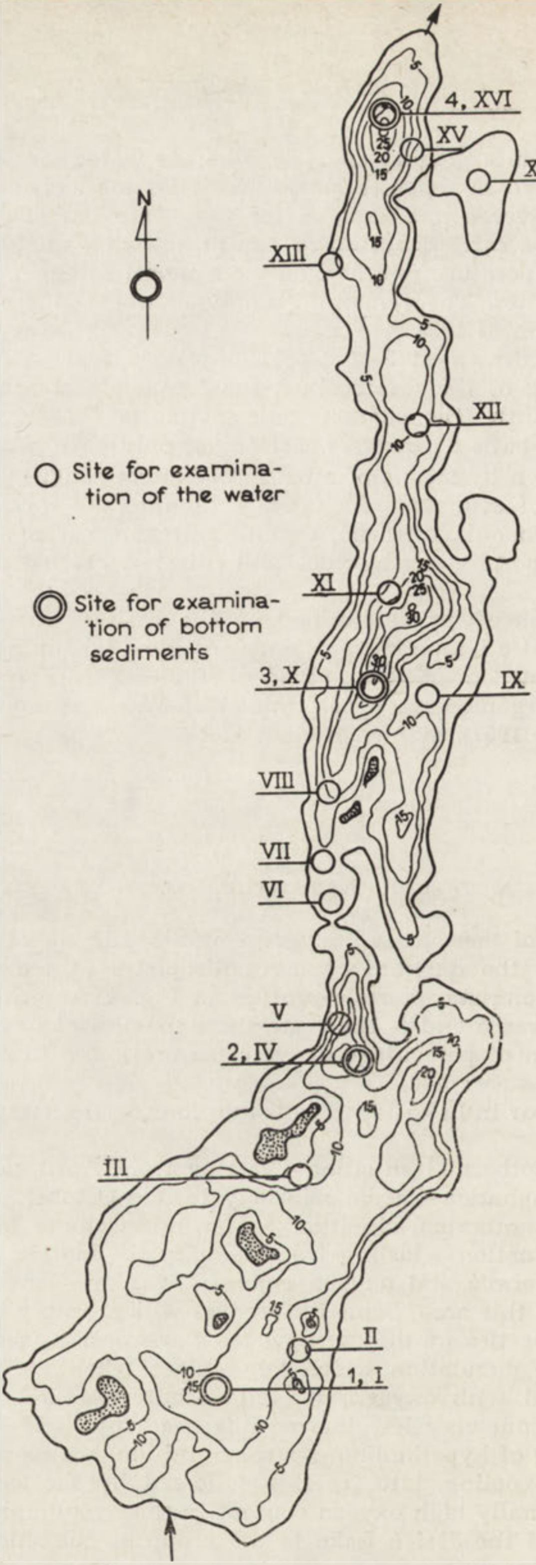

Fig. 2. Disposition of sites for examining the water, and bottom sediments in the Lake Jasień

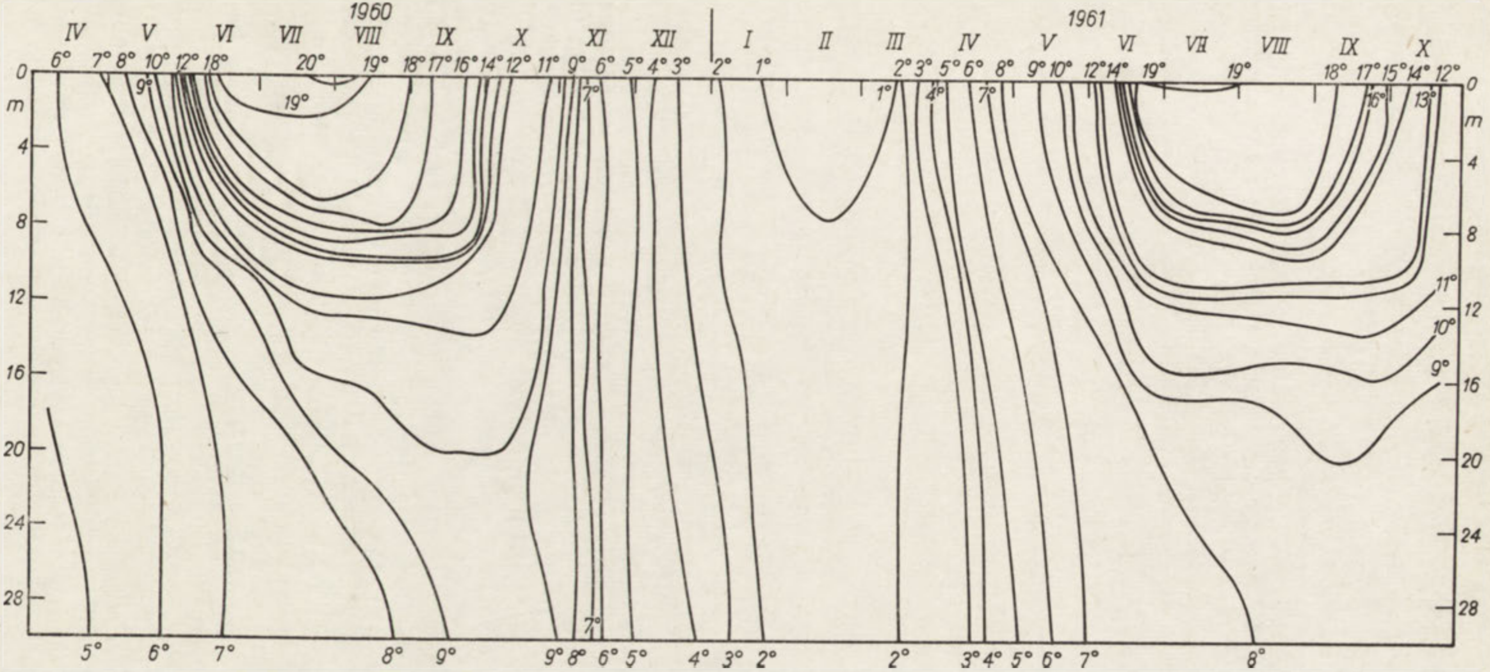

Fig. 3. Thermal stratification of water between April 1960 and October 1961

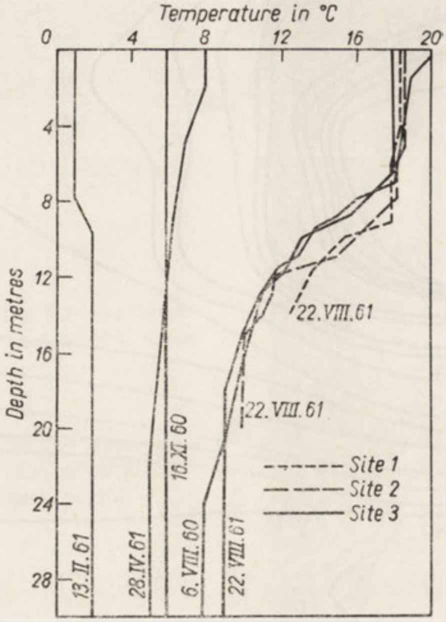

Fig. 4. Thermal profiles of water in the principal seasons

B. TURBIDITY, TRANSPARENCY, COLOUR, pH

The lowest grade of water turbidity has been stated to occur during winter stagnation, i.e. from 1 mg/l SiO_2 at a depth of 0—20 m to 7 mg/l SiO_2 at the bottom. In the spring of 1960 and 1961 turbidity displayed relatively large varieties. At the beginning of April 1960 the water turbidity oscillated from 3 mg/l SiO_2 at the surface, 7 mg/l SiO_2 at a depth of 22 m, to an amount of 20 mg/l SiO_2 at the bottom, but in the same period 1961, during strong winds raising great waves, turbidity reached its highest limit: 10 mg/l SiO_2, and near the bottom even up to 25 mg/l SiO_2. Such a high degree of turbidity 10 mg/l SiO_2 was observed only in the superior strata of epilimnion in summer, whereas in the autumn the water recovered its usual value, reaching 7 mg/l SiO_2 in its superior strata, 5 mg/l SiO_2 in the lower ones, and up to 20 ml/l SiO_2 at the bottom.

Transparency measured by a Secchi disk of 30 cm diameter, oscillated from 12 to 3.7 m in summer during blooming in the southern basin and 4.2 m in the northern one.

The water colour was lowest in winter i.e. 10 mg/l Pt, and highest — 30 mg/l Pt in the southern basin at the beginning of April 1961. In the remaining periods the water colour oscillated nearly always round 15 mg/l Pt, rising only in the surface strata during summer to 17 mg/l Pt.

In the period of summer stagnation the water displayed alkaline or weakly alkaline reaction from pH 8.0—8.2 at the surface, to 7.4—7.7 in the bottom strata.

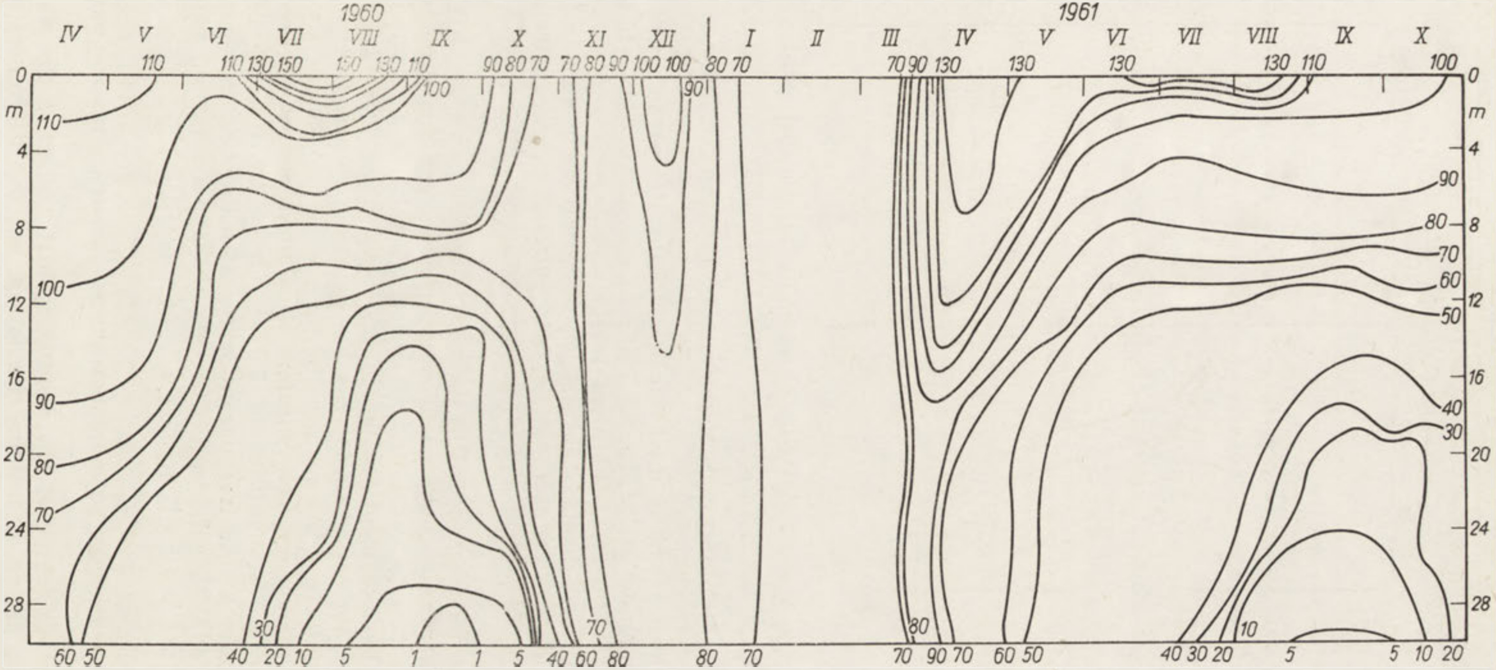

Fig. 5. The waters oxygen stratification between April 1960 and October 1961

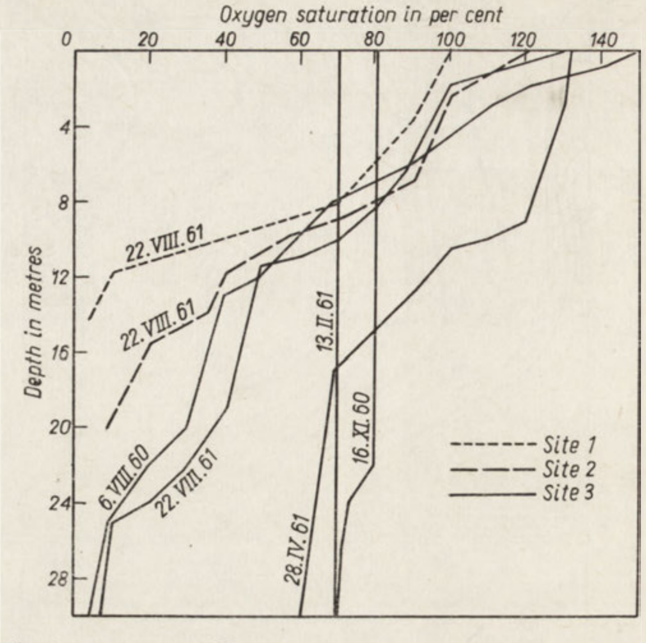

Fig. 6. Oxygen profiles of water in the principal seasons

C. TOTAL HARDNESS, NON-CARBONATE HARDNESS, CARBON DIOXIDE

The highest grade of total hardness could always be noted during the summer circulation period, i.e. 4.2—4.8 miliequivalent/l (11.8—13.4°n) in 1960, and: 4.9—5.2 meq/l (13.7—14.6°n) in 1961.

Total hardness displays only slight stratification in summer, told by its decrease told by its decrease in the surface stratum down to 7 m — 3.4—3.3 meq/l (9.5—9.2°n) and its increase in deeper strata, i.e. 3.5—3.8 meq/l (9.8—10.6°n).

Total hardness amounted in the winter season to 4.4 meq/l (12.0°n). Non-carbonate hardness oscillated in the period of autumn circulation and in winter from 2.0—2.4 meq/l, and in spring from 1.1 to 2.0 meq/l.

Alkalinity also displayed only slight oscillation from 2.1 to 2.5 meq/l. A single and highest alkalinity has been stated in April 1961, in the lowest hypolimnion strata of the northern basin.

During springtime and autumn circulation the water contained free carbon dioxide in quantities of 3-1—4.4 mg/l CO_2 and 3.5—4.7 mg/l CO_2. During summer in the transparent water of epilimnion the amount of free CO_2 dropped to 1.1—2.2 mg/l CO/, mainly on account of photosynthesis, whereas at the bottom it rose to 6.5—7.0 mg/l CO_2.

D. DRY RESIDUE, SUSPENDED MATTER, CHEMICAL OXYGEN DEMAND

The highest degree of dry residue could be noted in the water of Lake Jasień in the period of autumn circulation, but the differences noted in the two subsequent years were considerable. While in the autumn of 1960 the quantity of dry residue oscillated from 188 mg/l at the surface to 220 mg/l near the bottom, in the same period of 1961 it was respectively: 218 mg/l to 230 mg/l. Because of a complete mixing up of the water in autumn, a value of 220—230 mg/l should be accepted as the highest one reached in the lake. The maximum value was approached by the lake's water also in the spring of 1960, i.e. from 190 to 210 mg/l. In the same period of 1961, dry residue was lower, i.e. from 150 mg/l to 180 mg/l, which indicates a considerable influence of circulation intensity on the water total mineralization.

In the summer vertical stratification, much like the one of hardness could be noted; it was 145 mg/l at the surface, decreasing to 138 mg/l at a depth of 8 m, and growing again up to 180 mg/l at the bottom.

In the winter period the value of dry residue is up to 195 mg/l.

During the summer circulation period the total quantity of suspended matter was highest, oscillating from about 50 mg/l in epilimnion and metalimnion down to 30 mg/l in hypolimnion.

The share of volatile suspension in relation to mineral one was expressed by a ratio of 80:20% in epilimnion and 70:30% in hypolimnion.

The total content of suspended matter during the springtime homeothermic situation displayed considerable differences in the two successive years concerned. When in 1960 from the total quantity of suspension of about 25/mg/l in all the winter mass, only 5% forms part of the mineral one, in 1961 in the whole amount of 40 mg/l the ratio of volatile suspension to mineral one is reverse. This is the consequence of violent springtime circulation raised by strong winds proper to the season.

In summer the largest quantity of suspended matter gathers in metalimnion i.e. 48/mg/l, and epilimnion shows only 25 mg/l; 95% of the above is the volatile suspension.

In winter the quantity of suspension oscillates from 18 mg/l at the surface to 35 mg/l near the bottom; it is in 70—80 per cent a mineral one.

The amplitude of oscillations in chemical oxygen demand amounted from 4.5 mg/l O_2 in winter, to 7.5 mg/ O_2 in summer and autumn. Only in the bottom strata theresults were higher, presenting 9.0—11.3 mg/l O_2.

Table II

Certain determinations characterizing the content of organic compounds at 1 m depth, April 1961

Determination	Result	Site 1	Site 2	Site 3	Site 4
Colour	mg/l Pt	70	10	5	5
Chemical oxygen demand	mg/l O_2	9.8	6.5	5.6	9.2
Organic nitrogen	mg/l N	1.12	0.58	0.57	0.56
Albuminoid nitrogen	mg/l N	0.53	0.39	0.36	0.37
Loss on ignition	mg/l	80	40	42	36

The highest content of organic substance (tab. II) could always be noted in site 1, influenced by the inflow from the lake Obrowo, it was lower in site 2, characterizing waters flowing from the southern to the northern basin, finally it was the lowest in site 4, near to the outflow of the river Łupawa.

E. ION CONTENT: Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Fe^{3+}, Mn^{2+}, Cl^{-}, SO_4^{2-}.

The inflow from the Obrowo lake brings to Lake Jasień water containing 30—35 mg/l Ca, but water of the Łupawa river carries away 60—70 mg/l Ca from the northern basin. Fig. 7 shows the increase of concentration of calcium in both basins of the Lake Jasień; in the southern basin from 30 mg/l Ca to 50 mg/l Ca, and in the northern one up to 60—65 mg/l. A distinct vertical stratification of calcium content could be stated in summer (Fig. 8): from 53 mg/l Ca in site 2, and 59 mg/l Ca in site 3 at the surface, up to 60 mg/l Ca and 65 mg/l Ca near the bottom. Therefore parallel with the increase of concentration of calcium in waters flowing through the Lake Jasień, their biological decalcification takes place.

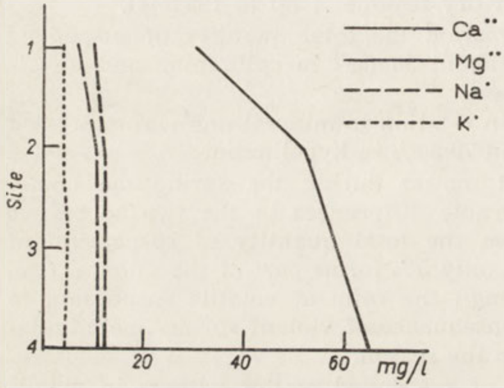

Fig. 7. Kations level stratification at water surface in July 1961

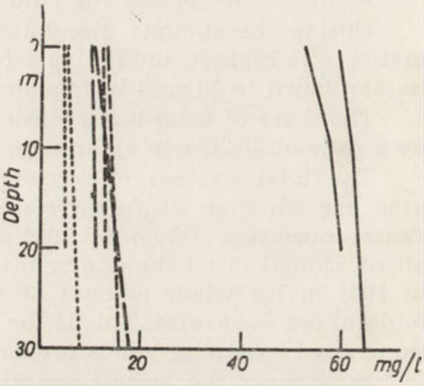

Fig. 8. Kations vertical stratification in sites 2 and 3, July 1961

In the period of spring andautumn circulation displacement of calcium and equalization of its content occur at a scale of 50—60 mg/l Ca.

Magnesium and sodium ions occurring in low quantities of about 10 mg/l and potassium 5 mg/l do not show distinct stratification. A relatively most considerable vertical differentiation is noted in summer for magnesium: 11 mg/l Mg near the surface, up to 18 mg/l Mg at the bottom in site 3.

The ratio Ca:Mg is in the Lake Jasień in mg/l — 65:10.

Iron appeared in quantities from 0.05 to 0.1 mg/l Fe^{3+}, increasing in site 3 to 0.5 mg/l Fe^{3+} at the bottom.

The inflow from the lake Obrowo carries water with a content of 0.3 mg/l Fe. The content of manganese oscillated also slightly: from 0.025—0.05 mg/l Mn^{2+}. The value of 0,1 mg/l Mn^{2+} has been stated only in winter in site 3 near the bottom, and systematically in the above mentioned inflow.

The content of chlorides oscillated between 12 to 16 mg/l Cl^{-} in spring, summer and autumn, dropping in winter down to 3—4 mg/l Cl^{-}. As the ratio Cl:Na nearly corresponds to the equivalent ratio $Cl^{-} = Na^{+}$, ions Cl^{-} penetrate into the water of Lake Jasień mostly as the dissolved salt NaCl. Sulphates, characterized by an oscillation amplitude from 17 to 30 mg/l SO_4^{2-}, did not either show distinct stratification in the summer months. Only in winter, in sites 3 and 4 the content of sulphates was higher in limits from 28—35 mg/l SO_4^{2-}.

F. INTERDEPENDENCE OF AMMONIUM, NITRITE AND NITRATE COMPOUNDS AND ORGANIC NITROGEN

The circulation of nitrogen substances has been graphically presented in the vertical section of site 3 (Fig. 9—12). A comparison of the system of curves in periods of spring and autumn circulation (Fig. 9, 11) confirms the above discussed differences existing in the intensity of circulating motions during spring and autumn. Nitrates reaching 0.20 mg/l N at the start of spring circulation, were nearly used out already in April over the surface layers in connection with the development of phytoplankton. This was accompanied by a growth of nitrogen, the maximum of which reached 0.7 mg/l N at a depth of 4 m. In deeper layers from down to 22 m, the quantities of organic N decrease down to 0.35—0.4 mg/l N, and grow again towards the bottom, reaching 0.5—0.7 mg/l N in deep places (Fig. 9).

Albuminoid nitrogen equalled about 50% of organic nitrogen in all the sites, and its fluctuation depended on the fluctuation of organic N.

The level of ammonia and nitrites was equalized over the whole lake at: 0.12 mg/l ammonia N, and 0.003 mg/l nitrite N, only bottom water showed in site 3 far higher value up to 0.20 mg/l ammonia N, and 0.021 mg/l nitrite N, which was the highest result for nitrate ever noted in the lake.

The period of summer stagnation was characteristic for a great consumption of assimilable forms of nitrogen. It was conceivable by a drop of nitrate nitrogen nearly in all the water mass. In so far as the quantity of nitrates at a depth of 10—14 oscillated in April between 0.15 to 0.20 mg/l N — it was in July only 0.06 mg/l N at the same depth (Fig. 10).

The curve of organic nitrogen reached in the summer two distinct maxima: 0.60—0.65 mg/l Nn at a level of 2 and 20 m. The increase of organic nitrogen in the higher epilimnion can be explained by the blooming of algae, whereas in hypolimnion it was due to the fall of dead particles of phytoplankton.

The starting decomposition process of organic substance is also proved by the growth of ammonia nitrogen from 0.12 mg/l N at a depth of 14 m to 0.20 mg/l N at 24 m. In that time the quantity of ammonia N was at the surface 0.02 mg/l. Nitrites remained at a level of 0.005 mg/l N, reaching a maximum of 0.008 mg/l N in metalimnion.

Albuminoid nitrogen making in April distinctly a half of all the organic nitrogen, indicated thus its vegetal origin, but in July the curve of albuminoid nitrogen correlated with organic nitrogen rising, however, to about 2/3 of its quantum, which would show the share of nitrogen deriving from the animal world.

Autumnal circulation, convexion and diffusion caused a nearly total mixing of the water and supplementation of the shortage of assimilable nitrogen in epilimnion. The content of nitrates reached the value from 0.22 mg/l N at the surface to 0.29 mg/l N near the bottom, and of nitrites respectively from 0.003 to 0.010 mg/l N (Fig. 11). Large differentiation of the quantity of ammonia: from 0.04 mg/l ammonia N in a 15 m high water column to 0.30—0.39 mg/l N at the bottom, may be explained by the advancing decomposition of protoplasm of sinking vegetal and animal bodies, which was accompanied by a slight increase of nitrites. The curve of nitrate content during winter stagnation (Fig. 12) reminds of a quite analogous curve from November. The advancing process of nitrification of nitrogenous substance

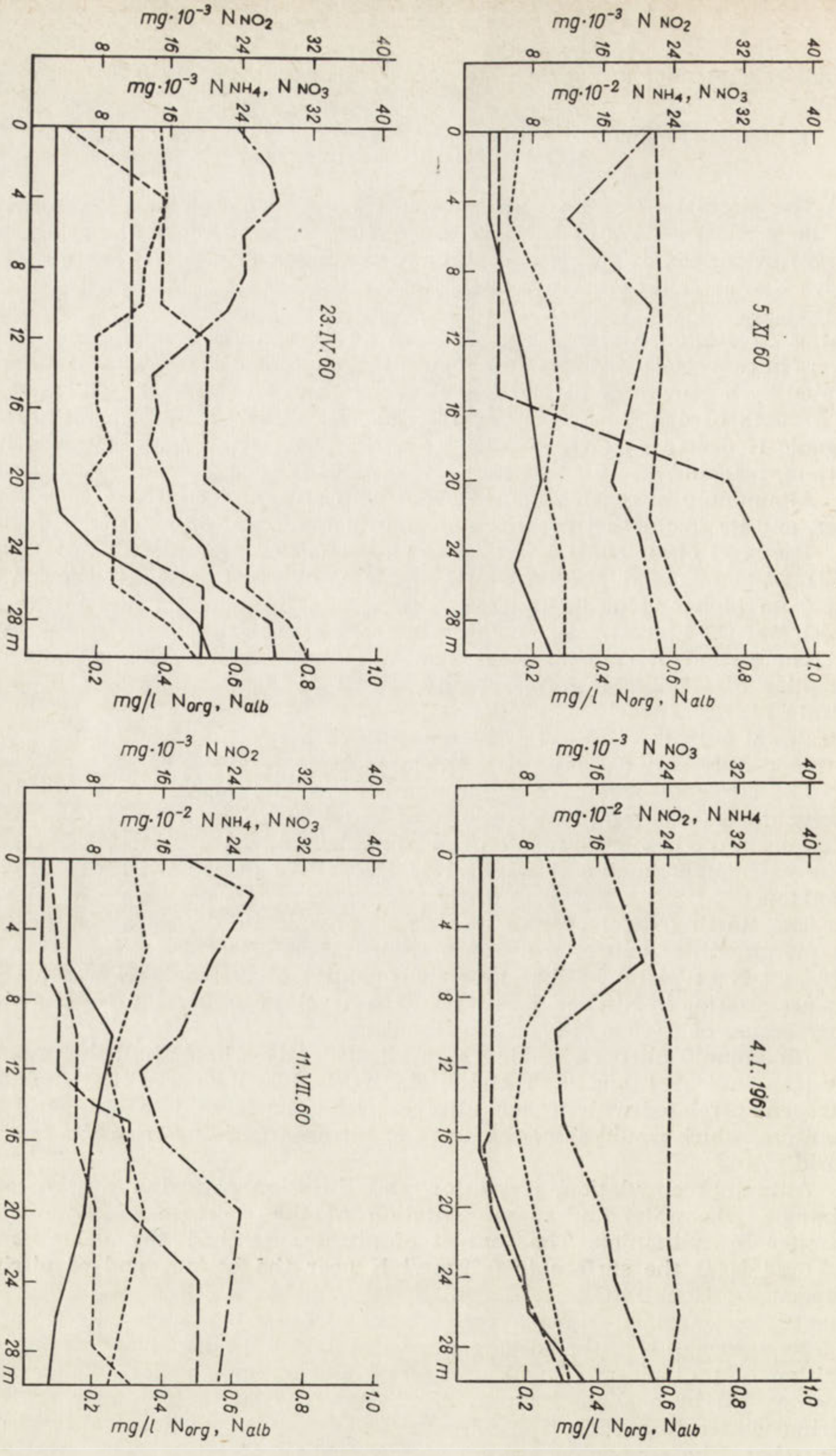

Fig. 9, 10, 11, 12. Stratification of nitrogen compounds in hypolimnion, was characterized by a drop in the content of ammonia nitrogen and growth of nitrite nitrogen. Ammonia showed an amplitude of fluctuation from 0.04 mg/l N at a depth of from 0 to 20 m up to 0.12 mg/l N at the bottom, and nitrites from 0.003 mg/l N at a depth of 0—17 m to 0.014 mg/l nitrite N in the bottom layer.

G. PHOSPHORUS AND SILICON COMPOUNDS

After Stangenberg (1937) it has been accepted that determination of phosphates in the surface water of lakes during their autumnal circulation characterizes adequately the entire affluence into the mentioned compound. Thus relatively small quantities of phosphorus have been found in the Lake Jasień, telling an amount of 0.12 in the southern basin and 0.10 mg/l PO_4^{3-} in the northern one.

In summer there occurred in epilimnion a shortage of phosphorus, connected with its consumption by phytoplankton, shown in table III.

Table III

Drop of phosphorus content during summer stagnation in 1961

Site	Depth m	Date of drawing samples			
		5.IV/. 61	21.VI/.61	12.VII/.61	23.VII/.61
1	0	0.10	0.02	0.01	0.01
	15	0.60	0.24	0.15	0.08
2	0	0.08	0.03	0.01	0.00
	20	0.62	0.20	0.12	0.09
3	0	0.06	0.01	0.00	0.00
	30	0.55	0.19	0.20	0.11
4	0	0.06	0.02	0.00	0.00
	25	0.60	0.22	0.18	0.08

For the sake of comparison the table shows also data from April 1951. Springtime maximum was characterized in the Lake Jasień by quantities from 0.06 to 0.10 mg/l PO_4^{3-} and 0.55—0.60 mg/l PO_4^{3-} near the bottom, which represents the highest data concerning the content of phosphorus noted in the lake. In the autumn and springtime circulation period the content of silicon amounted to about 10 mg/l SiO_2, but in summer and winter distinct stratification from 5 mg/l SiO_2 at the surface, up to 10—12 mg/l SiO_2 at the bottom could be noted.

H. MICROELEMENTS

No traces of arsenic, selenium, lead, zinc or chromium have been stated in the water of the Lake Jasień.

Copper, the physiological role of which is closely connected with the processes of formation and lasting of chrophyll, has been discovered only in the inflow from the Obrowo lake in a quantity of 0.001 mg/l, whereas in site 1 the presence of copper could not be confirmed.

Considerable quantities of iodine in the water are characteristic by values from 0.0084 mg/l J in site 4, to 0.0089 mg/l J in site 1.

Fluorine occurred in quantities from 0.2—0.3 mg/l F at the surface to 0.04 mg/l F near the bottom, which has considerable meaning to a sanitary estimate of the water quality.

Boron, indispensable element to the correct metabolism of plant organisms, occurred only in small quantities of 0.05 mg/l B in the water of the Lake Jasień.

I. ANALYSIS OF BOTTOM DEPOSITS

To the elaboration of a characteristic of bottom deposits in the Lake Jasień, Stangenberg's classification (1938) was applied, for it relied on the occurrence of particular components in prevailing quantities; thus silicate, calcareous, organic and mixed deposits could be distinguished.

Silicate sediments have been stated in sites I, II, X, in deeper profundal parts (9.0—30.0 m deep). They mostly contained silicate in quantities of 46.3—49.4%, besides that organic substance in 29.4—35.4%, considerable quantities of iron and aluminium: 5.80—15.6%, carbonates — 3.05—13.06%, low quantities of phosphates — 1.70—2.15% and of chlorides — 0.067—0.180%.

Ammonia content in silicate sediments oscilated from 12.60 to 86.15 mg%, nitrates from 0.22 to 1.70 mg%, organic nitrogen from 1340 to 1851 mg%. The content of total nitrogen was 1.3—1.9% and of organic carbon from 12.3—18.7%.

The ratio C:N oscillated from 8.6 to 13.0.

Sediments in sites VI, VII, XII, XV (depth from 1.0—3.5 m) placed in the littoral zone, have been determined as calcareous ones. When dry they had a white-grey tint and were distinguished by a very large content of calcium carbonate i.e. from 74.50 to 93.90%.

Out of the remaining components of calcareous sediments the following should be mentioned: organic substance 5.6—18.0%, silicates 0.1—5.9% and minute quantities of iron, aluminium, phosphates and chlorides, showing an amplitude of fluctuation respectively: 0.10—3.3%, 0.03—0.55% and 0.031—0.366%. The content of nitrogenous substance was very low. The range of fluctuation in ammonia was 0.02—0.03 mg%, of nitrates 0.06—0.20, of organic nitrogen from 278 to 554 mg%.

Total nitrogen oscillated between 0.2—0.5% and carbon from 1.6—5.8, giving a ratio C:N from 3.2 to 13.5.

Samples drawn from sites III, IV, V, VIII, IX, XII (depth 9.0—15.0 m) have been ranged to organic sediments. When fresh they had a black or greenish tint, but after drying they assumed grey-greenish tints of various shades.

The content of organic substance fluctuated from 41.8% to 50.4%, and the relatively considerable quantity of carbonates, from 7.00 to 21.80% and of silicates from 21.0 to 40.5%. The content of iron together with aluminium was from 5.20 to 13.20%, of phosphates from 0.12 to 2.12%, and of chlorides from 0.36—0.122%.

The characteristic feature of these sediments was a considerable content of nitrogenous and carbonic substance. Ammonia nitrogen occurred in limits of 15.0—140.0 mg%, nitrate nitrogen — 0.11—2.00 mg%, and organic nitrogen — 947—2177 mg%. The content of total nitrogen oscillated between 1.2—2.2% and of carbon between 17.7—24.8%.

Samples from sites IX and XVI, containing basic ions by about 30% have been classified as mixed sediments. The large fluctuation amplitude in the content of basic components in midlake sediments should be explained by extremely heterogenous sedimentation conditions of components created by a gutter lake with numerous partitions and bolts at the bottom.

4. DISCUSSION

The way of supplying a lake generates a number of specific limnological features which determine the great distinction of a springwater lake.

The chemical composition of the Lake Jasień water has been presented in the form of an ion balance (tab. IV), considering all items occurring in quantities larger than 0.001 milli equivalent per litre. The ion-equivalent water composition displays among cations a decided preponderance of calcium, which amounting to 2480 meq makes 64% of cations. Magnessium occurs in far smaller quantities making 0.812 meq, which is 21% of all cations, whereas sodium and potassium occurring in quantities: 0.416 and 0.117 meq are only 11% and 3% of cations content.

Table IV

Ion Balance of the Lake Jasień Water

Kations	mg equivalent	Anions	mg equivalent
Ca^{2+}	2.480	HCO_3^-	2.400
Mg^{2+}	0.812	SO_4^{2-}	0.624
Na^+	0.416	Cl^-	0.563
K^+	0.117	NO_3^-	0.219
Fe^{3+}	0.005	NO_2^-	0.001
Mn^{2+}	0.001	PO_4^{3-}	0.003
NH_4^+	0.008	CO^{2-}	0.002
		F^{-3}	0.015
Total:	3.839	Total:	3.827

The first place among anions is that of hydrogen carbonate — 62% — further sulphates — 16%, chlorides — 15% and nitrates — 5% of the anions' total content. According to Alekin (1956) the Lake Jasień may be ranged to the class of hydrogen carbonate waters of the calcium group, which can be approximately expressed by the ratio:

$$HCO_3^- < Ca^{2+} + Mg^2 < HCO_3^- + SO_4^{2-}$$

The high content of calcium and magnesium in the water, characteristic for ground waters, is one of the features of a springwater lake.

The rather high water hardness displays insignificant fluctuation round average values. The appearance of slight stratification in total hardness is connected in summer with the biological decalcification of epilimnion and calcification of hypolimnion (Stangenberg 1934, 1956), and on the other hand lack of differentiation of non-carbonate hardness is connected with the low susceptibility of chlorides and calcium sulphate to biological factors (Stangenberg 1950).

The dry residue of the examined lake water, when compared to other non-polluted lakes (Szmal 1959, Gromadska 1956) is characteristic for its high values, similarly as silicon, when compared with Ohle's (1934) data which correlates with the ways of supply of the Lake Jasień.

The rapid exhaustion of copper in the lake's water can be explained by its consumption by phytoplankton and its sorption by organic sediments covering the bottom. For Maksimow (1954) states that the capability of strongly decayed organic sediments to absorb copper ions can be so remarkable, that copper becomes no more assimilable to plants.

Considerable quantities of fluorine in the bottom water layers can be, according to Deesbach (1957), explained by contacts with layers of calcium carbonate. The total content of fluorine in the water of Lake Jasień can be considered rather high. Kajkov (1961) investigating 23 lakes in the Murmańsk Circuit did not find a reservoir with higher fluorine content than 0.05 mg/l F. Juday, Birge and Meloche (1938) determined the fluorine content in the lakes of the north-eastern area of Wisconsin to be in the limits of 0.08—0.51 mg/l F, and Mackereth and Heron (1954) found in England's lakes an average content of 0.15 mg/l F. Kniżnikow (1958) quoting on the other hand examples of particularly high fluorine content in lake waters, mentions one lake in Kazakhstan with 4 mg/l F.

Poor quantities of boron can be connected according to Maksimow (1954) with high content of calcium ion.

Distinct interdependence between the course of meteorological events (Fig. 1) and the forming of thermic stratification (Fig. 3) could be observed. The warming of surface layers in June 1960 moves very slowly down into the lake's depth, whereas the same event in 1961 occurs far quicker owing to stronger winds.

In contradistinction to bradimictic lakes (Passowicz 1938, Olszewski 1959), the thermocline of which embraces a much narrower layer of water, the Lake Jasień displays a thermocline far spread in the vertical sense, typical for tachymictic lakes (Olszewski 1953 a, 1953 b, Paschalski 1959). The protraction of thermocline is undoubtedly due to the water motion connected with its supply from bottom springs and turbulent motions caused by attrition of waters running towards the outflow, against those touching the current flowing across. Almost parallel to the thermocline there is also the oxycline, stretching vertically down, it had during its occurrence remained in the top layers of thermocline, e.g. in August 1960 and 1961 it could be found at a depth of 6—12 m. (Fig. 4,6). From the mutual situation of thermocline and oxycline the conclusion can be drawn that the index of depth reached by the waving motion of water prompted by the wind, will be the inferior limit of oxycline and not of thermocline, the deeper range of which should be explained by the water's thermal conductivity. This is in agreement with the investigation of Patalas (1956), carried out in the lake Zamkowy.

Chemical analysis of bottom sediments supplemented the lake's characteristics and confirmed a number of conclusions resulting from the water's physico-chemical analysis. The very large content of carbonate ion in calcarous sediments is here remarkable. Stangenberg and Żemoytel (1952) have not found more $CaCO_3$ than 47% in the littoral sediments of Charzykowskie lake. The far larger quantities of $CaCO_3$ in littoral than in profundal sediments can be explained by a more intense decalcification of water owing to the photosynthesis of algae in the vicinity of coasts grown over by plants and the decomposition of mollusk shells gathering here in large masses.

Organic sediments had here a rather individual picture of C:N ratio. When compared with other data (Kuzniecov 1939) showing in analogous sediments a content of carbon in the limits 25—55% of nitrogen 1—3%, values lower than the average, particularly in the case of carbon, have been stated in the Lake Jasień. This fact can, however, be explained by the character of mineralization of sediments in the Lake Jasień. The rate of organic sediment mineralization is here certainly considerable, on account of the large stock of oxygen in hypolimnion and the oxygen access to biopel. The leading role of mineralization in these conditions will be held by bacteria, fungi and actinomycetes, favoured by the relatively high temperature of hypolimnion waters; enzymatic processes would occur here in a lower degree. Intense mineralization processes explain the relatively smaller quantities of carbon in sediments and a certain shift of the ratio C:N to the advantage of nitrogen. The ratio C:N in organic sediments of the Lake Jasień was comprised in the limits from 10.1 to 20.9 by an equal median 14.9.

Table V

Content of organic substances in bottom sediments of the Lake Jasień estimated by various methods

Site	Type of sediment	Organic substances in % of dry mass determined as:		In relation to method restingon loss on ignition result: increased (+) diminished (−)
		Loss on ignition	C org ×1,724	
1	silicate	35,4	28,3	− 7,3
2		25,0	21,2	− 3,8
10		30,6	29,1	− 1,5
11		29,4	32,2	+2,8
6	calcareous	18,0	5,9	−12,1
7		14,1	2,7	−11,4
13		5,6	4,6	− 1,0
15		10,0	9,9	− 0,1
3	organic	49,4	42,7	− 6,7
4		43,5	33,4	−10,1
5		44,6	30,5	−14,1
8		44,0	32,4	−11,6
9		50,4	35,8	−14,6
12		41,8	38,4	− 3,4
14	mixed	39,4	20,6	−18,8
16		38,7	26,7	−12,0

A comparison of results of organic substance determination by two different methods (Tab. V) indicates a positive error of 2.6% values obtained by the method based on the loss on ignition. This is caused by an erroneous ranging of different varieties of crystallizing and chemically combined water among organic substances.

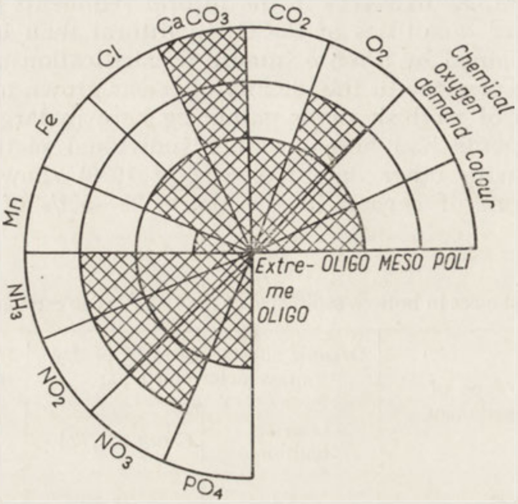

Fig. 13. Hydrochemical spectrum of the Lake Jasień

The degree of eutrophizing the reservoir is shown by the hydrochemical spectrum (Fig. 13). The reservoir's morphological properties, first of all its steep and deep gutter and the way of supplying the lake by springwater, inhibit the progress of traphization of the lake, which should as a whole be ranged among mesotrophic reservoirs; also processes of the lake's maturity are more advanced in the southern than in the northern part.

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5. SUMMARY

On the basis of detailed physico-chemical investigation of the water and fragmentary chemical analysis of bottom sediments, conducted during the period from April 1960 to October 1961, a hydrochemical characteristic of the Lake Jasień has

been elaborated. The lake supplied by bottom springs of substantial yield, belongs to the scheme of the Pomeranian Lake District, and forms the outflow reservoir of the river Łupawa.

The dynamics of the thermal and oxygen conditions distinguished the following features to be typically connected with the springwater-outflow character of the lake.

1) Spring circulation of mean duration, occurring while the lake water is not yet saturated with oxygen,

2) late occurrence and stabilization of summer stratification.

3) intense and long autumn circulation, strong cooling, short freezing and short lasting ice cover,

4) exceptionally good oxygenation of hypolimnion waters.

The chemical composition of the lake water, presented in the form of ion balance, draws attention to the high content of calcium and magnesium, typical for ground waters, which would be one of the features of a springwater lake. Littoral lime sediments were distinct for a exceptionally high content of $CaCO_3$ (74.5—93.9%), while the content of organic substance in gyttia amounting to 50%, showed an interesting picture of C:N ratio, indicating a shift to the advantage of nitrogen. This proves a great intensity of mineralization processes of organic matter, favoured by a high oxygen content and high temperature of hypolimnion water, which is one more characteristic of a springwater lake.

A distinct interdepedence between the course of meteorological phenomena and the shaping of termic stratifications has been observed. The Lake Jasień has a much diffused, in the vertical sense, thermocline, typical for tachymictic lakes, formed under the acting of mobile waters, connected with the springwater supply and turbulence caused by the outflow of the river Łupawa.

6. STRESZCZENIE

Na podstawie szczegółowych badań fizyko-chemicznych wody oraz fragmentarycznych analiz chemicznych osadów dennych prowadzonych od kwietnia 1960 r. do października 1961 r., opracowano charakterystykę hydrochemiczną jeziora Jasień. Zasilane źródłami dennymi o dużej wydajności jezioro wchodzi w skład Pojezierza Pomorskiego i stanowi zbiornik wypływowy rzeki Łupawy.

Dynamika układów termiczno-tlenowych wyróżniła jako cechy typowo związane ze źródliskowo-przepływowym charakterem jeziora:

1) średnio długą cyrkulację wiosenną, przebiegającą przy niepełnym nasyceniu tlenem wód jeziora,

2) późne powstanie i utrwalenie się uwarstwień letnich,

3) intensywną i długą cyrkulację jesienną, silne wychłodzenie późne zamarzanie i krótkie zlodzenie,

4) wyjątkowo dobre natlenienie wód hypolimnionu.

Skład chemiczny wody jeziora przedstawiony w postaci bilansu jonowego zwraca uwagę na wysoką zawartość wapnia i magnezu znamienną dla wód gruntowych, jako jedna z cech jeziora źródliskowego. Litoralowe osady wapienne wyróżniały się wyjątkowo dużą zawartością $CaCO_3$ (74,5—93,9%) natomiast zawartość substancji organicznej w gytii wynosząca około 50% charakteryzowała się ciekawym układem stosunków C:N wykazującym przesunęcie na korzyść azotu. Dowodzi to dużej intensywności procesów mineralizacji materii organicznej, którym sprzyja duża zasobowość tlenowa i znaczna temperatura wód hypolimnionu jako jeszcze jedna z cech jeziora źródliskowego.

Obserwowano wyraźną zależność między przebiegiem zjawisk meteorologicznych a kształtowaniem uwarstwień termicznych. Jezioro Jasień posiada bardzo rozwleczoną w układzie pionowym termoklinę, typową dla jezior tachymiktycznych, kształtującą się pod wpływem ruchów wód związanych z zasilaniem źródliskowym oraz turbulencją wywołaną odpływem wody rzeką Łupawą.

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VERTICAL DISTRIBUTION OF PERIPHYTON IN THE LAKE MIKOŁAJSKIE

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ABSTRACT

Seasonal changes in the appearance of littoral periphyton (chlorophyll and productivity) have been stated in the Lake Mikołajskie.

Three maxima in the amount of chlorophyll and productivity have been observed: in early spring (end of April), in summer (mid-August) and late autumn (November). The largest quantities of periphyton appear mostly near the surface, but in the summer period they could be found also deeper. At a depth of 2.0 m the quantity of periphyton is over twice lower than near the surface. There is considerable convergence between the dynamics of changes in littoral periphyton and seasonal changes in mid- lake phytoplankton.

Not many studies are concerned with vertical differentiation of periphyton. The papers of Maciolek (1962), Maciolek and Kennedy (1964), discuss periphyton stratification in the pelagial of high mountain lakes. The papers of Rehbronn (1964) and Meschkat (1934) are concerned with littoral, they do not, however, connect the amount of periphyton with its productivity. Research started in 1965—66 in the reed-covered parts of the Lake Mikołajskie had the direct aim of solving problem.

Investigations included periphyton developing on glass plates lowered for one month's period to depths of 0.5, 1.0, 1.5 and 2.0 m into the water of a reed-covered part of the lake, near the Hydrobiological Station in Mikołajki. Research included the period between March 25, 1965 and March 21, 1966. It was carried out using two series of plates, one of which was placed a fortnight later than the other. The size of periphyton's biomass has been analysed by a chlorophyll method (Szczepańska, 1967) similarly as that of plankton, and the gross production of oxygen excreted by periphyton in standard conditions * was calculated. There exists positive correlation (outside the blooms) between the amount of chlorophyll and the quantity of excreted oxygen (Pieczyńska, Szczepańska, 1966). Both methods parallelly applied become a mutual complementation and control.

By analysing seasonal changes in the amounts of periphyton at various depths (Fig. 1), we state that its intense development, starting immediately after the disappearance of the ice cover, lasts about one month. During its maximum the amounts of chlorophyll reach 9.5 $\mu g/cm^2$ at a depth of 0.5 m,

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* 10 hours' exposition, 1000 lux, temperature 20°C.

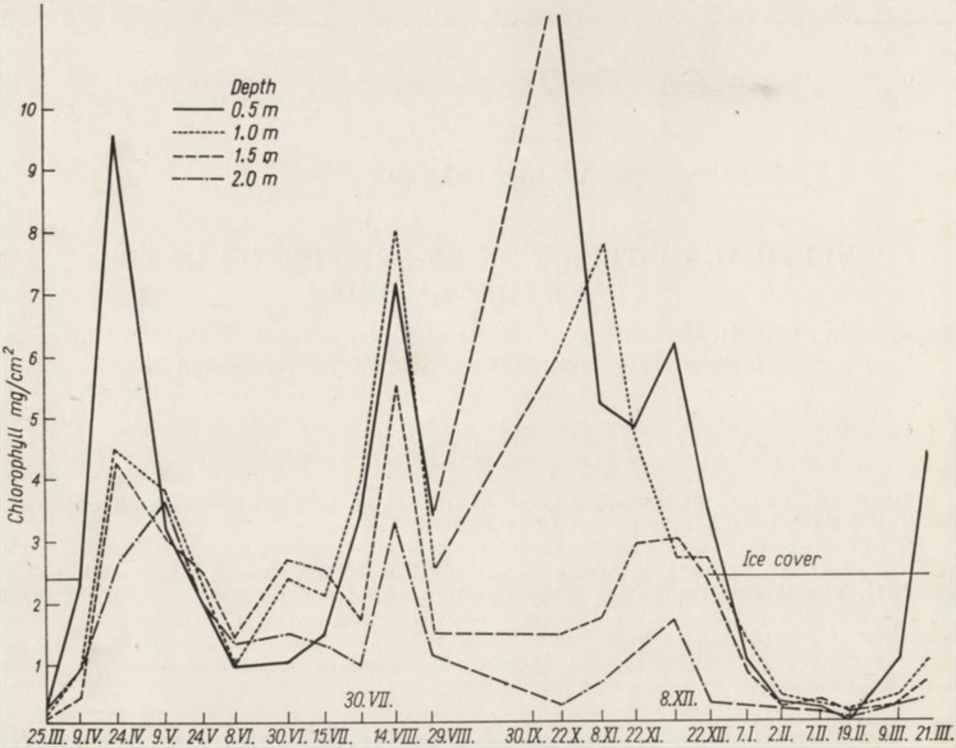

Fig. 1. Seasonal changes in chlorophyll amount of phragmithetum periphyton at different depth; Mikołajskie Lake, 1965—1966

while at a depth of 1.0 m they show only 4.5 μg/cm². They are steadily lower at deeper levels and display a value 2.6 μg/cm² at a depth of 2.0 m. The mean quantity examined in four levels amounts in that period to 5.2 μg/cm². In the second decade of May begins the decrease of its quantity remaining at a low level for about two months. Differences in the quantity of chlorophyll are at particular levels in this period quite insignificant and their mean in that time amounts to 2.02 μg/cm² for all the levels. The second greater increase of periphyton biomass starts at the end of July. The maximum of summer growth falls to mid-August, reaching then a maximum of chlorophyll content — 8.0 μg/cm², therefore somewhat lower than that of spring, but the mean value for the whole profile in that period is somewhat higher than the springtime maximum, reaching 5.9 μg/cm². The summer maximum appears at a depth of 1 m, and its drop is observed towards the end of August.

In the third decade of October begins the autumn increase of periphyton amount, it is the largest and lasts the longest. The mean of the period is for chlorophyll 6.8 μg/cm². The maximum quantity of chlorophyll amounted in this period, at a depth of 0.5 m, up to 19.3 μg/cm². Large quantities of chlorophyll in the autumn period can be observed during two months. In the period: end of December — beginning of January occurs a considerable winter drop of periphyton quantity. The amount of chlorophyll oscillates in the winter period in the limits of 0.1 to 1.0 μg/cm².

Springtime maximum of 1966 is somewhat accelerated in relation to the same, period of the previous year, starting in the third decade of March, while in 1963 in began round April 10.

During springtime and autumn maximum the quantities of chlorophyll at a depth of 0.5 m are much higher than at other depths. The gradient of chlorophyll amount during springtime maximum is 2.1 μg/cm²/m, during autumn climax — 7.9 μg/cm²/m. This indicates a considerably greater differentiation of autumn periphyton. From the first decade of June till mid-August the quantities of surface periphyton are smaller than in further depths.

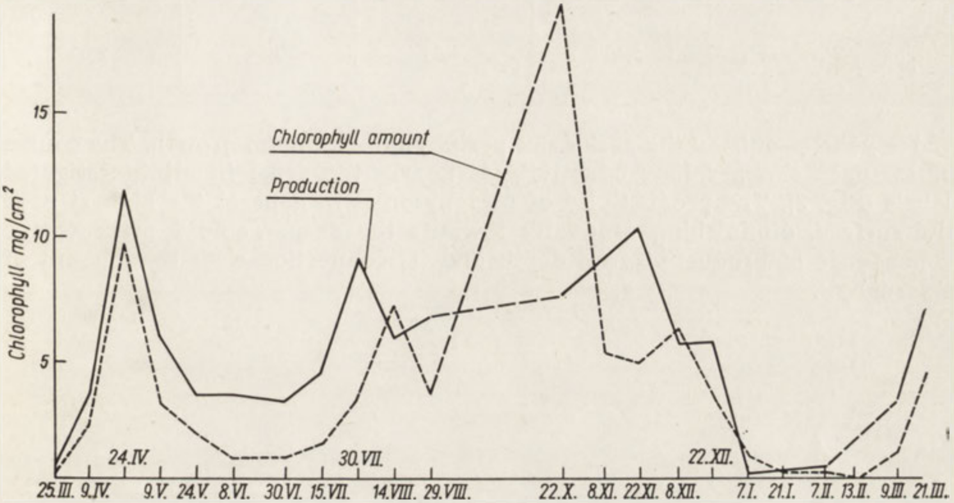

Fig. 2. Seasonal changes in chlorophyll amount and gross production — depth 0,5 m

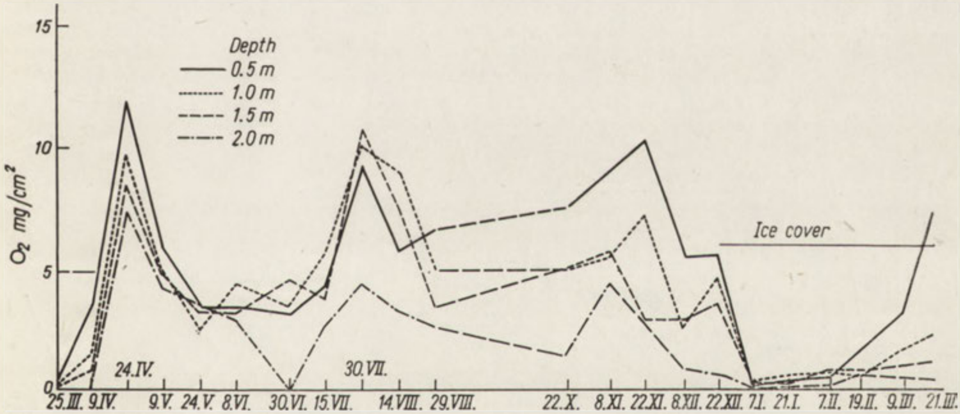

Fig. 3. Seasonal changes in gross production of phragmithetum periphyton at different depth

Seasonal changes in the quantities of chlorophyll correspond to the production of periphyton (Fig. 2). There is rather large concordance in the changes of the amount of chlorophyll and its productivity in the course

Table I

Mean annual quantities of chlorophyll and productivity

depth	0,5 m	1,0 m	0,1 m	2,0m
chlorophyll in μg/cm²	4,4	2,96	2,01	1,33
productivity 2 in μg/cm²	5,49	4,32	3,94	2,43

of the year — outside the periods of periphyton maximum growth. The course of seasonal changes in productivity is nearly the same in all investigated levels (Fig. 3). The productivity of periphyton is in general the highest near the surface, diminishing gradually towards the deep. Table 1 presents the dependence of productivity on depth and its connection with the amount of chlorophyll.

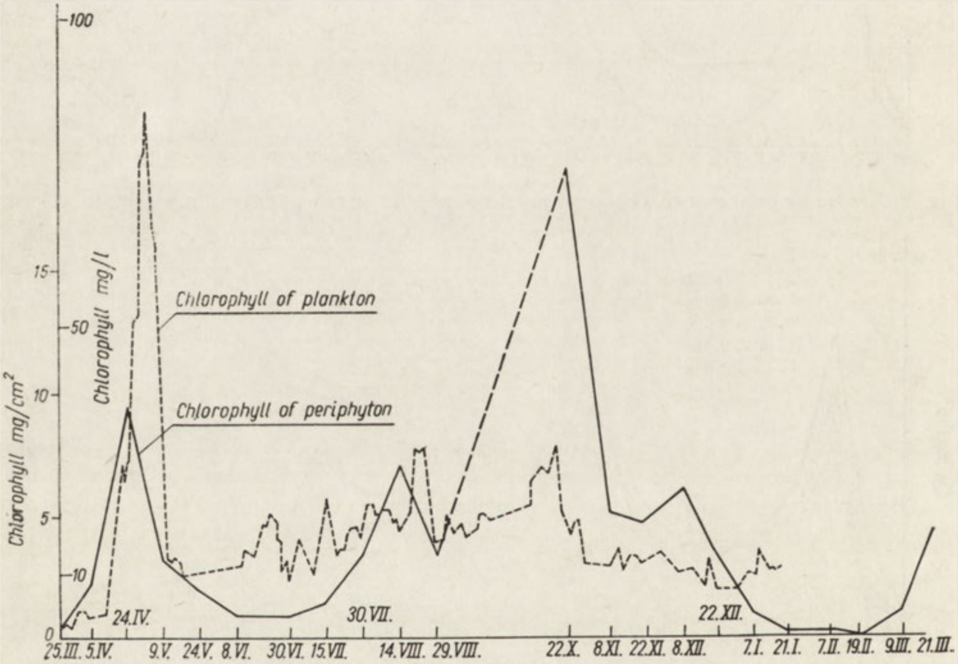

Fig. 4. Seasonal changes in periphyton amount of phragmithetum and of mid-lake

It can be stated that mean annual values for quantities of chlorophyll and productivity of surface periphyton are over twice as high as near the bottom. Meschkat (1934) explains the event of vertical differentiation occurring in periphyton by differences of light and water climate.

The production of periphyton is in general the highest at a distance of 0,5 m from the surface, however, in the period from end May to mid-August, when the chlorophyll gradients have a negative value, productivity is also larger at greater depths. Periphyton production is always lowest at a depth of 2 m. The highest gradient values fall to the periods of periphyton's maximum development, which would prove largest differentiation of production between surface and bottom in those periods. Means for all the year for the quantities of chlorophyll and oxygen excretion in the examined depths, indicate that these values decrease parallelly with the growing depths. However, by large quantities of chlorophyll, productivity is inclined to decrease. The above dependence is illustrated by Table No. 2.

Table II

Assimilation numbers and periphyton chlorophyll in the Mikołajskie Lake

Date	Chlorophyll amount in $\mu g/cm^2$	Assimilation number gCO_2/ /g of chlorophyll/ hours
Feb. 7, 1966	0.30	2.75
July 30, 1965	2.50	0.52
Oct. 22,1965	6.80	0.10
annual mean	2.50	2.07

The table shows that the assimilation number (amount gCO_2 assimilated by lg of chlorophyll during one hour) rapidly decreases. Szczepański and Szczepańska (1966) have stated a very high assimilation number only in very early stages of periphyton development. After this, lasting hardly a few days, period of high productivity, follows a period of decrease of the assimilation number and its subsequent stabilization at a rather equal level, along with a further growth of periphyton quantities.

The lack of correlation between the amount of chlorophyll and production ability during maximum development of periphyton has been stated by Pieczyńska and Szczepańska (1967). Low values of the assimilation number in that period confirm the author's conclusion. The comparison of dynamics in the changes of mid-lake plankton biomass (Szczepański 1966), with changes in periphyton, indicates considerable convergence.

Springtime and summer mid-lake plankton blooms accord with the periods of maximum periphyton development in littoral. Differences in the intensity of blooming can easily be noted. The greatest bloom of mid-lake plankton occurs in spring, maximum development of periphyton occurs in autumn. The above mentioned differences do not, however, shake the existence of connections occurring between the general dynamics of seasonal changes in the lake and the littoral periphyton.

SUMMARY

In the littoral of Lake Mikołajskie seasonal changes of dynamics and vertical periphyton differentiation have been investigated. Glasses were placed for a one month's period in depths of 0.5, 1.0, 1.5 and 2.0. After exposition, the size of periphyton biomass and its productivity were analysed by a chlorophyll method. Research was conducted all the year round.

Three maxima of periphyton development have been stated: end April, mid-August and November. In the June-July period the amount of periphyton and its pro-

ductions ability is relatively slow, as compared with the same in spring and autumn. Gradients of chlorophyll amounts and production have then negative values, which means that periphyton amounts are lower by the surface than deeper down. In the remaining periods gradients are positive.

Lowest amounts of periphyton were found at a depth of 2 m. Mean annual amounts of periphyton and its productivity indicate a uniform drop of periphyton amounts along with growing depth. At a depth of 2 m mean values of annual periphyton amounts are more than twice lower than at a depth of 0.5 m.

During the maximum periphyton development no positive correlation between the amount of chlorophyll and its production ability can be observed. There exists considerable convergence between the dynamics of periphyton changes in littoral and seasonal changes of mid-lake phytoplankton.

STRESZCZENIE

W litoralu jeziora Mikołajskiego badano dynamikę sezonową i pionowe zróżnicowanie perifitonu. Szkiełka umieszczono na okres 1 miesiąca na głębokościach: 0,5, 1,0, 1,5, i 2,0 m. Po ekspozycji, analizowano wielkość biomasy perifitonu metodą chlorofilową i jego zdolność produkcyjną. Badania prowadzono przez cały rok.

Stwierdzono wstępowanie trzech maksimów rozwoju perifitonu, w końcu kwietnia, w połowie sierpnia i w listopadzie. W okresie czerwiec—lipiec ilość perifitonu i jego zdolność produkcyjna jest stosunkowo niska w porównaniu z ilościami perifitonu na wiosnę i w jesieni. Gradienty ilości chlorofilu i zdolności produkcyjnej mają w tym okresie znaki ujemne, to znaczy że ilości perifitonu przy powierzchni są mniejsze niż głębiej. W pozostałych okresach gradienty są dodatnie. Najniższe ilości perifitonu stwierdzono na głębokości 2 m. Średnie roczne ilości perifitonu i jego zdolności produkcyjne wskazują na równomierny spadek ilości perifitonu wraz z głębokością. Na głębokości 2 m średnie wartości ilości perifitonu dla całego roku są przeszło dwukrotnie mniejsze, niż na głębokości 0,5 m.

Podczas maksymalnego rozwoju perifitonu nie obserwuje się dodatnich korelacji między ilością chlorofilu i jego zdolnością produkcyjną.

Istnieje duża zbieżność między dynamiką zmian perifitonu w litoralu a zmianami sezonowymi fitoplanktonu śródjeziornego.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	**15** (28)	2	183 ~~831~~—189	1968

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THE INFLUENCE OF DESICCATION AT DIFFERENT AIR HUMIDITIES ON HATCHABILITY OF *TRIOPS CANCRIFORMIS* (BOSC) EGGS

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ABSTRACT

The embryonic development of *Triops cancriformis* eggs accomplishing probably gastrulation after 6 days in water, attains the stage which is resistant to desiccation. Desiccation stops the development, but the development continues in the air at relative humidity of 100% until the pre-hatching stage is attained. However the eggs are not able to hatch under these conditions.

CONTENTS

1. INTRODUCTION

Numerous papers can be found in the literature which deal with the effect of different variable environmental factors on the hatchability of eggs in Branchiopoda. The majority of them pertains to Anostraca (for review of the more recent literature, see Nourisson 1964), and only a few which deal with Notostraca (e. g., Mathias 1937, Chaigneau 1959a, b, Hempel-Zawitkowska 1967).

Previous studies (Hempel-Zawitkowska 1967) have proved that the eggs of *T. cancriformis* are being laid in the stage of one cell (the ovum). The embryonic development, lasting in this species for 9—40 days under the laboratory conditions and following thoroughly outside the reproductive system of a female, involves a number of stages which differ greatly as to their response and resistance to temperature changes and desiccation. A supposition has been made that the developing embryo at early stages of the development up to 5 days after laying before the stage of gastrula has been attained, is not able to survive such environmental changes (desiccation and extremal temperatures) which are merely stopping the development in further stages. In order to verify this hypothesis in the present experiments, the eggs at different stages of embryonic development were air-dried at different relative humidities, and then, after replacing them into water, their development was observed and the hatchability ascertained.

2. MATERIAL AND METHODS

The eggs used in the experiments were gathered from the egg pouches of parthenogenetic females of *T. cancriformis,* collected from a fish pond (fry pond I) of the fish farm Łąki Jaktorowskie, near Warsaw in June 1966. Each season the pond has been under inundation for 6—8 weeks (May-July), and *T. cancriformis* was found to occur there each year in masses (Hempel-Zawitkowska 1968). Before the desiccation treatments were applied, the eggs had been kept in the pond water for different time at a temperature of about 20°C.

Three groups of eggs were used in the experiments: (1) the eggs which after being pressed out from the female's egg pouches, were kept in water for one day only, (2) those, which were kept for 3 days in water and, (3) for 6 days.

The portions of the eggs of about 100 in each group were placed on the slides between two sheets of filter paper and then kept in air-tight jars. The jars contained various saturated solutions of salts, forming a layer of 2 cm thick at the bottom, which were maintained constant relative humidities over the solution in the jars interiors (Winston, Bates 1960). The saturated solutions of the following substances, producing various relative humidities, were used.

$MgCl_2$	—33% R.H.
$Mg(NO_3)_2$	—55% „
NaCl	—76% „
KCl	—85% „
H_2O destil.	—100% „

Over each solution, there was an brass rack supporting the slides with filter paper and the eggs.

The eggs were kept in jars at the above listed relative humidities for 28 days at a temperature of about 20°C. After this period the eggs were transferred into tap water of ambient temperature, each group of the eggs from one jar to a separate glass container. Previous studies (Hempel-Zawitkowska 1967) showed that the aerated tap water had no harmful effect on the development and hatching of the Triops eggs. Later on, the time and percentage of hatchings were recorded. The hatchability was expressed as a cumulative percentage of eggs that have hatched to a given day of their stay in water. These observations on hatching were run for 30—60 days depending on the experimental series. The lack of hatching for at least 7 subsequent days in all the samples of one series was considered as the end of the hatching process. After this period the water was gently poured out of the containers, the remaining eggs were air-dried for 20 days, and then the containers refilled with water in order to check whether there were some diapausing, but still living eggs among them. The latter procedure showed that all the eggs which had not hatched during the first period of submerging in water did not hatch either in the second submerging period, proving thus that they were dead.

3. RESULTS

In all the experiments, except for the series with 100% R. H. treatment, clear differences were found in hatchability among the designed groups of the eggs (Figs 1 & 2). The highest percentage of hatching was observed in these eggs whose embryonic development seemed to be most advanced, that is, in the eggs which had been deprived of their water environment not earlier than after 6 days following their removal from the females' egg pouches

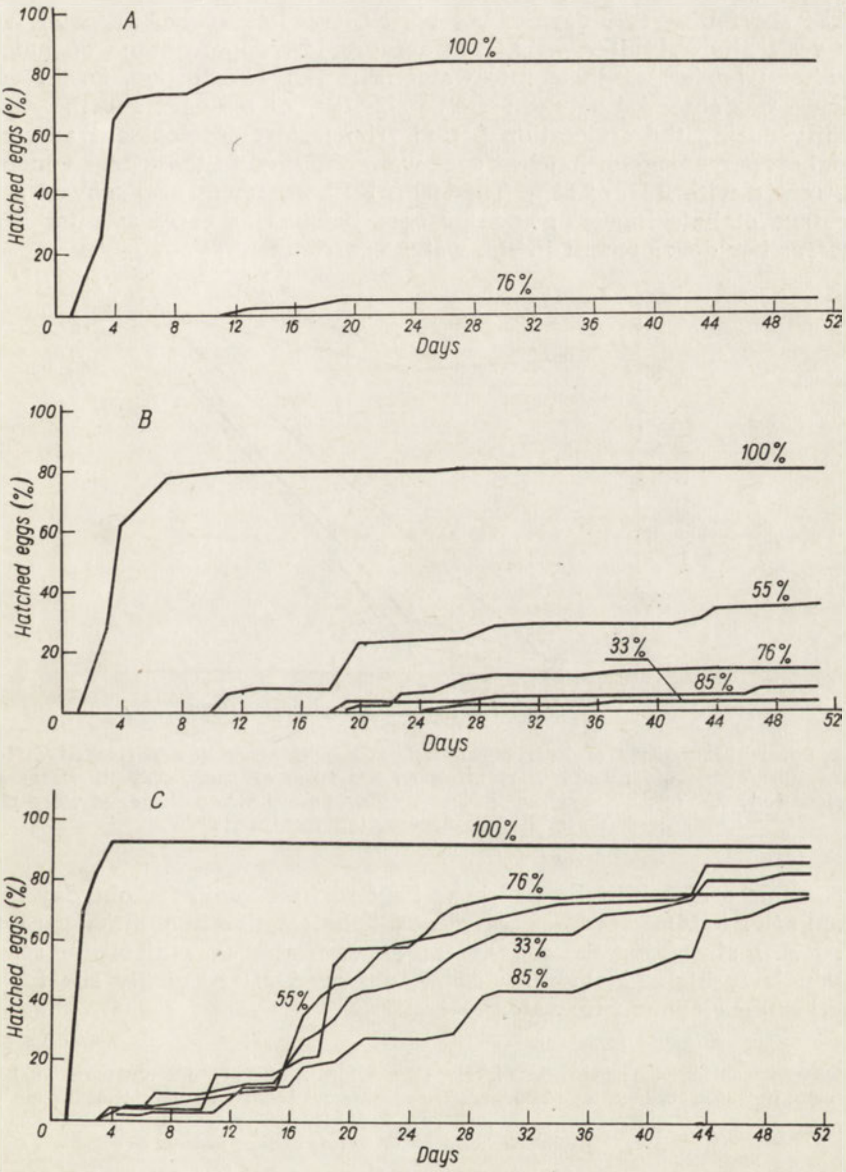

Fig. 1. The hatchability of the *Triops cancriformis* eggs after desiccation at different relative humidities of the air.

A. The eggs, after being taken out of the egg puches, had stayed 1 day in water prior to desiccation. B. Correspondingly, 3 days in water. C. Correspondingly, 6 days in water. Per conts above the curves refer to relative humidity of air in the experimental jars

(Fig. 1C). This percentage was high and uniform (75—88). The hatchability in the treatment with the water saturated air (100% R. H.) was even higher and amounted to 93%. These eggs transferred into tap water terminated hatching as early as after 4 days following the transfer, thus the development in this treatment was faster than that in the remaining ones.

The shortening to 3 days of the period eggs had stayed in water before their desiccation resulted in a considerably lower percentage of hatching eggs after transferring them into water (Fig. 1B). In addition, no direct dependence was observed between the hatchability of the eggs and the relative humidity during the desiccation period. The fastest development as well as the highest percentage of hatched eggs were observed in these eggs which had been treated with R.H. of 55%. The 100% R.H. treatment also showed a high percentage of hatchings (80) and the eggs hatched as early as after 7 days of the further development in the water environment.

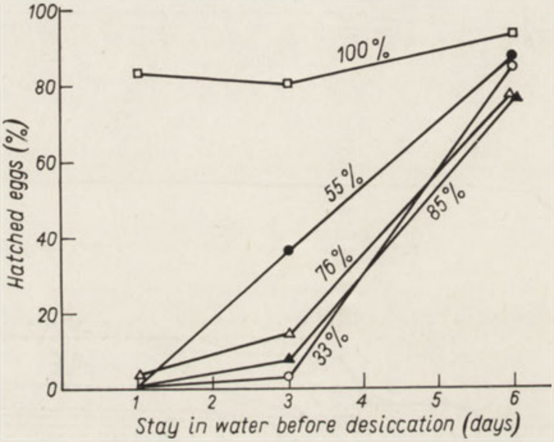

Fig. 2. Total hatchability of *Triops cancriformis* eggs after desiccation at different relative humidities of the air in relation to the time of their stay in water prior to desiccation. "O" on the abscissa indicates the moment when the eggs were placed in water before desiccation treatment

The eggs which initially had been kept in water only for one day hardly hatched after having been desiccated at all the relative humidities except for 100% R.H. Maintaining the eggs in the air saturated up with water resulted in a similarly high hatchability (85%), observed after equally short period of further development in water.

Table I

Cumulative percentage of hatched eggs of Triops cancriformis after; (1) different period of development in water, followed by (2) desiccation at different relative humidities of the air

Days between laying and desiccation	Relative humidity of the air in experimental jars				
	33%	55%	77%	85%	100%
1	0	0	3.8	0	83.2
3	3.0	35.9	14.4	7.6	80.9
6	84.0	87.2	77.2	76.0	93.0

The sum of hatchings for particular series (Table I, Fig. 2) corroborated the above described regularities. At any value, except for 100%, of the relative humidity during the desiccation period, the percentage hatchability was several times higher in these eggs which had stayed in water for 6 days prior to desiccation as compared with those whose corresponding period lasted only for 3 days. The sum of hatched eggs in 100% R.H. treatment was very high in all the instances, and the differences between the groups of the eggs were insignificant. Apart from the series run at R.H. of 100%, which revealed the highest per cent of hatching, the remaining series did not showed any clear dependence between the egg hatchability and the relative humidity of air, since the next to the highest percentage of hatched eggs was observed in 53% R.H. treatment, and the hatchings in 85% R.H. treatment were lower than in the remaining series.

4. DISCUSSION

Thoroughly different opinions can be found in the literature dealing with the effect of water shortage on the development and hatching of the eggs of Branchiopoda. Although the eggs have been never observed to hatch under natural conditions without, a prior, thorough or partial, drying off the water body, the undried eggs often did so under laboratory conditions. In spite of that, Mathias (1967) has put forward a hypothesis on existance of two categories of the eggs, (1) the eggs apt to immediate hatching and, (2) those which have to undergo the period of drought before they hatch. However, this view was not corroborated by Ncurisson (1964) who in his studies on *Chirocephalus diaphanus* Prev. has found only one category of the eggs which can develop both after desiccation and without it. According to the latter author, desiccation was not a determining factor for both the embryonic development and hatching in the Branchiopoda eggs, although the temperature was. On the other hand, it is evident from the experiments by Chaigneau (1959) that the eggs which had been dried for a longer period hatched better than those dried shortly. The opposite was found by Campan (1929), who reported on the most rapid hatching in those eggs which had been kept in wet mud before they were placed into water.

These quite contradictory opinions on the effect of desiccation on the development of eggs in Branchiopoda find some explanation in the results of the previous paper by one of us (Hempel-Zawitkowska 1967). They suggest that the degree of the embryonic development, and especially the given stage which undergoes desiccation is a factor differentiating the response of the eggs to desiccation. It was found that the eggs which were dried immediately after having been laid were not able to survive the drought period, whereas the eggs which had stayed in water until the gastrulation period was completed, after being dried and then replaced into water, hatched in a high percentage (about 90) within 2—5 days.

In all the available literature on the experiments with the eggs of Branchiopoda, the desiccation factor is not precisely defined, especially the information is lacking on the relative humidity of air at which the experiments were performed. Besides, which is of a vital importance for the analysis of desiccation effect on development and hatchability of Branchiopoda eggs, the earlier studies were carried out with bisexual species, in which the embryonic development starts already in the reproductive system of a female and the eggs laid are far advanced in their development. This would change fundamentally their response to desiccation, increasing their general resistance to extreme factors of the abiotic environment with the course of the development.

The results of the present paper confirm in general the above mentioned hypothesis (Hempel-Zawitkowska 1967). The eggs of *Triops cancriformis* can develop without the drought period, providing the development follows under static conditions and the abiotic factors of the environment do not deviate much from the optimum typical for the development of a given species. Such a trait can be defined as a considerable stenoplasticity of the development of the eggs of *T. cancriformis*.

Desiccation certainly is a factor which changes this type of reaction, causing, in fact, a significant broadening of the abiotic conditions range (oscillation in temperature, the drought period), within which a further development and eventual hatching can occur. The developing embryos of *T. cancriformis*, affected by desiccation, acquire thus eury-plasticity and a higher resistance to astatism and aperiodicity (sensu Klekowski 1966) in the outer environment conditions.

5. CONCLUSIONS

The embryonic development in *Triops cancriformis* starts after the egg has been liberated from the egg pouches of a female, can follow both in water and in the air saturated with water. In the latter, the development can reach the stage just preceding the hatching. However, the egg does not hatch under these conditions, although Dutrieu (1960) has observed the eggs of a related species, *Artemia salina* (Leach) to hatch on moist filter paper. That the eggs of *T. cancriformis* can reach pre-hatching stage at R.H. of 100%, is evident from the fact that they hatch shortly after transferring them to water (Fig. 1 & 2).

With all the remaining relative humidities, the embryonic development has probably ceased to follow at this stage at which the egg was deprived of the water environment. Further development will continue not earlier unless the egg is replaced into water. The eggs which had stayed in water for 6 days following their laying could attain the most advanced stage of the development, they probably completed gastrulation (Hempel-Zawitkowska 1967), surmounting in this way the critical stage in desiccation resistance. That is why these eggs, after 28 days of desiccation at different relative humidities, placed again in water, showed a very high percentage of hatching (Fig. 2). In the group of the eggs kept initially in water for 3 days, there were only a few which went through this critical stage mentioned, and these eggs were able to survive the desiccation period. The period of one day the other group of the eggs stayed initially in water was thoroughly insufficient and almost all the eggs of this group died under the impact of desiccation (Fig. 2).

6. SUMMARY

The eggs of *Triops cancriformis*, pressed out of the egg pouches, were initially kept in water for 1, 3 and 6 days at a room temperature, and later on, desiccated in the air at different relative humidities: 33, 55, 76, 85 and 100% R. H. After this

period, the eggs were placed again in water and their hatchability has been recorded for 30 days. The unhatched eggs were air-dried again for about 20 days and then replaced into water and checked for hatchability.

In all the relative humidity treatments, except for 100%, the percentage of hatched eggs was highest in these eggs which before desiccation had stayed in water for 6 days (Fig. 1, 2). It is most probable that these eggs had accomplished the gastrulation process, thus overcoming the critical stage in desiccation resistance. This stage was attained by much smaller proportion of the eggs which had been developing in water for 3 days. In 100% relative humidity treatment, the development of the eggs continued similarly to that in water until the pre-hatching stage was attained, but the eggs did not hatch under these conditions.

7. STRESZCZENIE

Jaja *Triops cancriformis* wydobyte z komór jajowych przetrzymywano w wodzie 1, 3 oraz 6 dni w temperaturze pokojowej, po czym 28 dni w powietrzu o wilgotności wzgl. 33, 55, 76, 85, 100%. Po tym okresie jaja zalewano wodą i rejestrowano przez 30 dni wylęgi. Jaja niewylęgnięte suszono na powietrzu ok. 20 dni, powtórnie zalewano wodą i notowano wylęgi.

Po pobycie we wszystkich użytych wilgotnościach wzgl. powietrza, prócz 100% w. w., wylęg był najwyższy u tych jaj, które przebywały przed suszeniem 6 dni w wodzie (rys. 1, 2). Jaja te zapewne zakończyły gastrulację przekraczając w ten sposób krytyczne stadium odporności na wysychanie; po 3 dniach rozwoju w wodzie znacznie mniejsza część jaj osiągnęła takie stadium. W 100% w. w. rozwój jaj przebiegał dalej tak samo jak w wodzie, aż do stadium przedwylęgowego, ale sam wyląg nie następował.

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