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MUTUAL RELATIONSHIPS OF MICROORGANISMS IN IŁAWA LAKES WATERS

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ABSTRACT

Among 166 investigated cultures of saprophytic bacteria isolated from waters of Iława lakes, 79% hindered the development of numerous water bacteria. Relatively the greatest number of antagonists was observed in winter, and the least in autumn. The most extensive antagonistic action was observed in spore-forming bacteria of the genus Bacillus; the least number of antagonists appeared among bacteria of the genera Aeromonas, Alcaligenes, and Micrococcus. The phenomenon of autoantagonism was observed in 9 strains of the genera Pseudomonas, Flavobacterium, Micrococcus, and Bacillus. 11 strains of the genera Aeromonas, Corynebacterium, Micrococcus and Bacillus stimulated the development of various types of bacteria.

CONTENTS

1. INTRODUCTION

Very few authors have studied the problem of mutual relationships between water microorganisms, and most of the existing works concern the antibiotic properties of *Pseudomonas fluorescens*. Thus, e.g., Lewis (1929) isolated from sea water a strain of *Pseudomonas fluorescens* which hampered the development of various spore-forming bacteria and soil fungi in laboratory conditions. Wallhäusser (1951 a, b) found a strong inhibition of growth of *Bacillus terrestris* and *Bacillus petasites* in the presence of *Pseudomonas fluorescens*. Later it was proved (Gräf 1958) that this property is a feature of individual strains. Klinge (1959) reported that only some strains of *Pseudomonas fluorescens* do secrete to their environments an ingredient hampering the development of other microorganisms.

According to Rosenfeld and ZoBell (1947) antibiotic substances are produced by various sea bacteria. Grien and Meyers (1958) observed antibiotic properties in 50% of actinomycetes isolated from rivers' estuaries at Florida banks, while Demny et al. (1961) proved antibiotic properties in 154 out of 1414 strains of actinomycetes isolated from salt marshes, sea water, littorals and estuaries. Kra-

silnikova (1961) investigated the influence of 326 strains of sea bacteria isolated at various depths of the Pacific Ocean upon *Escherichia coli, Staphylococcus aureus, Mycobacterium luteum* and *Saccharomyces cerevisiae;* she found antibiotic properties in 27 strains only. The highest percentage of antagonistic strains was found among the spore-forming bacteria.

A number of strains of fresh-water bacteria also reveals an antagonistic influence upon the development of microorganisms (Nižegorodova 1968). According to her, about 30% out of the 420 cultures of saprophytic bacteria isolated from the bottom sediments of the Danube delta acted antagonistically against the development of *Staphylococcus aureus, Escherichia coli, Actinomyces violaceus* and *Bacillus mycoides.* The maximum amounts of bacteria secreting the substances which hindered the development of the listed microorganisms were found in the grey and black muds, rich in organic compounds. In another study (Fedjanin 1968) it was proved that among 945 different strains of saprophytic bacteria, isolated from water of the Danube drawn at various spots, 97% hindered the development of *Pseudomonas tumefaciens,* 34% of *Escherichia coli,* 26% of *Staphylococcus aureus* and 16% of *Bacillus carotovorus.* The most extensive range of the antagonistic influence was observed in strains belonging to the genera Pseudomonas, Bacterium and Bacillus. This finding was corroborated by Kutelev (1968) who investigated the antagonistic action of 100 strains of bacteria isolated from water and muds of the reservoirs fed from the Danube.

There are very few data as to the stimulating action of water bacteria. The only fairly extensive approach can be found in Chodyniecki (1968); he found such action in some strains of the *Pseudomonas sp.* belonging to the IInd, IIIrd and IVth groups, in the *Vibrio sp.* and *Aeromonas sp.* According to Chodyniecki, the strains of the *Flavobacterium sp.* and some of those belonging to the *Aeromonas sp.,* were particularly sensitive to such stimulation, while neither of the strains belonging to the *Pseudomonas sp.* proved to be sensitive to the stimulating action of the other bacteria. However, the investigation was carried out on stored strains, and not on freshly isolated ones.

Ecologically interesting are the mutual relationships within the typical water microflora, and this is what the present paper deals with.

2. MATERIAL AND METHODS

The investigated strains of bacteria. The investigations were carried out on various strains of bacteria isolated from water of Iława lakes in winter (January), spring (April), summer (July) and autumn (October) of 1968 (Niewolak 1970). They were identified according with Bergey (1957) and the Shewan et al. (1960) scheme. All the bacteria were cultivated on broth agar with the pH 7.2. In general, 166 strains were studied, belonging to the genera: Aeromonas (13 strains), Vibrio (8 strains), Pseudomonas (16 strains), Achromobacter (2 strains), Alcaligenes (5 strains), Flavobacterium (3 strains), Xanthomonas (2 strains), Micrococcus (33 strains), Streptococcus (5 strains), Corynobacterium (7 strains), Arthrobacter (1 strain), Bacillus (65 strains), and to the family of Enterobacteriaceae (the genera Aerobacter and Escherichia — 6 strains).

Interaction of bacteria. The problem of interaction of water bacteria was studied by the Fredericq-Levine method as modified by Kjems and described by Klinge (1959). 24 hr broth cultures of the investigated strains were inoculated by means of a Pasteur pipette on Petri's plates 10 cm in diameter, with the agar substrate partly dried in 45°C, and then the plates were incubated during 48 hr in a temperature which was optimal for the given strain. The developed colonies were subsequently killed by 60 min exposition to the chloroform vapour, the plates were ventilated in a sterile container for 30 min, and finally poured with 3 ml of 0.7% broth agar contaminated with 0.001 ml of the 24 hr broth culture of the test strain of bacteria. The results were checked after 24 hr of incubation in the optimal temperature. If the action was antagonistic, areas of inhibition of the growth of test bacteria strains could be seen around the killed colonies; the breadths of these areas were measured in milimeters from the colonies' edges. A stimulating action was revealed by an area of stronger growth of bacteria around the killed colony.

3. RESULTS

The investigations concerning the relationships among the water microflora were carried out separately for each of the 4 groups of bacteria strains isolated in the four sampling terms. The results are presented in Tables I–IV.

Among 35 strains of various bacteria isolated in winter (Table I), 32 strains, or over 94%, revealed an antagonistic action. The number of sensitive strains was not much lesser, as it was 91.4%. The greatest range of antagonistic action was found in strains of the genus Bacillus. Some of them, e.g. *Bacillus sp.* No. 389 and *Bacillus pumilus* No. 391 inhibited about 45% of all bacteria tested in this group. Similar or not much lesser extents of antagonistic impact were revealed by *Micrococcus sp.* No. 371, *Micrococcus ureae* No. 385 and strain No. 363 of the Enterobacteriaceae family. Extents of the inhibition areas varied depending on the intensity of a strain's bactericidal influence. The most active were No. 363 strain of the Enterobacteriaceae family, and the species *Bacillus pumilus* No. 383. On the other hand, *Micrococcus candidus* No. 381, *Micrococcus varians* No. 398 and *Streptococcus salivarius* No. 388 proved to be inactive. In general, the ranges of antagonistic influence, and the bactericidal action measured by the extents of the growth inhibition areas, tended to be rather weak among the Coccaceae, as well as in the Corynebacterium-Arthrobacter group. *Corynebacterium sp.* No. 410 and *Bacillus sp.* No. 389 proved to be wholly immune to the influence of the investigated bacteria. The phenomenon of autoantagonism was observed in *Bacillus sp.* No. 396. Stimulation was observed in six cases only. Thus, *Micrococcus ureae* No. 385 stimulated the development of *Micrococcus sp.* No. 371; *Corynebacterium rathaiji* No. 374 stimulated the development of *C. humiferum* No. 364; *Bacillus sp.* No. 368 and 376 stimulated the development of strain No. 363 of the Enterobacteriaceae family, and of the species *Corynebacterium humiferum* No. 364.

In the second group of water bacteria (47 strains) isolated early in spring (Table II) just after the receding of ice, an antagonistic action was revealed by 83% of the strains. All of them were sensitive to one or more species of bacteria. The greatest range of antagonistic action was observed in the following strains: *Vibrio sp.* No. 526, *Bacillus subtilis* No. 489 and 518, *Bacillus cereus* No. 509 and *Bacillus sp.* No. 545. They inhibited 40 to 55% of all the bacteria used as test microorganisms. At the same time, these strains revealed the most intensive bactericidal influence. In their presence, the areas of inhibition of the growth of some strains were as large as 15 or 20 mm. On the other hand, completely inactive were *Vibrio sp.* No. 530, *Alcaligenes sp.* No. 492 and a few strains of the genus Bacil-

lus. Autoantagonism was observed in three cases, and stimulating properties were revealed only by *Bacillus coagulans* No. 490 with respect to *Vibrio sp.* No. 530.

Out of 44 strains of bacteria isolated in summer (Table III) antagonistic influence was observed in 84%, similarly as in spring. The most active, both as to the range and intensity of their antagonistic impact, proved to be *Aeromonas sp.* No. 731, *Pseudomonas sp.* group IV No. 761, and *Bacillus polymyxa* No. 753. They inhibited from 41 to 52% of all the investigated strains of bacteria, and the inhibition areas produced by them reached sometimes a few scores of milimeters. Highly active were also *Aeromonas sp.* No. 759, *Bacillus macerans* No. 703 and *Bacillus sp.* No. 715. Inactive strains were observed among the genera Aeromonas, Flavobacterium, Micrococcus and Bacillus. The phenomenon of autoantagonism was observed only in the single case of *Pseudomonas sp.* group IV No. 761, and stimulating action — in four cases: *Aeromonas sp.* 737 and 739, and *Micrococcus sp.* No. 735 stimulated the development of *Flavobacterium sp.* No. 711, and *Aeromonas sp.* No. 759 favoured the growth of *Vibrio sp.* No. 708. The few species immune from the influence of the products of metabolism of other bacteria belonged to the genera Aeromonas, Micrococcus and Bacillus.

Among 40 strains isolated in autumn (Table IV) 60% revealed antagonistic action. Particularly large range of such action was characteristic of *Micrococcus sp.* No. 855, *Bacillus sp.* No. 841, and *Bacillus firmus* No. 845. They inhibited as many as 40% of all the bacteria isolated at this term. At the same time these strains revealed intensive bactericidal impact expressed by the relatively largest growth inhibition areas observed in this group of strains. However, many species proved to be inactive, as e.g. *Pseudomonas sp.* I group No. 825, strains of the genus Alcaligenes, and a number of strains of the genera Micrococcus and Bacillus. A large part of the autumn sample, or as many as 20% of the strains, were also insensitive to the products of metabolism of other bacteria. Autoantagonism appeared in 5 cases, most remarkably in *Bacillus circulans* No. 850. Among the bacteria isolated in autumn no stimulant strains were observed.

In various strains of the same species of bacteria, both the bactericidal and bacteriostatic influence was often observed. They also differed in sensitivity. Strains No. 546 and 554 of *Bacillus pumilus* are a remarkable example of this phenomenon. Both strains inhibited different species or strains of bacteria. Besides, *Bacillus pumilus* No. 546 was much more active than No. 554. The same can be said about the strains of *Bacillus macerans* No. 702 and 703, and about many other ones, belonging to several systematic groups of bacteria and isolated in different seasons. In

Table I. Zones of growth inhibition (mm) of bacteria strains isolated in winter (January 1968)

Inhibiting strains \ Sensitive strains	No.	*Pseudomonas sp.* I gr.	*Pseudomonas sp.* I gr.	*Pseudomonas sp.* IV gr.	*Achromobacter sp.*	*Alcaligenes sp.*	*Enterobacteriaceae*	*Xanthomonas sp.*	*Micrococcus candidus*	*Micrococcus sp.*	*Micrococcus sp.*	*Micrococcus sp.*	*Micrococcus candidus*	*Micrococcus candidus*	*Micrococcus ureae*	*Micrococcus varians*	*Micrococcus candidus*	*Micrococcus flavus*	*Micrococcus varians*	*Micrococcus sp.*	*Streptococcus salivarius*	*Corynebacterium humiferum*	*Corynebacterium rathaiji*	*Corynebacterium sp.*	*Corynebacterium sp.*	*Arthrobacter simplex*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus pumilus*	*Bacillus sp.*	*Bacillus pumilus*	*Bacillus sp.*	*Bacillus megatherium*	*Bacillus megatherium*	*Bacillus sp.*	% of inhibiting strains
Pseudomonas sp. I gr.	387	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	8.5
Pseudomonas sp. I gr.	407	—	—	—	—	—	—	—	—	—	—	8	—	4	—	—	—	9	—	—	4	—	—	—	—	7	—	—	—	—	—	—	—	—	—	14.3
Pseudomonas sp. IV gr.	406	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.7
Achromobacter sp.	377	—	—	1	—	4	—	—	—	—	2	—	—	—	1	1	—	—	1	—	—	—	—	1	—	1	2	—	—	—	—	1	1	—	—	1	34.2
Alcaligenes sp.	375	—	—	—	—	—	1	—	—	—	—	4	—	3	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	5	14.3
Enterobacteriaceae	363	—	2	1	4	11	—	—	—	—	—	11	5	1	—	1	—	—	—	1	13	—	—	—	—	3	1	—	—	—	2	—	—	—	9	40.0
Xanthomonas sp.	403	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.7
Micrococcus candidus	365	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	5.7
Micrococcus sp.	366	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9
Micrococcus sp.	371	—	—	—	3	—	3	—	3	—	—	10	5	1	—	—	5	—	—	10	3	—	—	2	—	6	3	6	—	—	2	2	—	3	—	—	45.6
Micrococcus sp.	372	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	5.7
Micrococcus candidus	381	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus candidus	382	—	—	1	—	4	—	—	—	—	—	1	—	—	1	—	—	5	—	—	—	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	20.0
Micrococcus ureae	385	4	—	—	1	3	5	5	—	—	—	—	5	2	—	—	1	4	—	—	—	4	—	—	—	—	4	—	—	—	3	3	—	—	—	1	39.9
Micrococcus varians	392	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	3	—	—	—	—	1	8.5
Micrococcus candidus	394	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9
Micrococcus flavus	395	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	2	—	—	11.4
Micrococcus varians	398	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus sp.	402	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.5
Streptococcus salivarius	388	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Corynebacterium humiferum	364	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	4	—	2	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	14.3
Corynebacterium rathaiji	374	—	—	—	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	5	11.4
Corynebacterium sp.	399	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9
Corynebacterium sp.	410	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	1	—	—	—	—	—	—	—	—	—	5	—	8.5
Arthrobacter simplex	393	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9
Bacillus sp.	368	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	3	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.5
Bacillus sp.	376	—	—	—	—	—	—	—	—	3	—	—	—	3	—	—	—	—	2	—	1	—	—	—	—	—	2	—	—	—	—	—	2	—	5	20.0
Bacillus pumilus	383	—	—	—	5	—	3	—	—	—	1	7	—	—	—	1	5	—	—	—	6	—	—	—	—	—	6	—	—	—	12	—	3	—	2	31.4
Bacillus sp.	389	—	—	3	2	1	6	—	—	—	1	—	3	1	—	3	—	5	—	—	—	4	2	—	—	2	3	—	1	—	—	2	—	6	1	—	48.5
Bacillus pumilus	391	—	—	1	1	2	1	—	2	—	—	—	—	—	1	—	—	—	1	3	—	—	3	—	—	—	3	3	—	—	—	1	1	—	1	1	42.8
Bacillus sp.	396	—	2	—	—	—	1	—	—	—	3	—	—	—	—	—	3	—	4	2	—	—	—	—	—	3	—	—	—	—	5	2	—	—	2	2	31.4
Bacillus megatherium	397	—	—	—	1	—	3	1	1	—	—	—	2	—	—	—	—	4	—	4	4	—	1	—	—	—	6	—	—	—	—	10	—	5	—	4	37.1
Bacillus megatherium	401	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.7
Bacillus megatherium	405	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	8.5
Bacillus sp.	411	—	—	5	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.7
% of sensitive strains		2.9	5.7	20.0	22.8	25.7	22.8	8.5	8.5	2.9	8.5	20.0	22.8	37.1	8.5	8.5	20.0	25.7	20.0	25.7	22.8	8.5	17,1	14.3	0.0	22,8	37.1	5.7	2.9	0.0	14.3	22.8	8.5	20.0	11.4	37.1	91.4	94.3

— — no inhibition.

Table II. Zones of growth inhibition (mm) of bacteria strains isolated in spring (April 1968)

Inhibiting strains \ Sensitive strains	No.	*Aeromonas sp.*	*Aeromonas sp.*	*Aeromonas sp.*	*Vibrio sp.*	*Vibrio sp.*	*Vibrio sp.*	*Vibrio sp.*	*Pseudomonas sp.* I gr.	*Pseudomonas sp.* I gr.	*Pseudomonas sp.* II gr.	*Pseudomonas sp.* II gr.	*Pseudomonas sp.* II gr.	*Alcaligenes sp.*	*Enterobacteriaceae*	*Micrococcus varians*	*Corynebacterium sp.*	*Bacillus badius*	*Bacillus subtilis*	*Bacillus coagulans*	*Bacillus sp.*	*Bacillus megatherium*	*Bacillus polymyxa*	*Bacillus cereus*	*Bacillus sp.*	*Bacillus cereus*	*Bacillus sp.*	*Bacillus sphaericus*	*Bacillus subtilis*	*Bacillus popilliae*	*Bacillus sp.*	*Bacillus subtilis*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus pumilus*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus macerans*	*Bacillus pumilus*	*Bacillus pumilus*	*Bacillus sp.*	% of inhibiting strains
Aeromonas sp.	505	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	2.1
Aeromonas sp.	529	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	1	—	—	—	—	1	—	—	8.5
Aeromonas sp.	535	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	1	—	—	—	—	—	—	—	—	—	4.2
Vibrio sp.	500	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	6.3
Vibrio sp.	507	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	2.1
Vibrio sp.	526	—	—	—	—	—	—	—	—	—	—	—	—	—	1	7	—	3	1	—	—	9	—	—	3	—	15	3	—	—	—	12	9	3	4	—	—	—	4	5	4	—	10	—	—	8	2	5	40.3
Vibrio sp.	530	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Pseudomonas sp. I gr.	534	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1
Pseudomonas sp. I gr.	561	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	3	2	—	—	—	—	—	1	—	—	—	—	—	—	3	3	2	—	7	3	—	—	—	—	—	2	—	—	—	—	21.2
Pseudomonas sp. II gr.	508	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	2.1
Pseudomonas sp. II gr.	544	3	—	—	—	5	—	—	—	—	—	4	—	—	—	2	1	—	2	—	—	—	—	—	3	—	2	2	1	—	—	—	—	—	—	5	—	—	3	—	—	—	—	8	—	11	29.6
Pseudomonas sp. II gr.	560	—	—	5	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	3	—	—	8	—	—	—	—	—	—	—	—	—	—	10.5
Alcaligenes sp.	492	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Enterobacteriaceae	533	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—	1	—	5	—	1	—	12.7
Micrococcus varians	539	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	—	—	—	—	2	1	—	—	—	—	—	2	—	—	—	—	—	—	10.5
Corynebacterium sp.	513	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	3	—	4	—	—	—	—	—	—	—	—	3	—	—	8.5
Bacillus badius	488	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	—	1	1	—	—	—	—	—	—	—	—	—	—	2	12.7
Bacillus subtilis	489	1	—	5	—	6	—	—	—	—	1	—	—	—	5	7	11	2	—	—	—	—	—	3	8	—	15	—	—	—	1	12	20	2	—	5	3	—	2	5	2	—	12	7	—	9	—	—	48.9
Bacillus coagulans	490	—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	3	—	1	—	—	2	—	—	—	—	—	—	—	—	—	—	10.5
Bacillus sp.	494	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus megatherium	503	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus polymyxa	504	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	4	—	—	2	—	—	2	—	1	1	—	17.0
Bacillus cereus	509	—	5	—	—	20	—	10	2	2	—	—	—	—	—	8	11	1	—	—	—	4	—	—	8	2	18	8	—	—	—	15	7	7	3	—	—	2	4	13	3	—	10	2	—	5	5	3	55.3
Bacillus sp.	512	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	—	6.3
Bacillus cereus	514	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	1	1	1	—	—	—	—	1	—	—	—	—	—	—	12.7
Bacillus sp.	516	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus sphaericus	517	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1
Bacillus subtilis	518	1	1	6	—	11	—	—	3	—	—	4	1	—	—	7	—	—	—	1	—	10	—	—	—	—	15	—	—	—	—	12	11	—	4	2	—	—	5	6	2	4	10	—	1	10	3	7	50.9
Bacillus popilliae	520	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	2.1
Bacillus sp.	521	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus subtilis	523	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	2	1	—	5	—	—	3	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	12.7
Bacillus sp.	524	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus sp.	528	—	—	15	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	1	1	—	—	—	1	—	—	—	—	1	—	3	2	—	—	1	—	21.2
Bacillus sp.	531	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	2	—	—	—	4	—	—	—	1	—	—	—	5	—	—	—	—	14.8
Bacillus sp.	532	—	—	2	—	—	9	—	—	—	—	—	—	—	—	—	—	—	2	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	5	—	1	—	19.1
Bacillus sp.	536	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	13	10	2	7	4	—	—	3	3	3	—	2	—	—	6	—	—	25.1
Bacillus sp.	538	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	9	—	—	—	—	5	—	—	—	—	7	—	—	—	3	5	—	1	—	—	—	—	5	—	—	—	—	—	—	—	8	19.1
Bacillus sp.	541	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	4.2
Bacillus sp.	545	—	5	—	—	3	2	—	—	—	—	—	—	—	—	5	—	—	1	3	—	3	—	—	5	—	2	—	—	—	—	4	8	—	—	2	3	12	7	6	2	6	—	—	—	6	4	8	44.5
Bacillus pumilus	546	1	—	6	—	—	—	—	—	—	—	1	—	—	—	8	—	—	—	—	—	4	—	—	7	—	4	3	—	—	—	1	6	1	—	—	—	10	—	—	—	6	—	—	—	6	—	3	31.8
Bacillus sp.	547	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1
Bacillus sp.	548	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1
Bacillus sp.	549	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus macerans	551	—	—	—	—	—	—	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1
Bacillus pumilus	552	—	—	—	5	—	—	—	5	—	—	2	—	5	—	—	5	2	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24.8
Bacillus pumilus	554	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	1	—	—	12.7
Bacillus sp.	559	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1
% of sensitive strains		10.5	6.3	14.8	2.1	12.7	10.6	6.3	6.3	2.1	6.3	12.7	2.1	2.1	6.3	23.4	14.8	12.7	14.8	8.5	2.1	12.7	4.2	8.5	25.5	4.2	19.1	14.8	8.5	2.1	8.5	21.2	23.4	27.6	17.0	23.4	17.0	14.8	12.7	21.2	38.2	6.3	17.0	10.2	8.5	21.2	23.4	19.1	100.0	83.0

— — no inhibition.

Table III. Zones of growth inhibition (mm) of bacteria strains isolated in summer (July 1968)

Inhibiting strains \ Sensitive strains	No.	717 *Aeromonas sp.*	723 *Aeromonas sp.*	725 *Aeromonas sp.*	729 *Aeromonas sp.*	731 *Aeromonas sp.*	733 *Aeromonas sp.*	737 *Aeromonas sp.*	739 *Aeromonas sp.*	743 *Aeromonas sp.*	759 *Aeromonas sp.*	708 *Vibrio sp.*	765 *Pseudomonas sp.* I gr.	722 *Pseudomonas sp.* II gr.	730 *Pseudomonas sp.* II gr.	745 *Pseudomonas sp.* II gr.	756 *Pseudomonas sp.* II gr.	734 *Pseudomonas sp.* IV gr.	761 *Pseudomonas sp.* IV gr.	746 *Achromobacter sp.*	705 *Alcaligenes sp.*	711 *Flavobacterium sp.*	704 *Micrococcus colpogenes*	720 *Micrococcus sp.*	728 *Micrococcus ureae*	735 *Micrococcus sp.*	749 *Micrococcus sp.*	752 *Micrococcus conglomeratus*	754 *Micrococcus sp.*	712 *Streptococcus sp.*	714 *Streptococcus sp.*	750 *Streptococcus sp.*	755 *Streptococcus sp.*	751 *Corynebacterium sp.*	702 *Bacillus macerans*	703 *Bacillus macerans*	706 *Bacillus subtilis*	707 *Bacillus cereus*	713 *Bacillus sp.*	715 *Bacillus sp.*	721 *Bacillus sp.*	740 *Bacillus sp.*	744 *Bacillus sp.*	748 *Bacillus sp.*	753 *Bacillus polymyxa*	% of inhibiting strains
Aeromonas sp.	717	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.5
Aeromonas sp.	723	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Aeromonas sp.	725	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	4.5
Aeromonas sp.	729	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Aeromonas sp.	731	—	—	—	—	—	10	1	—	—	1	5	—	2	—	—	—	—	1	—	3	3	7	9	—	—	—	—	—	—	4	—	5	2	—	—	7	—	8	4	5	—	—	12	—	40.9
Aeromonas sp.	733	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.5
Aeromonas sp.	737	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	2	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	11.3
Aeromonas sp.	739	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	6.8
Aeromonas sp.	743	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.8
Aeromonas sp.	759	—	—	—	—	1	1	—	2	—	—	—	—	1	—	—	3	1	8	6	—	—	6	8	5	—	—	—	—	—	3	5	—	4	5	—	—	—	—	—	1	—	—	—	36.3
Vibrio sp.	708	—	—	8	—	2	—	—	2	—	3	—	—	—	4	—	1	—	5	1	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—	—	—	—	—	1	1	10	—	27.3
Pseudomonas sp. I gr.	765	—	—	—	—	3	2	3	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	4	—	—	3	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	15.9
Pseudomonas sp. II gr.	722	—	—	—	1	—	—	—	—	—	9	—	—	—	—	—	—	—	1	—	—	1	—	1	—	—	—	—	—	—	—	—	12	—	—	—	—	—	2	—	—	—	2	18.1
Pseudomonas sp. II gr.	730	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.8
Pseudomonas sp. II gr.	745	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	6	8	—	—	—	—	—	—	—	—	9.1
Pseudomonas sp. II gr.	756	—	—	—	—	—	—	2	—	—	1	—	—	—	—	—	—	—	14	1	2	—	—	—	—	—	—	—	—	2	—	—	—	1	—	—	—	—	—	1	3	—	—	20.4
Pseudomonas sp. IV gr.	734	—	—	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9.1
Pseudomonas sp. IV gr.	761	—	1	1	—	6	—	3	5	—	7	—	—	10	—	—	2	—	18	—	—	12	—	2	—	—	—	—	—	2	—	—	—	8	—	—	7	14	12	—	10	3	1	—	5	45.4
Achromobacter sp.	746	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	4.5
Alcaligenes sp.	705	—	—	—	—	—	2	—	—	—	1	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	9.1
Flavobacterium sp.	711	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus colpogenes	704	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus sp.	720	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus ureae	728	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus sp.	735	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.8
Micrococcus sp.	749	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.5
Micrococcus conglomeratus	752	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	6.8
Micrococcus sp.	754	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14	—	—	—	—	—	—	—	—	3	6.8
Streptococcus sp.	712	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.5
Streptococcus sp.	714	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	3	—	—	—	—	—	—	—	—	5	—	—	7	—	—	—	—	9.1
Streptococcus sp.	750	—	—	—	—	—	5	4	—	—	—	—	—	2	—	—	2	—	2	—	—	—	—	2	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	5	—	—	—	—	15.9
Streptococcus sp.	755	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.2
Corynebacterium sp.	751	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	6	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	1	—	—	1	13.6
Bacillus macerans	702	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	4	2	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	13.6
Bacillus macerans	703	—	—	—	—	6	—	—	—	—	3	—	—	6	—	—	—	—	—	—	—	9	12	3	—	—	—	5	2	—	—	—	13	7	—	—	6	10	12	8	10	—	—	—	4	36.3
Bacillus subtilis	706	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	9	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	9.1
Bacillus cereus	707	—	—	—	—	4	7	4	—	—	—	—	—	6	—	—	—	2	—	1	—	—	—	9	9	2	—	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	22.7
Bacillus sp.	713	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	1	—	1	—	—	—	—	2	—	—	—	2	—	—	—	—	—	3	—	—	—	—	—	—	8	—	—	2	—	18.2
Bacillus sp.	715	—	—	10	—	3	—	—	—	—	4	—	—	5	1	—	1	—	10	1	—	—	—	—	—	—	—	1	6	—	—	—	—	5	—	—	—	—	—	—	7	—	1	3	1	34.0
Bacillus sp.	721	—	—	—	—	—	1	—	—	—	2	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.8
Bacillus sp.	740	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus sp.	744	—	2	—	—	—	4	—	—	—	—	8	—	—	—	—	—	3	—	—	—	—	—	7	—	—	—	1	1	4	3	—	—	—	—	—	—	—	—	—	—	—	—	—	20.4
Bacillus sp.	748	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	2.3
Bacillus polymyxa	753	—	2	1	1	—	1	3	—	—	—	3	33	—	—	—	2	1	3	1	1	—	4	6	4	1	—	—	—	—	3	4	—	3	—	—	—	8	—	6	—	2	—	—	10	—	52.3
% of sensitive strains		0.0	13.6	18.2	4.5	18.2	25.9	15.9	9.1	0.0	27.2	9.1	4.5	0.4	4.5	2.2	22.7	15.9	31.8	15.9	6.8	13.6	11.3	38.6	13.6	4.5	0.0	31.8	18.2	6.8	9.1	0.0	20.4	29.5	2.2	0.0	11.3	4.5	9.1	6.8	22.7	4.5	11.3	13.6	18.2	90.9	84.0

— — no inhibition.

Table IV. Zones of growth inhibition (mm) of bacteria strains isolated in autumn (October 1968)

Inhibiting strains \ Sensitive strains	No.	*Vibrio sp.*	*Vibrio sp.*	*Vibrio sp.*	*Pseudomonas sp.* I gr.	*Alcaligenes sp.*	*Alcaligenes sp.*	*Enterobacteriaceae*	*Enterobacteriaceae*	*Enterobacteriaceae*	*Enterobacteriaceae*	*Flavobacterium sp.*	*Flavobacterium sp.*	*Xanthomonas sp.*	*Micrococcus sp.*	*Micrococcus sp.*	*Micrococcus candidus*	*Micrococcus colpogenes*	*Micrococcus candidus*	*Micrococcus candidus*	*Micrococcus varians*	*Micrococcus ureae*	*Micrococcus candidus*	*Micrococcus conglomeratus*	*Micrococcus sp.*	*Micrococcus ureae*	*Micrococcus sp.*	*Corynebacterium sp.*	*Bacillus firmus*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus luteus*	*Bacillus sp.*	*Bacillus coagulans*	*Bacillus firmus*	*Bacillus lentus*	*Bacillus coagulans*	*Bacillus circulans*	*Bacillus sp.*	% of inhibiting strains
Vibrio sp.	821	—	1	—	1	—	—	—	—	—	—	1	—	—	—	—	—	2	—	1	—	—	1	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	22.5
Vibrio sp.	856	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.5
Vibrio sp.	858	2	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	12.5
Pseudomonas sp. I gr.	825	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Alcaligenes sp.	811	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Alcaligenes sp.	822	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Enterobacteriaceae	804	—	—	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	5.0
Enterobacteriaceae	829	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.0
Enterobacteriaceae	844	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	—	—	—	1	—	2	—	—	—	—	1	—	—	—	—	—	—	—	—	—	10.0
Enterobacteriaceae	851	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Flavobacterium sp.	823	—	—	—	—	—	—	—	—	—	—	2	1	4	—	—	—	—	—	2	—	3	—	—	1	—	—	—	—	—	—	3	5	—	—	2	—	—	—	2	—	25.0
Flavobacterium sp.	838	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.5
Xanthomonas sp.	815	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	2.5
Micrococcus sp.	805	—	—	1	—	—	—	—	—	—	—	1	—	3	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	12.5
Micrococcus sp.	813	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus candidus	826	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	2.5
Micrococcus colpogenes	830	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus candidus	832	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus candidus	833	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus varians	835	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus ureae	839	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.5
Micrococcus candidus	840	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus conglomeratus	846	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	5.0
Micrococcus sp.	853	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus ureae	854	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Micrococcus sp.	855	—	—	1	—	—	—	—	—	—	1	2	—	2	—	—	—	1	1	—	1	4	—	—	1	—	1	—	—	1	—	—	4	2	—	1	1	2	—	—	—	40.0
Corynebacterium sp.	828	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	2.5
Bacillus firmus	806	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	2	—	—	1	—	—	—	1	—	1	6	—	—	1	—	—	2	—	2	22.5
Bacillus sp.	807	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	4	—	1	1	—	—	8	—	—	—	17.5
Bacillus sp.	810	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus sp.	816	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	10	5.0
Bacillus sp.	836	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	4	2	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	12.5
Bacillus luteus	837	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus sp.	841	5	—	—	—	—	—	—	—	—	—	2	—	2	—	—	3	—	2	7	—	—	2	—	—	—	3	—	—	—	15	—	—	—	2	3	—	3	2	—	32.5
Bacillus coagulans	843	—	—	—	—	—	4	—	—	—	—	2	—	—	—	—	—	5	2	—	—	—	—	—	—	—	3	—	—	—	7	—	—	—	—	3	—	3	2	—	22.5
Bacillus firmus	845	12	—	6	—	—	—	—	—	—	—	7	—	13	8	—	—	—	—	10	8	—	—	—	—	4	—	—	—	—	3	10	6	4	—	—	3	11	3	—	37.5
Bacillus lentus	847	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	1	—	—	—	—	—	—	—	—	2	—	—	—	1	1	—	—	12.5
Bacillus coagulans	848	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Bacillus circulans	850	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	7	—	—	—	10	—	7.5
Bacillus sp.	857	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
% of sensitive strains		7.5	2.5	15.0	2.5	0.0	5.0	0.0	0.0	2.5	17.5	5.0	17.5	2.5	0.0	5.0	0.0	5.0	7.5	15.0	10.0	17.5	2.5	12.5	10.0	0.0	17.5	0.0	5.0	5.0	0.0	15.0	22.5	7.5	7.5	15.0	10.0	10.0	17.5	17.5	10.0	80.0 60.0

— — no inhibition.

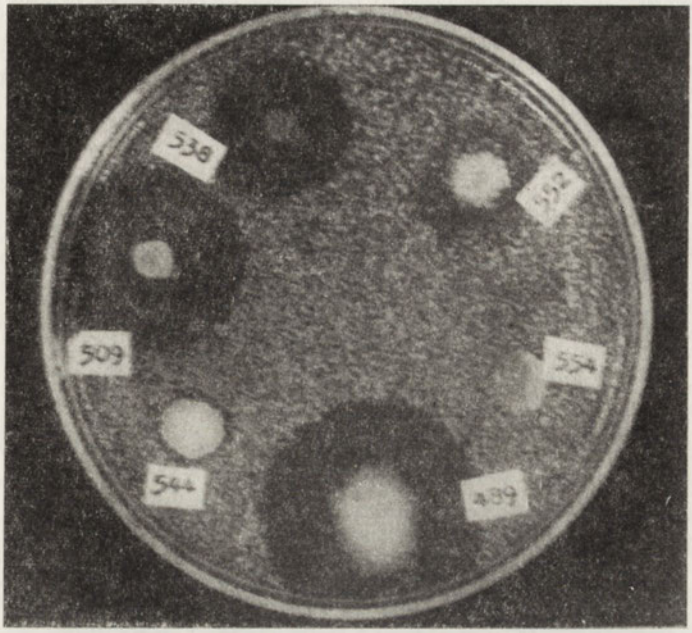

Fig. 1. Areas of growth inhibition of *Corynebacterium sp.* No. 513 under the influence of *Bacillus subtilis* No. 489, *Bacillus cereus* No. 509, *Bacillus sp.* No. 538, *Pseudomonas sp.* II group No. 544, *Bacillus pumilus* No. 552, *Bacillus pumilus* No. 554

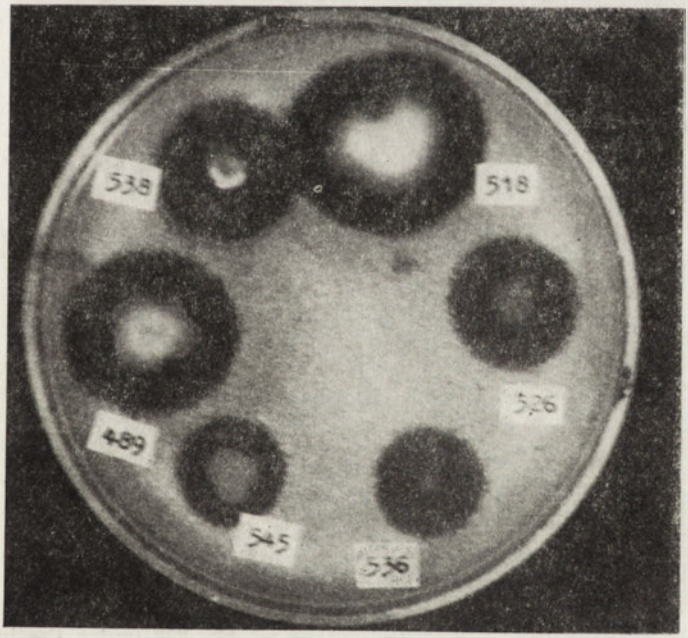

Fig. 2. Areas of growth inhibition of *Micrococcus varians* No. 539 under the influence of *Bacillus subtilis* No. 489, *Bacillus subtilis* No. 518, *Vibrio sp.* No. 526, *Bacillus sp.* No. 536, *Bacillus sp.* No. 538, *Bacillus sp.* No. 545

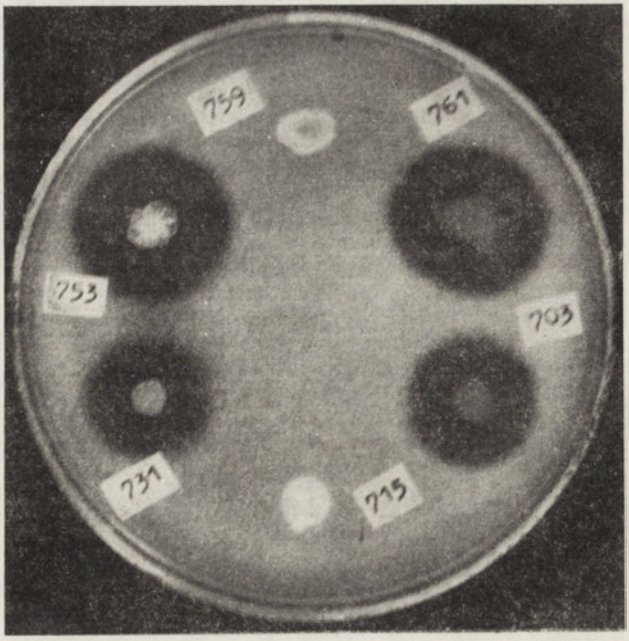

Fig. 3. Areas of growth inhibition of *Bacillus subtilis* No. 706 under the influence of *Bacillus macerans* No. 703, *Aeromonas sp.* No. 731, *Bacillus polymyxa* No. 753, *Pseudomonas sp.* IV group No. 761; *Bacillus sp.* No. 715 and *Aeromonas sp.* No. 759 — inactive

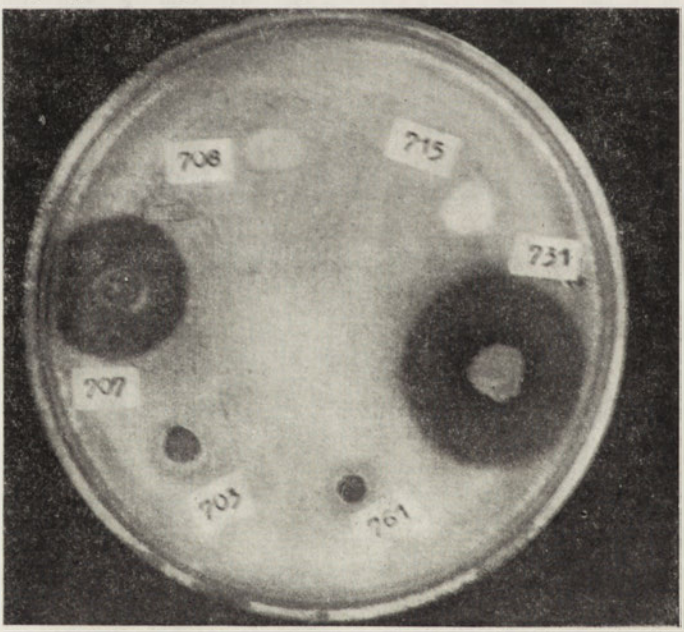

Fig. 4. Areas of growth inhibition of *Aeromonas sp.* No. 733 under the influence of *Bacillus macerans* No. 703, *Bacillus cerus* No. 707, *Aeromonas sp.* No. 731, *Pseudomonas sp.* IV group No. 761; *Vibrio sp.* No. 708 and *Bacillus sp.* No. 715 — inactive

many cases also the sensitivity of various strains of the same species of bacteria to the products of metabolism of other microorganisms was found to be different.

The antagonistic properties of bacteria are documented in Fig. 1–4; they present the areas of growth inhibition of *Corynebacterium sp.* No. 513, *Micrococcus varians* No. 539, *Bacillus subtilis* No. 706 and *Aeromonas sp.* No. 733 under the influence of a number of water bacteria isolated in spring and summer 1968.

4. DISCUSSION

In natural water reservoirs microorganisms constitute a complex biocenosis in which the several types of bacteria, fungi and other microorganisms remain in definite mutual relationships. There can be various types of interaction, but in water reservoirs they are essentially reduced to those of parasitism, metabiosis and antagonism. The latter phenomenon in bacteria has been known for almost 100 years, but it was only the practical application of the antibiotics which turned the attention of ecologists to this problem.

From the biological point of view, the most important are the results of antibiotic influences in water reservoirs. The present author has found a very frequent occurrence of this property among the microflora of Iława lakes. The antagonists were the most numerous in winter (94%), and the least numerous in autumn (60%). The broadest range of antagonistic action was generally typical for the spore-forming bacteria of the genus Bacillus, which sometimes inhibited as many as 30 to 55% of all the strains of water bacteria isolated in the given season. Similar results were also obtained by Fedjanin (1968) who investigated antagonistic action of bacteria isolated from the Danube water towards the phytopathogenic microorganisms. However, in opposition to the results of researches by Krasilnikova (1961), Nižegorodova (1968) or others, the antagonistic properties were quite widespread among the microflora of the Iława lakes. Besides the spore-forming bacteria, antagonistic strains occurred in the other systematic groups of Gram-negative rods and cocci, while their ranges of influence and its intensities measured by the extent of the areas of the growth inhibition of the test organisms were different. Relatively the least number of active strains was observed among the bacteria of the genera Aeromonas, Alcaligenes and Micrococcus. Strains of the genus Pseudomonas isolated from water of Iława lakes, in opposition to the data of Fedjanin (1968) and Chodyniecki (1968) revealed a rather small range of antagonistic action. Their activity was also relatively weak; the areas of growth inhibition of the test organisms

did not exceed 10 mm. Only one strain, No. 544, of the genus Pseudomonas revealed a more extensive range of antagonistic influence, but its intensity was low. Among the seven investigated strains of the genus Vibrio, only one proved to be inactive. Within this genus, two strains were even found to have wide ranges of antagonistic influence and high intensities of their products of metabolism. However, Chodyniecki (1968) did not observe any antagonistic influence of museum strains of bacteria belonging to the genus Vibrio upon water microorganisms. Probably the capacity to produce antibiotic substances by microorganisms is, as Gräf (1958) maintains, a property of individual strains, perhaps dependent upon their environmental conditions. The same can be said about the sensitivity of bacteria to the products of metabolism of microorganisms.

An antagonistic strain can often be in its turn inhibited by another antagonistic microorganism. This type of phenomenon was observed many times in the present research, and the most remarkable example was *Bacillus subtilis* No. 489. In laboratory conditions it inhibited the growth of a number of species of water bacteria, and it was itself inhibited by some strains of the genera Vibrio, Pseudomonas and Bacillus. Besides, some microorganisms which produce antibiotic substances in a laboratory can fail to do so in their natural water environments. E.g., Krasilnikova (1961) found a distinct influence of the type of a substrate on the antibiotic activity of several strains of bacteria. According to her, bacteria cultivated on proteins are the most active. In synthetic environments most of the strains investigated by her revealed fair growth but their antibiotic properties were weaker or lacking.

According to Chodyniecki (1968), the pH of the environment is another important factor determining the antibiotic activity of bacteria.

Some species or strains of bacteria which inhibited the growth of other microorganisms, produced autoantagonistic substances, inhibiting their own growth and multiplication (Wood 1965). Such bacteria were observed a few times during the present investigations, but their activity was rather small. The two exceptions were *Pseudomonas sp.* group IV No. 761 and *Bacillus circulans* No. 850 which inhibited remarkably the development of the cells of their sibling strains. It cannot be excluded that in specific environmental conditions antibiotic substances can change into stimulating ones; this is maintained by Wallhäusser (1951a). Anyway, some microorganisms do secrete stimulating products of their metabolism into their environments, as e.g. *Micrococcus ureae* No. 385, *Corynebacterium rathaiji* No. 374, and some others isolated from water of the Iława lakes.

Thus the effects of the antibiotic influences in the investigated reser-

voirs, very interesting for an ecologist, are determined jointly by many factors, among which an important role is played by the water environment conceived according with the following suggestion by Gessner (1955): "Water will be no longer considered as the source of food supply only, but as the space of active interaction as well"; its influence will be eventually explained by further investigations. Probably no far-reaching effects of antibiotics should be expected in natural reservoirs. More likely are local influences, apparent for example in the detritus, on algae or on other fixed particles of organic matter in water, where the most diverse species of bacteria survive in closed vicinity.

Acknowledgements

We wish to express our gratitude to Prof. Halina Karnicka, the Head of the Department of Technical Microbiology, Higher School of Agriculture in Olsztyn, for her important suggestions offered when this work was being prepared for print.

5. SUMMARY

The investigations were carried out separately for each of the four groups of strains of bacteria isolated in January, April, July and October 1968. In total, 166 strains of bacteria were investigated belonging to the genera Aeromonas, Vibrio, Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium, Xanthomonas, Micrococcus, Streptococcus, Corynebacterium, Arthrobacter and Bacillus, and to the family Enterobacteriaceae. The greatest proportion of antagonistic strains was observed in winter (94%), and the smallest one in autumn (60%). The widest range of antagonistic action was in general characteristic of the spore-forming bacteria of the genus Bacillus, which inhibited sometimes as much as 30 to 55% of all the water bacteria isolated in the given sampling season. The least number of antagonistic strains was observed among the genera Aeromonas, Alcaligenes and Micrococcus. Some strains produced autoantagonistic substances, but with the few exceptions (*Pseudomonas sp.* group IV No. 761 and *Bacillus circulans* No. 850) the intensity of their influence was small. Somewhat more than 6% of the investigated strains secreted to the environment products of metabolism stimulating the growth of some water bacteria.

6. STRESZCZENIE

Badania prowadzono oddzielnie na czterech grupach szczepów bakterii, izolowanych w styczniu, kwietniu, lipcu i październiku 1968 r. Ogółem przebadano 166 szczepów bakterii z rodzajów Aeromonas, Vibrio, Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium, Xanthomonas, Micrococcus, Streptococcus, Corynebacterium, Arthrobacter i Bacillus oraz z rodziny Enterobacteriaceae. Stwierdzono największe występowanie szczepów antagonistycznych zimą — 94%, najmniej zaś w jesieni — 60%. Najszerszym zasięgiem działania antagonistycznego charakteryzowały się z reguły bakterie przetrwalnikujące z rodzaju Bacillus, które hamowały do 30—55% ogółu wyodrębnionych w danym okresie bakterii wodnych. Najmniej antagonistycznych szczepów występowało wśród bakterii z rodzaju Aeromonas, Alcaligenes i Micrococcus. Niektóre szczepy bakterii wytwarzały substancje o własnościach autoantagonistycznych, jednakże poza nielicznymi wyjątkami (*Pseudomonas sp.* IV gr. nr 761 i *Bacillus circulans* nr 850) aktywność ich była niewielka. Nieco ponad 6% badanych szczepów uwalniało do środowiska metabolity o własnościach stymulujących rozwój niektórych bakterii wodnych.

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MACROFAUNA COMMUNITIES OF THE LITTORAL OF MIKOŁAJSKIE LAKE

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ABSTRACT

The paper discusses 4 types of macrofauna communities connected with various substrata accessible for colonization in shallow lake littoral. The tips of last year's reeds, broken under the water surface, are a new not described before habitat. They are colonized by a rich and almost monospecific community of Chironomidae larvae. Only three Chironomidae species are common for all the four communities.

CONTENTS

1. INTRODUCTION

In literature dealing with fauna of lake littoral the majority of papers discuss only one of its components — benthos (Rzóska 1936, Romaniszyn 1954, Anderson and Hooper 1956, Nocentini 1963), periphyton (Duplakov 1933, Meschkat 1934, Meuche 1939, Pieczyńska 1964), littoral macrofauna (Szczepańska 1958, Giziński 1958, Gurzęda 1959, Stańczykowska 1960, Wolnomiejski and Dunajska 1966), or fauna colonizing stones (Dusoge 1966). Several papers refer only to one systematic group, e.g. to the dominating in littoral Chironomidae larvae (Romaniszyn 1950, Kaftannikova 1967).

In littoral there are several different substrata such as, e.g., bottom sediments, stones, submerged parts of macrophytes and so on. Specific and typical communities are formed for each of the substrata. Interdependences between these communities have been observed on the simple example, i.e. in conditions where only two kinds of substrata accessible for colonization have been present — bottom sediments and monospecific community of macrophytes.

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2. TERRAIN DESCRIPTION AND METHODS

Investigations have been carried out on a chosen section of littoral in the NW part of Mikołajskie Lake (Masurian Lakeland). It is an eutrophic lake, of a surface area 470 ha, maximal depth 27.8 m, and the length of shore line 14,400 m. Limnological characteristic of Mikołajskie Lake is given by Szczepański (1958) and Paschalski (1960).

The littoral in the emergent vegetation zone of the investigated part was overgrown by a reed-belt (*Phragmites communis* Trin.) 30 m wide; the bottom was sandy and slimy, and the water depth 0.60 m. According to the classification of Bernatowicz and Zachwieja (1966) this type of littoral is a small lake phytolittoral.

Samples of benthos and plant macrofauna were taken every 5 days from 5 July till 9 September 1967. Benthos samples were taken using the tubular sampler of Lastočkin and Ulomskij (Ulomskij 1952) type, of a surface 10 cm². Each time a series of 10 samples was taken. The samples were rinsed on a sieve of a mesh size 0.2×0.2 mm and macroscopically sorted out.

Samples of plant fauna were taken by cutting the reed just near the botom with the help of an apparatus (Kajak unpublished), which consisted of a plexiglass tube with a movable blade at the end, which after cutting the reed tightly closed the opening, thus avoiding losses of fauna when pulling the reed out of water. The reed surface was measured after separating the periphyton. Then the samples were macroscopically sorted out.

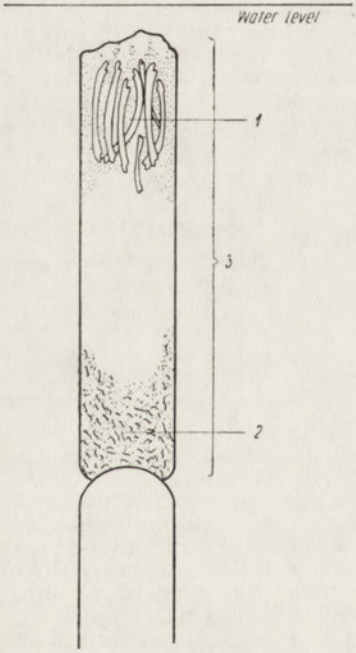

Fig. 1. The stalk in the oblong section. 1 — colonizing fauna, 2 — detritus, 3 — the part filled with water

The total of 548 samples was taken, and in that 248 benthos samples and 300 samples of fauna colonizing the reed. The fauna on reeds has been divided into three distinctly differing communities:

1. Community colonizing the outside surface of young reed (this year's),
2. Community colonizing the outside surface of old reed (last year's). Samples were taken from 10 August,
3. Community colonizing the interior of reed stalks. Samples were taken from 10 August.

The name "reed stalks" is used for the tips of old reed broken under the water

surface. Up to the first node they are filled with water and abundant with fauna, mainly Chironomidae larvae.

The cross-sectional areas of a broken reed tips has been assumed as a surface of substratum for the fauna of reed stalks. This is the area through which the organisms have a contact with the outside environment, and obtain the oxygen and food (analogicaly the number of e.g. benthic animals is related to 1 m² of bottom). The way of distribution of animals within the reed stalk (Fig. 1) is also in favour of this method of calculation.

Numbers of fauna were calculated per a surface unit of a colonized substratum (i.e. benthos — per 1 m² of bottom sediments, fauna colonizing the outside surface of reed — per 1 m² of submerged part of reed shoot, fauna colonizing the interior of reed stalks — per 1 m² of the cross-sectional area of reed) and per the surface unit of littoral. Number of fauna colonizing the reed per a unit of surface of the littoral was calculated considering the density of fauna per surface unit of the substratum and the area of this substratum per 1 m² of littoral.

On the basis of measurements of reed and their numbers per 1 m², the area of substrata accessible for colonization on 1 m² of littoral at a depth 0.60 m was estimated on the average as:

young reed	— 0.72 m²,
old reed	— 0.18 m²,
reed stalks	— 0.0005 m².

For bottom sediments the value 1.00 m² has been accepted, excluding the surface covered by reed stalks growing there, which was ca 0.003 m².

3. RESULTS

QUANTITATIVE AND QUALITATIVE COMPARISON OF THE ANIMAL COMMUNITIES

In all communities of the littoral fauna the most numerous are the Chironomidae larvae, which are 50% (in bottom sediments) to 95% (in reed stalks) of the total number of organisms. Oligochaeta in benthos are placed second according to number, and in fauna on reed — Trichoptera (Table I).

The communities of fauna colonizing various substrata differed also in their composition. Bryozoa, Spongiae, Hydrozoa and Gyrynidae occurred only in communities on reed, whereas broods of Chironomidae and cocoons of leeches were found only on young reed.

Oligochaeta occurred on young reed and in bottom sediments, however, on both substrata they were represented by various forms — on reed Naididae, mainly *Stylaria lacustris* L. and *Nais sp.* were found, in bottom sediments — Tubificidae: *Ilyodrillus hammoniensis* Mich. and *Tubifex barbatus* Grube. The total participation of Oligochaeta in benthic fauna was high — 30% of the total numbers of fauna, but in the community of fauna on reed — only 4%. Oligochaeta did not occur on old reed and in reed stalks.

Also observed were differentiations in the occurrence of Mollusca on various substrata, but these were not so distinct as of Oligochaeta. In bottom sediments Mollusca were first of all represented by *Dreisena polymorpha* Pall., and on reed by *Theodoxus fluviatilis* L. *D. polymorpha*

Table I. The participation of various animal groups in communities of littoral macrofauna (percentage of the total number of fauna of the given community)

Group	Community			
	Benthos	Fauna of young reed	Fauna of old reed	Fauna of reed stalks
Chironomidae	50	62	81	95
Oligochaeta	30	4	—	—
Mollusca	5	4	4	+
Hirudinea	4	2	1	+
Donacia sp.	3	—	—	—
Nematoda	1	+	+	+
Asellus aquaticus L.	1	+	+	—
Trichoptera	+	10	9	+
Heleidae	+	1	+	—
Bryozoa	—	+	+	—
Gyrynidae (larvae)	—	+	+	1
Elmidae	+	+	+	—
Ephemeroptera	+	+	+	—
Odonata	+	+	—	—
Turbellaria	2	+	+	—
Hydrozoa	—	+	—	—
Spongiae	—	+	+	—
Others	2	8	2	2

+ — less than 1%
— — not found

was also found on young reed, but in smaller quantities than in benthos, and in majority of instances these were young individuals.

The only representant of a typical benthic fauna, not found in any other community, were larvae and pupae of *Donacia sp.*

The fauna colonizing old reed differed from the fauna of young reed in composition, and especially by qualitative relations between particular components — e.g. Oligochaeta, Odonata, *Dreisena polymorpha* did not occur on old reed, and Hirudinea, Trichoptera and Turbellaria occurred in smaller numbers on old reed than on the young.

The quantitative participation of respective communities in the total number of littoral fauna is illustrated by referring their number to the surface unit of littoral. Such comparison shows that benthos is the most numerous community:

benthos	9000 ind./m²,
fauna of young reed	1000 ind./m²,
fauna of old reed	600 ind./m²,
fauna of reed stalks	330 ind./m².

With reference to 1 m² of colonized substratum the quantitative relations are unlike the previous ones, the most numerous community is the fauna of reed stalks:

benthos	9000 ind.,
fauna of young reed	1400 ind.,
fauna of old reed	3300 ind.,
fauna of reed stalks	330,000 ind.

The time changes in number of a benthic community and of a community of fauna of young reed are similar — in both these communities in the middle of July and at the beginning of September the numbers of fauna decreased (Fig. 2). An increase in number was observed in the communities of fauna of old reed and of reed stalks near the end of August.

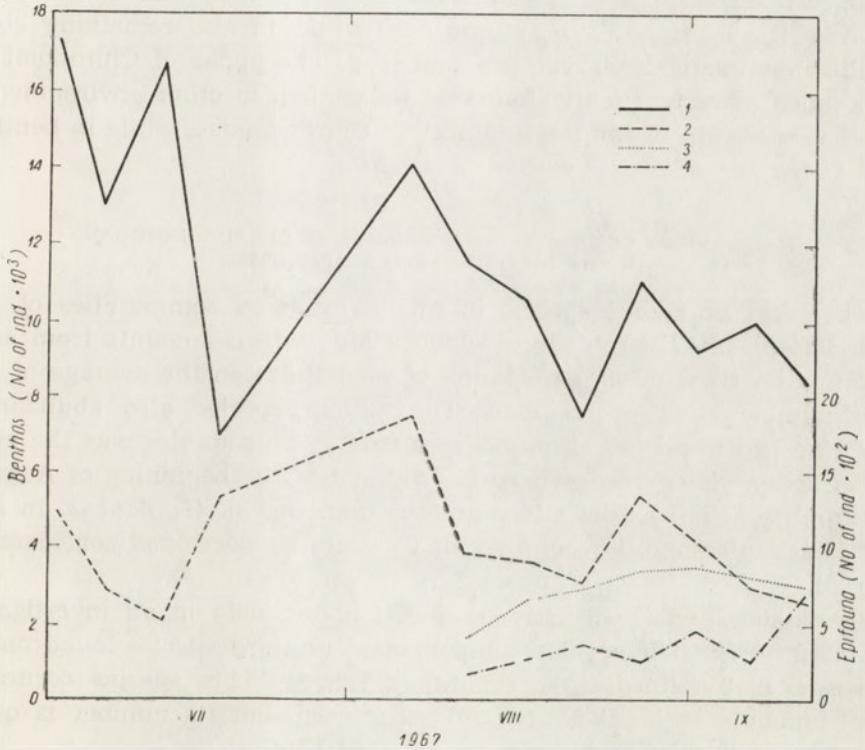

Fig. 2. Number of individuals per 1 m² of littoral surface in various communities of littoral fauna. 1 — benthos, 2 — fauna colonizing young reed, 3 — fauna colonizing old reed, 4 — fauna colonizing the interior of reed stalks

COMMUNITY COLONIZING THE INTERIOR OF REED STALKS

Fauna colonizing a reed stalk concentrated at the top of it and is 3–4 cm deep. Above the node there is a detritus layer, 1–3 cm thick (Fig. 1), in which Diatomeae, Cyanophyta, Chlorophyta, remains of plants

tissue, pine pollen, sand grains, Cladocera crusts and exuviae, dead individuals and faeces of Chironomidae were distinguished.

Larvae of Chironomidae were 95% of the total number of individuals of the fauna of reed stalks, and apart from them the following occurred: Mollusca, Hirudinea, Nematoda, Trichoptera and Gyrinidae (larvae).

It should be assumed that a reed stalk is only the site, and not the source of food for Chironomidae colonizing it, as the dominant species there *Glyptotendipes glaucus* Mg. is a typical filtrator (K a l u g i n a 1960, 1963). Also the presence of the cones of salivary secretion, the position of individuals inside the stalk (Fig. 1), and no traces of biting on the stalks prove the filtration character of feeding of Chironomidae in stalks.

More than half of the Chironomidae larvae occurring in stalk are the larvae of the IV stage (7 to 17 mm long) while in the remaining communities dominate the larvae 3–6 mm long. The pupae of Chironomidae were much more frequently found in stalks than in other environments, and they were 8% of the total number of Chironomidae, while in benthos only 2.5%.

COMPARISON OF THE SPECIES COMPOSITION OF CHIRONOMIDAE IN THE DISTINGUISHED COMMUNITIES

The most numerous species in the investigated communities of the littoral fauna is *Glyptotendipes glaucus* Mg., which amounts from 40% (in fauna on reed) to 90% (in fauna of reed stalk) on the average of the total number of Chironomidae. The second species also abundantly occurring in littoral is *Cricotopus silvestris* F. This species was the most numerous on young reed, where in July and at the beginning of August, the number of it was definitely greater than that of *G. glaucus*. In August and September the numbers of *C. silvestris* decreased considerably (Fig. 3).

G. glaucus and *C. silvestris* occurred numerously in all investigated communities as their regular components. The third species found on all substrata is *Tendipedini macrophtalma* Tshern. This species occurs in small amounts in benthos and on young reed, but its number is quite considerable in reed stalks and on old reed (Fig. 3).

The following species should be added to the list of Chironomidae species common for benthos and fauna colonizing the outside surface of reeds: *Tanytarsus lauterborni* Kieff., *Cryptochironomus pararostratus* Lenz, *Endochironomus tendens* F., *Pseudochironomus prasinatus* Mall., *Pentapedilum exsectum* Kieff. and *Corynoneura sp.*

Some of these species, apart from the fact of their occurrence on several substrata, definitely prefer one of them — e.g. *C. pararostratus* is most numerous on young reed, and *P. prasinatus* in bottom sediments.

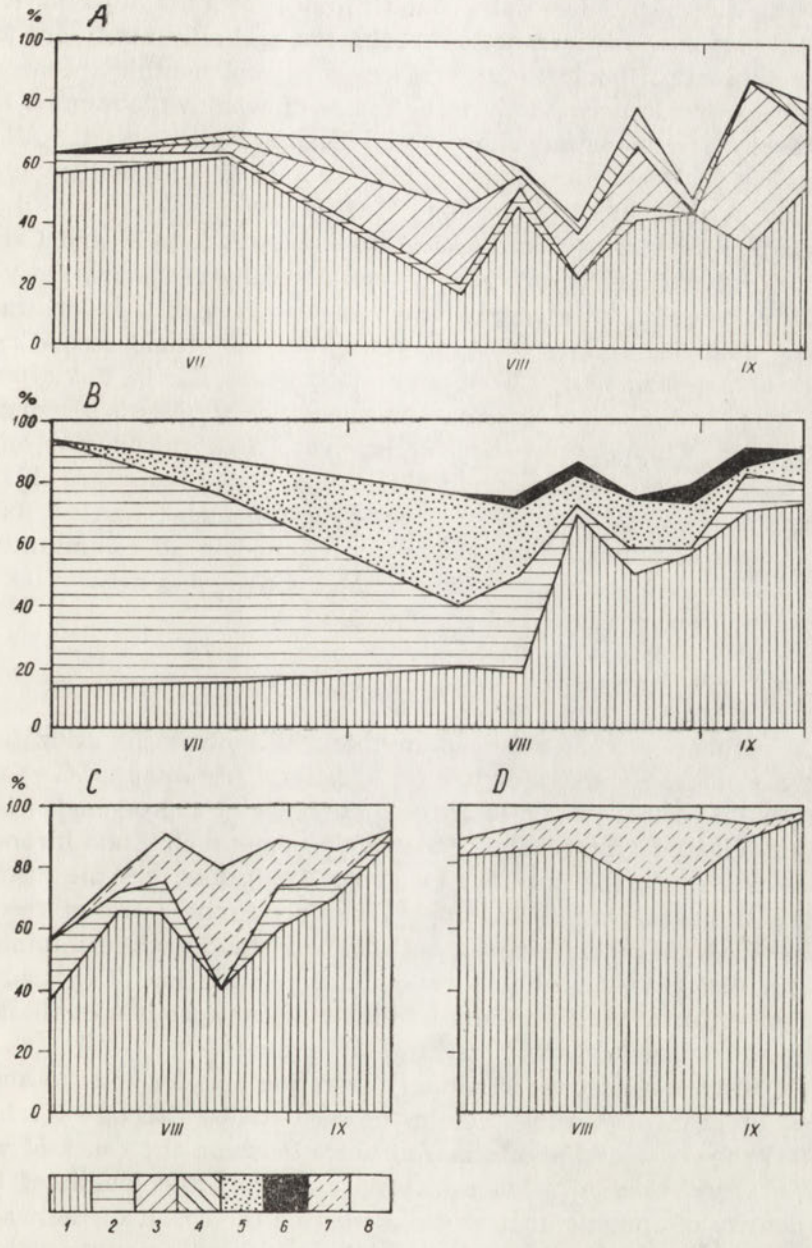

Fig. 3. Percentage participation of dominant species in communities of Chironomidae larvae colonizing various substrata in shallow lake littoral. 1 — *Glyptotendipes glaucus* Mg., 2 — *Cricotopus silvestris* F., 3 — *Pseudochironomus prasinatus* Mall., 4 — *Cryptochironomus defectus* Kieff., 5 — *Tanytarsus lauterboni* Kieff., 6 — *Endochironomus tendens* F., 7 — *Tendipedini macrophtalma* Tshern., 8 — other species. A — bottom sediments, B — young reed, C — old reed, D — the interiors of reed stalks

Tanytarsus mancus v. der Vulp. and *Cryptochironomus defectus* Kieff., which are very numerous in bottom sediments, and only rarely are found in other substrata, should be recognized as typical benthic species. The following species occurring only in bottom sediments: *Ablabesmyia lentiginosa* Freis., *Microtendipes chloris* Mg., *Tendipes plumosus* L., *Allochironomus* Kieff., Procladius Skuse appear sporadically in shallow littoral but are not typical for this lake zone.

Eukiefferiella bicolor Zett., *Endochironomus tendens* F. and *Cricotopus algarum* Kieff. are recognized as typical epiphytic species. On young reed the number of Chironomidae species was greater than on the old reed. The following species occur on young but not on old reed: *Cryptochironomus defectus* Kieff., *Tanytarsus lobatifrons* Kieff., *Eukiefferiella bicolor* Zett., *Polypedilum breviantennatum* Tshern., *Microspectra praecox* Mg. and Clinotanypus Kieff. However, *Stenochironomus gibbus* Fabr. and *Polypedilum convictum* Walk. were only found on old reed. The fauna of reed stalks is very poor in Chironomidae species, its majority consists of animals numerously represented in all communities of littoral fauna — *Glyptotendipes glaucus* Mg., *Cricotopus silvestris* F. and *Tendipedini macrophtalma* Tshern. (Fig. 3).

4. DISCUSSION

In literature there has not been anything till now on the utilization of the interior of reed stalks as a habitat of littoral macrofauna. Černovskij (1949) describes instances of the occurrence of Chironomidae larvae from the genera Glyptotendipes, Stenochironomus and Endochironomus in the dead stems of Scirpus. Kalugina (1960) describes the instances of mining the dead parts of macrophytes (among others also of reed) by *Glyptotendipes glaucus*. However, both these instances concern mining in the plant tissues, while the process of mass colonization of the substratum (the interior of reed stalks) described in this paper is a qualitatively different phenomenon than colonization by mining.

The Chironomidae cones of salivary secretion are close one to another and sometimes even join together in the reed stalks, although Kalugina (1959) showed that the minimal distance between the cones of young larvae of *Glyptotendipes glaucus* is approximately the length of larva. Great density of animals in reed stalks may be the result of the way the larvae get inside: in the second half of August great amounts of broods of *G. glaucus* were observed on reed just under the water surface, then the number of early larval stages of this species increased in the fauna on reed, as result of colonizing by young larvae the substratum to which the broods were attached. Only slightly bigger larvae (3–4 mm long)

actively or passively get into water. Part of the larvae, together with detritus gets inside the reed stalks, where they build their cones of salivary secretion. Only growing larvae fill the entire bore of the stalk.

Great number of Chironomidae and great participation of larvae of the IV stage and pupae in reed stalks may prove the inaccessibility of this habitat for predators, e.g. fish. And so, reed stalks may play in the lake the part of specific "reserve" of larvae of Chironomidae for new generations, despite e.g. greater pressure of predators in the lake.

The investigations showed a great differentiation of the density of littoral macrofauna on various substrata, and thus a different extent of utilization of substratum accessible for colonization. The surface is utilized the best in reed stalks, and the least — on young reed. However, with the reference to the entire littoral the numbers of a given community are not determined by the colonization density, but by the area of substrate accessible for colonization. In the investigated section of littoral the largest area accessible for the colonization is that of bottom sediments, and therefore the benthos number is the highest (9000 ind./m² of littoral). The fauna of reed stalks, despite its great density (330,000 ind./m² of substrate area) is hardly 3% of the total number of littoral macrofauna. This is a result of a small number of reed stalks per 1 m² of littoral (about 15).

The differences between communities of fauna of young and old reed, showed during the investigations, may be due to the different degree of periphyton development on both these substrata. Ruttner (1963) points to the fact that the longer the substrata are submerged in water the richer is the periphyton growth there, which allows for its wider utilization as a habitat and food base for epifauna. Kowalczewski (1965) found that periphyton biomass on old reed is about twice higher than on young reed, and contains more organic matter. Rich development of filamentous algae (*Cladophora sp.*) on old reed allows for wider utilization of this substratum by epifauna, and especially by Chironomidae larvae.

5. SUMMARY

During the investigations carried from 5 July till 9 September 1967 in shallow littoral of Mikołajskie Lake four types of macrofauna communities were distinguished: 1. benthos, 2. community colonizing the outside surface of young reed, 3. community colonizing the outside surface of old reed, 4. community colonizing the interiors of reed stalks. The name "reed stalks" is used for the tips of old (last year's) reed broken under the water surface and colonized by numerous fauna (Fig. 1). In the investigated period the larvae of III and IV stage and the pupae of Chironomidae were found in reed stalks. The participation of larvae of IV stage and pupae is greater in the fauna of reed stalks than in other communities.

The number of macrofauna colonizing the reed stalks in relation to the surface of colonized substratum is very high — 330,000 ind./m². However, because of the small number of reed stalks per 1 m² of littoral (about 15) the significance of this fauna is

not so great. With reference to a surface unit of littoral benthos is the richest community — 9000 ind./m².

The main component of all investigated communities are Chironomidae larvae, which are from 50% of the total number of fauna in benthos to 95% in reed stalks. As far as numbers are concerned, Oligochaeta are on the second place (in benthos), and also Trichoptera (in fauna on plants). Mollusca and Hirudinea were also numerous.

There are only three Chironomidae species common for all four macrofauna communities, and these are: *Glyptotendipes glaucus* Mg., *Cricotopus silvestris* F. and *Tendipedini macrophtalma* Tshern.

6. STRESZCZENIE

W badaniach prowadzonych w okresie 5.VII–9.IX. 1967 w płytkim litoralu jeziora Mikołajskiego wyróżniono 4 typy zespołów makrofauny: 1. bentos, 2. zespół zasiedlający zewnętrzną powierzchnię trzcin młodych (tegorocznych), 3. zespół zasiedlający zewnętrzną powierzchnię trzcin starych (zeszłorocznych), 4. zespół zasiedlający wnętrza źdźbeł trzcinowych. Nazwy „źdźbła trzcinowe" użyto w stosunku do górnych końców starych trzcin ułamanych pod powierzchnią wody, zasiedlonych przez faunę (Fig. 1). W badanym okresie w źdźbłach trzcinowych występowały larwy III i IV stadium oraz poczwarki Chironomidae. Udział larw IV stadium oraz poczwarek w faunie źdźbeł trzcinowych jest wyższy niż w pozostałych zespołach.

Liczebność makrofauny zasiedlającej źdźbła trzcinowe w odniesieniu do powierzchni zasiedlanego podłoża jest bardzo wysoka i wynosi 330 000 osobników na 1 m². Jednak ze względu na małą ilość źdźbeł trzcinowych przypadających na 1 m² litoralu (ca 15 sztuk) rola zasiedlającej je fauny w skali całego litoralu jest niewielka. W odniesieniu do jednostki powierzchni litoralu najbogatszym zespołem jest bentos (9000 osobników na 1 m²).

Głównym komponentem wszystkich badanych zespołów są larwy Chironomidae stanowiące od 50% całkowitej liczebności fauny w bentosie do 95% w źdźbłach trzcinowych. Na drugim miejscu pod względem liczebności znajdują się Oligochaeta (w bentosie) i Trichoptera (w faunie naroślinnej). Licznie występowały Mollusca i Hirudinea.

Tylko trzy gatunki Chironomidae są wspólne dla wszystkich czterech zespołów makrofauny: *Glyptotendipes glaucus* Mg., *Cricotopus silvestris* F. i *Tendipedini macrophtalma* Tshern.

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J. HEMPEL-ZAWITKOWSKA

RESISTANCE OF EGGS OF *ARTEMIA SALINA* L. TO LOW TEMPERATURES AS RELATED TO SEVERAL CHOSEN ENVIRONMENTAL FACTORS

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ABSTRACT

Eggs of *Artemia salina*, both dry and hydrated revealed a considerable resistance to temperatures —10, —25, —79, and —196°C. The degree of this resistance is dependent on dehydration of protoplasm, caused by desiccation or osmotic dehydration, but almost independent from the prior presence of eggs at anaerobic conditions.

CONTENTS

1. INTRODUCTION

The problem of resistance of animals to low temperatures was rather broadly studied, especially in tardigrades, nematodes and in resting stages of higher invertebrates, i.e., eggs or pupae. Recent studies dealt with processes following in cells at very low temperatures as well as factors preventing harmful effects of freezing. The review of this problem is given by T h o m a s (1963).

The present paper concerns the resistance of *Artemia salina* eggs to low temperatures (—10, —25, —79, and —196°C) in connection with the following factors: 1. anaerobic conditions, 2. desiccation, 3. water salinity. These factors affect the normal developmental cycle of *Artemia salina*, which occurs in brackish, astatic water bodies.

2. MATERIAL AND METHODS

The material for studies were commercial eggs of *Artemia salina*, imported from California. According to D u t r i e u (1960) they were all at an advanced stage of embryonic development — prenauplius stage. Samples of several hundreds eggs, held in glass vials, were frozen in electric freezers to temperature of —10 and —25°C, or placed in thermoses filled with crushed solid carbon dioxide at temperature of —79°C, or liquid nitrogen at temperature of —196°C.

The eggs were hatched in glass containers filled with sea water with addition of 15‰ NaCl, to which a portion of 100 eggs transferred from each sample (technique acc. to Dutrieu and Chrestia-Blanchine 1966). The eggs were allowed to hatch at temperature of +25°C, and the percentage of hatched larvae was ascertained. On the average, the eggs were hatching from 2 to 7 days after placing them in water.

Three types of experiments were performed:

I. The effect of submerging the eggs in oxygen-free water on their survival at low temperatures. The eggs were placed in sea water with addition of 15‰ NaCl, deprived of oxygen by prior boiling and aerating with nitrogen, and kept later in desiccators with nitrogen. The exposures to nitrogen lasted 3, 7, 14, 20, and 32 days. After each of these periods samples of eggs were exposed to low temperatures: —10°, —79°, and —196°C for the following periods: 1, 3, 7, 14, 20, and 30 days at —10°; 1, 2, 5, 24 hr at —79°; and 0.5, 1, 2, 3, 6 hr at —196°C.

II. The effect of desiccation of eggs on their survival at low temperatures. The eggs were covered with sea water for 3 hr in order to soak in it, then they were stretched on filter paper and placed in desiccators with a constant relative humidity of air (R. H.) amounting to 9, 33, and 76%. These humidities were obtained using the following chemicals: P_2O_5 — 9% R. H. (Charles and Hadgman 1953), saturated solution $MgCl_2$ — 33% R. H., saturated solution NaCl — 76% R. H. (Winston and Bates 1960). After 17 days of exposure the percentage of water content in eggs was ascertained by weight, by drying a portion of eggs at 99°C. The following results were obtained: the eggs dried at 76% R. H., 33% R. H., 9% R. H., and the commercial eggs contained: 16.6%, 6.6%, 2.9%, and 9.2% of water, respectively. The remaining eggs were divided into subsamples and kept at —79° and —196°, using the following time of exposure: 1, 5 and 24 hr for —79°C, and 1, 2, and 6 hr for —196°C. Control exposures consisted of dry, commercial eggs.

III. The effect of water salinity on survival of eggs at low temperatures. Commercial eggs were covered with water of the following salt concentrations: 1. distilled water, 2. sea water +15‰ NaCl of a total salinity of 48‰, and 3. sea water +30‰ NaCl of a total salinity of 63‰. The exposures to low temperatures lasted as follows: at temperature —10°C: 1 and 2 days, at temperature —25°C: 1, 2, 5, and 10 days, at temperature —79°C: 1, 5, 24, and 48 hr, at temperature —196°C: 1, 2 and 6 hr.

3. RESULTS

I. The effect of submerging the eggs in water deprived of oxygen on their survival at low temperatures.

Since both the effects of temperature —10°C, often found under natural conditions, and the results of experiments differ considerably from those of —79°C and —196°C, which can be considered as very low temperatures, they will be discussed separately.

Temperature —10°C. The presence of control eggs (not exposed to low temperatures) in oxygen-free atmosphere decreases their hatchability (Fig. 1). The presence of eggs in oxygen-free atmosphere followed by a direct exposure to a temperature of —10°C increases hatchability of eggs at a longer period of nitrogen atmosphere action. In series kept in nitrogen atmosphere for the same number of days, the shorter was the exposure to lower temperature, the higher was the hatchability of eggs. When the eggs were kept at —10°C for the same number of days, the better hatches were observed in those eggs which were previously kept longer at the nitrogen atmosphere. The best hatchability was of these eggs which were kept at the nitrogen atmosphere for 20 days.

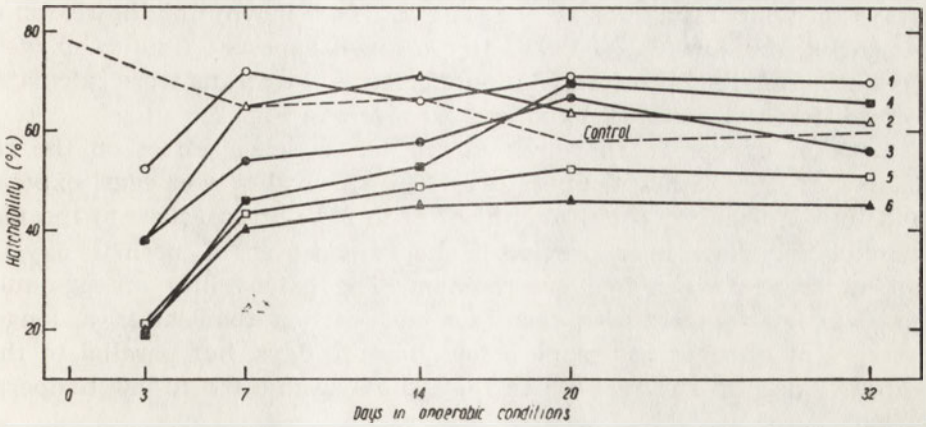

Fig. 1. Hatchability of *Artemia salina* eggs after exposures to anaerobic conditions and temperature of —10°C. 1 — 1 day at temp. of —10°C, 2 — 3 days at temp. of —10°C, 3 — 7 days at temp. of —10°C, 4 — 14 days at temp. of —10°, 5 — 20 days at temp. of —10°C, 6 — 30 days at temp. of —10°C

Temperatures −79° and −196°C. With the exposures applied, there is no clear dependence between the time of exposure and the percentage of eggs that hatched. It appears that with such short periods applied, the length of exposure time does not affect survival of eggs. What is important is the speed of freezing and defrosting. High differences that were found in the percentage of hatches between individual samples resulted most probably from the fact that despite of trying to freeze and defrost the eggs as quickly as possible, with manual transferr-

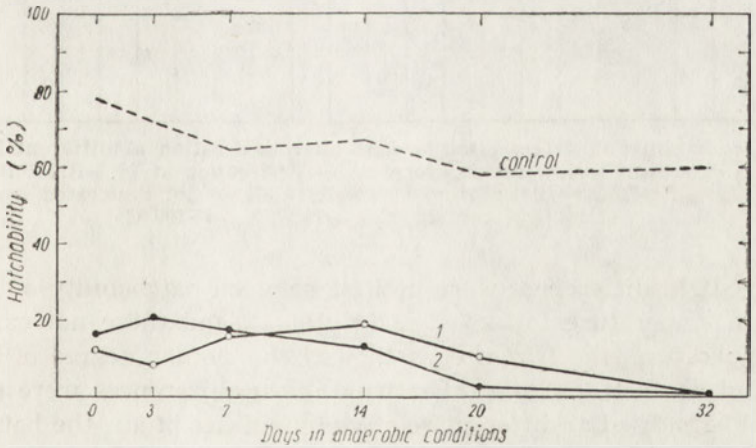

Fig. 2. Hatchability of *Artemia salina* eggs after exposures to anaerobic conditions and temperatures of —79°C (1) and —196°C (2)

ing of eggs, it was impossible to ensure equally rapid decreases or increases of temperature for all the samples. That is why for the reason of comparing the survival of eggs after a given exposure time to oxygen--free atmosphere and for a given temperature, the means were calculated from different periods of exposure, as given in Fig. 2.

Lack of oxygen in the environment has no clear effect on the resistance of eggs to low temperatures. The survival of wet eggs, exposed both to —79° and to —196°C is low, up to 20%, irrespective to the fact whether they have been previously kept in water of normal oxygen content or at oxygen-free environment. The hatchability of eggs subjected to low temperatures decreases not earlier than after a longer presence at nitrogen atmosphere for about 20 days, but parallel to this there is a drop in survival of control eggs, not subjected to low temperatures.

II. The effect of desiccation of eggs on their survival at low temperatures.

Similarly to the previous experiment, no dependence was found between the exposure time at given temperatures and the percentage of hatches, therefore the means were calculated for all the hatches at a given temperature (Fig. 3).

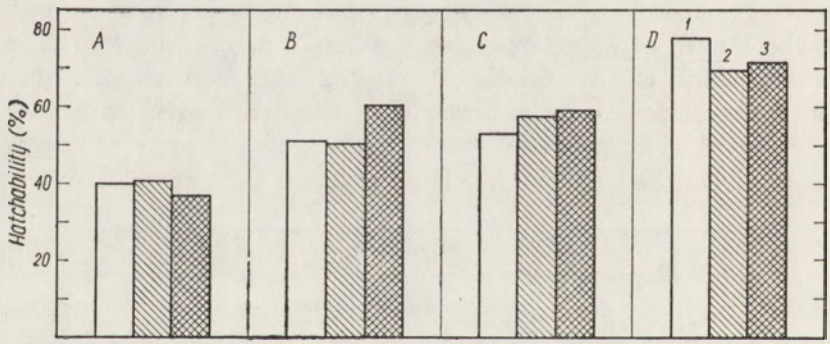

Fig. 3. Hatchability of *Artemia salina* eggs after desiccation at different humidities of air and exposures to low temperatures. A — desiccation at 76% R. H., B — desiccation at 33% R. H., C — desiccation at 9% R. H., D — not desiccated (commercial) eggs. 1 — control, 2 — —79°C, 3 — —196°C

Only slight differences were noticed between hatchability of eggs exposed to temperatures of —79° and —196°C, and those not exposed to low temperature; the frozen eggs showed the similar degree of hatching as that of controls. On the other hand, clear differences were observed between eggs dried at different relative humidities of air, the hatchability being higher the more desiccated were the eggs. Only commercial eggs do not confirm this regularity. Before exposure to low temperature these

eggs were not hydrated, neither there were dried at a given relative humidity of air. Their water content was 9.2% (thus, the intermediate value), but their hatchability was most intense.

III. The effect of water salinity on survival of eggs at low temperatures.

The intensity of hatching at a given temperature and salinity is presented graphically in Fig. 4. On the contrary to the previous experiment, there was a clear difference found in hatchability between frozen eggs and not frozen eggs, the difference vanishing with the increased salinity of water at which the eggs were frozen. When the eggs were frozen at a temperature of —10°C, the differences between hatches at different concentrations of salts are insignificant, they increase however when the eggs have been frozen at —25°C or lower temperature. There are also conspicuous differences in hatchability of eggs frozen at different temperatures, but with the same salinity of water.

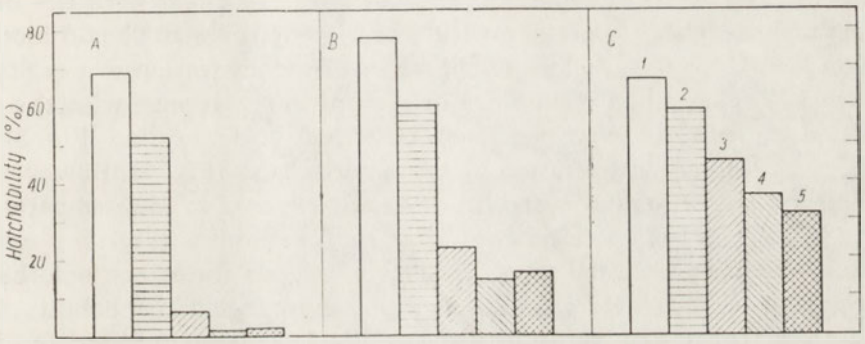

Fig. 4. Hatchability of *Artemia salina* eggs after exposures to low temperatures at different salinity of the environment. A — distilled water, B — salinity of 48‰, C — salinity of 63‰. 1 — control, 2 — —10°C, 3 — —25°C, 4 — —79°C, 5 — —196°C

In general, the survival of eggs after exposures to low temperatures is the highest at the highest salinity of water. The eggs submerged in distilled water survive only the freezing at —10°C, after exposures to remaining temperatures they even do not hatch at 10%.

4. DISCUSSION

Eggs of *Artemia salina* show rather high resistance to very low temperatures, both dry, as a resting stage, and hydrated, during the process of incubation. According to Whitaker (1940), dry eggs can endure a temperature of —190°C for 24 hr. Dutrieu and Chrestia-Blanchine (1967) have found that also eggs which were submerged in an advanced developmental stage (after the period of diapause) survive

temperatures of —79° up to 31 hr and —196°C up to 3 hr. According to these authoresses, the resistance of eggs depends on the stage of development: at the beginning of incubation the resistance is higher, at the end of incubation, just before hatching it is lower. These changes depend on the amount of trehalose which has protective value, and its amount decreases with incubation of eggs.

The increased cell osmotic pressure which decreases the freezing point end prevents from formation of ice crystals (literature review in: Thomas 1963), as well as the presence of protective substances, such as glycerol (literature review in: Rey 1959) saccharides such as glucose, saccharose, lactose — detected in bacteria, resistant to cold, and trehalose, found in *Trichiocampus populi*, an insect resistant to cold (Asahina and Tanno 1964).

The experiments carried out here aimed at finding which of the environmental factors increase resistance of *Artemia salina* eggs to low temperatures. As it is evident from the literature cited, both the desiccation and the higher osmotic pressure of the environment should increase resistance of the eggs to low temperatures by dehydration of developing embryos. The obtained results are in general in accordance with the supposition, both in the experiments on the effect of desiccation (Fig. 3) and that of increased concentration of the environment (Fig. 4). However, by comparing the content of water in dry eggs, exposed to low temperatures and the intensity of their hatches after such exposures (Fig. 3) it proves that so-called commercial eggs, in spite of higher water content that in the eggs dried at 9% R. H. and 33% R. H., show higher hatchability. This would confirm probably the hypothesis which was put forward by Dutrieu and Chrestia-Blanchine (1967) that trehalose plays role of a substance protecting from detrimental effects of freezing, since acc. to these authoresses the amount of trehalose decreases with the period of incubation. Therefore, the commercial eggs in the experiments presented here, in which incubation did not start, comprised probably more trehalose than the remaining eggs which were hydrated before desiccation, and started their development.

It seems that of the two factors protecting against detrimental effects of freezing, dehydration and trehalose, the latter is much more effective.

As it is evident from the experiments on the effect of osmotic pressure of the environment on resistance of eggs to cold, the hatchability depends clearly on this factor (Fig. 4). It can be also said that the boundary of harmfulness of low temperatures lies between —10° and —25°C, since the first temperature is less harmful even to eggs frozen in distilled water. It is in accordance with the level of eutectic point of plasm, —21°C, which changes with the ion composition, concentration, etc

(Thomas 1963). Protective factor in this experiment is not only osmotic dehydration of plasm by the environment of higher salt concentration, but also the increase of glycerol level in eggs with increasing concentration of outer environment, which has been observed by Clegg (1964) in experiments with *Artemia salina* eggs. The protective value of glycerol explains probably the high percentage of hatches of eggs submerged in the most concentrated environment after exposure to temperatures —79° and —196°C, this percentage being more or less equal to that of the eggs desiccated at 76% R. H.

From the comparison of hatchability of eggs after exposure to temperatures —79° and —196°C in all experiments it appears that in most cases temperature of —79°C is more harmful. This would converge with the view of Rey (1959) who stated that the least harmful effect is that of the temperature of liquid nitrogen, which causes freezing of all components, together with intercellular liquids, in one solid block and by this prevents from separation of liquid fractions which would caused osmotic disturbance.

The experiments on the effect of anaerobic conditions on the resistance to low temperatures did not give anticipated results. The results of numerous experiments carried out on bacteria showed (Thomas 1963) that the lethal effect of low temperatures was proportional to partial pressure of oxygen in the environment. Vacuum or nitrogen atmosphere were used for protection against oxygen. In the present experiments, the samples of eggs kept in the nitrogen atmosphere directly before freezing did not show any increased resistance to temperatures of —79° and —196°C, but on the contrary, after 32 days of anaerobic conditions their survival decreased completely (Fig. 2). This result is, in general, in agreement with the result of glycerol and trehalose contents analyses in eggs of *Artemia salina* at anaerobic conditions (Dutrieu and Chrestia-Blanchine 1966). These contents do not increase even when the eggs are kept for several months in nitrogen atmosphere. In the case of temperature of —10°C previous presence at anaerobic conditions increases slightly the hatchability of eggs after exposure to this temperature (Fig. 1). Such situation can be observed under natural conditions, when the eggs, lying on the bottom of a shallow reservoir among mud with a large quantity of decomposing organic substances are deprived oxygen for a longer period and at such conditions they undergo winter decrease in temperature.

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I wish to express my sincere gratitude to Prof. Janine Dutrieu for making it possible to carry out this work in the Department of General Physiology, University of Bordeaux, as well as for help in its performance.

5. SUMMARY

The resistance of *Artemia salina* eggs to low temperatures: —10°, —25°, —79°, and —196°C was examined in relation to: 1. previous stay at anaerobic conditions (Fig. 1 and 2), 2. degree of their desiccation (Fig. 3), and salinity of the environment in which they froze (Fig. 4). The intensity of hatches, expressed as per cent, was accepted as the criterion of evaluation. It was found that the eggs, both dry and hydrated, survived exposures to these temperatures, their hatchability being higher when they were more dehydrated either by desiccation or osmotically.

The eggs showed rather high resistance to anaerobic conditions. Exposure to nitrogen atmosphere for 32 days caused a small decrease in hatchability when the eggs were transferred to optimal conditions. Resistance to temperatures of —79° and —196°C was found to decrease after longer exposures to anaerobic conditions, the resistance to —10°C, on the contrary, showed a slight increase.

6. STRESZCZENIE

Badano odporność jaj *Artemia salina* na temperatury —10°, —25°, —79° i —196°C w zależności od: 1. uprzedniego przybywania w warunkach beztlenowych (Fig. 1 i 2), 2. stopnia wyschnięcia jaj (Fig. 3) oraz 3. zasolenia środowiska, w którym były mrożone (Fig. 4). Jako kryterium oceny przyjęto intensywność wylęgu przedstawioną w procentach. Stwierdzono, że jaja zarówno w stanie suchym jak i uwodnionym przeżywają ekspozycje w wymienionych temperaturach, a intensywność ich wylęgu jest tym większa im bardziej były odwodnione, przez wysychanie lub osmotycznie.

Jaja wykazują dużą odporność na przebywanie w warunkach beztlenowych. Ekspozycja w atmosferze azotu do 32 dni powoduje niewielki spadek intensywności wylęgu, po przeniesieniu jaj do warunków optymalnych. Odporność na temperatury —79° i —196°C po dłuższych ekspozycjach w warunkach beztlenowych maleje, natomiast odporność na temperaturę —10°C nieco się podnosi.

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J. HEMPEL-ZAWITKOWSKA

RESISTANCE OF EGGS OF *TRIOPS CANCRIFORMIS* (BOSC.) TO LOW TEMPERATURES AS RELATED TO SEVERAL CHOSEN ENVIRONMENTAL FACTORS

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ABSTRACT

Studies were made of hatchability of eggs of *Triops cancriformis* at low temperatures in relation to 1. previous stay in oxygen-free water, 2. degree of desiccation, 3. pre-freezing. It was found that resistance to low temperatures increases only after desiccation and pre-freezing of eggs.

CONTENTS

1. INTRODUCTION

Eggs of *Triops cancriformis*, which are also a resting stage of this crustacean inhabiting astatic water bodies, show a high resistance to external environmental conditions. Earlier studies on desiccation (Hempel-Zawitkowska and Klekowski 1968), on response to changes in osmotic pressure of the environment (Klekowski and Hempel-Zawitkowska 1968, Hempel-Zawitkowska 1969), and on resistance to strong doses of UV radiation (Hempel-Zawitkowska 1970) showed that these responses depend to a greater extent on the degree of progress in embryonal development.

In the present paper studies were made of the resistance of eggs to anaerobic conditions and to low temperatures, taking into account some other factors such as degree of desiccation or pre-freezing.

2. MATERIAL AND METHODS

Material for studies consisted of parthogenetic eggs collected near Warsaw in June 1969 and sent in oxygen-free water (by boiling) in closed flasks to Bordeaux where experiments were made. During this transportation, which lasted 7 days, the development was stopped at the minimum oxygenation of water, which was evident from a long period of incubation. After placing the eggs in water with full oxygen content

the incubation lasted 16 days. (Control hatches in water fully saturated with oxygen, as shown by previous experiments, occurred after 9–11 days of incubation).

Temperatures of —10°C and —25°C used in experiments were obtained in electric freezers, temperature of —79°C in a thermos with crushed solid carbon dioxide, and temperature of —196°C in a thermos with fluid nitrogen. Exposures of over 100 eggs were made in glass vials. After the exposures, the eggs were stored dry for 3 months and then allowed to hatch in tap water saturated with oxygen at a temperature of 18°C, and percentage of hatches was calculated.

Three types of experiments were made:

I. The effect of submerging the eggs in oxygen-free water on their survival at low temperatures. The eggs were submerged in pond water deprived of oxygen by boiling and ventilated with nitrogen for 1 hour, and later they were placed in desiccators with nitrogen atmosphere. Exposures to anaerobic conditions lasted 4, 6, 9, 15, and 29 days, later on the samples together with water in which they have been kept were subjected to temperatures of —79°C and —196°C for 1, 3, and 6 hrs. The eggs which were kept at anaerobic conditions but not exposed to low temperatures, were used as controls.

II. The effect of degree of desiccation of eggs on their survival at low temperatures. The eggs, after they had been kept for 12 days in water (thus approaching hatching), were put on a filter paper into desiccators maintaining constant relative air humidities (R. H.): 76, 33, and 9% R. H. These humidities were obtained over the following substances, 76% R. H. — saturated solution of NaCl, 33% R. H. — saturated solution of $MgCl_2$ (Winston and Bates 1960), and 9% R. H. — P_2O_5 (Charles and Hadgman 1953). Desiccation lasted for 8 days, then in a portion of eggs percentage of water content was defined by weight, which amounted to 66.8% of water in non-desiccated eggs, 14.1% — in eggs dried at 76% R. H., 8.7% in those dried at 33% R. H., and 2.7% in eggs dried at 9% R. H. The remaining eggs were subjected to low temperatures, with the following exposures applied: 1 day at temperatures of —10°C and —25°C, 1 hour at —79°C and —196°C. Two control samples of hatching were used: 1. eggs desiccated and not exposed to low temperatures and 2. hydrated eggs exposed to low temperatures.

III. The effect of pre-freezing on resistance of eggs at temperature of —196°C. The eggs after 20 days of stay in water with normal oxygen content at temperature of 20°C (being at pre-hatching stage) were dried for 2 weeks at open air, then divided into two groups: one of which was subjected to temperature of —10°C in order to pre-freeze them. Later the eggs, both pre-frozen and not pre-frozen, were subjected to temperature of —196°C with the following exposure times: 15 min, 30 min, 1, 2, 3, 4, and 5 hr. After exposures the eggs were stored dry for 3 months, then allowed to hatch and percentage of hatches was calculated. Desiccated eggs but not exposed to low temperatures were used as controls.

3. RESULTS

I. The effect of submerging the eggs in oxygen-free water on their survival at low temperatures.

Exposures to temperature of —79° and —196°C preceded by stay at anaerobic conditions were lethal for all samples. The control eggs, after exposures to anaerobic conditions hatched less, if they were longer exposed to these conditions. Per cent of hatches in individual samples was as follows:

Days without oxygen	0	4	6	9	15	29
% of hatches	78.4	79.9	77.4	83.8	52.4	9.0

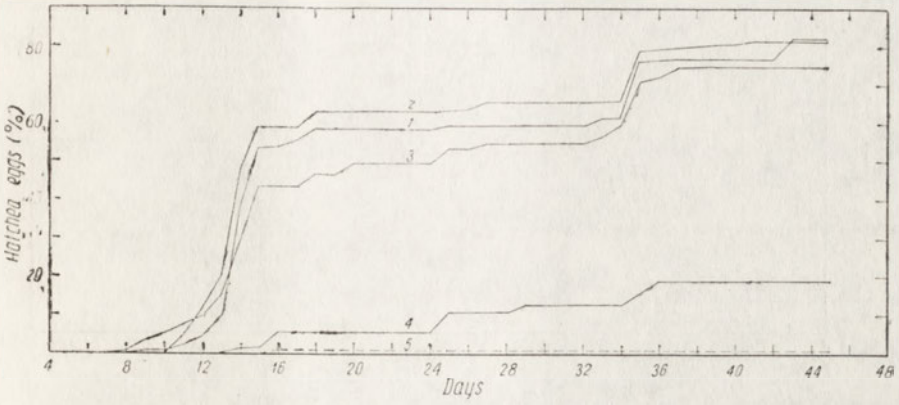

Fig. 1. Hatchability of *Triops cancriformis* eggs after desiccation at 76% relative humidity of air and freezing at different temperatures. 1 — —10°C, 2 — —25°C, 3 — —79°C, 4 — —196°C, 5 — control

II. The effect of degree of desiccation of eggs on their survival at low temperatures.

Hatchability of desiccated eggs is presented in Fig. 1–4. Exposures to all low temperatures applied were lethal only to hydrated eggs. The more the eggs were desiccated previously, the better was the hatchability of desiccated eggs (and not exposed to low temperatures). The same holds for eggs exposed to temperature of —196°C: the more they were desiccated, the higher was their resistance. The highest hatchability was of

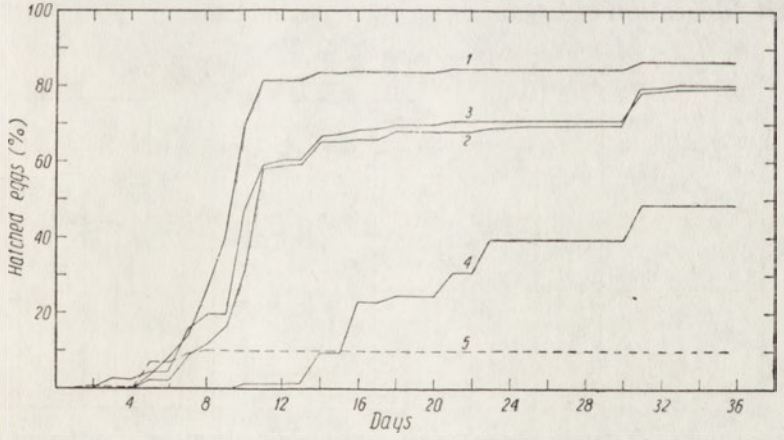

Fig. 2. Hatchability of *Triops cancriformis* eggs after desiccation at 33% R. H. and freezing at different temperatures. 1 — —10°C, 2 — —25°C, 3 — —79°C, 4 — —196°C, 5 — control

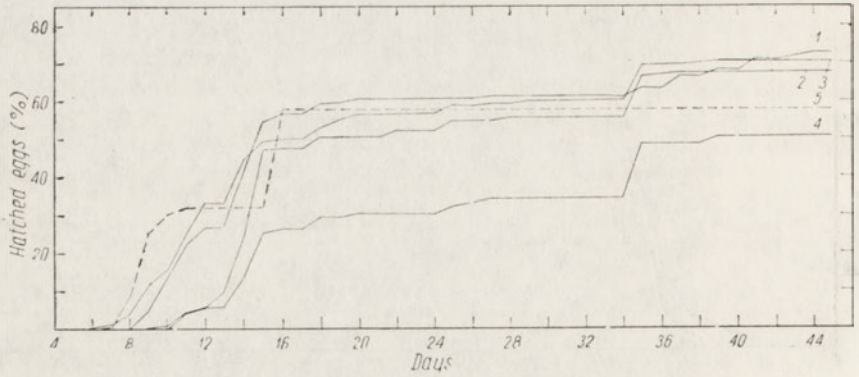

Fig. 3. Hatchability of *Triops cancriformis* eggs after desiccation at 9% R. H. and freezing at different temperatures. 1 — —10°C, 2 — —25°C, 3 — —79°C, 4 — —196°C, 5 — control

these eggs which were desiccated at 9% R. H., somewhat lower after desiccation at 33% R. H. On the other hand, the resistance of eggs to 3 remaining temperatures (—10°, —25°, and —79°C) is high, with the highest per cent of hatches after desiccation at 33% R. H. In general, the eggs after exposures to these temperatures show a very similar trend of the curve of hatchability which is much higher than the curve for control eggs, i.e., only desiccated and not frozen. The hatchability curve for eggs after exposure to —196°C has an intermediate value between that of the remaining temperatures and the control eggs. Apparently the best hatchability in the case of this temperature is obtained after thorough desiccation of eggs.

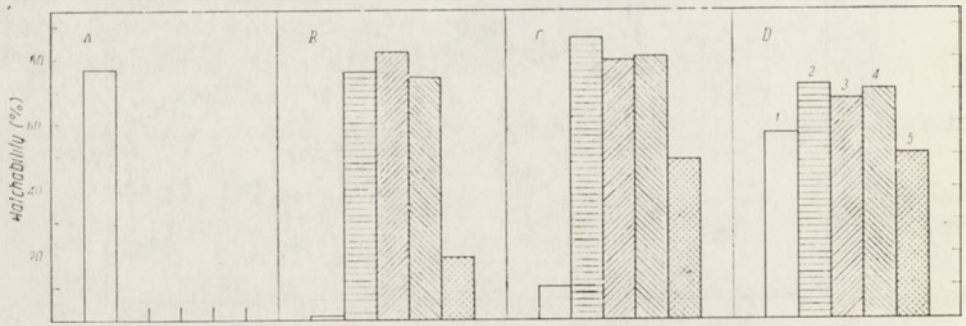

Fig. 4. Hatchability of *Triops cancriformis* eggs after desiccation at different relative humidities of air and exposures to low temperatures. A — eggs not desiccated (no hatching after freezing), B — eggs desiccated at 76% R. H., C — eggs desiccated at 33% R. H., D — eggs desiccated at 9% R. H. 1 — control, 2 — —10°C, 3 — —25°C, 4 — —79°C, 5 — —196°C

III. The effect of pre-freezing on the resistance of eggs to temperature of —196°C.

Hatchability of pre-frozen eggs and not pre-frozen eggs is shown in Fig. 5. The hatchability curves for eggs of the two series gather in two separate belts; hatchability of pre-frozen eggs is higher than that of not pre-frozen eggs, by 40%, on the average. There is no visible dependence

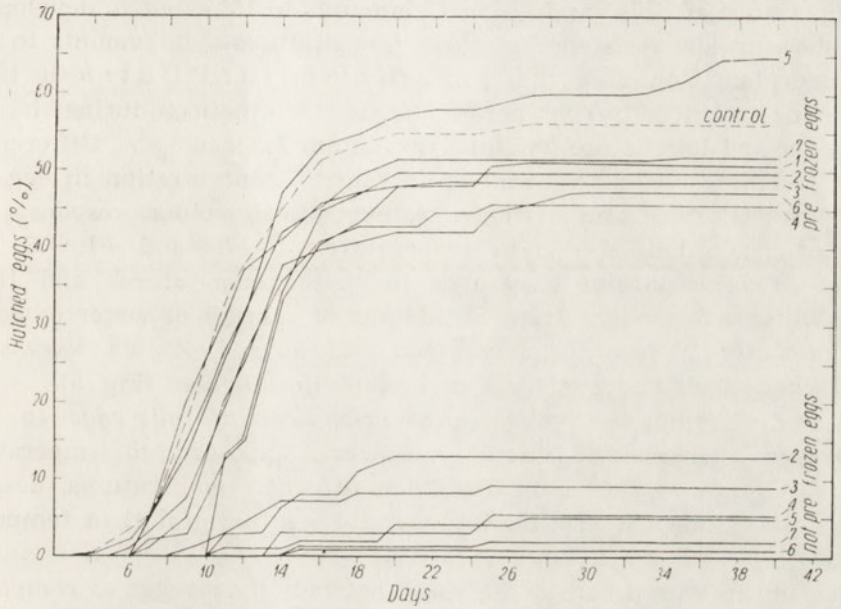

Fig. 5. Hatchability of *Triops cancriformis* eggs frozen at —196°C after and without pre-freezing at — 10°C. Exposures to temperatures of —196°C: 1 — 15 min, 2 — 30 min, 3 — 1 hr, 4 — 2 hr, 5 — 3 hr, 6 — 4 hr, 7 — 5 hr

between the intensity of hatches and the time of exposure both in pre-frozen eggs and not pre-frozen eggs; the observed differences are of random character and they probably depend on the speed of freezing and defrosting of a given sample.

Attention should be drawn to the fact that pre-frozen eggs start hatching earlier than not pre-frozen eggs.

4. DISCUSSION

The main factors protecting against the effects of low temperatures on protoplasm are: the increase in concentration of inner environment and the presence of some protecting substances, especially glycerol and saccharides (review of literature in: T h o m a s 1963). These both factors have a higher value in eggs of *Artemia salina* than in eggs of *Triops can-*

criformis since (leaving the genetic properties aside) brackish environment causes an increase in osmotic pressure in protoplasm of eggs and embryos, and, as it is evident from studies by C l e g g (1964), it increases the level of glycerol.

According to D u t r i e u and C h r e s t i a - B l a n c h i n e (1967) trehalose plays a protective role against the effect of freezing in eggs of *Artemia salina*. Its content in non-developing eggs, which are resistant to temperature of —79° and —196°C, amounts to 16%, but in developing eggs, showing low resistance to these temperatures — it amounts to 5%. As it is evident from an earlier paper (H e m p e l and D u t r i e u 1965) there is no trehalose in eggs newly laid. It is synthetized during the development and just before hatching its content reaches 3.5%. Differences in the trehalose content as well as in osmotic concentration in eggs of *Triops cancriformis* and *Artemia salina* explain other responses of hydrated eggs of both species to temperatures of —79° and —196°C. The eggs of *A. salina* survive exposures to these temperatures and their hatchability is dependent from the degree of salinity of water (even in distilled water 1.7% of eggs survived — H e m p e l - Z a w i t k o w s k a 1971), whereas all hydrated eggs of *T. cancriformis* died (Fig. 5).

After desiccation the resistance of *Triops cancriformis eggs* to low temperatures increases considerably. However exposures to temperature of —196°C show higher mortality than to other temperatures, despite the fact that this temperature is generally less harmful than temperature of —79°C (R e y 1959).

Freezing increased considerably the hatchability of eggs as compared to that of control eggs (desiccated at different relative humidities of air, but not frozen) most probably by a break in diapause, this break being caused by additional dehydration. The hatchability of control and frozen eggs has similar value only after the strongest desiccation at 9% R. H. at all temperatures examined.

Pre-freezing of eggs at temperature of —10°C before exposure to temperature of —196°C has caused a clear increase in resistance of eggs against this temperature (Fig. 5). The mechanism of this process depends on dehydration of intercellular fluids of the embryo and formation of ice crystals with simultaneous dehydration of protoplasm, causing by this an increase in intercellular osmotic pressure. This new state of hydration, analogous to the effect of strong desiccation, is a protection against harmful effects of freezing. Similar results of pre-freezing were obtained in studies by A s a h i n a and A o k i (1958) in which pre-freezing was applied to pupae of *Cnidocampa flavescens* (Walk), before they were frozen at temperature of fluid oxygen (—183°C), with the best results obtained at pre-freezing temperature of —30°C. Other temperatures of

pre-freezing were favourable for other animals. For example, —25°C for nematodes — plant parasites (Asahina 1959), since the speed of formation of intercellular ice crystals depends on hydration of protoplasm and on a number of other factors.

Lack of hatches in eggs exposed to temperature of —79°C and —196°C, after exposure to anaerobic conditions indicates that this factor does not increase resistance of eggs to very low temperatures, which was confirmed by experiments on eggs of *Artemia salina* (Hempel-Zawitkowska 1971). It is possible that elimination of oxygen as a factor which decreases resistance to low temperatures (Thomas 1963) does not compensate another harmful factor, such as high hydration of plasm in eggs frozen when submerged in water.

In general, it can be said that long presence at anaerobic conditions causes very slow development of eggs which indicates that many of them begin diapause which can be stopped by a very intense or repeated desiccation. This anaerobic condition brings a similar effect as desiccation (Hempel-Zawitkowska 1967), which, if occurs in an early developmental stage (in this experiment, the eggs just before being laid were exposed to anaerobic conditions since they were kept in closed bottles), causes a long-lasting diapause.

Acknowledgements

I wish to express my sincere gratitude to Prof. Janine Dutrieu for making it possible to carry out this work in the Department of General Physiology, University of Bordeaux, as well as for help in its performance.

5. SUMMARY

1. Eggs of *Triops cancriformis* showed a high resistance to temperatures of —10°, —25°, —79°, and —196°C, only when desiccated. Exposures to these temperatures of desiccated eggs stimulated their hatchability as compared with non-frozen eggs.

2. Pre-freezing of eggs before their exposure to temperature of —196°C increased their resistance to the latter temperature by about 40%.

3. With prolonged time of exposure of eggs to anaerobic conditions, their hatchability decreased. No effect was found of anaerobic conditions on resistance of eggs to low temperatures.

6. STRESZCZENIE

1. Jaja *Triops cancriformis* wykazują dużą odporność na działanie temperatur —10°, —25°, —79° i —196°C jedynie w stanie suchym. Ekspozycje w tych temperaturach jaj wysuszonych wpłynęły stymulująco na intensywność ich wylegu, w stosunku do jaj niemrożonych.

2. Wstępne chłodzenie jaj przed ekspozycją w temperaturze —196°C zwiększyło ich odporność na działanie tej temperatury o około 40%.

3. W miarę przedłużania ekspozycji jaj w warunkach beztlenowych zmniejsza się ich zdolność wylęgowa. Nie stwierdzono wpływu przebywania w warunkach beztlenowych na odporność jaj na niskie temperatury.

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POLSKIE ARCHIWUM HYDROBIOLOGII (Pol. Arch. Hydrobiol.)	18	3	303–323	1971

E. KAMLER

REACTIONS OF TWO SPECIES OF AQUATIC INSECTS TO THE CHANGES OF TEMPERATURE AND OXYGEN CONCENTRATION

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ABSTRACT

The reactions of larvae of the eurytherm, *Cloeon dipterum*, and of the stenotherm, *Perlodes intricata*, to the changes of temperature and of oxygen concentration were determined. The lethal O_2 concentrations for *C. dipterum* were lower, and their survival in similar conditions was higher than for *P. intricata*. The Q_{10} coefficient for *P. intricata* was markedly lower than the values expected from the Krogh's "normal curve". The working of the ventilatory systems of the two species was also analysed.

CONTENTS

1. INTRODUCTION

The investigation was carried out on larvae of two species with different environmental requirements, namely *Cloeon dipterum* (Linné), Ephemeroptera and *Perlodes intricata* (Pictet), Plecoptera. The study concerned the reactions of both species to the changes of temperature and of oxygen concentration. Special care was taken to obtain a systemic image of the reactions, and thus both factors were manipulated parallelly and various reactions were tested, like survival, O_2 lethal values, respiration and respiratory movements. The task of the study was also to establish, to what degree the physiological requirements of the animals were related to their habitats, and to try to explain the cause of their different resistance to high temperatures and low O_2 concentrations.

2. MATERIALS

The larvae of *C. dipterum* were collected from among the plants in a clay pit in a Warsaw suburb, and the larvae of *P. intricata* from stones of the Olczyski stream in Tatra Mountains. *C. dipterum* lives among the plants in pools, ponds, in the littoral of lakes, and in slow-running waters. Water temperature in such environments can assume high values (e.g., Gieysztor 1934 reported 30.6°C in a pool) and it is

apt to be changeable (e.g., Pattee 1965 observed a daily amplitude of 10.5°C in a reservoir inhabited by *C. dipterum*). Oxygen content is also highly changeable (e.g., air saturation of water can range from 10 to 170% in a pond — Lewkowicz and Wróbel 1971). On the other hand, *P. intricata* lives in stony mountain streams, with low water temperatures and high oxygen contents (Fig. 1). Figure 2 shows the present author's measurements of water temperature in the Olczyski stream (Tatra Mountains). It can be seen that both the annual and daily amplitudes are fairly small. The geographical distribution of both species is different, too. *C. dipterum* is a widespread species in lower parts throughout Europe, while *P. intricata* appears only in the mountains (Fig. 3). Both species are numerous in their habitats; e.g., *C. dipterum* accounted for 25.8% of all the Ephemeroptera larvae caught among the plants in pools near the Bug and Narew rivers (Kamler unpublished), and *P. intricata* constituted 5.8% of all the Plecoptera collected in Tatra Mountains (Kamler 1964).

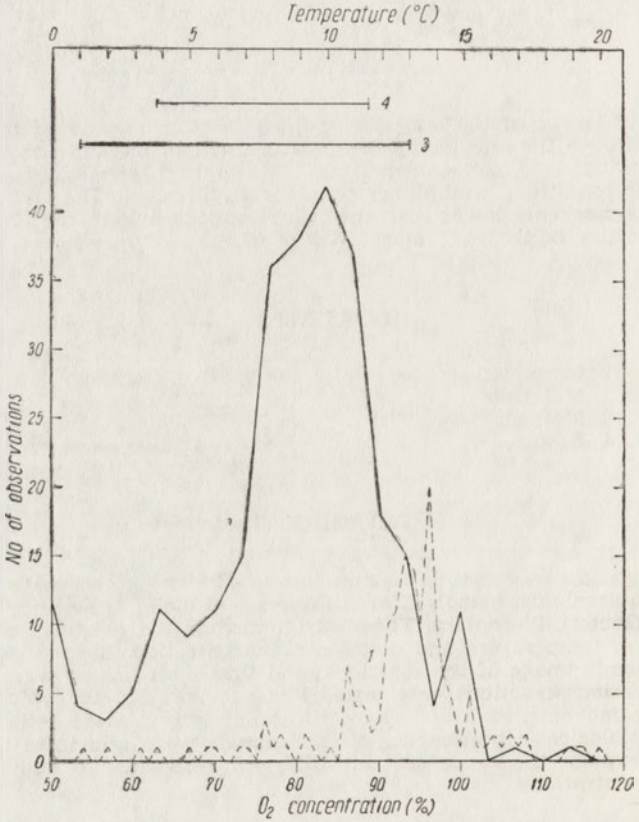

Fig. 1. Oxygen and temperature conditions of *P. intricata* larvae in their natural habitat. 1 — distribution of oxygen content, 2 — distribution of temperature in Tatra streams (compiled from Oleksynowa and Komornicki 1956, 1957a, b, 1960, 1965), 3 — temperature range of *P. intricata* after Wojtas (1964), 4 — temperature range of *P. intricata* after Pleskot (1951)

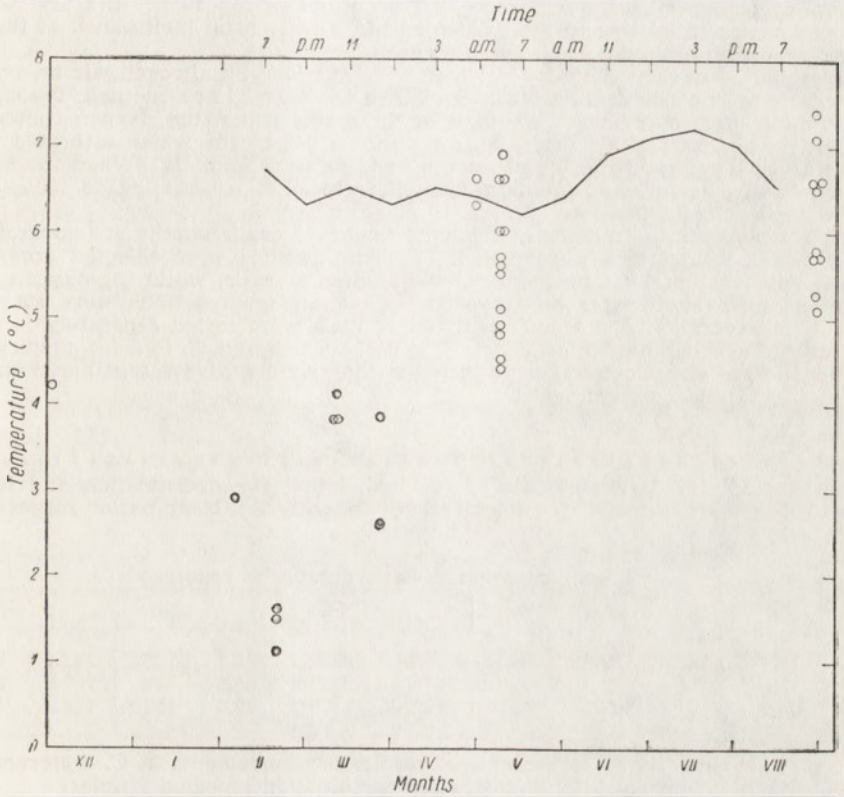

Fig. 2. Water temperatures in Olczyski stream. Points — seasonal temperature variation (winter–summer 1963, 1966, 1967, 1968, 1970), line — temperature changes over 24 hr, Aug. 27–28, 1963

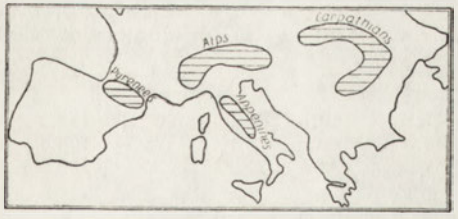

Fig. 3. Distribution of *P. intricata* in Europe

3. METHODS

Before the experiment the larvae had been placed for 24 hr in glass basins, water had been ventilated and the basins kept in a thermostat maintained at the required temperature with an accuracy of ±0.015°C.

The required oxygen contents in water were obtained by nitrogen, air or oxygen bubbling. Oxygen concentration was determined by Winkler's method, the standard deviation of measurements was 0.02 mg/l. In this paper, the oxygen content is expressed in per cent of air saturation of water, 100% means water saturated with air at a given temperature (°C) and atmospheric pressure (mm Hg). When the atmospheric pressure is not known (data from literature), it is assumed to have been 760 mm Hg.

Table I presents the scheme of the experiment. 15 combinations of temperatures and oxygen concentrations were tested. The temperatures were selected according with the environmental requirements of the studied animals, while the oxygen concentrations were similar for both species. The examined reactions were survival, lethal O_2 concentration and respiration; ten animals were tested separately in each temperature/oxygen combination (Table I, experiment 1a and 2). Besides, respiratory movements were investigated. For *P. intricata* they were analysed together with the other indices (Table I, experiment 2).

Table I. Scheme of the experiments

Experiment 1a. Measurements of survival, lethal O_2 concentration and respiration in *C. dipterum* (each O_2 concentration/temperature combination repeated 10 times)

Temp. (°C)	O_2 concentration (%) and number of readings									
	%	No.	%	No.	%	No.	%	No.	%	No.
5.5	15.4	341	29.3	638	56.5	893	104.3	726	142.2	896
15.5	12.0	86	32.3	196	64.4	348	101.2	342	137.6	368
25.5	25.2	61	47.3	111	68.9	157	99.6	104	156.3	138

Experiment 1b. Measurements of respiratory movements in *C. dipterum* (each O_2 concentration/temperature combination repeated 5 times)

Temp. (°C)	O_2 concentration (%)				
5.5	15.5	33.6	57.3	101.4	142.0
15.5	16.3	29.9	47.4	101.9	130.2
25.5	27.0	35.9	43.7	105.2	149.4

Experiment 2. Measurements of survival, lethal O_2 concentration, respiration and respiratory movements in *P. intricata* (each O_2 concentration/temperature combination repeated 10 times)

Temp. (°C)	O_2 concentration (%) and number of readings									
	%	No.	%	No.	%	No.	%	No.	%	No.
5.5	26.0	10*	47.1	159	69.1	257	113.2	278	194.0	576
10.5	33.6	23	42.5	39	76.1	105	108.3	190	133.5	201
15.5	25.8	10*	57.2	32	79.9	55	106.5	92	123.1	128

* Means the death of all animals before the first reading.

The procedure in experiments 1a and 2 was as follows:

1. The initial control bottle I was filled with water having the required temperature and O_2 concentration. Oxygen was fixed and titrated immediately.

2. Ten experimental bottles containing single animals were filled one by one and placed in a thermostat together with the respective final control bottles. The volume of the bottles used for *C. dipterum* was about 60 ml, and of those for *P. intricata* about 170 ml.

3. The initial control bottle II was filled and the oxygen content in it was measured. The oxygen content at the start of the experiment was calculated as a mean from the initial controls I and II.

4. The observations of survival and the readings of respiratory movements were continued during 1 min at regular time intervals until the death of the last animal; total numbers of the readings are quoted in Table I, experiment 1a and 2.

5. The oxygen contents in the experimental and final control bottles, lengths and weights of the animals were determined. Dry weights were determined after drying at 50°C. The latter data allowed to establish the lethal O_2 concentrations and O_2 consumption.

The part of the experiment concerning the role of O_2 concentration has a cross--reference pattern, as the results can be read either by comparing the animals' behavior in bottles with the different initial O_2 concentrations, or by examining the animals in bottles with the same initial O_2 concentration, since the oxygen content is gradually lowered by the animals themselves.

Respiratory movements of *C. dipterum* must have been measured separately (Table I, experiment 1b) because of the complex character of the movements, small size of the larvae, and their great mobility. 75 measurements were taken separately (3 temperatures and 5 oxygen concentrations, 5 repetitions). It was found that the best conditions of observation were obtained by placing single larvae in glass photometric cells of 10 ml capacity and 1 cm of the cell length. A cell was flushed twice with water supplied by means of a thin glass tube attached to the bottom, which was subsequently taken away. The cell was then closed with a glass plate covered with dense vaseline. Former tests by means of dyes had proved such a closure to be sufficiently tight. The vessels with animals were placed in constant temperature baths, and about ten minutes later the measurements started. The animals were watched under 8× lens during about 7 min. The records of ventilation periods (Fig. 4 A) and of rest periods (Fig. 4 B) were taken by means of a tapping key on a kymograph. Clock records every 10 sec (Fig. 4 C) were taken automatically. The ventila-

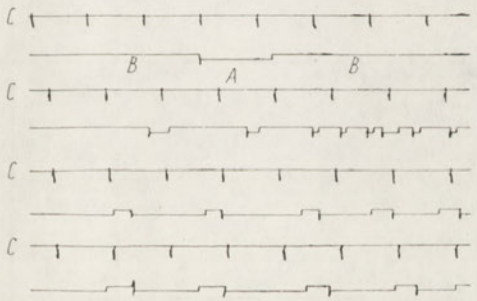

Fig. 4. Fragment of registration of gill movements of *Cloeon dipterum* (A), gills ventilating (B), gills motionless (C); clock records every 10 sec

tion and rest periods were then read from the graph with 0.1 mm accuracy, 0.1 mm being equal to $868 \cdot 10^{-6}$ min. Next, the percentage of ventilation time was calculated. Separate measurements of oxygen consumption allowed to calculate that during a 7 min period of measurement of the respiratory movements of *C. dipterum*, the oxygen content in the cells fell down by not more than 3% of its initial value. Besides, a statistical analysis of the gills' movements record was performed. The whole measuring period was divided into two halves. The percentages of ventilation times in the first and second halves were compared using the t-test, method of paired comparisons (Bailey 1959). In the first halves the mean was 43.07, and in the second ones it was 40.12; $t = 1.578$. This result is not significant at the 5% level which cor-

responds to $t = 2.042$. Thus it can be supposed that the measurement of the respiratory movements of *C. dipterum* has been carried out in more or less constant conditions.

Single gill movements of *C. dipterum* are so rapid and complex that they cannot be subject to visual observation, so that resort must have been made to cinematography. A 16 mm film, 32 shots per second, was realized in the Biological Institute for Inland Water Research of the Academy of Sciences, Borok, USSR. The full-grown larvae were placed in a perspex "artificial stream" with some gravel on the bottom. Two temperatures, 13 and 23°C, were tested in stagnant water and in water flow of 0.06 m/sec. The number of movements performed by the larvae in each series of measurements was counted simultaneously by two persons five to seven times during a slow projection of the film on a screen. From the 10 to 14 readings thus obtained the mean with its standard deviation was calculated, and coefficient of variation ranged in various series of measurements from 6.01 to 23.58%. Subsequently, the number of frames corresponding to the established number of gill motions was counted, and the frequency of single gill movements per 1 min was computed. Because of the great mobility of *C. dipterum* larvae, only a few sections of the film could be used for the readings of frequency of single gill movements, and thus the results must be considered as preliminary.

4. RESULTS

Survival

The results of measurements of survival of *C. dipterum* larvae are presented in Fig. 5. It can be seen that in higher temperatures the death

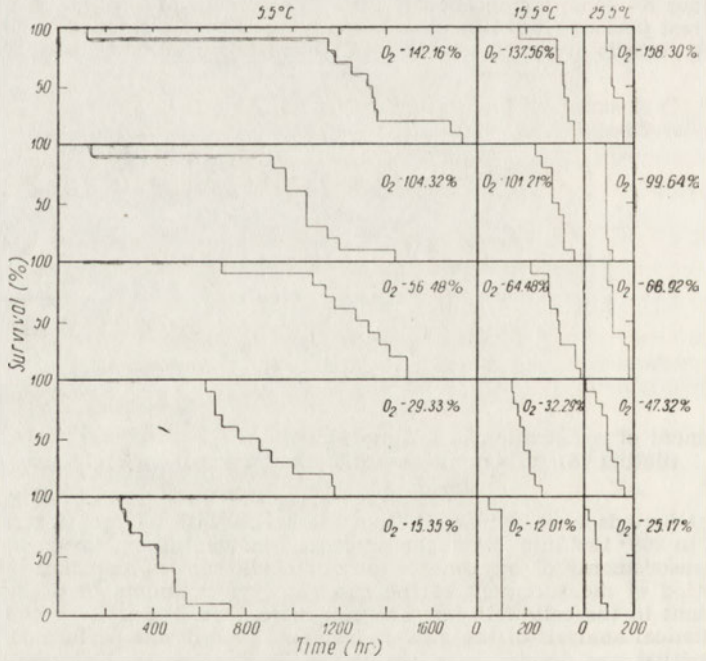

Fig. 5. Survival of *C. dipterum* larvae in different temperatures (°C) and initial O_2 concentrations (%)

of the larvae occurs earlier than in lower ones. It was also found that the larvae died earlier in those sets of experiments in which the initial O_2 concentration was low than in those in which there was much oxygen at the start of the experiment (by a set of experiments is meant here 5 or 10 repetitions of individual measurements taken at the same temperature and O_2 conditions; see Table I). Analogous results were obtained for *P. intricata* (Fig. 6).

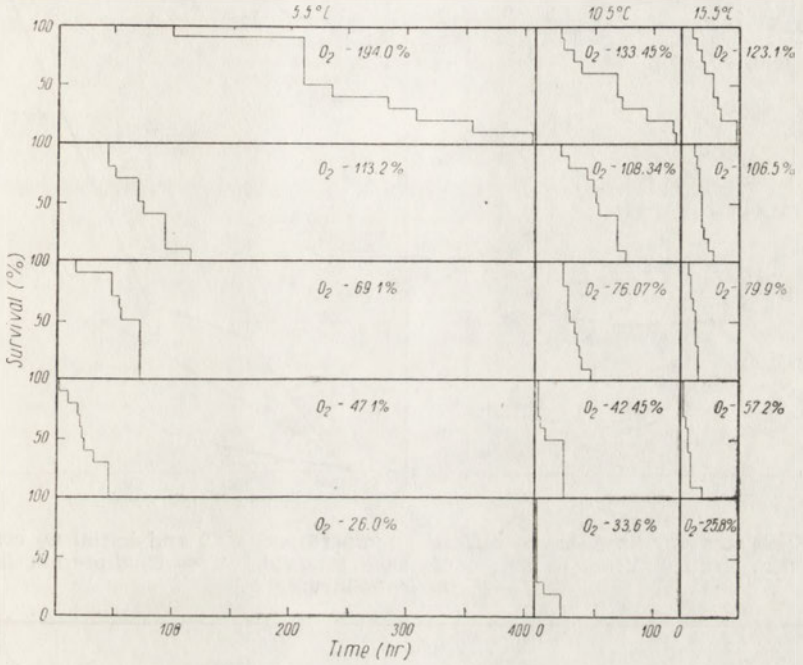

Fig. 6. Survival of *P. intricata* larvae in different temperatures (°C) and initial O_2 concentrations (%)

It can be seen from these illustrations that the survival times of separate individuals in one set were not identical. For a better comparison of the several sets of experiments, in each set the mean survival time for all the 10 individuals was computed together with the 95% confidence interval. The results for *C. dipterum* are presented in Fig. 7 A. Undoubtedly, there were significant differences in survival time of *C. dipterum* larvae, depending on temperature (5.5, 15.5 and 25.5°C). Such differences have been found at all the tested initial O_2 concentrations. In high initial O_2 concentrations (about 60% or more) the survival time did not change much with the change of O_2 concentration, but below this O_2 value the survival time falled down markedly with the decrease of the

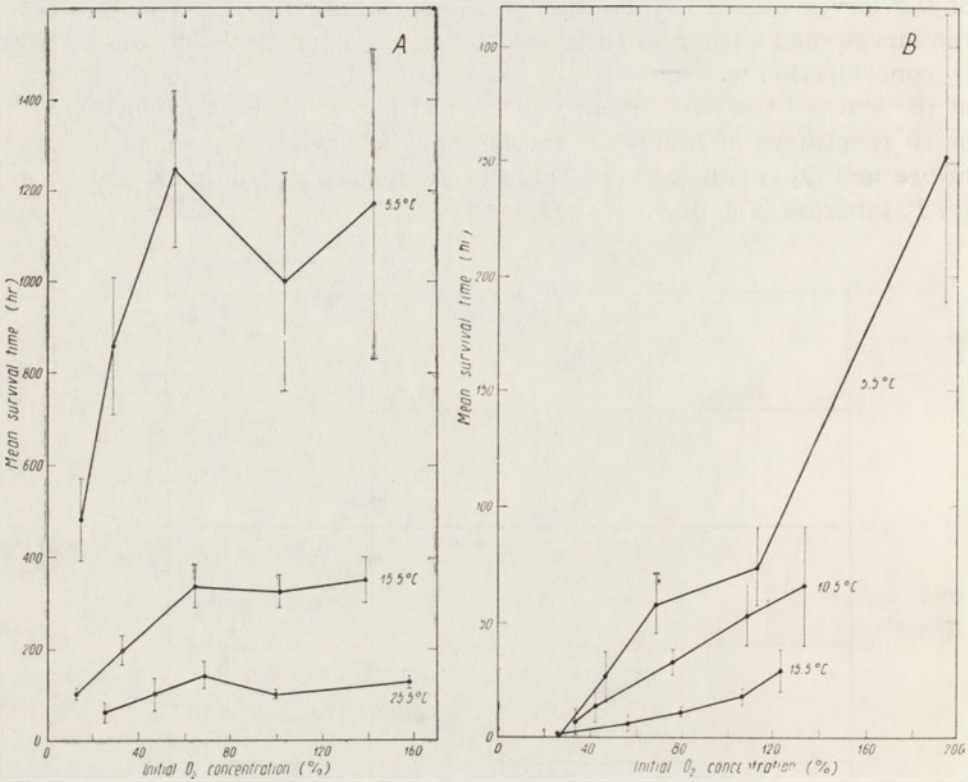

Fig. 7. Mean survival time (hr) in different temperatures (°C) and initial O_2 concentrations (%). Vertical lines — 95% confidence intervals. A — *C. dipterum* larvae, B — *P. intricata* larvae

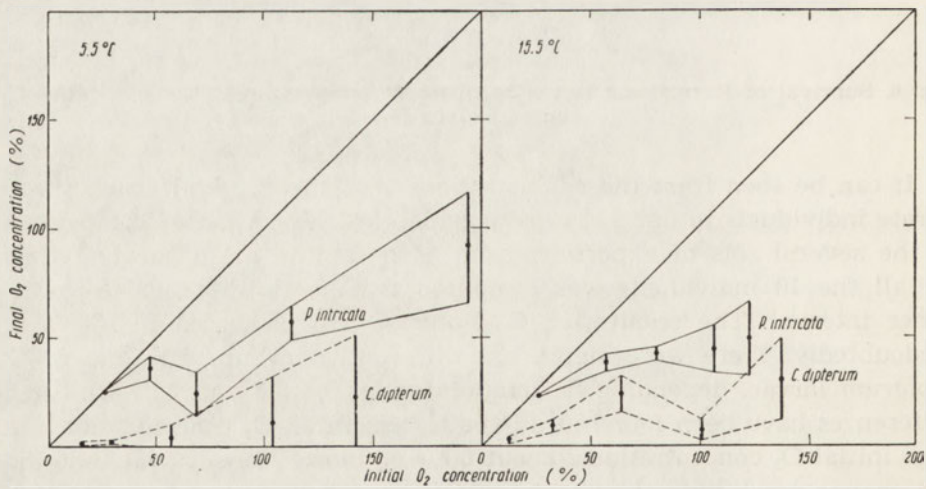

Fig. 8. Lethal O_2 concentrations of *C. dipterum* and *P. intricata* larvae. Points — means for 10 measurements, vertical lines — 95% confidence intervals

inital O_2 concentration; this effect was particularly remarkable in higher temperatures. A significant difference of the mean survival time of *P. intricata* larvae was also found in the tested temperatures 5.5°C, 10.5°C and 15.5°C (Fig. 7 B), but only at the initial O_2 concentrations higher than about 60%.

It is of course impossible to compare directly the survival times of *C. dipterum* and *P. intricata* because of the differences of the animals' weights, of the experimental vessels' capacities and of the oxygen consumption rates of both species of larvae. However, it is remarkable that at O_2 concentration 26.0% (temp. 5.5°C) and O_2 concentration 25.8% (temp. 15.5°C) all the larvae of *P. intricata* died very soon after they had been put in the vessel (Fig. 6, and 7 B), while the larvae of *C. dipterum* have been living much longer at a much lower initial O_2 concentration (12.0% at 15.5°C), as can be seen in Fig. 5 and 7 A.

Lethal O_2 concentrations

As it was stated above, the larvae of *P. intricata* transported from water rich in oxygen into water containing about 26% of oxygen at temperatures 5.5 and 15.5°C died almost immediately. At the medium tem-

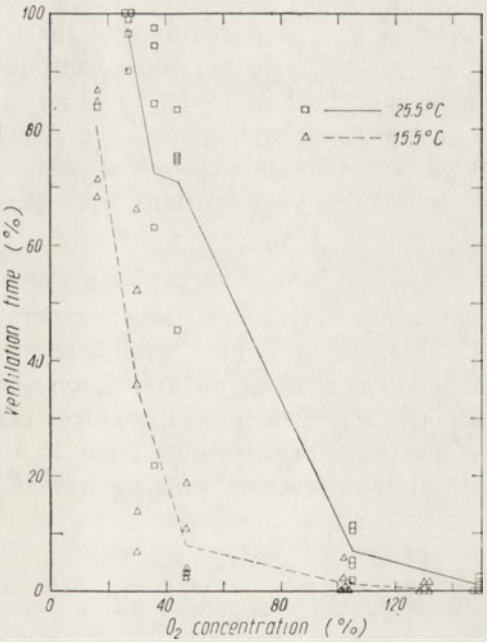

Fig. 9. Per cent of ventilation time in *C. dipterum* larvae in different O_2 concentrations at 25.5 and 15.5°C. Marks stand for the results of individual experiments, lines represent their averages

Table II. Lethal O_2

O_2 concentration at the start of experiment (%)	*Cloeon dipterum* (25.5°C)				
	25.2	47.3	68.9	99.6	158.3
Mean O_2 concentration at the end of experiment (%)	6.95	16.43	9.21	2.25	13.14
± 95% confidence intervals	±4.895	±9.390	±4.225	±1.000	±14.859

perature (10.5°C) the lowest O_2 content was somewhat higher, i.e. 33.6% (Table I). The mean survival time was 5.8 hr. Thus, it can be supposed that the lethal O_2 concentration for the *P. intricata* larvae, not adopted formerly to low oxygen concentrations, should be placed between 26 and 34%.

The lethal O_2 concentrations after exposure to an environment with decreasing oxygen content are presented in Fig. 8. For more ready comparison, we confronted only the results of experiments conducted at 5.5°C and at 15.5°C, in which both species were tested. It can be seen that in general larvae of *C. dipterum* died in lower oxygen concentrations than *P. intricata*. The areas defined by the 95% confidence intervals almost do not overlap. It is remarkable that the *C. dipterum* larvae are able to exhaust oxygen down to very low contents values, e.g. 0.02 ml O_2/l at 5.5°C, which is equivalent to 0.23% of air saturation. The results not presented in Fig. 8 are shown in Table II. The lethal O_2 concentrations seem to be fairly stable for each species; no clear relations with temperature or initial O_2 concentrations were observed.

Respiration

To compare oxygen consumption at various temperatures, we selected the results obtained for larvae of similar weight, exposed in water equilibred with air (Table I, experiment 1a and 2, one column before the last). For *C. dipterum* Q_{10} was 2.70 in temperatures ranging from 5.5 to 15.5°C, and 2.62 for the temperature range from 15.5 to 25.5°C. For *P. intricata* Q_{10} was 1.43 at temperatures ranging from 5.5 to 10.5°C.

Respiratory movements

In *C. dipterum* the frequency of single gill movements is high, 350 to 750 movements per 1 min (Table III). We did not observe a change of this frequency resulting from the change of the current speed from 0 to 0.06 m/sec, or from the change of temperature from 13 to 23°C.

concentrations

Perlodes intricata (10.5°C)				
33.6	42.5	76.1	108.3	133.5
29.50 ±3.910	27.72 ±7.210	29.30 ±8.290	46.61 ±13.760	64.83 ±8.150

The ventilatory activity of *C. dipterum* is intermittent and very irregular. E.g., successive ventilation periods and rest periods of an individual larva at 15.5°C and 47.4% air saturation of water were (min. 10^{-3}): ventilation 795, rest 13; v. 48, r. 16; v. 71, r. 8; v. 136, r. 98, and so on. This intermittency and irregularity can be observed in Fig. 4. In different environmental conditions, an animal changes the proportion of the ventilation and rest periods. In Figure 9, percentage of ventilation time in different O_2 contents at 25.5°C and at 15.5°C are presented. They increase with the increase of temperature and with the decrease of oxygen content. The increase of the proportion of ventilation time as the O_2 content decreases is not rectilinear: at higher O_2 contents, i.e. above 100% for 25.5°C, and above 50% for 15.5°C, it is less intensive than at lower concentrations. It can be supposed by extrapolating the data from Fig. 9 that at oxygen concentrations below 20% the gill movements are continuous.

Table III. Frequency of the single movements of gills per 1 min in different temperature/water current speed combinations

Temperature (°C)	Current speed (m/sec)				
	0.00			0.06	
23	478.2			724.6	
13	734.9 357.0	565.9 632.7	596.3 716.0	672.5	714.6

In *P. intricata*, the maximum observed frequency of the respiratory movements was 91 per minute. The respiratory movements frequencies at the tested temperatures and O_2 concentrations are shown in Fig. 10. The experiments conducted at 5.5 and 15.5°C had only 4 sets each, because of an almost immediate death of all the animals in the lowest O_2

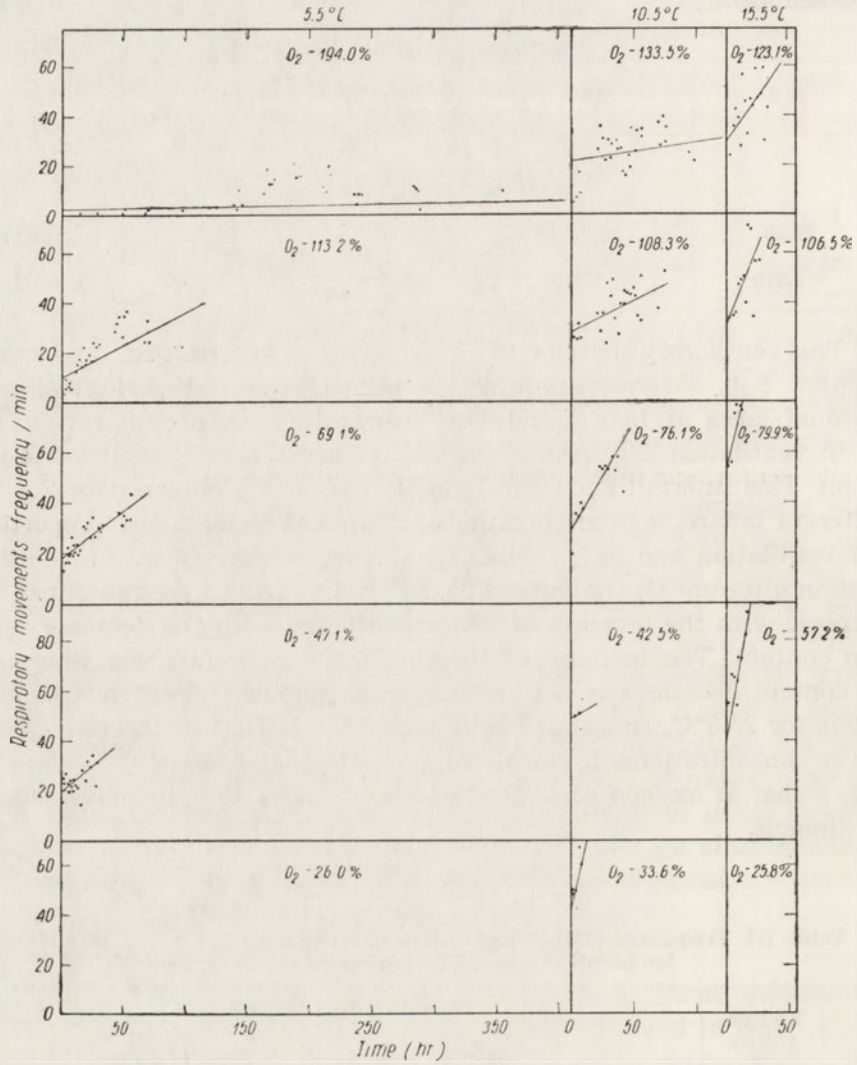

Fig. 10. Respiratory movement frequencies of *P. intricata* larvae in different temperatures (°C) and initial O_2 concentrations (%). Points — mean frequencies of 10 individuals in given time, lines — respective regression lines

concentrations, which was mentioned above. The respiratory movements frequency was proportional with temperature and reversely proportional with the oxygen content at the start of an experiment. A decrease of O_2 concentration brought about by its exhaustion from the experimental bottles by the animals themselves also caused an increase of frequency of the respiratory movements in all the sets of experiments. This increase can be described by regresions of the respiratory movements fre-

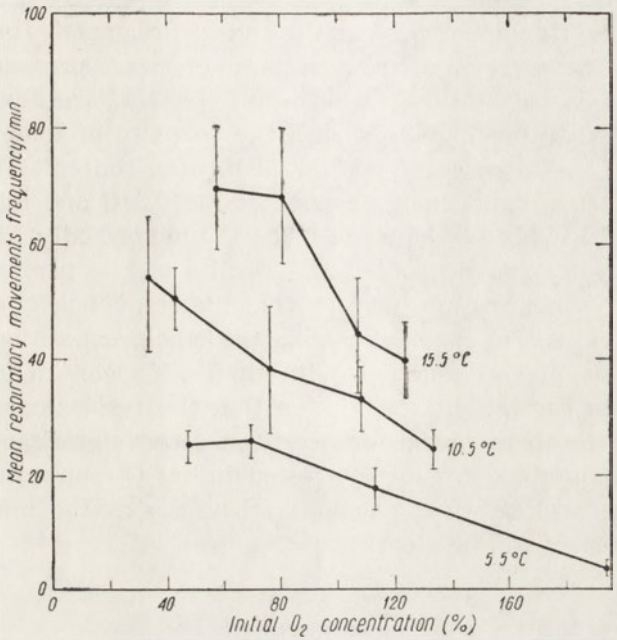

Fig. 11. Mean of all respiratory movement frequencies observed in all ten *P. intricata* larvae in each temperature (°C) and initial O_2 concentration (%). Vertical lines — 95% confidence intervals

quency/min on exposition time. The regression line at 5.5°C and 194% O_2 runs lower than might be inferred from the positions of the visible points, as 46 points in which the mean frequency was 0 could not be presented graphically on the drawing. The regressions were calculated

Table IV. Parameters of the regressions of the *P. intricata* larvae movements/min on time at different temperatures and initial O_2 concentrations

Temperature 5.5°C					
O_2 concentration (%)	26.0	47.1	69.1	113.2	194.0
a_0	—	19.5	17.45	9.87	2.63
a_1	—	+0.416	+0.372	+0.258	+0.006
Temperature 10.5°C					
O_2 concentration (%)	33.6	42.5	76.1	108.3	133.5
a_0	43.15	48.68	28.46	27.25	20.69
a_1	+2.247	+0.283	+0.863	+0.261	+0.077
Temperature 15.5°C					
O_2 concentration (%)	25.8	57.2	79.9	106.5	123.1
a_0	—	49.90	53.10	25.73	28.52
a_1	—	+2.225	+2.217	1.444	+0.680

by means of the formula: $y = a_0 + a_1 x$, where y stands for the respiratory movements frequency per 1 min and x designates the exposition time in hours. The regression equation parameters are presented in Table IV. It can be seen that the regression coefficients a_1 are always positive and usually their value increases with the increase of temperature and decreases with the increase of the initial oxygen content. The only exception is the set of experiments conducted at 10.5°C and initial O_2 concentration 42.5%, where a_1 equal to 0.283 is unexpectedly low.

For more ready comparison of the results of the particular sets of experiments, the mean respiratory movements frequencies performed by all the 10 individuals in each set during the whole exposition time were computed. Those means, together with their 95% confidence intervals, are presented in Fig. 11. It can be seen that the respiratory movements frequencies at the three tested temperatures differ significantly, and the difference is maintained for all the tested initial O_2 concentrations. The decrease of the respiratory movements frequency as the initial O_2 concentration increases can be clearly seen.

5. DISCUSSION

Fox and Simmonds (1933) used a method similar to the one employed in the present work to determine the survival time of *C. dipterum* and of *Baetis rhodani* living in cold streams. They exposed larvae in closed bottles at 16°C in higher and lower oxygen concentrations (6.4 to 7.4 ml O_2/l and 2.6 to 3.1 ml O_2/l respectively). The larvae of *B. rhodani* died after a few minutes in the lower oxygen concentration, while

Table V. Comparison

Species	Ref. No.	Temperature range (°C)				
		1-6.4	5-10	5.5-10.5	6.4-12.3	5-15
Perlodes intricata	1			1.43		
Perla abdominalis	2					
Perla marginata	3					
Pteronarcys californica	4		2.10; 2.19			1.3; 3.2
Acroneuria pacifica	4		1.19; 2.24			1.8; 1.9
Claassenia sabulosa	4					
Arcynopteryx signata	4					
Hexagenia recurvata	5	3.32			2.65	
Krogh's "normal curve"		5.59	3.65	3.47	3.31	3.22

1 — present paper,
2 — Pattee and Rougier 1969,
3 — Istenič 1963,
4 — Knight and Gaufin 1966,
5 — Morgan and Wilder 1936 (winter measurements).

the *C. dipterum* larvae survived for several hours. The authors found that the death of the larvae was not caused by CO_2 accumulation.

The lethal O_2 value for *Brachyptera risi* (Plecoptera), a species with similar ecological requirements as *P. intricata*, were determined by Madsen (1968) in standing water, at 10°C; his method was the same as the present one. The recalculated lethal O_2 value for *B. risi* is approximately 46% of air saturation of water, with a standard deviation equal to 7. The analogous value for *P. intricata*, determined at 10.5°C and 108.3% of air saturation at the start of the experiment was 46.6 (Table II), with a standard deviation 19.23, and thus it was almost identical.

Determinations of oxygen consumption performed in this work are not quite precise. The sources of the lack of precision in determining oxygen consumption by means of the closed-bottle technique were discussed in an earlier work (Kamler 1969). Hence, the present procedure was limited to a comparison of oxygen consumption at different temperatures.

Table V presents a comparison of Q_{10} values describing approximately the Krogh's "normal curve", Q_{10} values for *P. intricata* taken from the research data, and Q_{10} values for larvae of other Plecoptera (ref. No. 2–4) and Ephemeroptera (ref. No. 5), living in low and constant temperatures. The values describing approximately the Krogh's "normal curve" were read directly from the graph published in Ege and Krogh (1914) and computed, as the lists offered in the last mentioned paper and in Winberg and Pechen (1968) do not contain Q_{10} values for all the relevant temperature intervals, and besides, the Q_{10} value 2.63 for the 5°C to 15°C temperature range, quoted in Winberg and Pechen on page 66 as close to the "normal curve", seems to be

of Q_{10} values

5.5-15.5	11-15	10-20	11-20	15-20	15-25	20-25	15.52.5	30-20
	1.52-3.65	1.40 1.2-2.2 1.2-1.4 1.4-1.5 1.7	0.96-1.91	1.13-2.33	0.93-2.11 1.3-2.0 1.3; 1.3	0.65-2.22		0.55; 1.8 0.91-1.5
3.14	2.81	2.63	2.61	2.43	2.40	2.37	2.35	2.19

too low. There are many data in literature concerning the relationship between the metabolism of various non cold-adapted animals and temperature e.g., Winberg (1956) gave an extensive comparison for fish; he found these data to be highly consistent with the Krogh's "normal curve". Pattee (1965) quoted the Q_{10} value for *C. dipterum* measured by means of the closed-bottle (chemical) method as equal to 2.6, and obtained by means of the manometric method as equal to 3.4. Oxygen consumption was measured just before and 24 hr after the raising of temperature by 5°C, and the obtained values were adjusted to those obtained from the measurements of control animals at a constant temperature. The temperature intervals for these measurements were not stated in Pattee (1965), but according to his personal information, in the case of the closed-bottle method lower temperatures were 7 to 12°C and higher 9 to 14°C, while for the manometric method lower temperatures ranged from 3 to 8°C and higher from 9 to 14°C, but most measurements by the latter method were performed at 4 to 9°C. The available data do not allow for a full analysis of Pattee's results, but it can be supposed that his Q_{10} values for *C. dipterum* are not very remote from the Krogh's "normal curve". Also the Q_{10} values for *C. dipterum* obtained in the present work (2.70 at 5.5 to 15.5°C and 2.62 at 15.5 to 25.5°C) are close to the values expected from the Krogh's "normal curve" for the same temperature intervals. However, the Q_{10} value for *P. intricata* is markedly lower. In literature only a few data can be found for the cold-adapted Plecoptera and Ephemeroptera larvae, and it can be seen from the comparison in Table V that they are also much lower from those expected from the Krogh's "normal curve". There are, however, two exceptions: one out of the 31 Q_{10} values stated in Istenič (1963), 3.65 for the temperature interval 11 to 15°C, which is higher from the expected one (2.81), and one out of the 30 Q_{10} values stated in Knight and Gaufin (1966), 3.2 for the 5 to 15°C temperature interval, which is only slightly lower from the expected value (3.22). Scholander et al. (1953) proved the Q_{10} values to be in general consistent with those expected from the Krogh's "normal curve" for animals inhabiting steady warm environments (tropical seas, tropical rain forests), and for those living in cold but fluctuating environments (arctic terrestrial animals). The Q_{10} values quoted by these authors for temperate climate animals were also close to the "normal curve" values. However, the Q_{10} values for arctic fish and Crustacea living in steady cold environments were lower than the expected ones for the same temperatures. It is remarkable that also in the temperate climate there are some relatively steady cold environments, and the animals inhabiting them, as e.g. the Plecoptera and Ephemeroptera larvae, reveal much less vulnerability to

temperature changes than might be expected from the Krogh's "normal curve" within the range of low temperatures natural for them. Such discrepancy, however, is by no means surprising, if are reminded that the "normal curve" is an empirical device, and that it has not been based on measurements of cold-stenothermes. Prosser (1961), presents schematic graphs of log stabilized rates over a wide temperature range for cold-adapted and warm-adapted animals. He remarks that the most frequently encountered is such pattern of acclimatization (rotation and translation) in which the curve at low temperatures runs higher for the cold-adapted animals than for the warm adapted ones, and its slope is less steep, which is an evidence of lower Q_{10} values. However, we should not think that the Q_{10} is low for all the animals living in low temperatures. Prosser (1961) gives other examples besides the discussed one. Recently, Klekowski et al. (1970) found extremely high Q_{10} values at temperatures close to 0°C for an antarctic sea Amphipoda, *Paramoera walkeri*.

The anatomy of the respiratory apparatus and the mechanism of its work in *C. dipterum* has been fully described by Eastham (1958). *C. dipterum* is provided with 7 pairs of gills, in the first six pairs each gill consists of two lamellae. The gills are situated laterally along the abdomen. They move in metachronal rhythm, which causes water to flow between the gills and along the abdomen. Data on the role of gills in *C. dipterum* are contained in Wingfield (1939). He measured O_2 consumption at various oxygen concentrations by normal *C. dipterum* larvae and by those with amputated gills. The respiration of both groups was of the "independent" type; the critical oxygen concentration was 19% of air saturation of water for the larvae with their gills intact, but for those with amputated gills it was much higher, i.e. about 44% of air saturation. By comparing these results with analogous data obtained from observations of operated and unoperated larvae of *Baetis sp.* whose gills are motionless, Wingfield (1939) arrived at a conclusion that the main role of the gills does not consist in immediate withdrawal of oxygen from the environment but rather in causing water movements near the respiratory surfaces. Similarly, Eriksen (1963) proved that oxygen diffusion through the gills' surface was by no means more intensive than through any other respiratory surfaces. Thus the main role of the mobile gills consists in decreasing the oxygen gradients in the boundary layers around the respiratory surfaces.

An application of the cinematographic method allowed for the first time to estimate the rate of single gill movements in *C. dipterum*. This method was employed by Cukerzis (1966) for registration of the respiratory movements frequency of two species of Astacus. The present

results did not prove that *C. dipterum* modified the amount of oxygen near its body by slowing down or accelerating its gills movements. Hence, the mechanisms of adaptation to the changing environmental conditions had to be sought for in other behaviours. It turned out that the changes in the proportion of ventilation and rest periods could constitute such an adaptation. A pattern of respiratory behavior similar to *C. dipterum* can be observed in larvae of Chironomidae. Walshe (1950) took kymograph records of periods of irrigation of a tube, and of pauses in *Ch. plumosum*, at 15 to 19°C. Similarly as in *C. dipterum*, they were rather irregular; in normal larvae, the percentage of the time of irrigation of the tube increased, as oxygen contents in water fell down; this increase was more intensive at low oxygen contents than at higher ones. Such a "turning point" for *Ch. plumosus* is situated at about 20% of air saturation of water, i.e., at lower O_2 contents than that found for *C. dipterum* (about 50% at 15.5°C and about 100% at 25.5°C). This finding seems to be obvious, as *Ch. plumosus* is much more exposed to low oxygen concentrations than *C. dipterum*. In the latter species, a continuous motion of the gills can be expected below about 20% of air saturation. It was remarkable that Fox et al. (1937), who measured oxygen consumption of *C. dipterum* larvae at 10°C, found the critical O_2 content at about 18% of air saturation, and Wingfield (1939) at about 19% at the same temperature.

Knight and Gaufin (1963) describe the behavioral pattern of *Acroneuria pacifica* (Plecoptera) related with a decrease of oxygen content in their environment. Its part are respiratory movements consisting in "bendings" of the whole body. They found that the frequency of the respiratory movements increased as the oxygen content decreased, and the current speed fell down. The respiratory behavior of *A. pacifica* is similar to that of *P. intricata*. Knight and Gaufin (1963) observed in *A. pacifica* the maximum frequency of 92 undulations per minute; the same authors (1964) found it to be 145 per minute in *Pteronarcys californica*, and Pattee and Rougier (1969) who observed *Perla abdominalis* counted 117 movements per minute. All these figures are close to the maximum frequency of the respiratory movements noted in *P. intricata*, which is 91 per minute.

The results of the present work allow to suppose that one of the causes of the presented differences between the reactions of *C. dipterum* and *P. intricata* to temperature and oxygen contents can be the difference in the efficiency of their ventilatory systems. Very quick motions of the gills of *C. dipterum* can probably restore the oxygen supply in the boundary layers near the respiratory surfaces more efficiently and with lesser energy expense than the relatively slow move-

ments of the whole body performed by *P. intricata*. It is not thereby excluded, however, that regulation can occur parallelly at the tissue level in the circulatory system, or by blood system.

Acknowledgements

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6. SUMMARY

Laboratory experiments were carried out on an eurytherm, *Cloeon dipterum* (Ephemeroptera) and a stenotherm, *Perlodes intricata* (Plecoptera). 15 combinations of oxygen and temperatures were tested (3 values of temperature and 5 concentrations of oxygen); each combination was repeated 10 times. The investigated reactions were: survival, lethal concentrations of oxygen, oxygen consumption and respiratory movements frequency.

The larvae of *C. dipterum* live longer in low oxygen concentrations than those of *P. intricata*. The *C. dipterum* larvae can exhaust oxygen down to very low values, e.g. to as little as 0.02 ml O_2/l at 5.5°C. They exhaust oxygen to lower values than *P. intricata* at the same initial temperatures and oxygen concentrations. Q_{10} for the *C. dipterum* larvae is consistent with the Krogh's "normal curve" expectations, for *P. intricata* it is markedly lower. The gills of *C. dipterum* perform complicated respiratory movements at the rate ranging from about 350 to 750 per minute. No relationship between these movements and environment conditions was found out. Regulation consists in the change of the percentage of time occupied by ventilation. It increases with temperature and as the oxygen concentration decreases. Respiratory movements of *P. intricata* consist in the "bendings" of the whole body; their frequency increases as temperature rises up and oxygen concentration falls down. Their maximum observed frequency was 91 movements per minute.

It is suggested that the difference in the resistance of both species to high temperatures and low O_2 concentrations is connected, among other things, with the different efficiencies of their ventilatory systems.

7. STRESZCZENIE

Przeprowadzono eksperymenty laboratoryjne na eurytermicznym gatunku *Cloeon dipterum* (Ephemeroptera) i stenotermicznym gatunku *Perlodes intricata* (Plecoptera). Stosowano po 15 kombinacji tlen/temperatura (3 temperatury i 5 stężeń tlenu), każdą kombinację powtarzano 10-krotnie. Badano: letalne stężenia tlenu, przeżywalność, zużycie tlenu i częstotliwość ruchów oddechowych.

Larwy *C. dipterum* w niskich stężeniach tlenu żyją dłużej niż larwy *P. intricata*. Larwy *C. dipterum* mogą wyczerpywać tlen do wartości bardzo niskich, np. aż do 0.02 ml O_2/l w 5.5°C. Wyczerpują one tlen do wartości niższych niż *P. intricata* w tych samych temperaturach i wyjściowych stężeniach tlenu. Q_{10} larw *C. dipterum* jest zgodne z oczekiwanym z „krzywej normalnej" Krogha, zaś larw *P. intricata* — wyraźnie niższe. Skrzelotchawki *C. dipterum* wykonują skomplikowane ruchy oddechowe o częstotliwości rzędu 350–750 ruchów/min. Nie stwierdzono zależności tych ruchów od warunków środowiskowych. Regulacja polega na zmianie procentu czasu zajętego na wentylację. Jest on tym wyższy, im wyższa jest temperatura i im mniej tlenu zawiera woda. Ruchy oddechowe *P. intricata* polegają na „przysiadach" całego ciała; ze wzrostem temperatury i ze spadkiem zawartości tlenu w środowisku częstotliwość ruchów oddechowych wzrasta. Maksymalna zaobserwowana częstotliwość wynosi 91 ruchów/min.

Przypuszcza się, że różnica w odporności na wysokie temperatury i niskie zawartości O_2 związana jest u tych gatunków m.in. z różnicą efektywności ich systemów wentylacyjnych.

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Cena zł 17.—

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