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The journal publishes original works reporting experimental results, descriptive works and theoretical investigations in every sphere of hydrobiology. The article must contain original research not already published and which is not being considered for publication elsewhere. Papers will be published in the official Congress languages of Societas Internationalis Limnologiae (at present: English, French, Italian and German).

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THE PRIMARY PRODUCTION OF SZCZECIN BAY

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ABSTRACT

The data on the primary production of Szczecin Bay were obtained with the help of dark and light bottles method in the years 1964-1968. The seasonal changes of the production and destruction rate at various depths were analysed, as well as the differences in the production rate related to the water temperature and the extent of insolation. The gross yearly primary production of the bay was estimated.

CONTENTS

1. INTRODUCTION

The Szczecin Bay is a very interesting object for hydrobiologists. Although numerous observations allow to expect there very high production there is a complete lack of data concerning its primary production. One of the indexes of such production is oxygen oversaturation of bay's water, observed every year in spring and summer. The oxygen saturation occasionally reaches 190%. The other index is high biomass of phytoplankton, which in some years reached 760 ml under 1 m^2.

Estimation of production possibilities of Szczecin Bay is very important because of the constant intensification of commercial fishing in this area. The photosynthetic intensity is a good indicator of the production capacity of a water body. The phytoplankton is a direct food of certain fish species for a long time, or only during their larval development.

2. MATERIAL AND METHODS

The material concerning the seasonal changes of production rate was collected in the years 1964-1966. Apart from that two additional control series were made in May 1968.

The investigations were carried out with the help of light and dark bottles method, widely used and described in detail by Winberg (1960). This is a simple

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method, which allows to estimate not only the rate of oxygen production, but also the oxygen consumption for the metabolic processes. However, the quantitative estimation of organic matter produced in the unit of time on the basis of results obtained with the help of oxygen method is only approximate, and the results should be analysed carefully and critically.

The primary production measurements were made on two sites: one located in the central basin of the Szczecin Bay (site A), the other in the near shore region of the northern part (site "Wucyk"). Site A was representative for the central basin of the Szczecin Bay, but the results from site "Wucyk" cannot be transferred uncritically for the whole near shore zone of this water body. The latter was located in the mouth of the bay region, where the fluctuations of the salinity, not large but fairly frequent, could influence the particular plant and animal associations.

Only three series of measurements were made in 1964: in May, July and the first decade of September. In 1965 and 1966 the measurements were carried out from the end of April or the beginning of May till mid-November, once or twice a month.

On each of the sites the measurements were made at the following depths (meters from the surface):

site A — in 1964: 0.5, 2.5 and 5 m deep, in 1965 and 1966: 0.5, 1.5, 2.5 and 5.5 m deep;

site "Wucyk" — in 1964: 0.5 and 2.5 m deep, in 1965 and 1966: 0.5, 1.25 and 2.0 m deep.

In order to estimate the daily production in the whole water column under 1 m^2 all values from particular water layers were summed up.

Two pairs of bottles were exposed on each depth, previously filled with water from the same depth. The exposition time was 24 hr. The bottles were of 300 ml capacity, the dark ones made of opaque glass were additionally kept in black rubber protections.

The plankton was sampled with the Patalas sampler used for biomass determination, the water temperature was measured, and water samples were collected for the oxygen and chloride content at the beginning of each experiment.

Several additional control measurements were made apart from the mentioned ones. The every several days observations in various insolation conditions were made in September 1965 and two series of experiments with bottles exposed in two successive days in May 1968. These experiments were carried out in order to determine the differences of the production rate in short time intervals caused by various factors (e.g. the differences in solar radiation). The data on the solar radiation were supplied by the Laboratory of the State Institute of Hydrology and Meteorology in Świnoujście, and I would like to express my gratitude to the Director and the staff of this Institute. Unfortunately these data only estimate the number of hours with full sun in a day, and it is not always proportional to the quantity of solar energy reaching the water surface during a day.

Several observations on the share of nannoplankton in the total primary production were made also in 1965 and 1966. Therefore a pair of bottles on each depth was filled with water filtered through the bolting cloth No. 20 which stopped so-called net plankton, while the other pair of bottles was filled with non-filtered water. After 24 hr exposure the results were compared.

3. GROSS PRIMARY PRODUCTION AND ITS SEASONAL CHANGES

The production processes in Szczecin Bay begin in April, and they finish in the first or second half of November. The intensity of these processes is, however, very variable (Fig. 1).

In early spring (April and first half of May) the oxygen production in the whole water column does not exceed 2 g/m^2, and occasionally it does not even reach 1 g/m^2. This production varies from 2.96 to 10.75 g O_2/m^2 in the central part of Szczecin Bay from mid-May to the end of

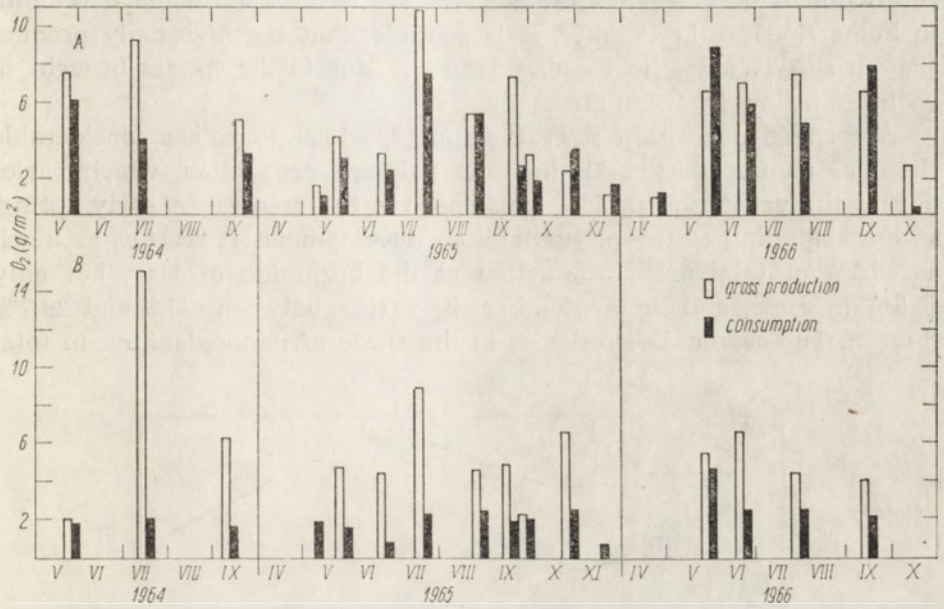

Fig. 1. The seasonal changes of daily production and consumption of oxygen in the water column under 1 m² in 1964-1966. A — central part of Szczecin Bay; B — near shore region of Szczecin Bay

September. The mean daily production in this period is 6.03 g O_2/m^2. The highest values reaching 10.75 g O_2/m^2 in the central part, and 14.97 g O_2/m^2 in the near shore part were observed every year in July. The primary production rate in the central part of water decreases beginning from October. In October in the years 1965-1966 it was from 2.36 to 2.56 g O_2/m^2/day and in November — from 0 to 0.89 g O_2/m^2/day.

Certain differences among the values of primary production measured the same day in the central part or in the near shore part were observed. The rhythm of seasonal changes of primary production in both these regions is slightly different. Although the highest rate of production in both regions is observed every year in July, the photosynthesis intensity in the near shore part is still high in October and sometimes even higher than in September, while in the central part the production rate considerably slows down in October. As already mentioned, site B was located in the northern part of the bay, where the inflow of Baltic water is the greatest (Wiktor and Zembrzuska 1959). Zembrzuska (1962), while analysing the species composition of phytoplankton, several times found there baltic species, which do not occur in the central part. The plankton biomass usually decreases in the central part of the Szczecin Bay in October, due to the inhibition of production processes. However,

the diatomes of the genus Chaetoceros reach their autumnal maximum in Pomorska Bay in October. It is possible, that the high daily production on site "Wucyk" in October 1965 was due to the species brought in with the inflow from Pomorska Bay.

According to several observations in 1965-1966, there is a considerable share of nannoplankton in the total primary production, which varies during the vegetation period. This share is the greatest in early spring, at the beginning of the phytoplankton development. It reaches as much as 88.2% of total gross production at the beginning of May, but only 46.8% in June, and in September it varies between 43.2 and 28.7% (Fig. 2). The seasonal differences in the share of nannoplankton in total

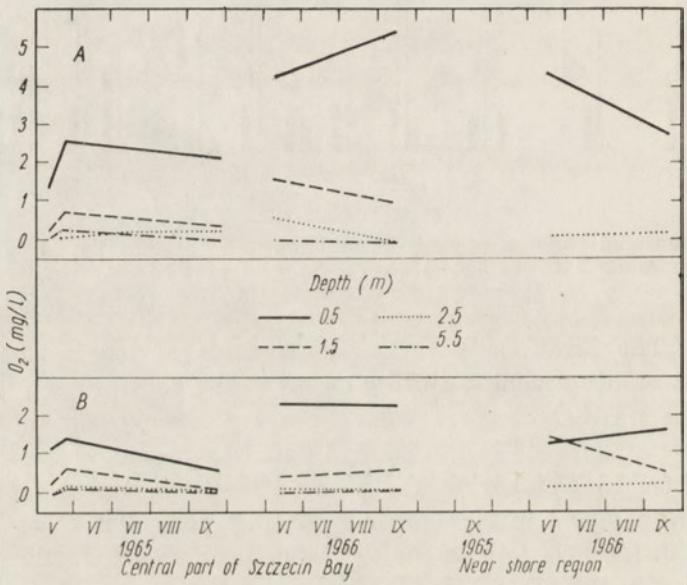

Fig. 2. The share of nannoplankton in daily oxygen production. A — total phytoplankton (not filtered sample); B — nannoplankton (filtered sample)

primary production were also observed by Czeczuga et al. (1968) in River Płoska, where at the depth of 0.5 m this share varied from 98.3% in June to 36% at the end of August.

The values of daily gross production in the water column under 1 m² result from the daily production in particular water layers. The production rate is not equal in the whole column. In waters of Szczecin Bay the photosynthetic activity decreases rapidly as the depth increases. The highest production was generally at the depth of 0.5 m. It varied from May to September from 1.30 to 5.50 mg O_2/l; and the average for this

period was 3.35 mg O_2/l (Fig. 3). A well marked decrease of the production rate was found at the depth of 1.5 m. Daily production in this depth varied from 0.2 to 3.1 mg O_2/l (1.06 mg O_2/l on the average), and from 13 to 71% (32% on the average) of daily gross production for the same

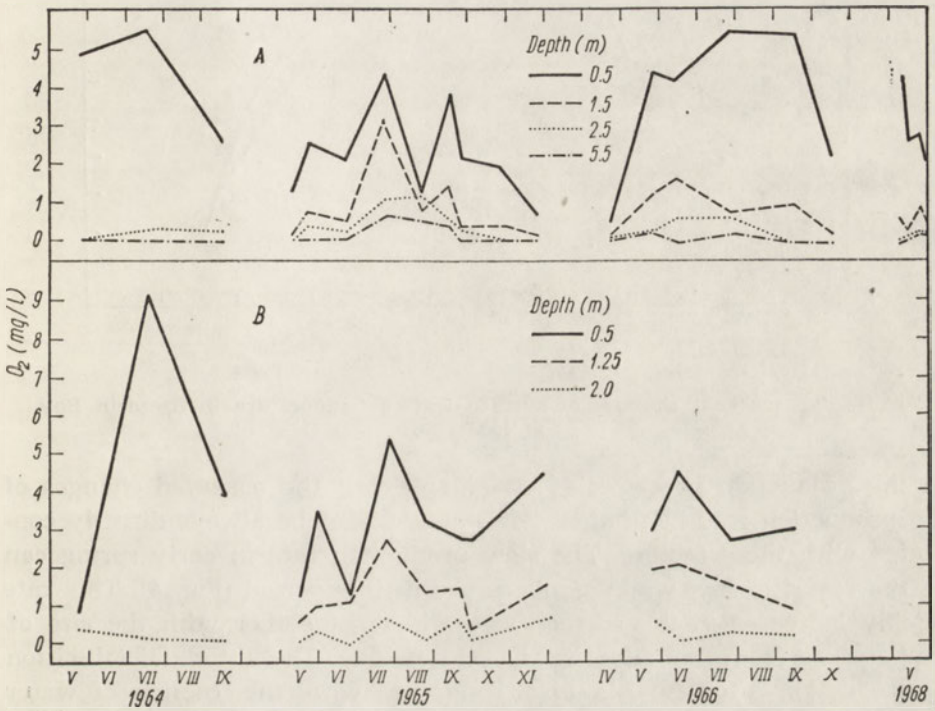

Fig. 3. The seasonal changes of oxygen production on particular depth layers. A — central part of Szczecin Bay; B — near shore region of Szczecin Bay

day at the depth of 0.5 m. In deeper layers, below 1.5-2.0 m the photosynthetic activity is very small, although occasionally the production processes take place in the whole water column, from the surface to the bottom in the central part.

Thus the main production zone in this water body is the near surface layer from 0 to 2.0 m deep. This layer covers 39 to 100% (78.7% on the average) of total gross primary production of the water column under 1 m^2 in the central basin.

The primary production rate depends on many factors, e.g. the water temperature, quantity of solar energy reaching the water surface, the composition and number of phytoplankton, nutrients content and many others.

The influence of water temperature and the degree of its insolation

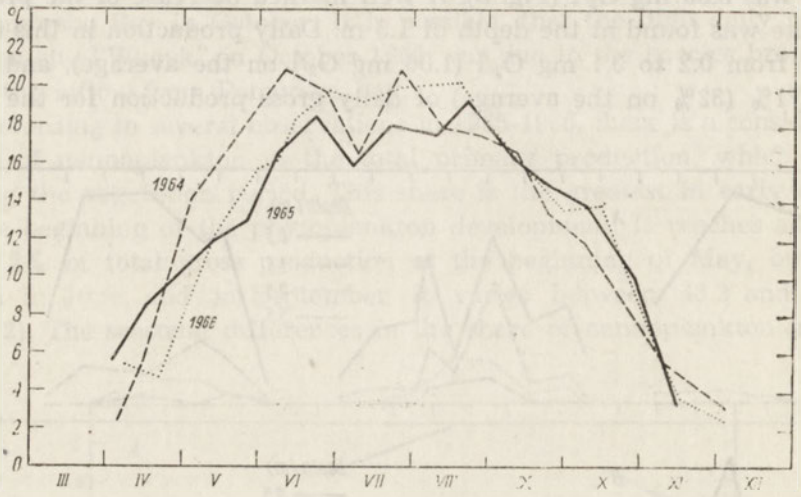

Fig. 4. The seasonal changes of surface waters temperature in Szczecin Bay in 1964-1966

on the production processes is undeniable, but the observed changes of the production rate in the body of water cannot be always directly connected with these factors. The slow production rate in early spring can be the result of low water temperature in this period (Fig. 4). This rate quickly increases in the surface water layers together with the rise of temperature from mid-April to the end of May. The autumnal inhibition of production processes is also connected with the cooling of water masses (end of October-November). In this period also important are the changes in solar radiation, i.e. smaller number of sun hours per day, and change of the angle of radiation on the water level.

Comparing the production data of the same months in successive years it can be noticed, that the lowest daily values were obtained in 1965, when the water temperature was slightly lower than in respective months in 1964 and 1966. However, there was no direct correlation between the water temperature and the daily production values. For an example, the highest values of daily production were usually in July — the period of a short lasting temperature decrease observed nearly every year. Also the production per day in September 1966 was almost equal to that in July this year (of the 0.5 m depth it was 5.45 and 5.50 mg O_2/l, respectively), however the differences of water temperature were quite considerable in the days of measurements (20°C in July and 16.2°C in September). Generally speaking, the water temperature — within certain limits — is not the only factor controlling the production rate.

Two observations were made in order to determine the range of changes of the production rate due to water insolation in September 1965. These observations were carried out in intervals, the first one was on the 23-24th September on a sunny day (9.6 hr of full sun), the second on 28-29th September on a cloudy day (3.8 hr of full sun).

The production rate on a sunny day was considerably higher than on a cloudy one. On cloudy day production on particular layers was only 29.0 to 53.7% of the production on sunny day, and the greatest difference in these rates was for the depth of 1.5 m. The compensation point was at the depth of 2.0 m on a cloudy day, while on a sunny one the oxygen production exceeded oxygen consumption in all layers (Fig. 5).

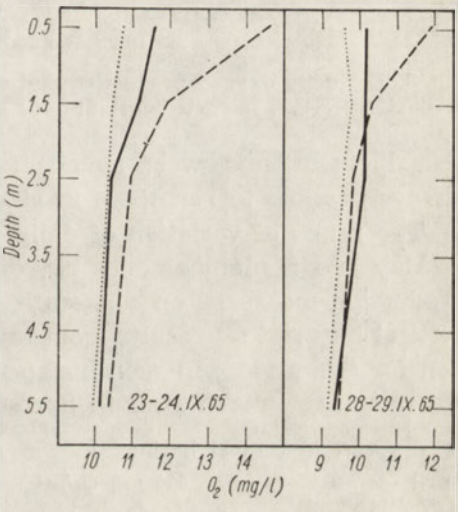

Fig. 5. The differences in oxygen production rate dependent on the insolation (23-24th September 1965 — sunny day, 28-29th September 1965 — cloudy day). — — — — — — oxygen production, ... oxygen consumption, ———— oxygen content in control sample

The results of measurements on consecutive days in May 1968 show that even in similar light conditions the production rate can considerably vary from one day to another. Both series of measurements were made on cloudy days. The differences in daily production measured on consecutive days were 54 to 77%, with the highest ones for the level of 1.5 m (Fig. 6). The reasons for these variations can be: e.g. quick depletion of nutrients, momentary changes in the CO_2 content, differences in the physiological state of algal cells, and many other ones which could not be included in present investigations. The above measurements were mainly carried out to find out to what extent all the collected results may be useful to analyse the seasonal variations of production rate, and to calculate the mean total yearly production. The measurements, as

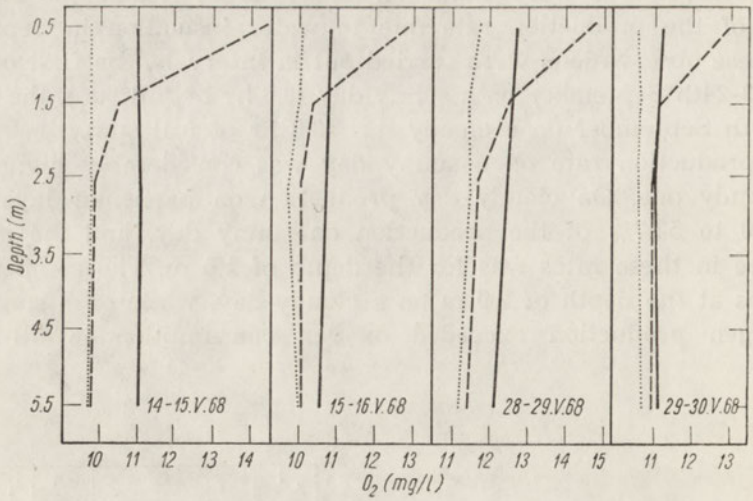

Fig. 6. The daily oxygen production and consumption in May 1968. — — — — oxygen production; ..... oxygen consumption; ——— oxygen content in control sample

already mentioned, were made once or twice a month, and it was not always possible to run them in similar insolation conditions.

The range of variation of daily primary production between the end of May and September does not differ from the range of variations of production measured on consecutive days of one month. Thus the arithmetical mean of all results from this period is the average daily production for this period. In such instance the average daily production in the water column under 1 m² in Szczecin Bay is:

a. in central basin:		b. in the near shore part:	
April	950 mg O_2/m^2	May-June and August-September	4260 mg O_2/m^2
May-June and August-September	5215 mg O_2/m^2	July	9375 mg O_2/m^2
July	9250 mg O_2/m^2	October	6560 mg O_2/m^2
October	2760 mg O_2/m^2		
November	445 mg O_2/m^2		

Assuming that 75% of the Szczecin Bay area is deeper than 2.5 m, the average daily production of the whole Polish part of Szczecin Bay in the water column under 1 m² is as follows:

April	950 mg O_2/m^2
May-June and August-September	4975 mg O_2/m^2
July	9300 mg O_2/m^2
October	2760 mg O_2/m^2
November	445 mg O_2/m^2

Thus the average daily primary production in Szczecin Bay from May to October is within the limits given by Winberg (1960) for

"relatively shallow, homothermic waters with well developed phytoplankton, very similar to typical eutrophic inland bodies of water" — and this applies to waters of this bay.

4. OXYGEN CONSUMPTION

In the water bodies apart from the production processes the destruction processes also take place, i.e. the decomposition of organic matter produced in this body of water and of that of allochtonous origin. The oxygen consumption accompanies these processes. They are generally determined as destruction, and this concept covers the process of oxygen consumption during the mineralization of non-living organic matter, and also the consumption during the metabolic processes of plants and animals. Contrary to the production processes, i.e. photosynthesis, which takes place only in light, the oxygen consumption takes place all day. In the oxygen method, the consumption is a difference in the oxygen content between the control sample and dark bottle after 24 hr exposure.

In order to determine the daily destruction in the water column under 1 m^2 the values for particular water layers were summed — analogically as for the estimation of daily primary production. Daily oxygen consumption varied in the Szczecin Bay from 990 mg O_2 to 9660 mg O_2/m^2, and certain seasonal changes were noticed. This consumption was low in early spring (April-first half of May) and amounted to 990-1010 mg O_2/m^2 (Fig. 1). The maximal destruction rate occurred usually in the second fortnight of May. The daily oxygen consumption varied within the limits of 2060-7670 mg O_2/m^2 from June to September. In November this rate clearly decreased, i.e. in 1965 it was only 1480 mg O_2/m^2. This lowering in November is probably connected with the fall of water temperature. The high rate of oxygen consumption in May is probably due to the increase of mineralization rate of non-living organic matter, caused by quick warming of water. It is possible that during the autumn and winter months, when the mineralization is slow due to the low temperature, there is a considerable accumulation of non-living organic matter which decomposes quickly after the spring increase of water temperature. This assumption can be supported by high values of daily oxygen consumption in May every year, however the plankton biomass was then still low, thus the respiration of zooplankton and phytoplankton could not cause such a great consumption.

Although the oxygen consumption takes place in all water layers, it is not equal in the whole water column (Fig. 7). However, the vertical differences observed at various depths at the same day are smaller than the differences in the production rate. Apart from that, certain water

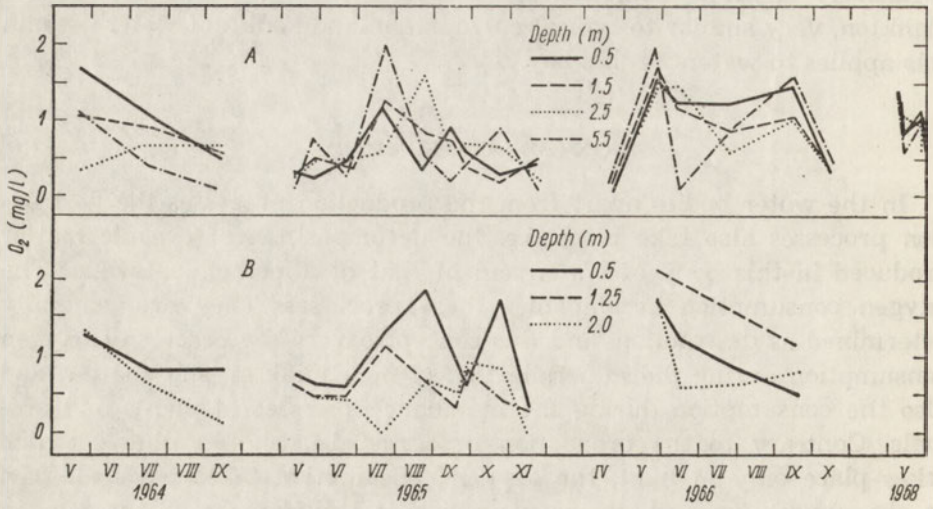

Fig. 7. The seasonal changes in daily oxygen consumption at particular depth layers. A — central part of Szczecin Bay; B — near shore region of Szczecin Bay

layers were distinguished, where the production was usually the highest — the maximal daily consumption of oxygen varied in depth and time. Usually during the vegetation season (May-September) the highest destruction rate was observed in the near surface water layers, but in early spring (April) and autumn (October and November) — in near bottom waters. However, it happened on several occasions in the period of May-September, that the destruction rate was higher in the near bottom water than near the surface. This occurred usually during the quick decrease of phytoplankton biomass.

5. PRODUCTION AND DESTRUCTION

The comparative data on daily oxygen production and consumption in the water column under 1 m² are presented in Fig. 1. Oxygen production during the whole period of investigations was usually higher in the near shore region (site "Wucyk") than its consumption, although the ratio of production to destruction varied within a year. Oxygen consumption ranges from 13.3 to 89.6% of its daily production in this region.

The oxygen consumption in the water column under 1 m² was in several cases higher than its daily production in the central part of the bay. This situation was observed in early spring at the beginning of the vegetative season, and usually near the end of this season at the time of production inhibition (October and November). However, during the

vegetation period (May-September) daily oxygen consumption was occasionally higher than production. This happened e.g. in the second fortnight of May in 1966 and 1968, and also in the second fortnight of September 1966. Such "minus oxygen ballance" in the water column under 1 m^2 is not necessarily due to the decrease of daily rate of production. It rather results from the increase of the destruction rate. The production rate was considerably high on e.g. days in May and September 1966, when the oxygen consumption was 152 and 118% of its daily production, respectively.

The ratio of xygen consumption to its production in particular water layers is different than in the whole water column.

In the near surface water the oxygen production is usually higher than the consumption from April to November. In deeper waters the situation is inverse. This is due to the decrease of oxygen production rate with the increase in depth, but the destruction rate in the whole water column is more uniform in Szczecin Bay. The compensation point is here at the depth 1.5 to 2.0 m. This is very rare that oxygen production exceeds its consumption in all layers, as e.g. on 21st September 1965. The daily oxygen consumption in surface water is usually from 26.7 to 89.6% of its daily production (46% on the average).

6. THE YEARLY PRIMARY PRODUCTION

In one of the above chapters the mean daily primary production rate for particular seasons is expressed in mg O_2/m^2. Assuming after W i n b e r g (1960) that 1 mg dry weight of plankton is equal 1.324-1.618 mg of oxygen (with the assimilation coefficient 1.25) it is possible to estimate the yearly primary production of Szczecin Bay in mg dry mass per the water column under 1 m^2, if the mean seasonal daily production values are multiplied by the number of days in this season, and the products are added. Thus obtained value is 612.5-761.3 g dry mass per 1 m^2 of the water surface (Table I).

Table I. The calculation of total yearly gross primary production in Szczecin Bay

Months.	Mean production (mg $O_2/m^2/24$ hr)	No. of days	Total production	
			mg O_2/m^2	mg dry wt/m^2
IV	950	30	28,500	17,610.0- 21,525.0
V-VI, VIII-IX	4975	122	606,950	375,089.0-447,415.0
VII	9300	31	288,300	178,181.8-217,744.0
X	2760	31	85,560	33,349.8- 64,619.5
XI	445	30	13,350	8250.0- 10,035.0
	Total	244	1022,660	612,480.6-761,338.5

These values should be treated as estimative ones, carefully, because they indicate rather the order of magnitude. The value of assimilation coefficient is not equal for all phytoplankton species, and the carbon content in dry phytoplankton mass also varies considerable. These data quoted by various authors are quite different.

The net production, i.e. gross production minus the amount of organic compounds used during production for metabolic processes, is much more difficult to estimate. Thus the amount of oxygen produced by photosynthesis within a day minus the amount of oxygen used in the same time for phytoplankton respiration is the index of net primary production. Unfortunately, the oxygen method gives the total daily oxygen consumption, i.e. the consumption for the metabolism of phyto- and zooplankton as well as the mineralization of non-living organic matter produced in the body of water or that of allochtonous origin. The latter can be of great significance in the waters of Szczecin Bay as the large amounts of this matter are brought in with the inflow of the Odra River. The yearly inflow of Odra amounts to 15.05 km^3 (Mikulski 1964), mean content of organic suspension is [1] 2.38 mg/l, and so 35,820 tons of organic suspension is brought to Szczecin Bay during a year. Therefore the oxygen consumption due to the mineralization processes of organic matter produced outside this body of water must be quite big.

Unfortunately the determination of the share of particular processes in the total daily oxygen consumption is impossible. On the basis of data of Vollenweider and Ravera (1958) a rough calculation of oxygen consumption due to the respiration of plankton crustaceans was made assuming that the mean oxygen consumption at a temperature 16-20°C is 4.1 γ for Cladocera (represented mainly by genus Daphnia and Bosmina) and for Copepoda — 1.4 γ per individual. These data are presented in Table II. These calculations show that the daily oxygen consumption due to the respiration of crustaceans is from 3.3 to 14.3% in the Szczecin Bay's waters, while in 1964, during an intensive development of zooplankton it amounted up to 73.5% of total daily destruction. These data are only approximate ones of course, and it is difficult to say how they differ from the real ratio in the bay's waters. It should be also remembered that crustaceans are only one of the zooplankton components and a not numerous one.

There are many data in the literature concerning biological production which take into account the share of plant respiration in the gross primary production. Odum (1954) e.g. for Silver Spring quotes 57.5%, Winberg (1960) assumes that this value is 15% on the average. The

[1] According to "The materials to the monograph on hydrobiology of Szczecin Bay", Part II (in Polish). Oddział Morski PIHM, Gdynia 1964.

Table II. The seasonal changes in daily oxygen consumption for the respiration of plankton crustaceans

Date	No. of ind./m²		Daily oxygen consumption mg/m²			% of total destruction
	Cladocera	Copepoda	Cladocera	Copepoda	Total	
25.V.64	30,000	50,000	123.0	70.0	193.0	3.3
9.VII.64	426,000	151,000	1746.6	211.4	1958.0	50.3
10.IX.64	574,000	48,000	2353.4	67.2	2420.6	73.5
12.V.65	2000	17,000	8.2	23.8	32.0	3.3
25.V.65	24,000	110,000	100.4	15.4	115.8	3.8
22.VI.65	16,000	13,000	65.6	18.2	83.8	3.9
20.VII.65	113,000	22,000	463.3	30.8	494.1	6.6
25.VIII.65	168,000	56,000	688.8	78.4	767.2	14.3
24.IX.65	72,000	9000	295.2	12.6	307.8	12.3
27.X.65	68,000	13,000	278.8	18.2	297.0	8.5
16.XI.65	20,000	10,000	82.0	14.0	96.0	6.4

result of O d u m seems to be too high for the studied region, as in the near shore part the daily oxygen consumption rarely exceeds 50% of its daily production.

If we would assume the losses for plant respiration as 15% on the average, the mean yearly net production of Szczecin Bay could be estimated as 520.6 to 647.1 g dry mass/m².

H ü b e l (1966) presented the yearly production of several brackish bodies of water from Ruggia in g C/m² on the basis of investigations in 1962 and 1963. These data allowed to calculate the quantity of organic substances produced in these bodies of water and they were compared with the values from Szczecin Bay. To calculate H ü b e l's data it was assumed after W i n b e r g (1960), that 1 mg dry mass of plankton equals 0.45 mg C.

The data presented in Table III show that the yearly primary production in Szczecin Bay is lower than in the most productive water bodies from Ruggia. This is somehow strange, as there are many data showing that the waters of the bay are if no more productive than Ruggia's water bodies then at least not less. This is proved by a large inflow of

Table III. Yearly primary production of brackish water bodies from Ruggia (acc. to H ü b e l 1966)

Yearly production	Water body				
	Kl. Jasmun der Bodden	Gr. Jasmun der Bodden	Breeger Boddan	Breetzer Bodden	Rassover Strom
g C/m²/year (H ü b e l 1966)	900	670	420	235	110
g dry wt/m²/year	1980	1474	924	743	242

fresh waters with large amounts of nutrients to Szczecin Bay, while Ruggia's water bodies are supplied with fresh water only to a small extent. Also higher salinity of the latter creates unsuitable environmental conditions for the development of several freshwater species which develop in masses in Szczecin Bay. The Szczecin Bay is also characterized by higher fish production.

However, all estimations of the amount of produced organic matter based on the results of oxygen method should be approached very carefully and critically. Also these factors cannot be always fully justified, as they considerably differ in the papers of many authors. The best conclusions may be reached when applying the same method of determining the primary production rate for all bodies of water.

7. SUMMARY

The measurements of primary production rate carried out in the Szczecin Bay in 1964-1968, using oxygen method of light and dark bottles, showed, that the production processes took place there from April till the first fortnight of November. The mean daily production rate changed as following:

Months	Central basin (mg O_2/m^2)	Near shore region (mg O_2/m^2)
April	950	no data
May-June	5215	4260
July	9250	9375
August-September	5215	4260
October	2760	6560
November	445	0

The highest daily production rate was found at the depth of 0.5 m from the surface. The production at 1.5 m amounted to 13-71.6% (32% on the average) of the one at 0.5 m. In deeper water layers the production rate was much lower. The share of nannoplankton in total phytoplankton production was 88.2% in May, 46.8% — in June, and 28.7 — 43.2% in September.

The oxygen consumption (destruction) was very low during the cold seasons, but it increased considerably in the warm ones. The highest consumption was observed in the second fortnight of May. The differences in the destruction rate in particular layers of water are lower than these of production. The highest oxygen consumption during the warm season was observed in near surface waters, but during the early spring and autumn in the near bottom ones. Oxygen production near the surface is greater than destruction, while near the bottom it is just the opposite. The compensation point in waters of Szczecin Bay is at the depth of 1.5 to 2.0 m.

Assuming after Winberg (1960) that 1 mg O_2 is equal to 0.618-0.792 mg dry mass of plankton, the gross yearly production of Szczecin Bay was estimated as 612.5-761.3 g dry mass per 1 m^2 of the water surface.

8. STRESZCZENIE

Obserwacje tempa produkcji pierwotnej fitoplanktonu przeprowadzone na Zalewie Szczecińskim w latach 1964-1968 metodą tlenową tzw. „jasnych i ciemnych butelek" wykazały, że procesy produkcyjne w tym zbiorniku trwają od kwietnia do

pierwszej połowy listopada. Średnie dobowe tempo produkcji zmieniało się następująco:

Miesiące	Basen Centralny (mg O_2/m^2)	Rejon przybrzeżny (mg O_2/m^2)
Kwiecień	950	brak danych
Maj-czerwiec	5215	4260
Lipiec	9250	9375
Sierpień-wrzesień	5215	4260
Październik	2760	6560
Listopad	445	0

Największe tempo produkcji dobowej obserwowano w warstwie wody 0,5 m od powierzchni. Produkcja mierzona na poziomie 1,5 m stanowiła od 13 do 71,6% (średnia 32%) produkcji warstwy 0,5 m. W głębszych warstwach wody tempo produkcji było już nieznaczne. Udział nannoplanktonu w procesie produkcji pierwotnej całego fitoplanktonu stanowił w maju 88,2%, w czerwcu 46,8%, we wrześniu od 28,7 do 43,2%.

Zużycie tlenu (destrukcja) przez organizmy planktonowe było w chłodnych porach roku nieznaczne, silnie wzrastające w ciepłych porach roku. Największe zużycie obserwowano w drugiej połowie maja. Różnice w tempie destrukcji w poszczególnych warstwach słupa wody są znacznie mniejsze niż obserwowane przy produkcji. W ciepłej porze roku największe zużycie tlenu obserwowano w przypowierzchniowych warstwach wody, natomiast wczesną wiosną i jesienią — w warstwach przydennych. W warstwach przypowierzchniowych produkcja O_2 dominuje nad destrukcją, w przydennych stosunek ten jest odwrotny. Punkt kompensacyjny leży w wodach Zalewu Szczecińskiego na głębokości 1,5-2 m.

Przyjmując za Winbergiem (1960), że 1 mg O_2 jest równoważny 0,618-0,792 mg suchej masy planktonu, oszacowano roczną produkcję brutto fitoplanktonu Zalewu Szczecińskiego na 612,5-761,3 g suchej masy na 1 m^2 powierzchni zbiornika.

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ALLELOPATHY AMONG THE AQUATIC PLANTS

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ABSTRACT

The pot experiments were carried out on the interrelations of the aquatic plants. The decrease of reed production was generally accompanied with the increase of the production of associated plant species, and inversely. It was found, that the type of soil modifies the growth of monospecies culture, and it influences the character of interrelations among the plants in mixed cultures.

CONTENTS

1. INTRODUCTION

The numerous observations of allelopathy among the terrestrial plants allow to expect the occurrence of such a phenomenon among the aquatic plants. There are several papers dealing with the chemical interrelations of the terrestrial plants (Nowiński 1961, Rademacher 1957, Börner 1968 — the reviews). There is, however, a general lack of papers dealing with allelopathy among the aquatic plants (McNaughton 1968).

This paper aimed at investigation of the allelopathy phenomenon as related to reed and its associated species.

2. METHODS AND MATERIAL

The main methods of studying allelopathy are the field observations, cultures of various plant species, isolation of allelopathic substances and control of their action. The generally applied method is the bi-species culture (Mann and Barnes 1950, 1952, Börner 1958), and the culture on the extracts from the investigated plants (McNaughton 1968, Patrick and Koch 1958).

The introductory culture experiments in 1968 were carried out in order to find which plant species can have an influence on the growth of reed. This allowed to plan the experiments for the season of 1969.

All cultures were run outdoor. The soil for each series of experiments was sieved on the sieve with the mesh size 1 mm and well mixed. The soil in the pots was covered by a thin layer of tap water, the level of which was controlled. The following pot cultures were established:

Experiment A: a culture of *Phragmites communis* Trin with *Typha latifolia* L. on sand, peat, garden soil, profundal sediment from an eutrophic lake.

A total of fifteen plastic buckets of the capacity 5 l each were used for each type of the soil, i.e. for one series. Each bucket contained 5.3 kg of soil. The plants were cultivated in the following way: buckets No. 1 to 5 had each 10 seedlings of *Phragmites communis*, from No. 6 to 10 — 5 seedlings of *Phragmites communis* and 5 of *Typha latifolia*, No. 11 to 15 — 10 seedlings of *Typha latifolia*. A total of 60 buckets were used for all soils. The experiment was carried out from the 15th May to 30th September 1969. The seeds were germinated in the sand germinating bed. Seedlings about 1 cm long were planted in the buckets.

Experiment B: a common culture of various plant species on the same soil, i.e. on the lake mud. The following sets were cultivated: *Phragmites communis* with *Schoenoplectus lacustris* (L); *Phragmites communis* with *Equisetum limosum* L.; *Schoenoplectus lacustris* with *Equisetum limosum*. Each plastic bucket of the capacity 5 l contained 3.6 kg of the lake mud. The plants were cultivated in the following way: buckets from No. 1 to 5 — 4 plants of *Phragmites communis*, No. 6 to 10 — 2 plants *Phragmites communis* and 2 *Schoenoplectus lacustris*, No. 11 to 15 — 4 plants of *Schoenoplectus lacustris*, No. 16 to 20 — 2 plants of *Phragmites communis* and 2 *Equisetum limosum*, No. 21 to 25 — 4 plants of *Equisetum limosum*, and No. 26 to 30 — 2 plants of *Equisetum limosum* and 2 of *Schoenoplectus lacustris*. A total of 30 buckets were used. The planted seedlings of *Phragmites communis* were one year old, and about 10 cm long. *Schoenoplectus lacustris* and *Equisetum limosum* were brought from a lake, reasonably well developed; particular plants were planted with a small part of rhizome. The experiment was carried out from the 26th June to 30th September 1969.

Experiment C: a common culture of *P. communis* and *Carex Hudsoni* (Bennet) on the garden soil. A series was composed of 15 earthen pots of the capacity 10 l with 11.4 kg of soil in each. The distribution of plants was as follows: in pots No. 1 to 5 — 4 *P. communis* plants, No. 6 to 10 — 2 *P. communis* and 2 *C. Hudsoni*, No. 11 to 15 — 4 *C. Hudsoni* plants. The reed seedlings were one year old, about 25 cm long, and they were planted with a small part of rhizome. *C. Hudsoni* was planted in small clumps of plants of the same size, separated from the sedge growing on the shores of Lake Mulik. The experiment was carried out from the 26th June to 30th September 1969.

Experiment D: the culture of *P. communis* on the lake mud with the addition of aerial parts of *T. latifolia*, *T. angustifolia*, *Heleocharis palustris* (L) Sch., *Glyceria aquatica* (L). Wahlb, *S. lacustris* (L) Palla, *Acorus calamus* L. The cultures were kept in plastic boxes of the capacity 0.8 l. Each box contained 600 ml of soil composed of the mixture of mud and plant material dried and milled in the ball mill (the control experiment: mud without the addition of plant material). The mud was mixed thoroughly with the plant material placed in boxes and covered with water. Five 1 cm each reed seedlings were planted in each box. Group Da: The soil in each box composed of 300 ml of mud and 300 ml (about 10 g dry weight) of milled plant mass. The boxes contained: from No. 1 to 5 — mud with milled *T. latifolia*, No. 6 to 10 — mud with *T. angustifolia*, No. 11 to 15 — mud with *H. palustris*, No. 16 to 20 — mud with *G. aquatica*, No. 21 to 25 — mud with *S. lacustris*, No. 26 to 30 — mud with *A. calamus*, No. 31 to 35 — only mud (the control). Group Db: the soil in each box composed of 500 ml of mud and 100 ml (about 3 g dry mass) of milled plant mass. The boxes contained: No. 1 to 30 — as in the group Da, No. 31 to 35 — mud and milled stems of *P. communis*, No. 36 to 40 — mud and milled *P. communis* flowers, No. 41 to 45 — only mud (the control). A total of 80 boxes were used. Experiment Da was carried out from the 14th to the 30th July 1969, experiment Db — from the 31st July to the 10th October 1969.

In the experiments A, B and C the height of plants and the number of shoots were monthly estimated. Five highest plants were measured in each pot, then the mean height per pot was calculated and the mean for all repetitions. Whenever there was less than 5 shoots in one pot all shoots were measured. The aerial and underground plant parts from the experiments A, B and C were collected on the 30th of September 1969. The roots and rhizomes were separated from the soil by washing on the sieve with the mesh size 1 mm. All material was dried at 82°C for 48 hr and weighed. The obtained results were statistically analysed.

The estimation of production of one seedling in experimental conditions allows to compare the dependence of production on the type of soil, as well as the influence of other plants.

The aquatic emergent plants which develop from the seeds do not reach the

size of fully grown plants during the first year. In the experiment A the plants reached their maximum height already at the beginning of September but they did not grow more during this month (Table I).

Table I. The growth of plants (in cm) on mud (means from 5 highest shoots). Experiment A

Plant	Date				
	15.V	17.VI	17.VII	6.IX	30.IX
P. communis	1.0	5.4	38.6	57.3	56.5
T. latifolia	1.0	5.9	32.9	45.2	45.1

In experiment C last year seedlings were used, and the mean height of 5 highest shoots at the end of the vegetative season was 67.4 cm. Also in this case the plants reached their maximum and did not grow any further. This allows to compare the results of various series, as the growth was accomplished in all experiments.

The vegetation season of 1969 was characterized by low precipitations, quite a number of sunny days, and the mean summer temperature was higher than usual.

3. RESULTS

THE AERIAL PARTS

Experiment A

The final results of experiment A (Table II) show the tendency of reed to a considerable increase of the number of shoots in favourable growing conditions.

Ten seedlings cultivated on mud produced from 49 to 62 shoots. The mean from 5 repetitions — 55.6 shoots per 10 seedlings.

However, in the same conditions the number of shoots of the cattail increased only slightly (11-14 per 10 seedlings).

The mass of one shoot of reed in the culture on garden soil was 0.197 g, while one shoot of cattail — 1.02 g, i.e. 5 times more. The biomass of both species collected from one bucket shows smaller differences, as both the increase of number and the increase of single plant biomass are considered.

In the conditions of extremely poor substratum (washed sand) both reed and cattail were in very bad conditions. The increases were insignificant. A certain reduction of number was observed. 10 seedlings of the cattail produced in one pot only 5.8 shoots — nearly a half was reduced. The reed was more resistant — 10 seedlings produced 8.4 shoots per pot.

In good conditions the reed growing with cattail increased in number 2.5 times on garden soil, and 3.6 times on mud, while the relative in-

crease of the reed number without cattail was 3.3 times and 5.6 times, respectively.

The biomass of reed shoot growing with cattail on garden soil was more than two times lower than of reed without cattail. The biomass of a single shoot of reed on mud was 1.6 times lower in the presence of cattail than without it.

However, in both variants of the experiment the cattail was growing better with reed than without it. The biomass of one shoot in the mixed culture was 1.56 times higher on mud, and 1.74 times higher on garden soil.

The differences are even clearer when estimating the total plant production per 1 bucket (Table II).

The biomass produced by one seedling greatly depends on the type of soil. One reed seedling produces from 12.4 mg dry weight on sand to 964 mg on lake mud. One cattail seedling produced from 3.6 mg on sand to 1228 mg on garden soil. Thus the production range is great, and for reed it is nearly 78 times, depending on the soil, and even more for cattail i.e. nearly 340 times. The reed growing with cattail has a lower production, but that of cattail with reed is even better.

Experiment B

The results obtained in this experiment are presented in Table III. In this experiment 10 cm seedlings were used instead of younger ones, and therefore stronger plants were obtained. This is shown by the comparison of reed with those from experiment A. The reed shoots had twice higher mass (347 mg as compared with 170 mg from experiment A), although the height of shoots was even slightly lower.

The reed cultivated with bulrush or with horsetail had lower production than reed alone. One seedling from monospecies culture produced 6 shoots, from the culture with horsetail — 5 shoots, and with bulrush — 4.6 shoots. The height is also given in the same order — 50.7 cm reed alone, 46.6 cm reed with horsetail, 35.6 cm — with bulrush.

One seedling of reed cultivated without any other plants produced 2.07 g dry weight of aerial parts, but the one cultivated with bulrush produced in the same time 1.31 g dry weight, i.e. 37% less. The influence of horsetail was weaker, as the production decrease per one seedling was 12%. Both plant species cultivated with reed increased their biomass in the same time — bulrush 49%, and horsetail 20% as compared with the same species in their monospecies cultures. The bulrush cultivated with horsetail increased its biomass 8%, and at the same time the biomass decreased 11% per one seedling of horsetail.

Table II. The growth of reed and cattail on various soils. Experiment A — 15th May-30th September 1969

Type of soil	Plant species	Variant of culture	Number of shoots per pot		Mean height (cm)	Mean dry mass per pot (mg)	Mean dry mass of 1 shoot (mg)	Mean biomass produced by 1 seedling (mg)	The ratio of biomasses produced by 1 seedling of competing plant species $\frac{a_b}{a}$ or $\frac{b_a}{b}$
			initial	final					
Sand	*Phragmites communis*	a	10	8.4	9.5	124	15	12.4	1.13
	Phragmites communis	a_b	5	6.3	8.2	70	11	14.0	
	Typha latifolia	b_a	5	2.8	3.9	18	6	3.6	1.00
	Typha latifolia	b	10	5.8	3.9	36	6	3.6	
Peat	*Phragmites communis*	a	10	11.0	8.2	148	13	14.8	0.92
	Phragmites communis	a_b	5	6.8	7.8	68	10	13.6	
	Typha latifolia	b_a	5	4.6	5.6	82	18	16.4	1.82
	Typha latifolia	b	10	9.0	4.0	90	10	9.0	
Garden soil	*Phragmites communis*	a	10	33.2	47.5	6550	197	655.0	0.33
	Phragmites communis	a_b	5	12.4	33.0	1080	87	216.0	
	Typha latifolia	b_a	5	6.4	53.3	10,720	1675	2144.0	1.75
	Typha latifolia	b	10	12.8	49.2	12,280	959	1228.0	
Lake mud	*Phragmites communis*	a	10	55.6	57.3	9640	170	964.0	0.39
	Phragmites communis	a_b	5	18.0	39.4	1880	104	376.0	
	Typha latifolia	b_a	5	5.2	55.1	8280	1591	1656.0	1.40
	Typha latifolia	b	10	11.6	45.2	11,820	1020	1182.0	

a — *Phragmites communis* — monospecies culture;

b — *Typha latifolia* — monospecies culture;

a_b — biomass of *Phragmites communis* cultivated together with *Typha latifolia;*

b_a — biomass of *Typha latifolia* cultivated together with *Phragmites communis.*

Table III. The growth of various aquatic plants on mud.
Experiment B — 26th June-6th October 1969

Plant species	Variant of culture	Number of shoots per pot		Mean height (cm)	Mean dry mass per pot (mg)	Mean dry mass of 1 shoot (mg)	Mean biomass produced by 1 seedling (mg)	The ratio of biomasses produced by 1 seedling of competing plant species
		initial	final					
Phragmites communis	a	4	23.8	50.7	8280	347	2.07	
Schoenoplectus lacustris	b	4	11.4	67.4	7780	682	1.95	
Equisetum limosum	c	4	67.6	42.0	6960	103	1.74	
Phragmites communis	a_b	2	9.2	35.6	2620	285	1.31	$a_b/a = 0.63$
Phragmites communis	a_c	2	10.0	46.6	3670	367	1.83	$a_c/a = 0.88$
Schoenoplectus lacustris	b_a	2	8.4	74.6	5820	692	2.91	$b_a/b = 1.49$
Schoenoplectus lacustris	b_c	2	7.0	66.3	4200	571	2.10	$b_c/b = 1.08$
Equisetum limosum	c_a	2	30.0	42.0	4160	139	2.08	$c_a/c = 1.20$
Equisetum limosum	c_b	2	21.4	38.5	3100	144	1.55	$c_b/c = 0.89$

a — *Phragmites communis* — monospecies culture;
b — *Schoenoplectus lacustris* — monospecies culture;
c — *Equisetum limosum* — monospecies culture;
a_b — *Phragmites communis* cultivated together with *Schoenoplectus lacustris*;
a_c — *Phragmites communis* cultivated together with *Equisetum limosum*;
b_a — *Schoenoplectus lacustris* cultivated together with *Phragmites communis*;
b_c — *Schoenoplectus lacustris* cultivated together with *Equisetum limosum*;
c_a — *Equisetum limosum* cultivated together with *Phragmites communis*;
c_b — *Equisetum limosum* cultivated together with *Schoenoplectus lacustris*.

Thus the reed decreased its production in the presence of both bulrush and horsetail.

The horsetail production in the presence of bulrush was lower, but in the presence of reed — higher. The bulrush production increased in the cultures with reed and horsetail.

Experiment C

The results obtained in this experiment are presented in Table IV.

One year old seedlings used in this experiment gave the highest increase of reed production, as one seedling produced 5.15 g dry mass. This increase was mainly due to the increase of shoots number — 15.4 per seedling, as compared with 6 per seedling in experiment B. However, the dry mass of one shoot did not differ substantially from this value in experiment B.

Carex Hudsoni, the second species used in this experiment produced 8.6 g dry mass per seedling. This species had a visibly positive influence on the reed production, as a single seedling of reed growing with it pro-

Table IV. The growth of reed and sedge on garden soil.
Experiment C — 16th May-6th October 1969

Plant species	Variant of culture	Number of shoots per pot		Mean height (cm)	Mean dry mass per pot (mg)	Mean dry mass of 1 ind. (mg)	Mean biomass produced by 1 ind. (mg)	The ratio of biomasses produced by 1 seedling of competing species
		initial	final					
Phragmites communis	a	4	62.0	67.4	20,600	330	5150	1.52
Phragmites communis	a_b	2	34.2	76.5	15,600	460	7800	
Carex Hudsoni	b_a	2	×	45.4	11,460	×	5730	0.67
Carex Hudsoni	b	4	×	48.4	34,400	×	8600	

× — It was impossible to separate a single individual of *Carex Hudsoni*.

a — *Phragmites communis* — monospecies culture;

b — *Carex Hudsoni* — monospecies culture;

a_b — *Phragmites communis* cultivated together with *Carex Hudsoni*;

b_a — *Carex Hudsoni* cultivated together with *Phragmites communis*.

duced 7.8 g dry mass. The reed in monospecies culture produced 5.15 g. Thus in the mixed culture the reed production was over one and a half times higher that in the reed monospecies culture. This production increase was caused mainly by the increase of shoots biomass, as their number slightly increased. In such situation the production of *Carex Hudsoni* was considerably lower. This is the only case among all investigated plant species, when the reed production clearly increased, but the competing plant decreased its production.

Experiment D

The reed seedlings planted on mud with the addition of plant material did not develop. 10 g dry mass of plant material per 300 ml of mud in experiment A, as well as 3 g per 500 ml in experiment B was too much. Due to the addition of plant material very strong fermentation processes took place in the mud. In the first experiment nearly all seedlings withered. In the second experiment with lower mass of added plant material, the seedlings which survived were several times smaller than the control ones, and the necrosis were clearly visible.

Thus the introduction of non-living plant material had much stronger influence than the mixed cultures with plants.

THE UNDERGROUND PARTS

The dependence of aerial parts of plants on the type of soil were presented above (Experiment A). Table V presents the dependence of

Table V. The share of each part of plant in production of macrophytes

Type of soil	Plant species	Plant parts		
		aerial (g per 1 pot)	underground	
			(g per 1 pot)	(% of the total)
Sand (Experiment A)	*P. communis*	0.12	0.78	86.6
	T. latifolia	0.04	0.35	89.7
Garden soil (Experiment A)	*P. communis*	5.24	18.78	74.1
	T. latifolia	11.82	30.88	72.0
Lake mud (Experiment A)	*P. communis*	9.64	23.89	71.3
	T. latifolia	11.82	27.94	70.5
Lake mud (Experiment B)	*P. communis*	8.27	18.61	69.2

the development of underground parts on the type of soil. The reed on mud produced in the experiment A 2.5 g of roots and rhizomes per 1 g dry weight of aerial parts, and in experiment B — 2.2 g, respectively. In the cultures on garden soil — 3.6 g of roots and rhizomes. The cattail growing on mud produced 2.4 g of roots and rhizomes per 1 g dry weight of aerial parts, and on garden soil — 2.5 g. Much greater share of the underground parts was found in the cultures on sand: 1 g dry mass of aerial parts of reed produced 7.3 g of roots and rhizomes, while cattail — 3.3 g.

4. DISCUSSION

Apart from a great variety of aquatic plants, the beds of emergents are usually monospecific. The beds with several species of plants are relatively rare. This phenomenon cannot be explained by the environmental conditions.

The replacement of one species by another, which later form the monospecies aggregation, can be observed during the succession changes. The observations concerning the withdrawal of certain plants from the stands previously occupied by them, and the expansion of certain species causing the disappearance of species formerly growing in this place (Haslam 1968), as well as own observations allow to expect certain mechanisms causing these changes. Nowiński (1961) quotes the results of many authors concerning the translocations of terrestrial plant species. The perennials with a vegetative growth with clones expanding in all directions are observed to wither in their central parts. Such development is usually caused by soil intoxication due to decomposition of organic litter of such clone (Nowiński 1961). In some years such

clone divides on the outside into a number of separate descendant plants, and the centre is inhabited by other species. The so-called "internal crop-rotation" is observed on the meadows, i.e. particular plant species change their place of growth on a given area (L i e t h 1960). Such phenomena, as e.g. slowing down or stimulation of the plant growth, caused by the activity of chemical substances derived form plants are called after M o l i s c h (1937) allelopathy. Thus, apart from the microclimatic differences, the type of soil or human differences in the plant cultures as manuring, utilization and so on, the specific forms of growth of particular species and their allelopathic interactions decede about the differentiation of plant associations (K n a p p 1960).

In agriculture the knowledge of allelopathic influences is very important as in many cases it helps to increase the crop production, or at least to prevent their decrease by application of proper crop-rotation preventing the so-called "soil sickness" i.e. the contamination of it by products of metabolism and plant decomposition, but not the lack of necessary nutrients.

It is obvious, that the allelopathic substances can act differently in field conditions than in the laboratory. K n a p p (1960) says, that the laboratory results can be applied in the natural conditions for natural associations. However, there are many obvious examples of allelopathic interrelation influences found on various not buffered nutrients in other laboratory arrangements, which would never be found in nature. The phenology plays a very significant part in the investigations of allelopathy. H o l z and R i c h t e r (1960) while investigating the alcaloids content in *Equisetum palustre* found their rapid decrease during the first frost. The type of substratum is also one of the factors influencing allelopathy (K n a p p 1960).

In the majority of cases it is difficult to separate direct influence of plants on other plants from that of other ecological factors. However, the influence of certain chemical substances produced by plants can be distinguished. These substances can have a bad or stimulating influence on the plant development.

The influence of various plant species is strongly connected with the environmental conditions. The plant production on various soils of plants influencing other plants is different (Fig. 1). The growth of reed and cattail on sand and peat is very poor, but is much better on the lake mud and garden soil. The influence of plants on other plants in the cultures on sand is not easily noticed. A slight increase of the biomass of reed in the culture with cattail can be only observed (Fig. 2) when compared with reed biomass without cattail.

On the peat soil the growth of reed in the cultures with cattail was

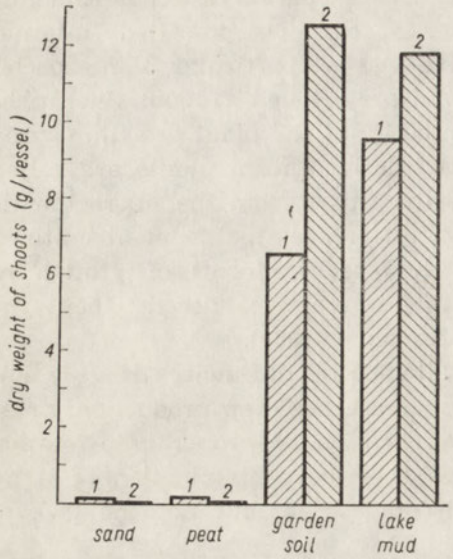

Fig. 1. Growth of *Phragmites communis* Trin. and *Typha latifolia* L. on different soils. 1 — *P. communis* Trin.; 2 — *T. latifolia* L.

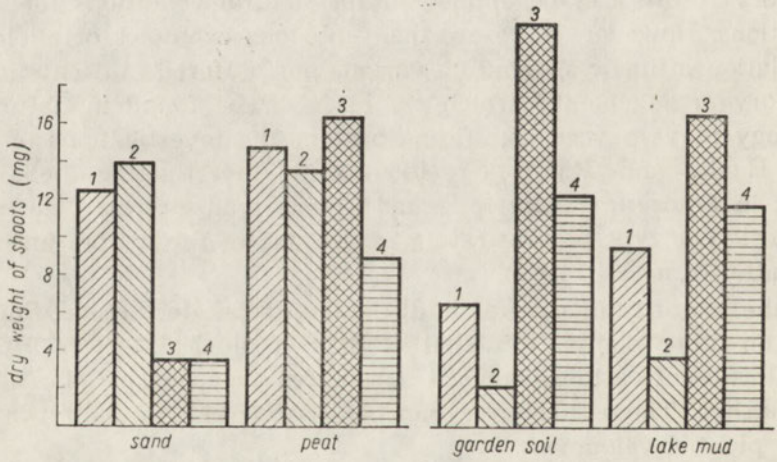

Fig. 2. Interaction of plants. Dry mass of aerial part produced by 1 seedling. 1 — *P. communis* Trin. — monospecies culture; 2 — *P. communis* Trin. cultivated together with *T. latifolia* L.; 3 — *T. latifolia* L. cultivated together with *P. communis* Trin.; 4 — *T. latifolia* L. — monospecies culture

slightly poorer as compared with the control one, but the cattail growing with reed shows a visible biomass increase compared with the cattail control (Fig. 2).

The differences between the biomass of these two species are easily distinguished in the cultures on garden soil and mud, both in mono-

species culture and in the mixed one (Fig. 2). The reed grows much better on the mud than on the garden soil (Table II), and for the cattail both environments are equal.

On the garden soil the biomass of reed in the cultures with cattail is 67% lower than in the monospecies reed culture. On the other hand biomass of cattail in the cultures with reed is 75% higher.

On the mud the production of reed in the cultures with cattail is 61% lower than in the monospecies reed culture. The cattail cultured with reed shows the 40% production increase as compared with cattail in monospecies culture. Thus the type of soil determines the way of penetration of chemical substances and their inactivation. Knapp (1960), while carrying out the experiments on the germination of *Lolium multiflorum* in various condensated nutrients with the addition of 0.1% of cumarine in distilled water, and on the soil (Humusboden) obtained in the latter the highest germination and the lowest in water with cumarine.

The visible increase of cattail production in the cultures with reed (40 and 75%) with a simultaneous decrease of reed production under the influence of cattail (67 and 61%) cannot be explained by accidental factors.

The production of reed cultured with bulrush and horsetail also decreased (Table III). The reed production was less influenced by bulrush than by cattail, and it was even less influenced by *Equisetum limosum* (Table VI). Among the above plant only *Carex Hudsoni* stimulated the reed production.

Table VI. The change of reed production under the influence of associated plants

The associated species	Reed production (% of the control)
Typha latifolia	39
Schoenoplectus lacustris	63
Equisetum limosum	88
Carex Hudsoni	152

The influences of plants are bi-directional, and the changes of production of plants associated with reed should be also noticed. All investigated species changed their production in the presence of reed (Table VII). The increase of associated plant production is generally accompanied by the decrease of reed production, and inversely.

In the case of sedge, bulrush and horsetail these changes are inversely proportional. However, the cattail slows down the reed growth much

Table VII. The influence of reed on the production of associated plant species

The associated species	Production (% of the control)
Carex Hudsoni	67
Equisetum limosum	120
Typha latifolia	140
Schoenoplectus lacustris	149

more than it could be the result of the increase of its production both on garden soil and on mud.

The bulrush in the cultures with horsetail slightly increases the production — 8% (Table III), at the same time slowing down the growth of horsetail in 11% as compared with the control.

Mann and Barnes (1949), Börner et al. (1959), Knapp (1960) and others in the experiments with the terrestrial plants frequently observed the stimulating or inhibiting influence of some plants on other. The phenomenon of joint stimulation or inhibition at the same time is very rare. In these investigations such situation did not happen.

The plants cultivated on various soils produced different biomass of aerial parts (Table II). The underground parts were also different depending on the type of substratum (Table V). The obtained results are slightly higher than the amount of roots per the mass unit of the aerial part of reed given by Szczepański (1969). This author says, that the reed produces 2 g of underground parts per 1 g of aerial parts. The values obtained in this paper are within the limits of results given in the already mentioned paper. An increase of the underground parts is probably caused by the poor substratum.

Börner et al. (1959) in their experiments on germination of flax in various nutrient concentrations found that the growth of roots is inversely proportional to the nutrients concentration.

Although the trophy of the environment has a decisive influence on the macrophytes production, it is not the only factor influencing it. The competition among various plant species is also of a great importance in regulating this production.

Among the numerous competition mechanisms, the allelopathic influences are very important, as due to them the production in certain environmental conditions can be considerably increased or decreased. Better knowledge of this phenomenon in the lake littoral would allow to learn more about the complicated ecological processes which take place in this zone.

5. SUMMARY

The experiments on the allelopathic influences among aquatic plants were carried out in 1969. The following plant species were cultivated together: 1. *Phragmites communis* and *Typha latifolia*, 2. *Phragmites communis* and *Schoenoplectus lacustris*, 3. *Phragmites communis* and *Equisetum limosum*. It was found, that in the mixed cultures the plant production was different than in their monospecific cultures. The decrease of reed production was accompanied by the increase of other species production, and inversely.

The influences of plants on other plants depend on the type of soil.

The growth of reed and cattail improves on better soil, such as mud and garden soil, but not on peat and sand (Table II). The growth of underground parts also depends on the type of substratum; the growth was best on sand, then on garden soil and mud (Table V).

Dead plant material introduced to the substratum inhibited the growth of reed.

6. STRESZCZENIE

W sezonie wegetacyjnym 1969 r. przeprowadzono doświadczenia nad wielkością oddziaływań allelopatycznych między roślinami wodnymi. Hodowano wspólnie: 1. *Phragmites communis* z *Typha latifolia*, 2. *Phragmites communis* z *Schoenoplectus lacustris*, 3. *Phragmites communis* z *Equisetum limosum*. Stwierdzono, że w hodowlach mieszanych produkcja roślin różniła się od produkcji tych roślin w monokulturze. Spadkowi produkcji trzciny towarzyszy wzrost produkcji rośliny towarzyszącej i odwrotnie (Tab. VI i VII).

Wzajemne oddziaływanie roślin są zależne od rodzaju gleby.

Rozwój trzciny i pałki jest lepszy na glebach żyźniejszych — muł jeziorny i ziemia ogrodowa, słabszy zaś na torfie i piasku (Tab. II). Rozwój części podziemnych zależy od żyzności gleby — największy jest na piasku, mniejszy na ziemi ogrodowej, jeszcze mniejszy na mule (Tab. V). Martwy materiał roślinny wprowadzony do gleby oddziałuje hamująco na rozwój trzciny.

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THE INFLUENCE OF ALCOHOL EXTRACTS OF SOME ALGAE (CHLORELLA AND SCENEDESMUS) ON AQUATIC MICROORGANISMS

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ABSTRACT

Alcohol extracts of algae of the genera Chlorella and Scenedesmus inhibit the development of about 20% of water microorganisms. Particularly vulnerable are Gram-positive bacteria of the genera Micrococcus and Bacillus. Among the Gram-negative bacteria, the greatest number of vulnerable strains can be found in the Pseudomonas group and in the Enterobacteriaceae family. Chlorella extracts have more extensive scope of antagonist action than Scenedesmus extracts. The latter, however, are more active and they inhibit the growth of bacteria in larger areas.

CONTENTS

1. INTRODUCTION

A number of algae species produce in light bactericidal or bacteriostatic substances. A detailed list of the latter can be found in Sieburth (1964). Some of them inhibit the development of saprophytic bacteria, to some others pathogenic ones are vulnerable, and thus they seem to have sanitary significance (Sieburth 1962, Sieburth and Pratt 1962). In inland waters, particularly important in this respect are species belonging to the Chlorella and Scenedesmus genera. According to Levina (1961), these organisms inhibit the development of bacteria of the coli and salmonella groups. Also Telitčenko et al. (1962) and Steblyuk (1968) showed the lethal impact of Chlorella and Scenedesmus upon salmonellas. Alcohol extracts of these algae act toxically against Gram-positive bacteria, too. According to Krzywicka (1966), the growth of *Bacillus subtilis* was completely inhibited after 16 hr of exposition in light to properly processed alcohol extracts of *Chlorella vulgaris*. These bacteria also die in the environment of similar extracts of *Chlorella pyrenoidosa* (Matusiak et al. 1965).

It seems interesting from an ecological point of view, to what extent the above mentioned algae are apt to inhibit the development of plant microorganisms living in water. No clear data can be found in literature. Only Burkholder et al. (1960) and Duff et al. (1966) offer some general remarks, but the main concern of their works are antibiotic properties of sea algae and bacteria.

The program of microbiological research of the Iława lakes includes a study of various factors influencing the development of plant microorganisms of those

waters; phytoplankton seems to be one of such factors. The present paper concerns the influence of alcohol extracts of a few species of the Chlorella and Scenedesmus algae upon Iława lakes plant microorganisms.

2. MATERIAL AND METHODS

Algae. The following algae were used for study: *Chlorella vulgaris* A23, *Chlorella pyrenoidosa* A82, *Chlorella ellipsoidea* A25, *Scenedesmus obliquus* A125, *Scenedesmus quadricauda* A119, and *Scenedesmus acuminatus* A124 from the Pratt collection in Prague, received from the Department of Botany, Higher School of Agriculture in Lublin. The algae were cultivated in 25°C, in steady bulb light (4000 Lux), in Erlenmayer flasks containing a proper mineral medium, through which sterilized air with 5% of CO_2 was pumped.

For the Chlorella cultures, the following mineral medium was used (after Matusiak et al. 1965): KNO_3 — 1.0 g; KH_2PO_4 — 0.135 g; $MgSO_4 \cdot 7H_2O$ — 0.5 g; $FeSO_4 \cdot 7H_2O$ — 0.003 g; natrium citrate — 0.0057 g; solution of microelements (H_3BO_3 — 2.137 g; $MnCl_2 \cdot 4H_2O$ — 1.810 g; $ZnSO_4 \cdot 7H_2O$ — 0.220 g; $(NH_4)_2MoO_4$ — 0.002 g; $CuSO_4 \cdot 5H_2O$ — 0.070 g; $Co(NO_3)_2 \cdot 6H_2O$ — 0.080 g; NH_4Vo_3 — 0.010 g; distilled water — 1000 ml) — 1 ml; distilled water —1000 ml; pH 6.8-6.9.

The Scenedesmus algae were cultivated according to Meffert (1960) on the following medium: $(NH_4)_2HPO_4$ — 0.272 g; $MgSO_4 \cdot 7H_2O$ — 0.18 g; KH_2PO_4 — 0.14 g; microelements according to Arnon (Krauss 1953), ferrum according to Rhode (1948), distilled water — 1000 ml; pH 6.8.

Preparation of alcohol extracts. 14 days old cultures were centrifuged during 20 min at 2000 r.p.m.; the clear liquid was then sipped from above the sediment, 1000 mg of which was put into Erlenmayer flasks, where 10 ml of 96% ethyl alcohol was poured on it. It was then left in a cool at 4°C until complete evaporation occurred. The sediment thus prepared was soaked with sterilized water in the amount of 1 ml/100 mg of fresh cell mass of algae.

Bacteria. Bacteria strains isolated during 1967-1968 from water of Iława lakes (Niewolak 1970) were used for study. They were identified by means of the Bergey's (1957) key and the Shewan's et al. (1969) scheme.

Experiments. 1 ml portions of 24 hr bacteria cultures were inoculated on broth agar, on Petri plates. After solidification, rings of filtration blotting paper 5 mm in diameter and permeated with 0.05 ml of an algae alcohol extract were put upon the plates. After 24-48 hr of incubation of bacteria at room temperature, the extent of the area of inhibition of bacteria growth was measured (in mm) from the ring's rim to the outer rim of the growth area.

3. RESULTS

From among 768 various bacteria strains, isolated from Iława lakes water, 162 strains (21.1%) proved more or less vulnerable to one or more species of the investigated algae. The greatest number of vulnerable strains (126, i.e. 77.7%) was found among the Gram-positive bacteria, mainly from the Micrococcus and Bacillus genera (Table I and II), while there was only 36 vulnerable strains (22.3%) among the Gram-negative bacteria of the Enterobacteriaceae family, and the following genera: Aeromonas, Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium, Xanthomonas, Vibrio, Corynebacterium, and Arthrobacter (Table III). Only 11 strains of bacteria were vulnerable to all the investigated species of algae: *Micrococcus varians* No. 290, 392 and 398, *M. flavus* No. 395, *Streptococcus sp.* No. 714, *Staphylococcus* No. 83, *Bacillus sp.* No 23 and 215, *B. firmus* No. 98, *Aeromonas sp.* No. 442 and Flavobacterium No.

711. The majority yielded to antagonistic action of one, two or rarely three or four species of algae. Chlorella extracts acted upon much greater number of bacteria strains than Scenedesmus extracts. E.g., 30 strains of Gram-negative rods and of the Corynebacterium genus (Table III) reacted to Chlorella extracts, while only half as many reacted to Scenedesmus extracts. Similarly, among the studied Gram-positive rods (Table II), 60 strains reacted to Chlorella extracts, and from about a dozen to 21 strains reacted to Scenedesmus extracts. Also among the cocci (Table I), more strains reacted to Chlorella than to Scenedesmus extracts.

It seems, however, that Scenedesmus extracts were more intensively active than Chlorella extracts; it can be observed most clearly in Table I, where the largest areas of growth inhibition of the cocci, reaching 5 mm and more, appear much more often in presence of Scenedesmus extracts (10 times) than in presence of Chlorella extracts (4 times). Figure 1 presents the areas of growth inhibition of some Gram-positive bacteria in presence of alcohol extracts of the investigated algae.

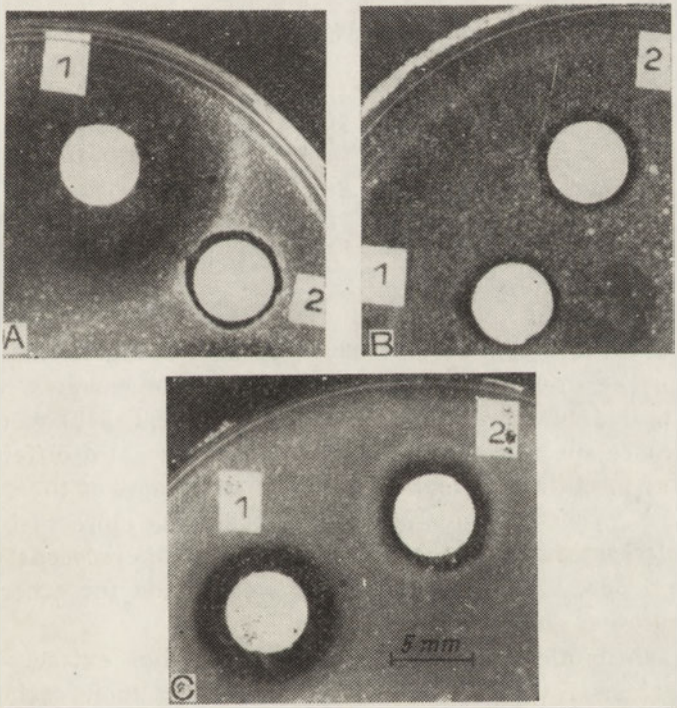

Fig. 1. Areas of growth inhibition of bacteria under the influence of alcohol extracts of algae. A — *Bacillus sp.* No. 23 culture: 1 — *Chlorella vulgaris* extract, 2 — *Chlorella pyrenoidosa* extract; B — *Bacillus megatherium* No. 397 culture: 1 — *Chlorella vulgaris* extract, 2 — *Chlorella pyrenoidosa* extract; C — *Micrococcus candidus* No. 345 culture: 1 — *Chlorella vulgaris* extract, 2 — *Chlorella pyrenoidosa* extract. Outside of growth inhibition areas there is seen growth stimulation areas

Table I. Growth inhibition areas of Gram-positive cocci

Strains of bacteria	No.	Date of isolation	*Chlorella*			*Scenedesmus*		
			vulgaris	*pyrenoidosa*	*ellipsoidea*	*obliquus*	*quadricauda*	*acuminatus*
Micrococcus varians	34	IV 1967	1	0.5	3	—	—	—
caseolyticus	53	V	—	1	0.5	—	—	—
sp.	55	V	0.5	2	—	—	—	—
sp.	72	V	—	1	1	—	—	—
caseolyticus	73	V	3	—	3	— s	3 s	—
candidus	107	VII	—	—	5	5	7	7
sp.	168	VIII	—	0.5	—	0.5 s	—	—
sp.	190	IX	1	0.5	1	—	—	—
ureae	205	IX	0.5	1	0.5	—	— s	— s
freudenreichii	213	IX	0.5	1	—	—	0.5	—
luteus	260	XI	1	0.5	—	—	—	—
ureae	261	XI	—	0.5	—	—	—	—
freudenreichii	275	XI	—	0.5	0.5	0.5	0.5	—
varians	290	XI	0.5	0.5	0.5	0.5	0.5	0.5
sp.	294	XI	0.5	1	0 5	—	0.5	—
freudenreichii	296	XI	1	0.5	2	1	2	3
luteus	301	XI	0.5	0.5	0.5	—	—	—
conglomeratus	322	XII	0.5	0.5	1	—	2	3
conglomeratus	329	XII	1	2	1	1 s	— s	—
candidus	345	XII	2.5 s	1.5 s	0.5	—	—	2
varians	346	XII	2	2	2	2	—	—
conglomeratus	354	XII	0.5	—	0.5	—	5	3
varians	359	XII	1	1	—	2	1	2
candidus	365	I 1968	—	—	5	—	3	—
sp.	366	I	2	—	1	2	5	5

— — lack of growth inhibition; s — growth stimulation.

Various strains of the same species often reacted differently. E.g. *Micrococcus varians* No. 34 and 539 was developing normally and did not react to the presence of Scenedesmus alcohol extracts, while other strains of the same species (No. 290, 346, 359, 392, 398) were inhibited by one or more species of these algae. There were also differences as to the extent of inhibition areas between various strains of the same species of bacteria. E.g., *Micrococcus varians* No. 290 was more resistant to the influence of Scenedesmus alcohol extracts than *Micrococcus varians* No. 359, 392 and 398. Similar observations were made for other species of bacteria and other genera of algae.

Besides antibiotic properties, a part of alcohol extracts of the investigated algae revealed stimulating influence upon certain bacteria species. This was indicated by a formation of a characteristic growth nimbus immediately around the blotting paper ring permeated with an alcohol extract of the proper algae (when the action was not antagonistic), or at the outer rim of the inhibition area (in vulnerable cultures). The

under the influence of alcohol extracts of algae (in mm)

Strains of bacteria	No.	Date of isolation	*Chlorella*			*Scenedesmus*		
			vulgaris	*pyrenoidosa*	*ellipsoidea*	*obliquus*	*quadricauda*	*acuminatus*
Micrococcus sp.	371	I 1968	1	1	1	—	—	—
candidus	381	I	—	—	2	— s	5	2
ureae	385	I	1	2	3	—	—	—
varians	392	I	1	0.5	3	1	2	4
candidus	394	I	—	—	1	—	1	1
flavus	395	I	2	1	8	3	7	6
varians	398	I	3	2	3	2	8	6
caseolyticus	427	III	0.5	1	—	—	—	—
conglomeratus	428	III	2	1	—	—	1	2
sp.	455	III	3	1	2	1	1 s	—
candidus	464	III	—	0.5	1 s	— s	0.5 s	—
sp.	482	III	1	1	1	—	—	—
varians	539	IV	1	2	1	—	—	—
sp.	591	V	—	—	—	—	1	1
sp.	658	VI	0.5	2	— s	— s	—	—
rubens	685	VI	0.5	0.5	0.5	0.5	—	—
sp.	735	VII	0.5	0·5	0.5	—	—	—
conglomeratus	752	VII	0.5	1	—	—	—	—
sp.	754	VII	0.5 s	0.5 s	0.5 s	0.5 s	0.5 s	0.5 s
Streptococcus salivarius	388	I 1968	5	1	—	—	—	5
sp.	714	VII	0.5 s	0.5 s	0.5 s	0.5 s	0.5 s	0.5 s
Sarcina ureae	76	VI 1967	4	—	—	—	—	4
flava	103	VII	3	3	4	—	1	4
Staphylococcus aureus	83	VII	1	2	1	0.5	0.5	1
The number of vulnerable strains:			38	40	36	17	25	21

stimulation phenomenon could be observed among both Gram-positive and Gram-negative bacteria. At Figure 1C, a growth nimbus can be seen beyond the inhibition areas of the *Micrococcus candidus* No. 345, around the blotting-paper rings permeated with an extract of *Chlorella vulgaris*.

4. DISCUSSION

Ecological significance of antibiotic substances secreted by algae in water environment has been much disputed in recent years. Because of their selective influence upon a number of pathogenic bacteria, even some sanitary importance is ascribed to them. Such role can be performed by the Diatomae *Skeletonema costatum* in sea waters and in river estuaries, while a similar influence in inland waters is ascribed to several species belonging to the Chlorella and Scenedesmus genera. It can be seen from the present work that these algae reveal a relatively wide scope of antagonistic influence. Alcohol extracts from these orga-

Table II. Growth inhibition areas of Gram-positive rods

Strains of bacteria	No.	Date of isolation	*Chlorella*			*Scenedesmus*		
			vulgaris	*pyrenoidosa*	*ellipsoidea*	*obliquus*	*quadricauda*	*acuminatus*
Bacillus sp.	12	IV 1967	1	0.5	1	—	—	—
sp.	23	IV	5	1	4	7	6	8
megatherium	71	V	2	2	—	—	—	—
megatherium	97	VI	2	2	0·5	—	—	—
firmus	98	VI	3	4	5	2	4	3
sp.	142	VIII	0.5	1	—	—	—	—
sp.	158	VIII	0.5	0.5	—	—	—	—
sp.	162	VIII	—	1	—	0.5	—	—
brevis	184	IX	0.5	0.5	—	—	—	—
sp.	185	IX	1	2	2	1	1	—
sp.	191	IX	0.5	1	—	—	0.5	—
sp.	192	IX	0.5	1	—	—	—	—
sp.	194	IX	0.5	1	2	—	—	—
sp.	196	IX	0.5	1	—	—	—	—
sp.	210	IX	—	1	—	—	0.5	—
sp.	211	IX	0.5	1	1	—	—	—
sp.	215	IX	0.5 s	0.5 s	0.5 s	0.5 s	0.5 s	0.5 s
sp.	220	IX	—	0.5	0.5	—	—	—
sp.	221	IX	0.5	0.5	—	—	—	—
sp.	222	IX	0.5	0.5	0.5	0.5	—	—
sp.	224	IX	0.5	0.5	—	—	—	—
sp.	229	IX	1	2	2	—	—	—
sp.	249	IX	1	2	2	—	—	—
sp.	255	IX	—	—	—	0.5 s	0.5 s	0.5 s
sp.	269	IX	—	—	—	2	2	2
sp.	272	XI	0.5	1	—	—	—	—
sp.	280	XI	—	0.5	0.5	0.5	0.5	0.5
sp.	303	XI	1	2	—	—	—	—
pumilus	312	XII	1	1	0.5	—	—	—
sp.	313	XII	0.5	0.5	0.5	— s	— s	— s
pumilus	319	XII	0.5 s	0.5 s	0.5 s	— s	— s	— s
subtilis	320	XII	1	2	1	—	—	—
sp.	322	XII	0.5	1	—	—	—	—
sp.	349	XII	0.5	0.5	—	—	—	—
sp.	368	XII	2	—	2	—	— s	— s

— — lack of growth inhibition; s — growth stimulation.

nisms inhibit the development of many different species of Gram-positive bacteria, as well as of a number of Gram-negative ones. Among the Gram-positive bacteria, species belonging to the Micrococcus and Bacillus genera proved to be particularly vulnerable. Vulnerable strains occurred also among streptococci, sarcinae and staphylococci, and among the species of Corynebacterium. Bactericide influence of algae upon streptococci was also found by Burkholder et al. (1960), Jorgensen and Nielsen (1961), Saz et al. (1963), Duff et al. (1966), and recently also by Postolica (1968) and Stangenberg (1968). All

under the influence of alcohol extracts of algae (in mm)

Strains of bacteria	No.	Date of isolation	*Chlorella*			*Scenedesmus*		
			vulgaris	*pyrenoidosa*	*ellipsoidea*	*obliquus*	*quadricauda*	*acuminatus*
Bacillus brevis	382	I 1968	2	2	2	—	—	—
sp.	389	I	1	1	1	—	—	—
megatherium	397	I	0.5	1	1	—	—	—
megatherium	401	I	1	—	3	—	2	—
sp.	424	III	1	0.5	0.5	—	—	—
licheniformis	434	III	2	1	1	1	0.5	—
sp.	437	III	0.5	—	1	—	—	—
sp.	445	III	—	1	1	0.5	—	—
sp.	447	III	0.5	0.5	1	0.5	—	—
sp.	465	III	1	0.5	0.5	0.5	—	—
sp.	470	III	0.5	2	—	—	—	0.5
pumilus	478	III	1	1	1	—	—	—
subtilis	489	IV	1	1	1	—	—	—
polymyxa	504	IV	—	1	1	—	—	—
sp.	512	IV	1	1	1	—	—	—
sp.	516	IV	2	1	3	1	3	3
subtilis	518	IV	2	2	2	1	—	1
subtilis	523	IV	1	1	1	—	—	—
sp.	528	IV	1	0.5	—	—	—	—
sp.	536	IV	1	1	1	—	—	—
sp.	538	IV	1	2	1	—	—	—
sp.	540	IV	1	1	—	—	—	—
sp.	549	IV	2	1	3	—	4	4
pumilus	552	IV	1	1	2	—	—	—
sp.	563	VI	1	2	1	0.5	—	—
sp.	566	VI	0.5	0.5	—	0·5	— s	—
sp.	667	VI	2	1	—	—	—	—
sp.	674	VI	0.5	1	—	—	—	—
macerans	702	VII	— s	0.5 s	0.5 s	— s	0.5 s	—
polymyxa	703	VII	1	2	1	0.5	—	—
subtilis	706	VII	0.5	0.5	0.5	0.5	—	—
sp.	721	VII	0.5	0.5	0.5	—	—	—
sp.	748	VII	1	1	—	—	—	—
polymyxa	753	VII	0.5 s	0.5 s	1 s	0.5 s	—	—
mycoides	678	VII	2	2	1	3	2	2
The number of vulnerable strains:			61	65	46	21	15	11

these authors maintain consistently that various algae inhibit the development of bacteria strains not only in laboratory, but in natural water environments as well. Perhaps antibiotic substances secreted by algae play some role in their survival. It is possible that some restriction of growth of noxious and other bacteria can be advantageous for the algae, perhaps in the competition for nutritious ingredients.

According to D u f f et al. (1966), the selective action of algae towards Gram-positive bacteria, and in particular towards the Staphylococcus, and the lesser vulnerability of Gram-negative bacteria, which has been

Table III. Growth inhibition areas of Gram-negative rods and of the Corynebacterium

Strains of bacteria	No.	Date of isolation	*Chlorella*			*Scenedesmus*		
			vulgaris	*pyrenoidosa*	*ellipsoidea*	*obliquus*	*quadricauda*	*acuminatus*
Enterobacteriaceae	63	V 1967	1	2	0.5	0.5	0.5 s	—
	117	VIII	0.5	2	1	0.5	0.5	1
	186	IX	1	0.5	—	—	—	—
	206	IX	0.5	1	—	—	—	—
	344	XII	0.5	1	1	0.5	—	—
	429	III 1968	—	—	0.5	—	—	0.5
	455	III	2.5	1	2	1	1	—
	483	III	—	0.5	0.5	—	—	—
Aeromonas sp.	48	V 1967	1	1	1.5 s	— s	— s	— s
sp.	60	V	2	0.5 s	0.5 s	—	—	—
formicans	237	XII	1	2	2	—	—	—
sp.	442	III 1968	0.5	0.5	0.5	0.5	0.5 s	0.5 s
sp.	475	III	1	0.5	0.5	0.5	— s	—
sp.	733	VI	0.5 s	—	— s	0.5	— s	—
Pseudomonas sp. I gr.	33	IV 1967	—	1 s	0.5 s	—	—	— s
sp. I gr.	277	XI	0.5	1	1	— s	— s	— s
sp. I gr.	293	XI	0,5	1	0.5	—	—	—
sp. II gr.	156	VIII	0.5	0.5	—	—	—	—
sp. II gr.	173	VIII	1	0.5 s	—	—	— s	—
sp. II gr.	281	XI	0.5	0.5	0.5	0.5	—	—
sp. II gr.	503	V 1968	2	3	8	5	— s	7
sp. II gr.	722	VII	0.5	0.5	0.5	—	—	—

— — lack of growth inhibition; s — growth stimulation.

frequently observed, can have some ecological import. On the ground of similar observations, S a z et al. (1963) suggested that Gram-negative bacteria might be the primary plant microorganisms of the water. Such view seems to be corroborated by the present results. Gram-negative bacteria isolated from Iława lakes were inhibited by some species of algae, but the number of vulnerable strains in this group was almost four times lesser than among the Gram-positive species. Also the growth inhibition areas of those microorganisms in presence of alcohol extracts of the studied algae were in general less extensive than with the Gram-positive bacteria. Among the Gram-negative bacteria, the greatest number of vulnerable strains was found, besides the Pseudomonas group, among the Enterobacteriaceae family, in which many pathogenic species are known. Already a few years ago the lethal influence of the Chlorella and Scenedesmus algae upon some of them, and upon the group coli and salmonellae in particular (including the *Salmonella typhi murium)* has been shown experimentally by Soviet researchers (L e v i n a 1961, T e l i t č e n k o et al. 1962).

The bactericide factor in the investigated algae is supposedly chlorel-

and Arthrobacter genera under the influence of alcohol extracts of algae (in mm)

Strains of bacteria	No.	Date of isolation	*Chlorella*			*Scenedesmus*		
			vulgaris	*pyrenoidosa*	*ellipsoidea*	*obliquus*	*quadricauda*	*acuminatus*
Pseudomonas sp. III gr.	309	XII 1967	—	0.5	3	1	2	2
sp. III gr.	449	III 1968	1	—	—	—	0.5	0.5
sp. IV gr.	188	IX 1967	0.5	0.5	—	—	—	—
Achromobacter sp.	576	V 1968	1	1	1	1	1	1
Alcaligenes sp.	341	XII 1967	—	1	—	—	1	2
Flavobacterium sp.	711	VI 1968	0.5	0.5	0.5	0.5	0.5	0.5
Xanthomonas sp.	377	XII 1967	0.5	—	—	—	—	4
sp.	403	XII	0.5	—	—	—	—	—
sp.	450	XII	1	—	0.5	2	3	3
Vibrio sp.	526	VI 1968	2	2	2	—	—	—
sp.	649	VI	2	2	3	—	5	3
sp.	676	VI	—	0.5	—	1	1	0.5
sp.	680	VI	—	—	1.5	—	2	3
Corynebacterium sp.	308	XII 1967	0.5	—	—	—	1	—
sp.	314	XII	—	0.5	0.5	—	—	—
humiferum	364	I 1968	1	2	2	—	—	—
rethaiji	374	I	—	—	1	—	2	3
sp.	460	III	—	0.5	0.5	0.5 s	—	—
sp.	569	V	1	1	1	—	—	—
sp.	751	VII	0.5 s	0.5 s	0.5	—	—	—
Arthrobacter tumefaciens	216	IX 1967	1 s	0.5 s	0.5 s	— s	0.5 s	— s
The number of vulnerable strains:			33	34	31	15	16	15

line, found by P r a t t et al. (1944) in *Chlorella vulgaris* cultures. According to S p o e h r et al. (1949), it belongs to aliphatic acids with a long carbon chain. Besides chlorelline, another substance is pointed to, active in presence of light and supposedly belonging to the chlorophyllides, emerging as a result of saponification of the phytol group in chlorophyll by the chlorophylase enzyme. Such light-activated chlorophyllides, and similar substances, were discovered by J o r g e n s e n (1962) in *Chlorella vulgaris, Scenedesmus quadricauda,* and *Chlamydomonas reinhardi* cells by means of chromatography. This author suggested that chlorophyllides play a role of a bactericide factor in surface waters; it is possible that they can play such role also in the Iława lakes, as it is corroborated by the present laboratory experiments, as well as by the data concerning the influence of living and dead algae cells of the Chlorella and Scenedesmus genera upon plant microorganisms of these waters (N i e w o l a k 1971). However, the bactericide role of the mentioned algae in lake waters ought not to be overestimated, as the number of vulnerable strains of bacteria has been relatively small.

Another interesting phenomenon that has been observed, is a stimu-

lating action of alcohol extracts of the investigated algae towards certain bacteria isolated from Iława lakes water. An analogous observation was made by Burkholder et al. (1960), who worked with various extracts of sea algae *Coaniaulax temarensis* and *Dyctyopteris plegiogramma*, and some unidentified bacteria isolated from sea water. According to Duff et al. (1966) an emergence of the growth nimbus immediately beyond the inhibition area, is a result of the fact that in certain algae extracts, there are separate stimulating substances besides bactericides, whose activity can be observed at the fringes of the inhibition area. It can also be postulated that chlorelline or other similar substances produced by algae can have a stimulating effect, if their concentration is small, according with Arndt's law (Rippel-Baldes 1955).

5. SUMMARY

The research concerned the influence of alcohol extracts of algae: *Chlorella vulgaris, Ch. pyrenoidosa, Ch. ellipsoidea, Scenedesmus obliquus, S. quadricauda,* and *S. acuminatus,* upon plant microorganisms of Iława lakes. Out of 768 strains of water bacteria investigated, only 21% revealed some vulnerability to one or more species of the studied algae. Among the vulnerable strains, more than 77% were Gram-positive bacteria belonging to the Micrococcus and Bacillus genera. A bulk of vulnerable strains were sensitive to antagonistic action of one or two species of algae; vulnerability to three or more species was less frequent. Chlorella alcohol extracts had a much wider scope of antagonistic action than the analogous Scenedesmus specimens. However, the latter acted more strongly and in general they produced larger growth inhibition areas. Various strains of the same bacteria species often reacted differently. There were also differences in the extent of growth inhibition areas between several strains of the same bacteria species. Besides antibiotic properties, alcohol extracts of the investigated algae sometimes revealed stimulating action towards some strains of water bacteria. This phenomenon was observed with respect to both the Gram-positive and Gram-negative species.

6. STRESZCZENIE

Badano wpływ wyciągów alkoholowych glonów *Chlorella vulgaris, Ch. pyrenoidosa, Ch. ellipsoidea, Scenedesmus obliquus, S. quadricauda* i *S. acuminatus* na mikroflorę jezior iławskich. Spośród 768 przebadanych szczepów bakterii wodnych zaledwie 21% wykazywało mniejszą lub większą wrażliwość na jeden lub więcej gatunków badanych glonów. Wśród szczepów wrażliwych większość (ponad 77%) stanowiły bakterie Gram-dodatnie z rodzajów Micrococcus i Bacillus. Znaczna część wrażliwych szczepów bakterii podlegała działaniu antagonistycznemu jednego, dwóch, rzadziej trzech lub więcej gatunków badanych glonów. Wyciągi alkoholowe Chlorelli wykazywały znacznie większy zakres działania antagonistycznego niż analogiczne preparaty Scenedesmus. Te ostatnie były jednak bardziej aktywne i dawały na ogół większe strefy zahamowania wzrostu. Różne szczepy tego samego gatunku bakterii częstokroć reagowały odmiennie. Różnice dotyczyły także wielkości stref zahamowania wzrostu poszczególnych szczepów jednego gatunku bakterii. Oprócz właściwości antybiotycznych wyciągi alkoholowe badanych glonów wykazywały niejednokrotnie działanie stymulacyjne wobec niektórych szczepów bakterii wodnych. Zjawisko to występowało zarówno u bakterii Gram-dodatnich jak i gatunków Gram-ujemnych.

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THE INFLUENCE OF LIVING AND DEAD CELLS OF *CHLORELLA VULGARIS* AND *SCENEDESMUS OBLIQUUS* ON AQUATIC MICROORGANISMS

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ABSTRACT

Four different ways of behaviour of microorganisms have been found, in presence of living and dead cells of *Chlorella vulgaris* and of *Scenedesmus obliquus*: 1. dying of microorganisms in living and dead cultures of algae — *Micrococcus ureae*; 2. dying of microorganisms in living cultures of algae, and development in killed cultures — *Bacillus mycoides, Escherichia coli, Vibrio sp.*; 3. dying of microorganisms in living and dead cultures of algae in the initial period of joint cultivation, and development in the later period — *Azotobacter sp., Pseudomonas fluorescens, Rhodotorula sp.*; 4. intensive development of microorganisms in killed cultures of algae, and dying in living cultures in the initial period of joint cultivation, followed by development in the later period — *Aeromonas sp.*

CONTENTS

1. INTRODUCTION

Researchers have been long concerned with mutual relationships between phytoplankton and microorganisms. There is a widespread opinion that secretions of living plankton can be used by microorganisms (Reincke 1903 a, b, Roberg 1930, Aleev 1934, Aleev and Mudrecova 1937, Gorjunova 1954, Lefevre and Farrugia 1958). Other researchers, however, like Marshall and Orr (1930) and Stokes (1940) are not sure if phytoplankton does at all secrete organic substances in sufficient amount. Intensive development of bacteria in presence of algae is likely to be accounted for by dying of the latter and the consequent enrichment of the environment with easily assimilable organic substances (Waksman and Hotchkiss 1937, Steeman-Nielsen 1955, Lanskaja and Markianowič 1960). However, various algae show bactericidal properties. According to Razumov (1948), the development of saprophytic bacteria is often inhibited in water in presence of living cells of the algae Cyanophyceae. Steeman-Nielsen (1955) reports that inland species of Chlorella and sea species of Thalassiosira produce in light antibiotics, inhibiting the development of bacteria. The lethal influence of Chlorella and Scenedesmus upon bacteria

belonging to the coli and salmonella groups was shown by Levina (1961), Telitčenko et al. (1962), Steblyuk (1968) and Postolica (1968). Bactericidal strains also appear among some of the Diatomae, Cyanophyceae, Chlorophyceae and Thallophyta living in sea (Sieburth 1959, 1960, 1964 a, b, Burkholder et al. 1960). To some of these algae, and to the *Skeletonema costatum* in particular, even sanitary significance is ascribed (Sieburth and Pratt 1962). A similar role in sewage technology can be performed by *Anabaena variabilis, Scenedesmus quadricauda,* and *Navicula radiosa,* which have bactericidal influence upon the tubercular bacilla (Alekseeva et al. 1969).

Though extensive data have been collected about the mutual relationships between algae and bacteria, some issues still remain open; among them the problem of nutrition of bacteria on survival secretions of algae is particularly important.

In this work, the influence of living and dead cells of *Chlorella vulgaris* and of *Scenedesmus obliquus* upon some species of saprophytic bacteria and yeast isolated from lake water was investigated.

2. MATERIAL AND METHOD

Algae. There were used the algae *Chlorella vulgaris* Beijerinck and *Scenedesmus obliquus* (Turp.) Krüger from the Pratt collection in Prague, received from the Department of Botany Higher School of Agriculture in Lublin. The algae were cultivated at 25°C in steady bulb light (4000 Lux) in Erlenmayer flasks containing a proper mineral medium, through which sterilized air with CO_2 was pumped.

For the *Chlorella vulgaris* culture, the following mineral medium was used (after Matusiak et al. 1965): KNO_3 — 1.0 g; KH_2PO_4 — 0.135 g; $MgSO_4 \cdot 7H_2O$ — 0.5 g; $FeSO_4 \cdot 7H_2O$ — 0.003 g; natrium citrate — 0.0057 g; solution of microelements (H_3BO_3 — 2.137 g; $MnCl_2 \cdot 4H_2O$ — 1.810 g; $ZnSO_4 \cdot 7H_2O$ — 0.220 g; $(NH_4)_2MoO_4$ — 0.002 g; $CuSO_4 \cdot 5H_2O$ — 0.07 g; $Co(NO_3)_2 \cdot 6H_2O$ — 0.08 g; NH_4Vo_3 — 0.01 g; distilled water — 1000 ml) — 1 ml; distilled water — 1000 ml; pH 6.8 — 6.9.

Scenedesmus obliquus was cultivated according to Meffert (1960), on the following medium: $(NH_4)_2HPO_4$ — 0.272 g; $MgSO_4 \cdot 7H_2O$ — 0.18 g; KH_2PO_4 — 0.14 g; microelements according to Arnon (Krauss 1953), ferrum according with Rhode (1948), distilled water — 1000 ml; pH 6.8.

Bacteria. 7 strains of bacteria were used: *Micrococcus ureae* No. 385, *Aeromonas sp.* No. 422, *Pseudomonas fluorescens* No. 293, *Escherichia coli* No. 63, *Bacillus mycoides* No. 528, *Vibrio sp.* No. 530, *Azotobacter sp.* No. 925, and one strain of yeast *Rhodotorula sp.* No. 828. These strains were isolated from Ilawa lakes water in 1967-1968 (Niewolak 1970) and sampled randomly. All bacteria except the Azotobacter were cultivated on broth agar, and the Azotobacter was cultivated on Fiodorov medium with mannit; the yeast was fed on malt agar.

Experiment. Young, 14 days old cultures of algae were divided into 2 parts. One of them was inoculated immediately on 1 ml of bacteria suspension, while the other had been formerly killed in an autoclave during 10 min at 0.8 atm, at room temperature. The bacteria suspension for inoculation was prepared by washing 24 hr bacteria cultures and a 48 hr yeast culture on slant agar with 3 ml of physiological salt, and by dissoluting them in the 1/10 ratio. After inoculation, the cultures were kept at 25°C, and the number of cells of the investigated bacteria was counted (by the cultivation method) after 2, 4, 6, 8, 10, 12, 24, 48 and 96 hr.

3. RESULTS

Four different ways of behaviour of the investigated bacteria in presence of the living and dead cells of *Chlorella vulgaris* and *Scenedesmus obliquus* were found, they are represented in Fig. 1-8.

1. Dying of bacteria in both the living and dead cultures of algae (Fig. 1). The process of dying of bacterial cells of *Micrococcus ureae*

began immediately after they had been introduced into living and dead cultures of *Chlorella vulgaris* and *Scenedesmus obliquus*. After 24 hr the curve of dying had an almost logarythmic shape. Later the process of cells dying was inhibited, probably because of an adaptation to the changed environment. Differences were observed in the rate of dying of bacteria, related to the species and physiological state of the algae. *Micrococcus ureae* died more quickly in living and dead cultures of *Chlorella vulgaris* and slower in cultures of *Scenedesmus obliquus*.

2. Dying of bacteria in living cultures of algae, and development of bacteria in autoclave-killed algae cultures (*Bacillus mycoides, Escherichia coli, Vibrio sp.* — Fig. 2-4). In living algae, intensive dying of bacteria was observed, while in killed algae cultures, after two hours during which a part of cells died, an increase of bacteria populations occurred. Both processes (dying and development of *Bacillus mycoides*) were particularly intensive during the first 24 hr. Intensiveness of dying of bacteria in living cultures of *Chlorella vulgaris* and of *Scenedesmus obliquus* was almost identical. In dead cultures, *B. mycoides* developed better on *Scenedesmus obliquus* remmants. Similar results were obtained for *Escherichia coli* (Fig. 3) and *Vibrio sp.* (Fig. 4). Dying of

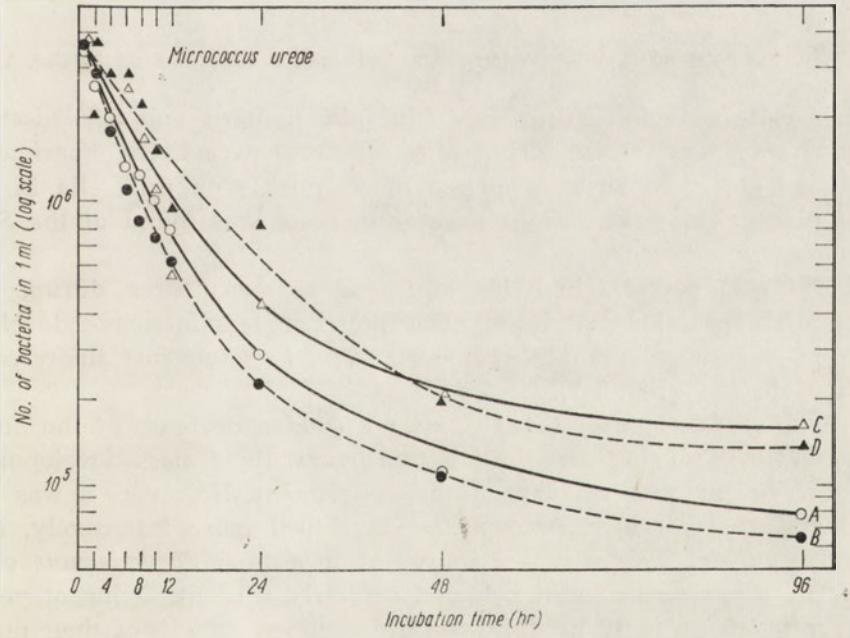

Fig. 1. Survival of *M. ureae* in algal cultures. A — survival of bacteria in living cultures of *Ch. vulgaris*; B — survival of bacteria in killed cultures of *Ch. vulgaris*; C — survival of bacteria in living cultures of *S. obliquus*; D — survival of bacteria in killed cultures of *S. obliquus*

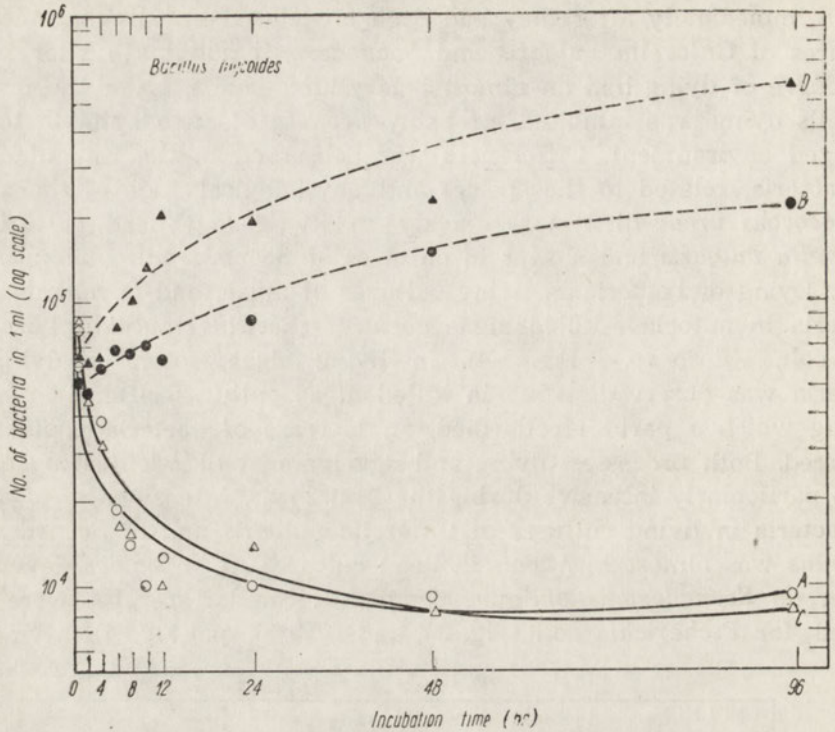

Fig. 2. Survival of *B. mycoides* in algal cultures. Denotations as in Fig. 1

bacteria cells in living cultures of *Chlorella vulgaris* and *Scenedesmus obliquus* was very slow during the research period. In experiments with dead algae, bacteria developed more quickly on Chlorella in the initial phase, while after 24 hr greater increase was found in the *Scenedesmus obliquus* culture.

3. Dying of bacteria in living and dead algae cultures during the initial hours of joint cultivating, and more or less intensive development in the later period (*Azotobacter sp., Pseudomonas fluorescens, Rhodotorula sp.* — Fig. 5-7).

In case of Azotobacter (Fig. 5), after a sudden decrease of the number of bacteria during the initial two hours, their mass development was observed in both versions of the experiment. However, it was the killed cultures in which Azotobacter developed more intensively, and *Chlorella vulgaris* was a better source of food than *Scenedesmus obliquus*. The Rhodotorula yeast behaved similarly (Fig. 6). Dying of yeast cells continued up to 12 hr, followed by a sudden growth of their number. Yeast developed better in both the living and dead Chlorella cultures, than in Scenedesmus.

4. Intensive development of bacteria in autoclave-killed algae cul-

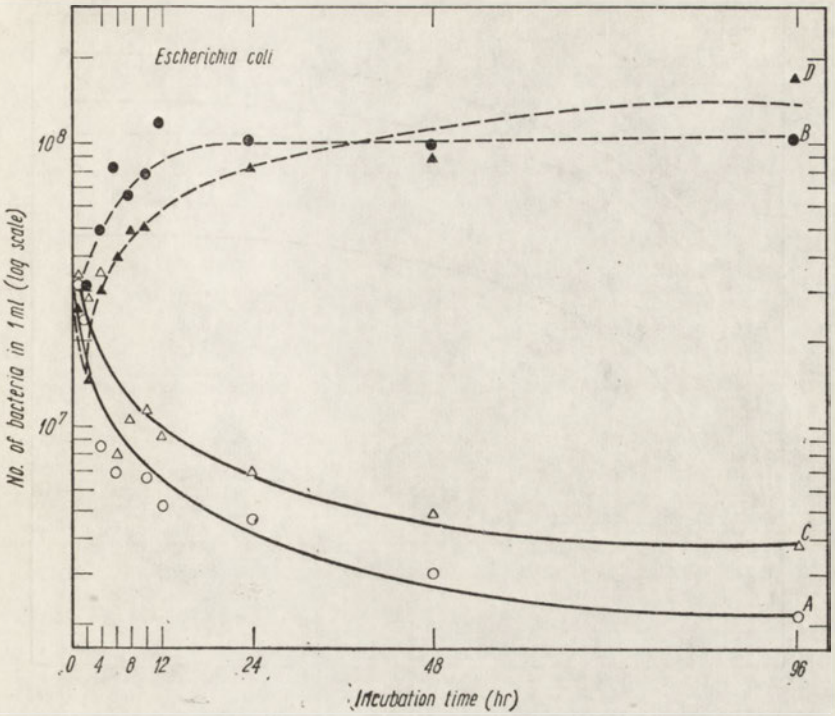

Fig. 3. Survival of *E. coli* in algal cultures. Denotations as in Fig. 1

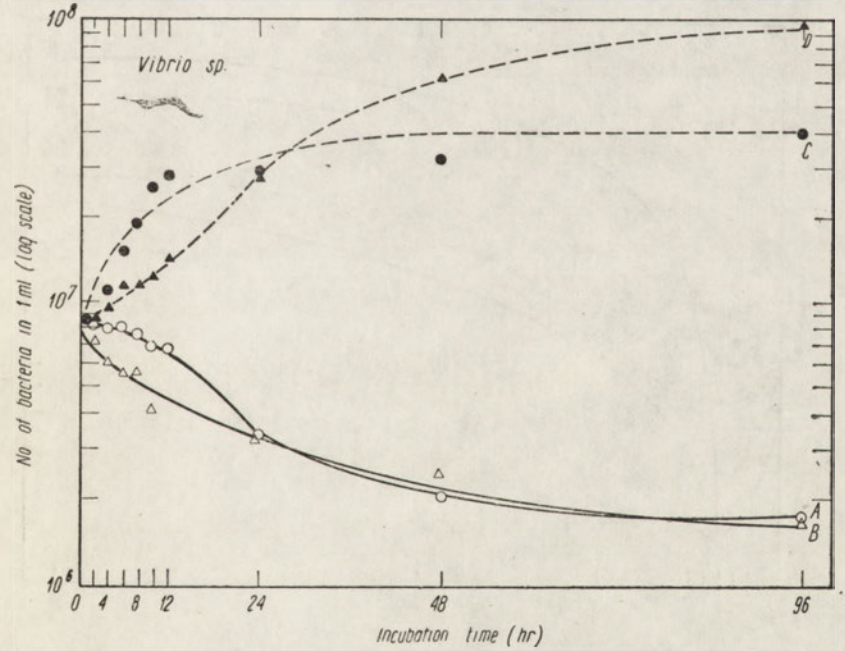

Fig. 4. Survival of *Vibrio sp.* in algal cultures. Denotations as in Fig. 1

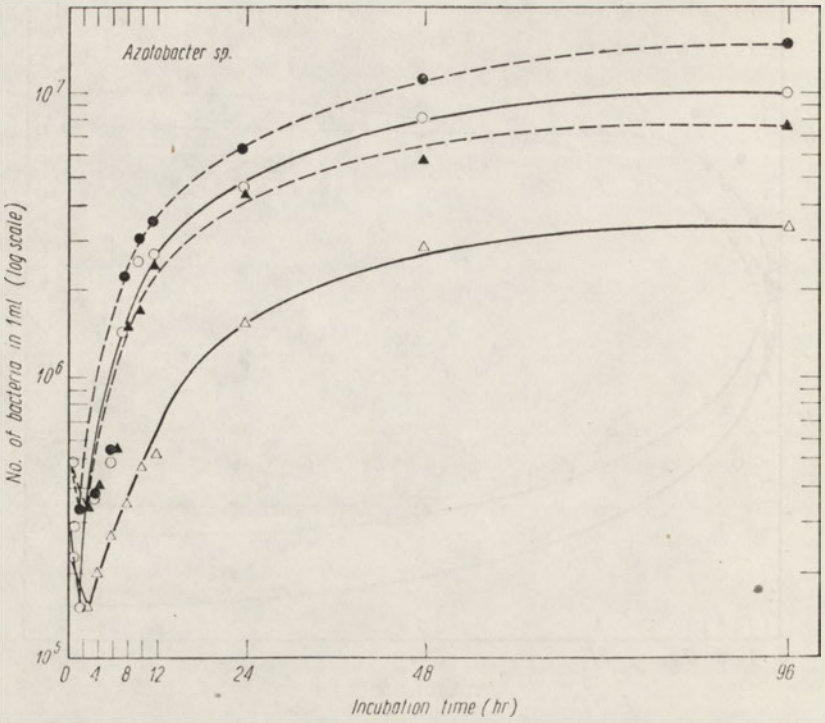

Fig. 5. Survival of *Azotobacter sp.* in algal cultures. Denotations as in Fig. 1

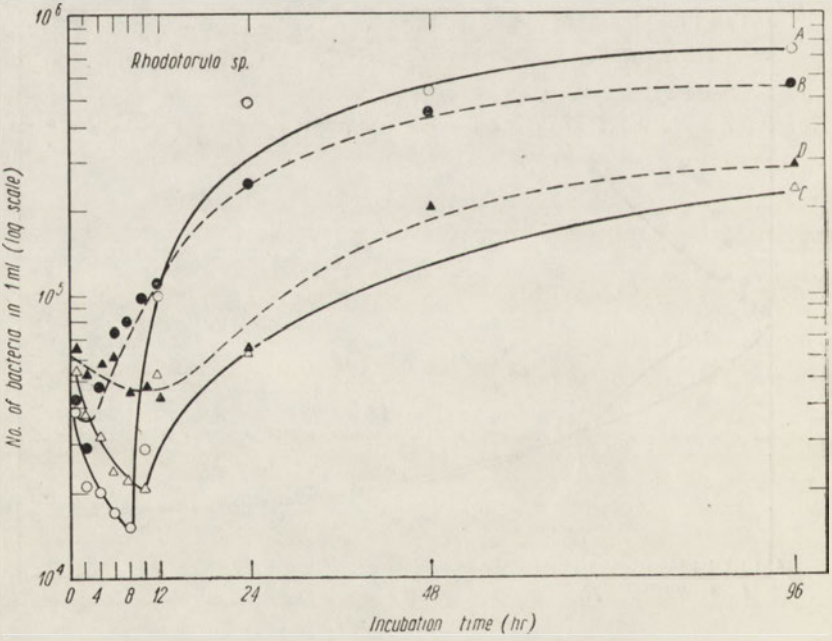

Fig. 6. Survival of *Rhodotorula sp.* in algal cultures. Denotations as in Fig. 1

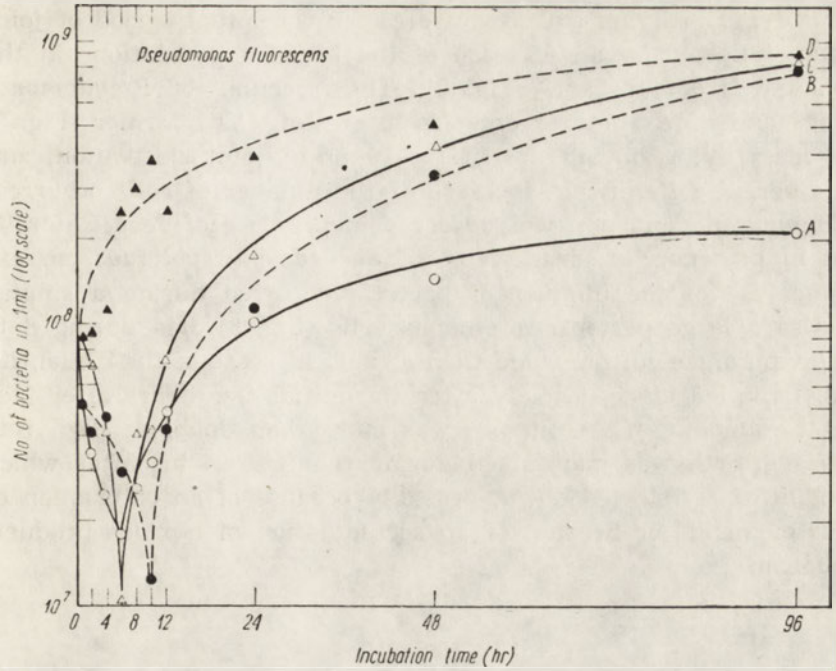

Fig. 7. Survival of *P. fluorescens* in algal cultures. Denotations as in Fig. 1

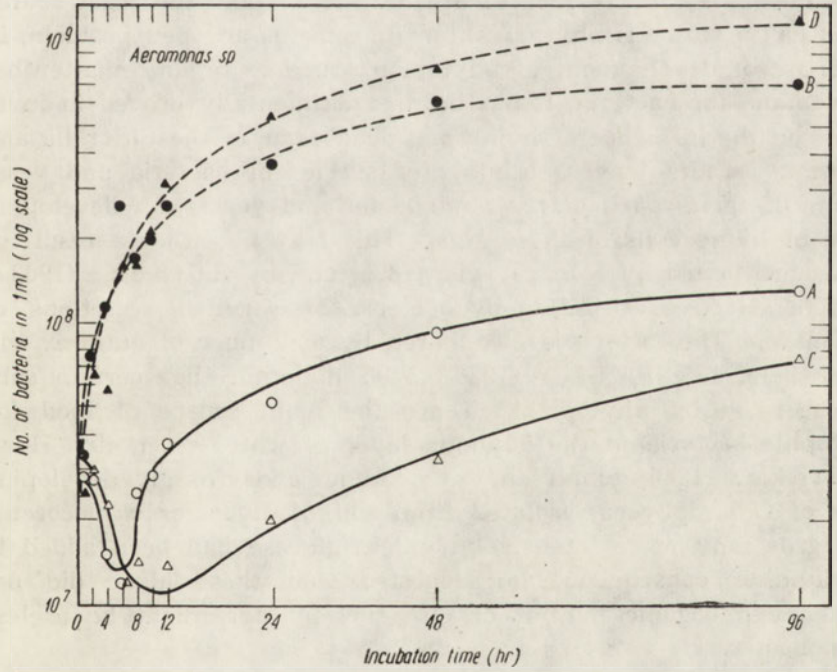

Fig. 8. Survival of *Aeromonas sp.* in algal cultures. Denotations as in Fig. 1

tures, and dying in living cultures of algae in the initial period of joint cultivating, followed by an increase of the bacteria populations in the later phase (*Aeromonas sp.* — Fig. 8). The reaction of *Pseudomonas fluorescens* and of *Aeromonas sp.* was interesting. The former (Fig. 7) revealed mass dying during the first 6-10 hr of joint cultivation, and after this period a significant increase of the number of cells occurred. An analoguous phenomenon was observed in presence of dead *Chlorella vulgaris*. In presence of dead cells of *Scenedesmus obliquus*, an explosive increase of the number of bacteria occurred during a similar period. Also a large part of Aeromonas cells (Fig. 8) died during 8 to 12 hr in living algae cultures, and then a rapid increase of their quantity occurred. In killed algae, already after the initial two hours after inoculation the amount of Aeromonas was more than doubled. High rate of bacteria growth was maintained during the first 24 hr, after which it was significantly slowed down, probably because of an exhaustion of nutritious elements, or because of an accumulation of noxious products of metabolism.

4. DISCUSSION

The main factor responsible for the growth of heterotrophic plant microorganisms is the presence of organic matter, and the main source of the latter in water is phytoplankton. It remains an open problem, in which phase of development of phytoplankton does organic matter become available for bacteria. It has been experimentally proved in investigations on the influence of living and dead algae of the Chlorella and Scenedesmus genera upon a number of species of bacteria and yeast that some of them, particularly Azotobacter and yeast, can develop in presence of living cells of these algae. This may be either a result of symbiosis mentioned by Kossowič (1896) and by Reincke (1903 a, b), or of a selective utilization by bacteria of survival secretions of phytoplankton. The latter view is shared by a number of authors, and some of them, like Pütter (1926), even maintain that certain substances secreted by phytoplankton are the main source of food for heterotrophic bacteria in the euphotic layer of water reservoirs. However, Stokes (1940) found that in young and strongly developing cultures of Chlorophyceae isolated from soil, *Azotobacter chroococcum* did not grow any more intensively, unless glucose had been added to the medium. In consequence, he suggested that these algae did not secrete enough organic matter, or else such matter might be useless for Azotobacter.

However, particularly favourable conditions for bacterial growth

occur after dying of phytoplankton. In dead, autoclave-killed cultures of *Chlorella vulgaris* and *Scenedesmus obliquus*, all the investigated species of bacteria except the *Micrococcus ureae* developed more or less intensively. Similar observations have been made long ago with Azotobacter. Benecke and Keutner (1903) believed that the dying cells of phytoplankton were perfect food supply for Azotobacter living in sea waters. Keutner (1905) proved that *Azotobacter chroococcum* could be found mainly on phytoplankton remnants, where the conditions of development were excellent for it because of food abundance. The relationship between the development of bacteria and phytoplankton was also confirmed by Butterfield and Purdy (1931), Waksman et al. (1933), Collins (1957), Manujlova (1959), and by others. According to these authors, intensive development of phytoplankton is connected with an inhibition of the growth of bacteria, while its mass dying after the blooms is favourable to the development of bacterial populations because of an increase of organic matter concentration.

In living cultures of *Chlorella vulgaris* and *Scenedesmus obliquus*, the species *Micrococcus ureae, Escherichia coli, Bacillus mycoides* and *Vibrio sp.* revealed very rapid dying, probably because bactericidal substances of the chlorelline type, or chlorophyll derivates, were secreted to the medium (Pratt et al. 1944, Steeman-Nielsen 1955, Levina 1961, Telitčenko et al. 1962, Sieburth 1964a, b) During the first 24 hr of joint cultivation with the algae, the process occurred with logarythmic acceleration in case of *Micrococcus ureae* and *Escherichia coli*. Later, the rate of cell dying of all the vulnerable bacteria was much slowed down, so that after 96 hr their survival was still a few per cent.

Investigating the influence of *Chlorella vulgaris, Scenedesmus quadricauda*, and *Ankistrodesmus anquistus*, upon microorganisms, Steblyuk (1968) found that *Escherichia coli* was more resistant to the action of antibiotic substances of these algae than *Salmonella typhi murium, S. enteritidis gärtneri* and *S. cholerae suis*. The latter were almost completely killed after 24 hr of joint cultivation with the mentioned algae, while the growth of *E. coli* was less inhibited. In other algae cultures, particularly in *Stratonostoc linckia* (Roth) Elenek, *Polyporthrix tenuis* (Kütz.), *Phormidan uncinatum* (ag.) Gomm., the *E. coli* was completely killed only after 7 to 17 days, and *Salmonella typhi, Shigella sonnei, Proteus vulgaris* and *Staphylococcus aureus* revealed lesser resistance (Postolica 1968).

In presented studies more resistance to bactericidal action of substances secreted to the medium by cultures of *Chlorella vulgaris* and *Scenedesmus obliquus* was shown by *Pseudomonas fluorescens, Aero-*

monas sp., *Rhodotorula sp.* and *Azotobacter sp.* The three first mentioned species began to die on mass scale during the initial period of joint cultivation with the algae, but after a few hours of adaptation to the peculiar environmental conditions they started to develop more or less intensively, and a significant increase of the number of cells could be soon observed. The process of adaptation took only two hours in case of Azotobacter, after which the amount of these bacteria has been increasing logarythmically. It is possible that the *Azotobacter sp.*, and some other species of the investigated bacteria, produce some peculiar enzymes, able to make up for the noxious activity of the algae metabolism products.

The results here obtained prove that the mutual relationships between algae and bacteria are very complex and diversified. They depend in large measure upon the kinds of interacting organisms.

5. SUMMARY

The study concerned the influence of living and dead cells of *Chlorella vulgaris* and of *Scenedesmus obliquus* upon some species of saprophytic bacteria (*Micrococcus ureae*, *Aeromonas sp.*, *Pseudomonas fluorescens*, *Escherichia coli*, *Bacillus mycoides*, *Vibrio sp.*, *Azotobacter sp.* and the *Rhodotorula sp.* yeast), sampled randomly from among a few hundreds species isolated from Iława lakes water.

Four different ways of behaviour of microorganisms have been found, in presence of living and dead cells of *Chlorella vulgaris* and of *Scenedesmus obliquus*: 1. dying of microorganisms in living and dead cultures of algae (*Micrococcus ureae*); 2. dying of microorganisms in living cultures of algae, and development in killed cultures (*Bacillus mycoides*, *Escherichia coli*, *Vibrio sp.*); 3. dying of microorganisms in living and dead cultures of algae in the initial period of joint cultivation, and development in the later period (*Azotobacter sp.*, *Pseudomonas fluorescens*, *Rhodotorula sp.*); 4. intensive development of microorganisms in killed cultures of algae, and dying in living cultures in the initial period of joint cultivation, followed by development in the later period (*Aeromonas sp.*).

6. STRESZCZENIE

Badano wpływ żywych i martwych komórek *Chlorella vulgaris* i *Scenedesmus obliquus* na niektóre gatunki bakterii saprofitycznych: *Micrococcus ureae*, *Aeromonas sp.*, *Pseudomonas fluorescens*, *Escherichia coli*, *Bacillus mycoides*, *Vibrio sp.*, *Azotobacter sp.* i drożdże *Rhodotorula sp.*, wybrane losowo spośród kilkuset szczepów wyodrębnionych z wody jezior iławskich. Stwierdzono 4 różne sposoby zachowania się badanych drobnoustrojów w obecności żywych i martwych komórek *Chlorella vulgaris* i *Scenedesmus obliquus*: 1. obumieranie drobnoustrojów w żywych i martwych hodowlach badanych glonów (*Micrococcus ureae*); 2. obumieranie drobnoustrojów w żywych hodowlach glonów oraz intensywny rozwój w hodowlach zabitych (*Bacillus mycoides*, *Escherichia coli* i *Vibrio sp.*); 3. obumieranie drobnoustrojów w żywych i martwych hodowlach glonów w początkowym okresie wspólnej hodowli oraz rozwój w późniejszym okresie (*Azotobacter sp.*, *Pseudomonas fluorescens*, *Rhodotorula sp.*); 4. intensywny rozwój drobnoustrojów w hodowlach glonów zabitych i obumieranie w żywych, w początkowym okresie wspólnej hodowli oraz wzrost liczebności w okresie późniejszym (*Aeromonas sp.*).

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THE APPLICATION OF BIOLOGICAL INDICATORS TO EVALUATE THE DAIRY SEWAGE TREATMENT IN BIOLOGICAL DITCHES

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ABSTRACT

The investigation of species composition of organisms in two biological ditches purifying dairy sewage were done. The investigations concerned the sewage inflow and outflow, activated sludge and periphyton. The activated sludge was studied more thoroughly. On the basis of the organisms community and morphological features the activated sludge from Bochnia was evaluated as a good one, but the one from Garwolin was found to be overloaded. Good sediment had the saprobity of the order of αm. Sessile Protozoa — *Vorticella convallaria* were abundant, free-swimming and crawling Protozoa were not so numerous. The overloaded sediment had the saprobity of p-αm. The filamentous fungi of the genus Mucor, and also Flagellata were very numerous there. Therefore the possibility of applying certain biological indicators was suggested for evaluation of the quality of both municipal and dairy sewage.

CONTENTS

1. INTRODUCTION

The biological ditches, called also circulation ditches are used for the sewage-treatment from small settlements, and industrial wastes from small industrial and dairy plants.

The decomposition of organic substances to mineral salts takes place in the ditches with the help of activated sludge composed of yellow-brown flocks with the diameter of several mm. These flocks are formed of the groupings of zoogleal bacteria, various species of Protozoa, Rotatoria, and Bacillariophyta, Cyanophyta and Chlorophyta which settle on them. The activated sludge appears automatically after few days of ditch's usage. It is assumed, that suspensions and colloids from sewage are adsorbed and coagulated on the surface of flocks, and consequently they

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are submitted to the activity of microorganisms (Sladeček 1958, Bolotina 1960, Pasveer 1961).

The dairy sewage is characterized by considerable changes in the amount of waste load. Physico-chemical investigations are not adequate for the control of plant work, as they allow only to estimate the state of sewage at the moment of sampling. This is often not characteristic for the investigated environment, and it may even lead to wrong conclusions. The biological investigations, on the basis of indirect concluding, allow to characterize the environment. The composition of biocenosis communities provides information not only on the environmental conditions at the moment of sampling, but also on the conditions during the period of establishing of this specific biocenosis.

The aim of the paper was to study the species composition of organisms in the investigated ditches, to determine the saprobity of activated sludge and to value the effectiveness of sewage-treatment on the basis of biological indicators considering their usefullness for dairy sewage. The physico-chemical indicators were also studied for comparison with the biological evaluation.

2. MATERIAL AND METHODS

A. TECHNOLOGICAL CHARACTERISTICS OF STUDIED PLANTS

The investigated biological ditches were built according to the project of Czesław Zabierzewski, a civil engineer from Biuro Projektowo-Konstrukcyjne Centralnego Związku Spółdzielni Mleczarskich in Warsaw. These sewage-treatment plants are in the Dairy Co-operatives in Garwolin in Warsaw province and in Bochnia in Cracow province. In both places the same procedure of sewage-treatment was applied during the investigations. The network of channels leads the sewage to the chamber with bars, where on the steel rods larger litter is stopped. After the preliminary cleaning the sewage inflowed to the circulation ditch.

In both investigated plants the ditches were working with some intervals, as the so-called accumulative ditch. The work cycle of such accumulative ditch is as follows; the sewage inflow to the ditch takes place as the sewage outflows from the plant. The investigations show that the amount of sewage inflow, and the concentration of organic substances are differentiated during a day. It was found, that the largest amounts of sewage and largest loads of contamination flow into the ditch in the period between 11 a.m. and 4 p.m. The sewage inflow causes a gradual filling of the ditch until the predicted maximal level of filling. In the ditch the sewage is stirred with the help of Kessener's brushes (cage rollers made from steel flat bars) run by electric motors. The brushes cause also the aeration of suspension of sewage and activated sludge and they move it, thus causing the circulation. The rate of flow should exceed 0.3 m/sec, to prevent the sedimentation of activated sludge on the bottom of the ditch. Early in the morning, before the plant starts to work, the brushes are stopped for 1 hr. During this hour the activated sludge settles at the bottom thus cleaning of the upper layer of sewage. Then the outflow leading to the receiver is opened. Each time about 1/3 of the total ditch capacity is removed, and the whole cycle starts again.

The circulation ditch in Bochnia can be characterized by: total length — 125 m, trapezoid shape with the base about 2 m, and the slope of the banks 1:1, the used capacity 280 m^3, maximal height of filling — 0.8 m, 2 aerating brushes, the stoppage of sewage in the ditch during the investigations was about 4 days and the volumetric load — about 180 g/m^3. Diagram of the Bochnia ditch is shown in Fig. 1.

The circulation ditch in Garwolin can be characterized by: the total length — 310 m, the width — 3.10 m, used capacity — 1060 m^3, maximal height of filling — 0.85 m, 3 aerating brushes, period of sewage stoppage in the ditch — 3 days, and the volumetric load — 312 g/m^3. The diagram of the ditch in Garwolin is shown in Fig. 2.

B. BIOLOGICAL METHOD OF STUDIES

The same method was applied for both ditches. The samples were collected from the following places:

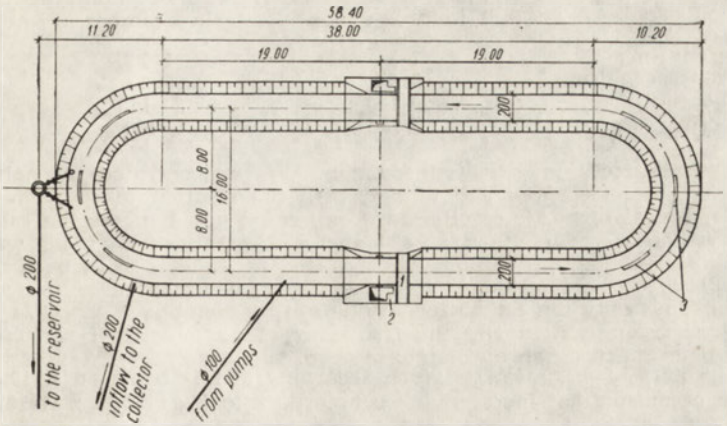

Fig. 1. The diagram of biological ditch in Bochnia. 1 — platform; 2 — cage type brush; 3 — brick guide L = 2.00 m

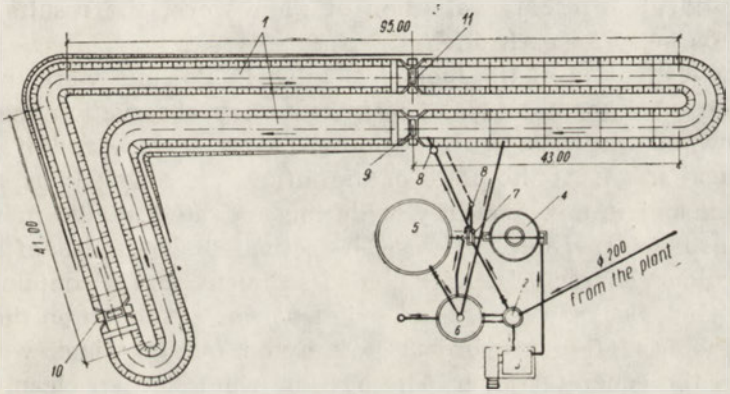

Fig. 2. The diagram of biological ditch in Garwolin. 1 — biological ditch; 2 — catch basin; 3 — sewage pumps; 4 — Imhoff's settler; 5 — reserve tank; 6 — secondary settler; 7 — distributor; 8 — flow collector; 9 — aerating brush No. 1; 10 — aerating brush No. 2; 11 — aerating brush No. 3

1. Inflow of sewage — from the collector located behind the pumps, during the inflow to the ditch;
2. Activated sludge — behind the brush located near the inflow;
3. Outflow — from the collector at the outflow;
4. Periphyton — from various places in the ditch.

The free swimming bacteria were counted under the microscope on slides, but Protozoa and other organisms in a modified Sedgwick-Rafter biological chamber of a capacity 0.5 ml.

In the investigations the estimative and quantitative methods were applied. This first method, used for the investigations of the activated sludge (not condensed) was based on the four steps scale:

Occurrence frequency	Number of bacteria in 1 field of vision	Number of organisms in chamber	Denotation
Sporadic organisms	1-5	1-5	+
Not numerous organisms	5-20	5-20	++
Numerous organisms	20-100	20-100	+++
Very numerous organisms	>100	>100	++++

The quantitatively investigated sediment was concentrated by half an hour sedimentation, then decanted and diluted with the solution taken from above the sediment in a ratio 1:1. All results were expressed per 1 ml of the sample. (The Bacteriophyta and Cyanophyta were not analysed quantitatively. This was because of the possibility of a considerable error caused by small size of bacteria and the fact that Chroococcus was included in the flocks, what obscured the microscopic observation. Thus only the estimative results are presented).

The species composition and the frequency of occurrence of periphyton organisms was determined by an estimative method.

The saprobity of organisms is given according to Liebmann's (1962) system, and of the organisms not included there according to Kolkwitz (1950) and Hanuška (1956).

3. RESULTS

A. BIOLOGICAL RESULTS

Because of the basic different in the composition of biocenoses of both ditches, and of different evaluation of their work, the results are presented separately for each ditch.

The investigation of the biological ditch in Bochnia were carried out on the 8th of December 1964. The introductory observation showed that the inflow stream was lightly coloured, very turbid, and it produced intense acid smell. At the place of the inflow the sewage was stirred by the brush, and it mixed evenly with the activated sludge in the ditch without producing visible streams. The activated sludge was of a yellow-brown colour, characteristic for a good sediment, and it contained small, easily settling flocks. The residual sediment was not found on the bottom. Small amounts of yellow-brown periphyton overgrowings were developing on the concrete slopes. The outflow water looked clean, and was as transparent as the clean water. This allowed to presume that the ditch works well.

The microscopic investigations of the inflowing sewage showed the presence of large amounts of free-living bacteria (Table I).

While analysing the activated sludge it was found, that the flocks

Table I. The sewage inflow

Ditch	Estimative No. of Bacteriophyta in 1 ml
Bochnia	++++
Garwolin	+++

were composed of groupings of bacteria, colloids and other substances, and also of large amounts of colonies of Cyanophyta from the genus

Table II. The activated sludge

Organisms	Saprobity	Estimative number in 1 ml of sediment	Number in 1 ml of concentrated sediment
Biological ditch in Bochnia			
Bacteriophyta			
Bacteriophyta non det.		++++	
Cyanophyta			
Chroococcus sp.		++++	
Oscillatoria formosa	αm	+	300
Bacillariophyta			
Nitzschia palea	αm	++++	3780
Protozoa			
Ciliata			
Aspidisca costata	βm	++	870
Chilodonella uncinata	αm	++	580
Cinetochilum margaritaceum	o-p	++++	2330
Hemiophrys agilis		+++	870
Holosticha brevis		+	290
Lionotus sp.		+++	1160
Metopus palaeformis f. attenuata		++	580
Opercularia sp.		+	150
Oxytricha sp.		+	300
Paramecium caudatum	αm	+++	1160
Vorticella convallaria	αm	++++	16,000
Vorticella sp.		+	300
Flagellata			
Ancyromonas contorta	o	+++	2030
Cercobodo ovatus	αm-βm	+++	2330
Mastigella commutans	o	+	290
Peranema trichophorum	p-o	+	150
Flagellata non det.		+++	870
Rhizopoda			
Amoeba limax	p-αm	++	580
Rotatoria			
Epiphanes senta	αm	+	20
Biological ditch in Garwolin			
Bacteriophyta			
Bacteriophyta non det.		++++	
Fungi			
Mucor sp.	p-αm	++++	1775
Protozoa			
Ciliata			
Colpidium colpoda	p-αm	+++	73
Vorticella microstoma	p	++	29
Flagellata			
Monas archabdomonas	p-αm	++++	1513
Cercobodo radiatus	p	++	15

Chroococcus. Cyanophyta were also present as individual organisms separated from the flocks (Table II).

On the flocks the large amounts of sessile Protozoa — *Vorticella convallaria* L. were present, and they dominated among all sediment Protozoa. Apart from that *Vorticella sp.* and *Opercularia sp.* were found sporadically.

Small numbers of free-swimming and crawling Protozoa were observed among the flocks. *Cinetochilum margaritaceum* Perty was most frequent among them, then *Paramecium caudatum* Ehrb. and *Lionotus sp.* Less frequently were found the following species: *Aspidisca costata* Duj., *Hemiophyrs agilis* Pen., *Chilodonella unicata* Ehrb., *Metopus palaeformis f. attenuata* Kahl, *Oxytricha sp.* and *Holosticha brevis* Stokes. Among the other Protozoa there were: Flagellata — *Ancyromonas contorta* Lemn., *Cercobodo ovatus* Lemn., *Mastigella commutans* Meyer, *Peranema trichophorum* Ehrb., *Flagellata non det.* and Rhizopada — *Amoeba limax* Duj.

Among the Rotatoria, *Epiphanes senta* Müll. was present.

Among the Cyanophyta, apart from the *Chroococcus sp.* described above, *Oscillatoria formosa* Bory was present in a small number.

Among the Bacillariophyta, *Nitzschia palea* Kütz. was present in a relatively large number.

The saprobity of 13 species was estimated out of the total of 16 determined species of organisms. On the basis of a clear dominance of alfa-mezosaprobical organisms (αm) the same range was accounted for the whole sediment environment.

The number of free-living bacteria decreased in the outlet and *Vorticella f. telotroch, Mastigella commutans* and *Nitzschia palea* (Table III) occurred sporadically.

In the periphyton (Table IV), *Nitzschia palea* and *Vorticella f. telo-*

Table III. The sewage outflow

Ditch	Organisms	Estimative number in 1 ml
Bochnia	Bacteriophyta	++
	Mastigella commutans	+
	Nitzschia palea	+
	Vorticella f. telotroch	+
Garwolin	Bacteriophyta	++
	Colpidium colpoda	+++
	Flagellata non det.	++
	Monas archabdomonas	++++
	Small flocks	++++

Table IV. The periphyton

Ditch	Organisms	Estimative number in 1 ml
Bochnia	*Lionotus sp.*	+++
	Nitzschia palea	++++
	Oscillatoria formosa	+
	Oxytricha sp.	+
	Paramecium caudatum	+
	Vorticella sp. f. telotroch	++++
Garwolin	*Chlorella vulgaris*	++++
	Flagellata non det.	+
	Monas archabdomonas	+
	Mucor sp.	++++
	Oscillatoria formosa	+
	Navicula minima var. atomoides	+
	Nitzschia palea	+

troch occurred abundantly, as well as some other organisms from the active sediment, probably casual components, which were present in small number.

The biological ditch in Garwolin was investigated on the 19th of March 1965. The sewage inflow was of a dirty white colour, wery turbid, and it produced an unpleasant acrid smell characteristic for condensed dairy sewage. The sewage inflow was well mixed with the activated sludge by brush in the ditch, without producing visible sewage streams. The activated sludge was of a light yellow colour non-typical for good sediment, and it contained small slowly settling flocks. This allowed to expect the occurrence of filamentous fungi in the activated sludge. The residual sediment was not found on the bottom. The periphyton green-yellow overgrowings were developing on the concrete parts of the ditch. The outflow was colourless and it produced no smell, but minute white and opalescent suspensions were present there.

The microscopic observations showed the occurrence of relatively large amounts of spheric forms of free swimming bacteria (Table I).

The flocks were the basic element of the active sediment and amounted to about 6400 per 1 ml. The average length of a flock was about 213 μ, the average width — 15 μ. The flocks were composed of zoogleal groupings of bacteria with filamentous fungi from the genus Mucor, which occurred in large numbers. The free-living bacteria and Flagellata — *Monas archabdomonas* (Fisch) H. Meyer. were also very numerous. *Cercobodo radiatus* (Klebs), and among Ciliata — *Colpidium colpoda* and *Vorticella microstoma* Ehrb. were less numerous.

The organisms dominating in the sediment were p-αm saprobical, while the rest of them — polysaprobical. On this basis the sediment was estimated as poly-alfa-mezosaprobical with the predominance of polysaprobity.

The outflow (Table III), even after an hour of sedimentation, contained as already mentioned minute suspensions which consisted of very small bacterial and fungal flocks. The free-living bacteria occurred in a small number. *Monas archabdomonas* was very abundant, *Flagellata non det.* were not numerous, but *Colpidium colpoda* was relatively abundant.

The periphyton (Table IV) developed relatively well on the concrete parts of the ditch on the level of sewage surface, close to the aerating brushes. It consisted of large amounts of fungi of the genus Mucor, and of algae — *Chlorella vulgaris* Beyer. The other algae — *Navicula minima var. atomoides* (Grün), *Nitzschia palea, Oscilatoria formosa,* the Protozoa — *Monas archabdomonas* and *Flagellata non det.* occurred in small numbers. The obtained results are presented in the tables.

B. PHYSICO-CHEMICAL RESULTS

The investigations in Bochnia were carried out on the 8th and 9th December 1964, when the temperature of surrounding varied within the limits −5 to +5°C. The temperature of sewage in the ditch was always above +5°C, apart from the relatively low temperature of surroundings. The investigations showed a very high degree of sewage purification, and for some indicators it was even above 99%.

The results presented in Table V are the arithmetic mean values of the sampling made in hour intervals. It can be generally said, that the

Table V. Biological ditch in Bochnia. Mean results of the two day physico-chemical investigations

Date	Type of sewage	Oxygen content (mg/l)	O.D. (mg O_2/l)	BOD-5 (mg O_2/l)	Suspension content (mg/l)
8.XII.64	sewage inflow	7.2	161.0	205.0	253.0
	sewage outflow	10.4	8.0	7.1	—
9.XII.64	sewage inflow	5.6	740.0	310.0	446.0
	sewage outflow	10.0	10.0	3 0	—

biological sewage-treatment ditch in Bochnia worked very efficiently. The results on the reduction of contamination and the parameters of work allow to conclude, that such ditches can be successfully applied for dairy sewage-treatment in Poland.

The investigations in Garwolin were carried out on the 19th and 20th March 1965. The temperature of the surrounding varied then within the limits 6-10°C. Although the investigations showed a relatively high reduction of sewage contamination the effect of treatment was considerably worse than that in Bochnia.

The results presented in Table VI are the arithmetic mean values of the sampling made in hour intervals. These results point to the over-

Table VI. Biological ditch in Garwolin. Mean results of the two day physico-chemical investigations

Date	Type of sewage	Oxygen content (mg/l)	O.D. (mg O_2/l)	BOD-5 (mg O_2/l)	Suspension content (mg/l)
19.III.65	sewage inflow	1.5	320.0	647.0	315.0
	sewage outflow	2 0	60.0	41.7	96.0
20.III.65	sewage inflow	3.1	438.0	760.0	223.0
	sewage outflow	3.5	24.0	30.7	97.0

loading of the activated sludge with the oorganic wastes, and therefore the treatment results are worse.

It can be generally said, that in both of the investigated plants, apart from low winter temperatures, very good effects of treatment were obtained. The ditch in Bochnia worked with a reasonably low load. All organic wastes as well as the activated sludge there underwent the processes of biological oxidation. The oxygen content in the sewage was very high due to the good aerating system. The ditch in Garwolin during the investigations was overloaded, which apart from the same time of sewage stoppage, caused a high condensation of organic wastes in the outflow. Although three aerating brushes were installed, the oxygen content in the sewage was low, which together with a rise of temperature could cause the oxygen depletion. In such conditions not all activated sludge is oxidized, and the excess of formed sediments must be occasionally disposed of, which complicates the procedure.

4. DISCUSSION

The species composition and saprobity of organisms were different in both ditches. On the basis of these data it was found that the ditch in Bochnia worked well due to proper sewage-treatment. The ditch in Garwolin worked worse, as the treatment efficiency was not as good.

Both sewage inflows were very turbid and produced an unpleasent acid smell. The microscopic observations showed differences between the

two inflows. In Bochnia ditch there was more bacteria than in Garwolin one. A large number of bacteria is not always a result of higher contamination. In very condensed wastes the bacteria have worse development conditions, and this is reflected in their abundance. Such situation could have place in this instance. It is also possible that at the moment of sampling in Garwolin ditch the sewage could be less condensed as the load of contamination varied in time.

The association of organisms in activated sludge was basically different in both ditches. The following general indicators of biological control of sediment can be presented:

A well working sediment is characterized by a large number of sessile Protozoa from the genera Vorticella, Opercularia, Carchesium and a small number of free swimming and crawling Protozoa. The flocks of such sediment are small and they easily settle. In the badly working sediment the Flagellata and Sarcodina are present in large numbers. Often a large number of free-living bacteria from the genus Spiryllum will occur, and also the filamentous bacteria and fungi, causing the so-called swelling of sediment, i.e. an increase of flocks' surface, thus making then settling difficult (Baines et al. 1953, Reynoldson 1942, McKinney and Gramm 1956).

The above indicators were based mainly on the investigations of activated sludge in municipal sewage, but their application for dairy sewage was not studied. The biological investigations of the active sediment purifying the dairy sewage are not advanced. The only authors who investigated this problem were Meinecke (1961), and Kalisz et al. (1963).

The activated sludge from Bochnia had a yellow-brown colour characteristic of a good sediment, and it easily settled. Large amounts of sessile Protozoa of the genus Vorticella were present in it, as well as small amounts of free-swimming Protozoa. Thus the organisms occurred according to the parameters of well working sediment, as given in the biological indicator of the sediment control.

The activated sludge from Garwolin had a light yellow colour, and it hardly settled. Flagellata and filamentous fungi occurred there in masses. Such biocenosis, according to the biological indicator, proves about the overloading of the sediment.

The groupings of organisms found in the sediment suggest thus a possible application of the discussed biological indicator to evaluate the broader variety of wastes than the municipal wastes.

The sediment can be also qualitatively characterized by the determination of saprobity of its organisms. The sediment is determined as a good one, if the alfa-mezosaprobes are the dominants. In the over-

loaded sediment the polysaprobes (L i e b m a n n 1962) are the dominants. According to the system of saprobes the environment of the activated sludge in Bochnia was determined as alfa-mezosaprobical. The domination of organisms with such saprobity was the basis for such a conclusion. Organisms which are characteristic for oligosaprobical zone were also found in this sediment. Their survival in the sediment confirms the conclusion about not overloading of the sediment, and thus of its proper exploitation.

In Garwolin the dominating organisms were within the range of saprobity from polysaprobical to alfa-mezosaprobical, and the remaining ones were polysaprobical. The saprobity of the sediment was thus determined as p-αm with the dominance of polysaprobical elements. This confirmed the conclusion of the overloading of sediment.

In the outlets of both ditches the number of bacteria was greatly reduced. In less purified Garwolin sewage the considerable quantities of microorganisms and small flocks were found, which due to the occurrence of filamentous fungi were slowly settling. In well purified sewage from Bochnia, the organisms were only sporadic, and no suspension occurred.

The aim of periphyton investigations was to find its species composition, and to determine, whether the overgrowings develop to such an extent, that they can slow down the circulation of activated sludge. The observations, however, did not confirm that. The dominants in periphyton occurred also in masses in the activated sludge, what allowed to presume that periphyton was functionally connected with the activated sludge and it did not establish a separate biocenosis.

The results of biological investigations were confirmed by physico-chemical analyses. According to it, the biological ditch in Bochnia worked with a small load of sewage, and the effectiveness of treatment was very high. The sewage load in Garwolin was large, and the treatment was worse.

5. SUMMARY

The biological and physico-chemical investigations of two dairy sewage biological purification ditches were made.

The biological ditch in Bochnia (Fig. 1) worked with the low load of sewage. The ditch in Garwolin (Fig. 2) was overloaded with sewage.

The paper aimed at a study of groupings of organisms occurring in these two differently working ditches, and to find out whether the biological characteristic of activated sludge is sufficient to evaluate the purification treatment efficiency.

The organisms in activated sludge were studied most thoroughly (Table II), and also the organisms in inflow (Table I), outflow (Table III) and in periphyton (Table IV).

It was found that in the active sediment in Bochnia there were mainly large amounts of sessile Protozoa from the genus Vorticella, and a small number of free

swimming and crawling Protozoa. The sediment was yellow-brown, it easily settled, and the saprobity was of the order of αm.

It was found that in the activated sludge in Garwolin mainly Flagellata and filamentous fungi from the genus Mucor occurred. The sediment was light-yellow, it settled hardly and the saprobity was of the order of p-αm.

Thus the sediment was different depending on the degree of sewage load. Similar properties and groupings of organisms are found in municipal sewage treatment. They are considered the indicators of good or bad quality of the sediment. It has been proposed to apply these indicators for both the municipal and dairy sewage.

A different effect of treatment was observed in the outflow. In Bochnia outflow the microorganisms occurred only sporadically, and no suspension was present. In Garwolin one, it carried large quantities of microorganisms and minute suspension of hardly setting flocks "swelled" due to the presence of filamentous fungi.

The biological evaluation was confirmed by the physico-chemical investigations (Table V and VI).

6. STRESZCZENIE

Przeprowadzono badania biologiczne i fizykochemiczne dwóch rowów biologicznych oczyszczających ścieki mleczarskie. Rów biologiczny w Bochni (Fig. 1) pracował w warunkach niskiego obciążenia ładunkiem zanieczyszczeń; rów w Garwolinie (Fig. 2) — w warunkach nadmiernego przeciążenia.

Celem pracy było poznanie zespołów organizmów zasiedlających te dwa różnie działające rowy i stwierdzenie, czy na podstawie biologicznej oceny osadu czynnego można sądzić o efektywności oczyszczania. Szczególną uwagę zwrócono na organizmy osadu czynnego (Tab. II), ale badano je również w ściekach dopływających (Tab. I), ściekach odpływających (Tab. III) i peryfitonie (Tab. IV).

Stwierdzono, że w skład osadu czynnego z Bochni wchodziły głównie bardzo duże ilości Protozoa osiadłych z rodzaju Vorticella i niewielkie ilości Protozoa wolno pływających i pełzających. Osad posiadał żółto-brązową barwę, łatwą opadalność i saprobowość rzędu αm. W skład osadu czynnego rowu w Garwolinie wchodziły głównie masowo występujące Flagellata oraz grzyby nitkowate z rodzaju Mucor. Osad posiadał barwę żółto-białą, trudną opadalność i saprobowość rzędu p-αm.

Charakterystyka osadu czynnego była więc różna w zależności od stopnia jego obciążenia. Podobne cechy i ugrupowania organizmów spotykane są w osadzie czynnym powstającym przy oczyszczaniu ścieków bytowo-gospodarczych. Są one uznane jako wskaźnikowe dla oceny dobrej i złej jakości osadu. Zasugerowano, że wskaźniki te mogą być stosowane jako wspólne dla oceny jakości osadu czynnego zarówno ścieków bytowo-gospodarczych jak i mleczarskich.

Różny efekt oczyszczania przejawił się także w obrazie odpływu. Odpływ w Bochni zawierał sporadycznie występujące mikroorganizmy i nie posiadał żadnej zawiesiny, a w Garwolinie niósł ze sobą znaczne ilości mikroorganizmów oraz drobną zawiesinę trudno opadających kłaczków „spuchniętych" przez obecność w nich grzybów nitkowatych.

Ocena biologiczna została poparta wynikami badań fizyko-chemicznych (Tab. V i VI).

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LETHAL TEMPERATURES OF ROACH FRY (*RUTILUS RUTILUS* L.) FROM LAKES WITH NORMAL AND ARTIFICIALLY ELEVATED TEMPERATURE

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ABSTRACT

Reactions to high temperatures of incubation have been compared for roach fry from natural spawning grounds in a lake with normal temperature (Ślesin) and in an artificially elevated one (Licheń), as well as for roach fry incubated in different temperatures in laboratory conditions. No significant differences between lethal temperatures for both populations have been found, while these temperatures depended upon incubation temperatures. An additional acclimation of fry in elevated temperatures caused an appriopriate increase of their lethal temperatures. When fry was kept in sublethal temperatures for 100 hr, a somewhat lesser mortality were observed in the groups from the heated Lake Licheń.

CONTENTS

1. INTRODUCTION

The investigation of thermal tolerance of fish from the Konin group of lakes was intended to bring an answer to the question, whether the heating of water during several years has brought about any changes in reactions to high temperatures among fish population.

Two populations of roach fry have been studied, coming from the Licheń and Ślesin lakes, belonging to the Konin group of lakes, connected by the Warta-Gopło channel.

The Licheń Lake, with 153.6 ha of surface, has been heated by warm water from the Konin power station since March 1958. The differences between temperatures of water taken and returned by the power plant are from 6 to 12 degrees C [1] in summer, and from 10 to 16 degrees C in winter. The amount of water returned to the lake has been increasing, as new generating units were set in motion, from 3.3 m^3/sec in the first half of 1958, up to 29 m^3/sec in the second half of 1964. During the next years there was no more increase, and there was even some decrease in winters, down to below twenty m^3/sec.

[1] The phrase "degrees C" denotes temperature differences, and the symbol "°C" denotes actual temperature values.

The extreme temperatures of the surface layers of Lake Licheń waters during these years were 6.6°C and 31°C.

The Ślesin Lake, with 148.1 ha of surface, is not affected by heated effluents, and its temperatures are typical for an inland lake under this latitude. Its extreme water temperatures during the research period were 0.5°C and 24°C.

2. METHODS

Lethal temperatures were found by the method of gradual heating of water, starting from the acclimation temperature (Tsukuda and Katayama 1957, Hutchinson 1961, Heat 1963, Shkorbatov 1964, Fry 1967, Horoszewicz 1969). In the 1967 field experiments series, in which fry was drawn from its natural spawning grounds, the actual temperature reported there was assumed in each case as the acclimation temperature. Freely swimming fry, in the final phase of resorption of the yolk sac, was transported with water directly from spawning grounds into 250 cm³ Witt's flasks, submerged in 12 l aquariums as their water coating. There were 5 fry in each flask. The rates of temperature increase, and lethal temperatures of each individual were recorded. In the several samples, the ranges of temperatures under which the first and the last individual died, were from 0.5 to 7.5 degrees C. A similarly large range of lethal temperatures, approaching 7 degrees C, was observed in roach fry by Cocking (1959), when the temperature increased at a rate of only 1/20°C per hr. In the series of experiments now reported, the medium rate of temperature increase ranged from 0.10 to 0.16°C per min, and it was not found to be related to either the range nor the extent of lethal temperatures in the studied sample (Fig. 1). The fairly extensive range of lethal

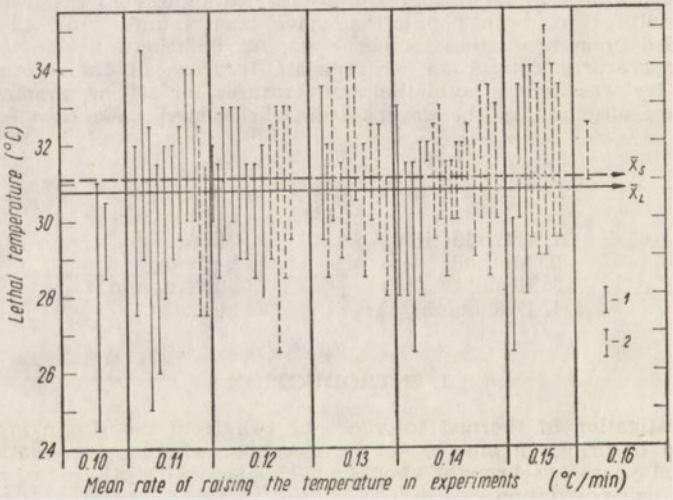

Fig. 1. The range of lethal temperatures of the samples, under different rates of increase of water temperature. 1 — Lake Licheń samples; 2 — Lake Ślesin samples; $\bar{x}_S$ — mean lethal temperature for Lake Ślesin roach fry; $\bar{x}_L$ — mean lethal temperatures for Lake Licheń roach fry

temperatures may have as its cause the large divergence of development phases in roach fry drawn from their natural spawning grounds. This is indicated by the next series of measurements, taken in 1968 on roach fry incubated in laboratory in temperatures from 12.0 to 15.0°C, 18.0 and 23.0°C, from spawn of a single female from Lake Licheń. In this fry the range of lethal temperatures did not exceed 2.1 degrees C in any group, though under the most uniform conditions of incubation of homogeneous spawn, some divergence of development phases are observed in the larvae (Emeljanov 1965). The need to secure homogeneous fry with

a known "thermic history" for comparisons emerged, besides methodological considerations, mainly from some assumptions concerning the actual research problem, which will be discussed in the relevant sections.

Lethal temperatures were established for a total of 239 roach fry from Lake Ślesin spawning grounds, 209 fry from Lake Licheń, and 30 of fry from the latter lake, incubated in laboratory. Besides, for each of these groups mortalities were recorded (percentage of dead larvae) after 50 and 100 hr of exposition to sublethal temperatures.

3. RESULTS

A. MORTALITY OF ROACH FRY FROM NATURAL SPAWNING GROUNDS IN LETHAL TEMPERATURES

In 1967 lethal temperatures were established during the periods of mass spawning of roach, which occurred in both lakes at similar water temperatures (13.3-18.6°C and 15.0-18.5°C) but at different periods, i.e. between April 6 and 13 in Lake Licheń and between May 25 and 31 in Lake Ślesin.

Mortality of roach fry from both of the compared lakes in lethal temperatures are shown as cumulative curves No. 1 and 2 in Fig. 2. As

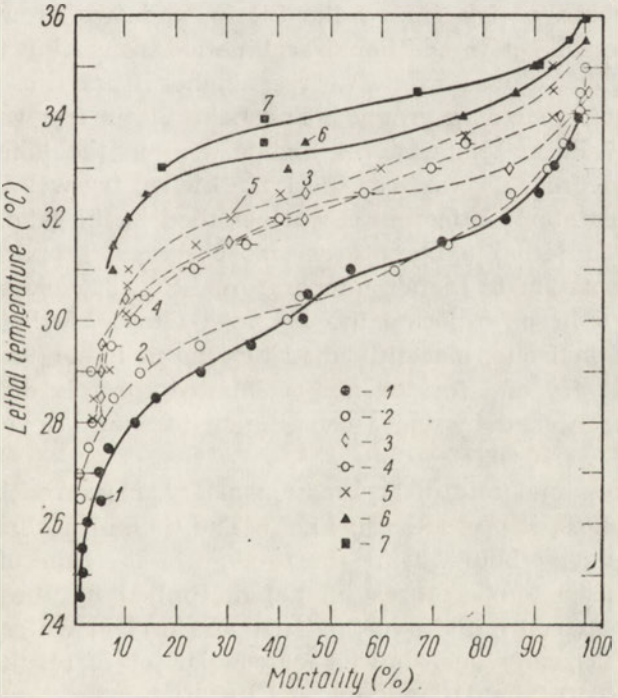

Fig. 2. Distribution of mortality rates for roach fry in lethal temperatures. The continuous line — Lake Licheń fry: 1 — from natural spawning grounds; 6 — additionally acclimated during 24 hr; 7 — during 36 hr. The dotted line — Lake Ślesin fry: 2 — from natural spawning grounds; 3 — additionally acclimated during 6 hr; 4 — during 14 hr; 5 — during 17 hr

it was found that these distributions deviate somewhat from normal, discrepancies were checked by the Smirnov-Kolmogorov consistency test λ for nonparametric distributions. The discrepancies between the distributions were found to be insignificant: $\lambda = 1.02$, at this level of λ, the Q function value was 0.7508 and the probability P was 0.2492.

The discrepancies between mean lethal temperatures and standard deviations for roach fry were similarly found to be insignificant: for the heated Lake Licheń they were $\bar{x}_L \pm SD = 30.8 \pm 1.91$, and for Lake Ślesin $\bar{x}_S \pm SD = 31.1 \pm 1.58$.

The high consistency of results obtained at different times for two different populations, when each of them was acclimated to similar temperature ranges 13.3-18.6°C and 15.0-18.5°C resulting from similar thermic histories of the respective spawning grounds, points only to the generally familiar relationship between lethal and acclimation temperatures.

B. MORTALITY IN LETHAL TEMPERATURES OF FRY EXPOSED TO ADDITIONAL ACCLIMATION

Three groups of fry from Lake Ślesin, and two groups from Lake Licheń, were subject to additional acclimation, consisting in short expositions to temperatures a few degrees higher from those actually observed in their spawning grounds. The Lake Ślesin fry was kept during 6, 14 and 17 hr in temperatures higher than in the lake (respectively 2.5, 3.0 and degrees C), whereas the Lake Licheń fry was kept during 24 and 36 hr in water 5.5 degrees C warmer than in the lake. In all groups an increase of lethal temperatures was observed, proportionate to the time of acclimation to higher temperatures (Fig. 2, curves No. 3-7). Already after 6 hr of exposing the fry to a temp. 2.5 degrees C higher than the original one, mortality distributions in lethal temperatures for this group of fry and for the not acclimated controls differed significantly, when checked by the λ consistency test (at λ level 1.99, Q was 0.9993 and P was 0.0007).

The process and rate of acclimation of fry, measured by lethal temperatures values, is presented in Fig. 3. The time of acclimation is given in terms of degree/hours, being the product of the time of exposing the fry to increased temperatures (in hr) multiplied by the difference (in degrees C) between initial and final temperatures of water for the given fry group. Such approach allowed for comparison of results for 5 groups of fry from both populations, exposed to different acclimation temperature during different times. It turned out that acclimation of fry to new temperatures was similar for both populations during the period of study, and it was analogous to what could be found in literature for

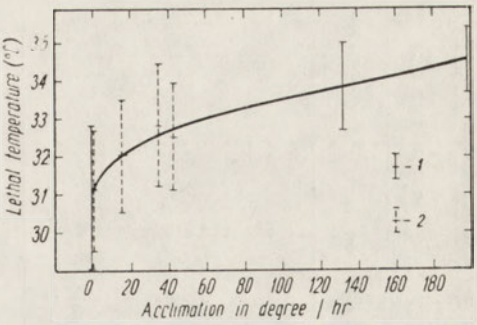

Fig. 3. The relationship between lethal temperatures and acclimation. 1 — samples of fry from Lake Licheń; 2 — samples of fry from Lake Ślesin

other species of fish (Sumner and Doudoroff 1938, Doudoroff 1942). However, the acclimation process was not completed for any group, and lethal temperatures did not reach the values appropriate for the given acclimation temperatures, i.e., the lethal temperature which would remain stable under prolonged exposure of fry to acclimation temperatures.

C. LETHAL TEMPERATURES FOR FRY ACCLIMATED TO FIXED TEMPERATURES

To obtain fry fully acclimated to a given temperature, i.e., with metabolism fixed at the level corresponding to the acclimation temperature, spawn from a single female from Lake Licheń was incubated in laboratory at three different temperatures: changing from 12 to 15°C, and stable 18.0 and 23.2°C. Mean lethal temperatures for the three groups of fry were $\bar{x}_{12} = 29.9°C$, $\bar{x}_{18} = 34.7°C$ and $\bar{x}_{23} = 35.5°C$, respectively, and were eventually considered as reference levels for all groups of fry in field experiments, when lethal temperatures were compared with acclimated temperatures.

D. A COMPARISON OF LETHAL TEMPERATURES AND ACCLIMATED TEMPERATURES FOR ALL GROUPS OF ROACH FRY

The recorded lethal temperatures for all groups of fry are listed in Table I, from lowest to highest. Within such a rank order of lethal temperatures, and thereby of acclimation temperatures, 5 groups of fry from natural spawning grounds exposed to additional acclimation to temperatures of 18, 19 and 22.2°C were ranked above the roach group incubated in laboratory in 18°C, which confirmed the view that the process of acclimation in the 5 above mentioned groups of fry was not completed.

Comparison of lethal temperatures of fry from natural spawning grounds, and thus acclimated to environment with temperatures fluctuating within the range of 3 to 5 degrees C, with results recorded for

Table I. Comparison of mean lethal temperatures of roach fry acclimated to different temperatures

Origin of fry	Temperatures of acclimation (°C)	Mean lethal temp. and Standard Deviations (°C) $\bar{x} \pm SD_{\bar{x}}$	The number of:	
			samples	individuals
L. Licheń — laboratory incubated	fluctuating, 12.0-15.0	29.9 ± 0.53	2	10
L. Licheń — natural spawning grounds	fluctuating, 13.3-18.6	30.8 ± 1.91	28	139
L. Ślesin — nat. sp. gr.	fluctuating, 15.0-18.5	31.1 ± 1.58	32	159
L. Ślesin — nat. sp. gr.	fluctuating, 15.0-18.5 + 6 hr in 18°C	32.0 ± 1.50	4	20
L. Ślesin — nat. sp. gr.	fluctuating, 15.0-18.5 + 14 hr in 18°C	32.5 ± 1.42	6	30
L. Ślesin — nat. sp. gr.	fluctuating, 15.0-18.5 + 17 hr in 19°C	32.9 ± 1.63	6	30
L. Licheń — nat. sp. gr.	fluctuating, 13.3-18.6 + 24 hr in 22.5°C	33.8 ± 1.14	6	29
L. Licheń — nat. sp. gr.	fluctuating, 13.3-18.6 + 36 hr in 22.5°C	34.5 ± 0.86	6	30
L. Licheń — lab. inc.	steady, 18.0	34.7 ± 0.74	2	10
L. Licheń — lab. inc.	steady, 23.2	35.5 ± 0.30	2	10

Table II. Mortality of roach fry in sublethal temperatures

Origin of fry	Temperature (°C)			Mortality (%)			Size of sample (No. of ind.)
	of acclimation	of exposition	control	after 50 hr	after 100 hr	resulting from temp. increase	
L. Licheń — natural spawning grounds	fluctuating 13.3-18.6	30.0	—	43.0	68.0	60.0	50
		—	16.6	5.0	8.0	—	50
L. Ślesin — natural spawning grounds	fluctuating 15.0-18.5	29.5	—	62.0	94.0	72.0	50
		27.0	—	49.0	96.0	74.0	50
		—	16.7-22.0	11.0	22.0	—	50
L. Licheń — laboratory incubated	fluctuating 12.0-15.0	26.0	—	20.5	53.1	40.0	145
		—	13.0-15.0	7.5	13.1	—	145
	steady, 18.0	29.0	—	13.5	98.5		204
		—	18.0	4.5	×	×	204
	steady, 23.2	30.0	—	33.5	93.7	69.1	240
		—	23.2	9.5	24.6	—	240

× — No control data have been recorded because of a damage in the relay circuit.

fry from controlled acclimation temperatures, showed that fry in the phase of resorption of the yolk sac in both lakes was acclimated rather to the lower than to the mean values of the range of fluctuating temperatures recorded on the spawning grounds. An analogous process of acclimation to fluctuating temperatures of natural environment during spring was observed for *Ameiurus nebolosus* from the Opeongo Lake in Canada, under different research methods (B r e t t 1944). During his whole year observations, the author found that acclimation to lower values of the range of changes was characteristic only of the spring season, when water temperatures increase rapidly and steadily. In summer, however, when mean daily temperatures are more stable, lethal temperatures indicate acclimation of fish to maximal observed temperatures.

E. MORTALITIES OF FRY IN SUBLETHAL TEMPERATURES

Roach fry from both of the compared lakes, acclimated to a similar temperature range, was exposed to stable sublethal temperatures during 50 and 100 hr. Control groups of fry were kept in their former temperatures, or in the environment temperature, as in the Ślesin field study. The mortalities in experimental groups, from which mortalities found in the respective control groups during the same period were deducted (Table II), were considered as the effect of high temperature influence. Some differences in mortalities for fry from the two lakes, suggest a little higher resistance to high temperatures in the Lake Licheń population. It means that fry from Lake Licheń could survive a few hours longer in warm water than fry from Lake Ślesin. However, the number of experiments was too small to prove this hypothesis.

4. DISCUSSION

Results of experiments did not reveal clear differences in reactions to high temperatures between roach fry from Lake Licheń, being at least the third, or perhaps even the fifth generation incubated in the heated lake, and roach fry from Lake Ślesin with its normal temperature variations. A shift of the spawning period allowed the Lake Licheń roach to reproduce at water temperatures virtually identical with those in Lake Ślesin, and also with those recorded in roach spawning grounds in many other lakes, like the Mazurian Lake Białe (W i l k o ń s k a and Z u r o m s k a 1967).

The temperature range from 12 to 16°C was the longest prevailing one in natural roach spawning grounds in the Uchinskoye reservoir near Moscow; in laboratory experiments, roach spawn rendered the

largest percentage of normally incubated larvae within those temperature values (Fig. 4). Spawn incubated above 20°C rendered greater incidence of development abnormalities of larvae, as well as higher mortalities (Rezničenko et al. 1962).

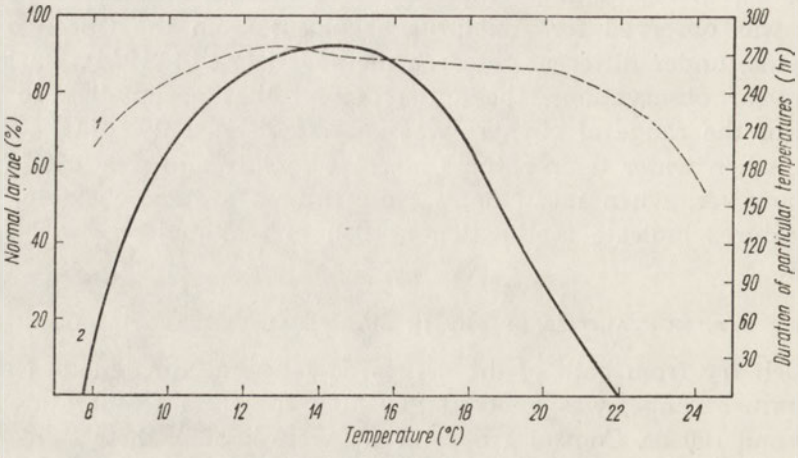

Fig. 4. Survival of roach larvae at different incubation temperatures and the duration of particular temperatures in natural roach spawning grounds. 1 — survival for normal larvae; 2 — the lengths of influence of different temperatures in natural spawning grounds (after Rezničenko et al. 1962)

Similarly high losses (22.0 and 24.6%) were recorded in control fry groups, kept in temperatures above 20°C during 100 hr (cf. Table II). No differences were observed in this respect between larvae from both of the compared lakes, even though the Lake Licheń fry was the progeny of a female acclimated to the heated environment, and the fry's embryonic development was carried out at 23.2°C. These facts seem to prove that in roach from the heated lake, no changes occurred as to the temperature range optimal for the propagation and embryonic development. Also the times of incubation for Lake Licheń spawn in a number of different temperatures was generally consistent with data obtained under similar temperature conditions for roach spawners from unheated spawning grounds (Kokurewicz 1970).

The somewhat smaller death rate of the Lake Licheń roach fry, observed in sublethal temperatures after 100 hr, with the other indices revealing no differences, could be related with the higher rate of water temperature increase immediately after mass incubation in this lake. Hubbs and Armstrong (1962) found that larvae of *Etheostoma spectablile* (Agassiz) — Percidae from Missouri and Arkansas streams survived better in high temperatures than larvae from Texas; they consider that faster temperature increase was responsible for their find-

ing. Both populations studied by them propagate under rather similar temperature conditions, but at different periods, and after three weeks since the spawning, temperatures in Arkansas streams are one to two degrees C higher from those recorded in Texas.

The situation with respect to temperature in the compared lakes of the Konin group was quite similar. After two weeks since the mass incubation of roach, water temperature in Lake Licheń was in average 1.5 degrees C higher than in Lake Ślesin, and after a month the difference increased to about 2 degrees C. While the spawning temperatures of roach were the same in both lakes, the Lake Licheń roach lived after two weeks in water heated to 21.5°C, while the Lake Ślesin fish had a month of time to acclimate to an almost identical temperature of 21.8°C.

On the other hand, when reactions to high temperatures are compared, we have to be aware of the differences in development and growth of individuals in the two populations, increasing every day, and having as its underlying cause the different.

5. CONCLUSIONS

A comparison of reactions to high temperatures of roach fry from a lake with normal temperatures, and from an artificially heated one, revealed no differences in temperature tolerance of the two populations. Lethal temperatures for both of them changed consistently with the adaptation temperatures. In sublethal temperatures, a somewhat smaller mortalities were found for the heated lake population, which could be caused by an earlier acclimation of fry to the higher rate of water temperature increase, characteristic of that lake.

6. SUMMARY

A comparison of lethal temperatures was made for roach fry from natural spawning grounds in a lake with normal fluctuation of temperatures (Ślesin), and in an artificially heated one (Licheń), as well as for fry incubated in laboratory at different temperatures. No significant differences in lethal temperatures of both populations were found, and the values of these temperatures depended on acclimation temperatures, in this case identical with incubation temperatures (Table I). An additional acclimation of fry in elevated temperatures resulted in both populations an increase of lethal temperatures proportional to the duration of acclimation (Fig. 2 and 3). Keeping the fry for 100 hr in sublethal temperatures allowed to observe a somewhat lesser mortalities in the samples from the heated Lake Licheń (Table II). However, in control groups from both populations kept in temperatures over 20°C, the amount of losses was similar, which proved that there were no clear changes with respect to the optimal and tolerable propagation temperatures in roach from the heated lake.

7. STRESZCZENIE

Porównano temperatury letalne wylęgu płoci z naturalnych tarlisk w jeziorze o normalnej termice (Ślesin) i sztucznie ogrzanym (Licheń) oraz wylęgu z ikry inkubowanej w różnych temperaturach. Nie stwierdzono istotnych różnic pomiędzy temperaturami letalnymi obu populacji, a wysokość tych temperatur zależała

od wysokości temperatur adaptacji, które w tym przypadku pokrywały się z temperaturami inkubacji (Tab. I). Dodatkowa adaptacja wylęgu w podwyższonych temperaturach wywoływała u obu populacji podniesienie temperatur letalnych odpowiednio do czasu trwania adaptacji (Fig. 2 i 3). Przetrzymywanie wylęgu w temperaturach subletalnych przez 100 godzin pozwoliło zaobserwować nieco mniejszą śmiertelność w grupach pochodzących z ogrzanego jeziora Licheń (Tab. II). Natomiast podobna u obu populacji wielkość strat w grupach kontrolnych, gdzie temperatura przekraczała 20°C, potwierdziła brak uchwytnych zmian w zakresie optymalnych i tolerowanych temperatur rozrodu płoci w ogrzanym jeziorze.

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L. SZLAUER

FISHING OF LAKE ARTHROPODA INTO LIGHT TRAPS

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ABSTRACT

Water Arthropoda were caught into 18 lm and 1000 lm light traps in the Ińsko Lake (North-Western Poland). Draughts into 1000 lm trap reached 90 ml of volume and approached a million of individuals during three hours. Better results were obtained before than after 10 p.m. Increase of the brightness of light caused great increase in the amount of light-trapped Cladocera, and small increase of Copepoda. Particular species differed largely in their attraction to light, which also depended on the zone of draught. Light trap is a convenient device for mass hauling of water Arthropoda in densely overgrown areas and from over the bottom. It yields draughts containing animals only, without other material.

CONTENTS

1. INTRODUCTION

Light has been recently widely used for fishing. Besides practical advantages, it makes possible important scientific observations. Borisov (1955) and Nikonorov (1963) proved that reactions to light in different fish species vary extensively. There are also other factors influencing this reaction, like sex and age of fish, and temperature, transparency and flow rate of water (Privolnev 1956, Nikonorov 1963).

Assuming that light fishing for water invertebrates may provide as ample observations as in the case of fish, a research scheme has been designed to establish the changes in quality and quantity of draught during a prolonged period of light fishing at the same spot, as well as to define the influence of the brightness of light on the reactions of Arthropoda. A similar study was performed by the present author at an earlier date (Szlauer 1969).

2. METHODS

The research has been made in the mesotrophic lake Ińsko during August and September 1968. As fishing implements, light traps were used, described in Szlauer (1969). As white light sources there were used 18 lm battery-fed bulbs, or 1000 lm (100 W) bulbs fed with 220 V current. Most often, a single 1000 lm

light trap was used, and the draughts were repeated a number of times at one or two days intervals. However, to establish the influence of brightness of light upon the amount of animals draughted, two traps were used simultaneously. At the same time, nets were used to define the composition of the fauna in the research area. In both net and trap fishing, animals were condensed by means of gauze with 0.2 mm holes. The research consisted of a number of series of draughts which will be described presently.

1. The littoral and profundal light trap fishing at one spot to establish the influence of prolonged intake upon the amount of draught. In the littoral, eight draughts were made between August 24 and September 10. The spot was the muddy bottom at the depth of 2.5 m, near a thicket of Myriophyllum. The 1000 lm light trap was left there for three hours (from 7 to 10 p.m.), after which the draught was hauled out. The draughts were repeated at two days intervals. Profundal draughts were made in the same way, three times between September 12 and 16, at 23 m of depth, 500 m from the shore.

2. The influence of the length of time of draught upon the amount of intake. The first series of draughts was made in the littoral zone (2.5 m of depth) between September 6 and 11. A 1000 lm light trap was installed at 7 p.m. and left until 5 a.m. next morning. The draught was taken out twice, at 10 p.m. and at 5 a.m. The seven-hours draught was re-calculated so as to be comparable with the three-hours intake. The series consisted of three trials. In the second, methodically identical series of three draughts made between September 25 and 30, the light trap was submerged at 5 m below water surface, 500 m from the shore, in the pelagic zone. The trap was anchored to a buoy so as to prevent wave pitching. Light was allowed to beam out only through side openings, as in bottom-anchored traps.

3. The influence of the intensity of light upon the amount of animals draughted; defining the degree of the animals attraction to light. Draughts were made on September 21 and 22 in the littoral (3 m of depth) and from September 24 to 29 in the pelagic zone. Light traps 18 lm and 1000 lm, as well as nets, were used in both series. Fishing went on for three hours each time, from 7 to 10 p.m. Light-trap hauls were repeated not less than three times in each series. To facilitate comparison of results, mean amounts of animals draughted into one trap during three hours were computed.

During the research period, physical and chemical measurements of water were taken. The epilimnion temperature on August 23 was 18°C and decreased to 14°C at the end of research. Visibility of Secchi's circle was 5.2 m on August 23, decreased to its yearly minimum of 4.1 m on September 7, and increased again to 5.5 m until September 30. Oxygen content above the profundal zone bottom (23 m of depth) measured on September 27 was 5.6 mg of O_2 per 1 l, and the temperature at this depth was 7.6°C.

3. RESULTS

Table I presents the results of repeated light-trap draughts at one spot in the littoral zone. In the littoral intake, there were both coastal species, and those typical for the pelagic plankton. In neither of them any decrease in the amount draughted, caused by the prolonged period of draught, was found (Table I). However, there were rather large irregular fluctuations of the intake. They were much more marked for the pelagic than for the coastal species. This is shown under the last heading in Table I, which shows the relations of maximum and minimum intakes (as the minimum, the lowest amount other than zero was considered). Those relations ranged from 4.5 to 65.0 for littoral species, and from 9 up to as many as 2400 for pelagic ones. For many species of pelagic Crustacea, the amounts of draught ranged from zero up to scores

Table I. Number of individuals caught into 1000 lm light trap, 7 p.m.-10 p.m. littoral zone

Species	No. of individuals								Ratio $\left(\frac{\text{max.}}{\text{min.}}\right)$
	24.VIII	26.VIII	28.VIII	30.VIII	1.IX	6.IX	8.IX	10.IX	
Littoral species									
Sida crystallina O.F.M.	2300	4290	7000	43,230	9750	12,210	7050	8100	18.8
Eurycercus lamellatus O.F.M.	3000	1650	300	1650	1320	0	1150	2400	10.0
Camptocercus rectirostris Schoedler	1250	660	300	3630	330	330	1150	2400	12.1
Acroperus harpae Baird	6500	660	100	3630	990	660	400	4100	65.0
Alonopsis elongata G. O. Sars	8750	3960	0	4950	0	1320	150	400	58.3
Alona sp.	6750	1650	200	11.550	1320	1650	3000	6000	57.7
Eucyclops macrurus G. O. Sars	2250	990	1300	660	330	660	500	100	22.5
Eucyclops macruroides Lilljeborg	4000	2310	3500	6930	1320	660	950	700	10.5
Ostracoda non-det.	2750	2640	4000	11,880	11,550	8580	5250	3900	4.5
Hydracarina non-det.	1750	990	500	2640	1320	1320	1100	400	6.6
Tendipedidae non-det. (pupa)	1500	180	670	540	710	240	240	372	8.3
Trichoptera non-det. (pupa)	200	150	480	200	350	60	66	66	8.0
Pelagic species									
Daphnia longispina O.F.M.	1250	660	0	14,850	6930	1320	0	400	37.1
Bosmina coregoni crassicornis Lill.	322,000	47,500	1300	602,000	1200,000	359,000	500	226,500	2400.0
Bosmina longirostris O.F.M.	6750	3630	0	55,000	27,000	2310	150	9150	366.6
Bythotrephes longimanus Leydig	0	330	0	1320	2970	660	0	500	9.0
Leptodora kindtii Focke	750	0	0	1320	1320	330	0	100	13.2
Eudiaptomus gracilis G. O. Sars	6500	7260	0	0	14,190	43,890	200	300	319.4
Eurytemora lacustris Poppe	3750	1980	0	330	0	330	0	100	37.5
Heterocope appendiculata G. O. Sars	8000	58,740	4300	14,190	9900	27,390	100	7800	587.4
Mesocyclops leuckarti Claus	500	1980	0	3630	4290	0	50	1700	86.0
Thermocyclops oithonoides G. O. Sars	0	13,860	200	660	94,380	1650	50	300	1888.0
Total littoral species	41,000	20,130	18,350	91,490	29,290	27,690	21,006	28,938	4.9
Total pelagic species	349,500	135,940	5800	693,300	1360,980	436,880	1050	246,850	1296.1

of thousands. Also in the series of pelagic draughts, no decrease of amount was found (Table II). Indeed, there was even some increase in three consecutive draughts, both in the first and the second half of night. This is illustrated in Table II. However, the profundal data were dif-

Table II. Number of individuals caught into 1000 lm light trap during 3 hr, pelagic zone

Species	No. of individuals caught							
	25-26.IX		28-29.IX		29-30.IX		Total	
	before 10 p.m.	after 10 p.m.	before 10 p.m.	after 10 p.m.	before 10 p.m.	after 10 p.m.	before 10 p.m.	after 10 p.m.
Sida crystallina	625	158	380	124	1135	1245	2140	1527
Daphnia longispina	12	4	12	0	50	277	74	281
Daphnia cucullata	25	28	15	16	75	255	115	299
Bosmina coregoni crassicornis	3310	910	6045	1071	16,535	38,095	25,890	40,076
Leptodora kindtii	25	4	37	0	412	407	474	411
Bythotrephes longimanus	25	0	37	0	12	13	74	13
Eudiaptomus gracilis	72	13	12	9	25	54	109	76
Eurytemora lacustris	300	2	102	32	525	83	927	117
Heterocope appendiculata	400	54	365	14	210	468	975	536
Mesocyclops leuckarti	37	17	27	14	0	18	64	49
Thermocyclops oithonoides	50	20	12	33	0	0	62	53
Tendipedidae non-det. (pupa)	38	1	61	4	95	4	194	9
Total	4919	1211	7105	1317	19,074	40,919	31,098	43,447

ferent (Table III). For most profundal species, the amounts hauled fell down notably in consecutive draughts. In particular, the effect was remarkable for *Bosmina longirostris*, *Pallasea quadrispinosa*, and *Eurytemora lacustris*. However, it was not observed for species draughted in small amounts, and for the Tendipedidae nymphs.

It was remarkable for the littoral zone that during the second half of night, material decrease of the amounts draughted was observed for the species typical for that zone, as well as for those which had floated there from the pelagic area (Table IV). Some exceptions from this rule were observed, but only at the beginning of the research. The decrease of draughts during the second half of night was also characteristic for the pelagic fauna. However, it was observed only on September 25 and 28 trials. In the last trial (September 29) just the opposite was recorded (Table II). The Tendipedidae nymphs should be noted, as their amount fell regularly during the second half of each night.

Table III. Number of individuals caught into 1000 lm light trap, 7 p.m.-10 p.m., profundal zone

Species	No. of individuals			
	Light trap draught			Net draught
	12.IX	14.IX	16.IX	16.IX
Daphnia cristata longiremis G. O. Sars	3630	70	50	14
Simocephalus vetulus O.F.M.	260	230	25	14
Bosmina longirostris O.F.M.	71,700	2770	50	0
Bosmina coregoni crassicornis Lill.	7700	690	100	14
Eurycercus lamellatus O.F.M.	40	30	125	0
Eurytemora lacustris Poppe	23,000	2350	75	0
Cyclops scutifer G. O. Sars	2340	990	25	28
Megacyclops gigas Claus	1260	590	775	243
Thermocyclops oithonoides G. O. Sars	0	200	75	43
Eucyclops serrulatus Fischer	0	230	150	14
Ostracoda non-det.	0	0	0	57
Pallasea quadrispinosa G. O. Sars	17	0	0	0
Hydracarina non-det.	620	2110	275	0
Tendipedidae non-det. (pupa)	24	42	27	0
Total	111,091	10,302	1752	427

To facilitate analysis of results, two coefficients were introduced in Table V, covering brighter and dimmer light draughts, and net hauls, viz: (1) The A:B ratio, expressing the relation of the percentage individuals caught to the 18 lm light trap (A) to the percentage of individuals caught into the net (B). The ratio defines the degree of attraction by 18 lm light; (2) The C:D ratio, being the relation of draughts to brighter (C) and dimmer (D) light traps. It shows, how many times more effective the brighter light trap was.

It can be seen from the C:D coefficient that all species light-trapped in the littoral reacted much more strongly to brighter light (Table V). The effect was most remarkable for the Cladocera, and most of all for the pelagic Cladocera, like *Bosmina coregoni crassicornis, Bosmina longirostris, Daphnia longispina.* It was also observed for *Bythotrephes longimanus,* but the C:D coefficient could not be computed, as the D draught was zero for them. The littoral Copepoda, in opposition to the Cladocera, showed much lesser increase of attraction to the brighter light. Also the pelagic fauna was more effectively trapped by brighter light (Table V). The effect was much more marked for the Cladocera than for the Copepoda in this zone, too, with one exception of *Eurytemora lacustris.*

The Table V data allow for comparison of the increase of draughts caused by the use of brighter light for the same species in different

Table IV. Number of individuals caught into 1000 lm light trap during 3 hr, littoral zone

Species	No. of individuals caught							
	6–7.IX		8–9.IX		10–11.IX		Total	
	before 10 p.m.	after 10 p.m.	before 10 p.m.	after 10 p.m.	before 10 p.m.	after 10 p.m.	before 10 p.m.	after 10 p.m.
Littoral species								
Sida crystallina	12,210	4683	7050	2558	8100	4880	27,360	12,121
Eurycercus lamellatus	0	851	1150	21	2400	215	3550	1087
Camptocercus rectirostris	330	1561	1150	21	2400	344	3880	1926
Acroperus harpae	660	2554	400	75	4100	322	5160	2951
Alonopsis elongata	1320	993	150	0	400	0	1870	993
Alona sp.	1650	2270	3000	107	6000	64	10,650	2441
Eucyclops macrurus	660	142	500	107	100	0	1260	249
Eucyclops macruroides	660	284	950	11	700	43	2310	338
Ostracoda non-det.	8580	3405	5250	548	3900	559	17,730	4512
Pelagic species								
Daphnia longispina	1320	851	0	11	400	0	1720	862
Bosmina coregoni crassicornis	359,700	141,225	500	795	22,650	3547	382,850	145,567
Bosmina longirostris	2310	1290	150	21	9150	0	11,610	1311
Bythotrephes longimanus	660	568	0	0	500	0	1160	568
Leptodora kindtii	330	426	0	0	100	0	430	426
Eudiaptomus gracilis	43,890	18,163	200	0	300	0	44,390	18,163
Eurytemora lacustris	330	142	0	11	100	21	430	174
Heterocope appendiculata	27,390	5250	100	0	7800	64	35,290	5314
Mesocyclops leuckarti	0	0	50	0	1700	43	1750	43
Thermocyclops oithonoides	1650	142	50	32	300	64	2000	238
Total littoral species	26,070	16,743	19,600	3448	28,100	6427	73,770	26,618
Total pelagic species	437,580	168,057	1050	870	43,000	3739	481,630	172,666

Table V. Comparison of degree of attraction by 18 lm light trap and degree of effectiveness of brighter (1000 lm) light trap, in littoral and pelagial zones. A — % in 18 lm trap draughts; B — % in net hauls; C — 1000 lm trap draughts; D — 18 lm trap draughts

Species	Littoral		Pelagial	
	A : B	C : D	A : B	C : D
Cladocera				
Daphnia cristata longiremis	—	—	0.02	10.0
Daphnia cucullata	—	—	0.37	12.7
Leptodora kindtii	—	—	1.62	51.6
Sida crystallina	0.15	15.8	—	95.1
Daphnia longispina	—	96.4	0.26	16.6
Bosmina coregoni crassicornis	28.70	164.6	2.14	15.9
Ceriodaphnia sp.	0.07	44.8	—	—
Bosmina longirostris	5.38	116.9	—	—
Eurycercus lamellatus	2.55	56.8	—	—
Camptocercus rectirostris	0.36	7.5	—	—
Acroperus harpae	0.05	26.8	—	—
Alona sp.	2.07	21.2	—	—
Copepoda				
Cyclops scutifer	—	—	0.19	0.02
Eudiaptomus gracilis	2.64	2.8	0.37	1.9
Eurytemora lacustris	4.04	1.0	0 21	14.2
Heterocope appendiculata	—	10.7	2.73	3.2
Mesocyclops leuckarti	0.11	10.0	0.21	6.9
Thermocyclops oithonoides	0.34	8.1	0.02	5.0
Megacyclops viridis	1.82	15.3	—	—

environments. It can be seen that for almost all pelagic species the increase was much greater in the alien littoral than in their native pelagic zone (*Daphnia longispina, Bosmina c. crassicornis, Eudiaptomus gracilis, Heterocope appendiculata, Mesocyclops leuckarti* and *Thermocyclops oithonoides*).

The amounts of light-trapped animals were not only counted, but also their volumes were measured (bottom deposits). Traps with 1000 lm bulbs during the first half of night (before 10 p.m.) yielded largest volumes. Here are some of the largest volumes hauled after 3 hr of action of the light trap, quoted by way of example: August 26 — 90 ml, August 30 — 80 ml, September 1 — 80 ml, September 20 — 90 ml, September 21 — 75 ml. It must be emphasized that light draughts contained living animals only. There was no litter or phytoplankton in properly hauled draughts. Of course it was the most numerous, as well as the largest species (Tendipedidae nymphs and Ephemeroptera larvae) which contributed most to the draught volumes.

The A:B coefficient (Table V) allowed to rank the found species by their attraction to 18 lm light. In the ranking, only littoral species were considered in littoral draughts, and only pelagic species in pelagic draughts. The decreasing attraction rank lists are as follows:

The littoral zone: *Eurycercus lamellatus, Alona sp., Megacyclops viridis, Camptocercus rectirostris, Sida crystallina, Ceriodaphnia sp., Acroperus harpae.*

The pelagic zone: *Heterocope appendiculata, Bosmina coregoni crassicornis, Leptodora kindtii, Daphnia cucullata, Eudiaptomus gracilis, Daphnia longispina, Eurytemora lacustris, Mesocyclops leuckarti, Cyclops scutifer, Daphnia cristata longiremis, Thermocyclops oithonoides.*

Two species can be added at the beginning of the pelagic list: *Bythotrephes longimanus* and *Megacyclops gigas.* They were very strongly attracted by light, which was proved by their high incidence in light draughts, while they were totally absent from net hauls, which reflects their rarity in the environment.

4. DISCUSSION

One of the most remarkable results was that though draughts were repeated several times at the same spots, and scores or even hundreds of thousands of animals were caught each time, no decrease of the amounts draughted into light traps was found neither in the littoral, nor in the pelagic zone. I could hardly be assumed that so ample hauls had not influence the supply of animals in the trap's neighbourhood. Thus, the efficiency of draughts ought rather to be regarded as the result of reconstruction of animal supply during the one or two days intervals between draughts. This can be accounted for by active transportation of animals, as well as by horizontal motions of the masses of water which are typical for the littoral and epilimnion. Probably it was the latter factor which was decisive. This factor seems also to be responsible for the large and irregular fluctuations in the numbers of pelagic animals caught in the littoral (Table I). Tides of pelagic waters, bringing pelagic animals, depended on irregular shifts of wind strength and direction.

In the profundal, a decrease of the amounts draughted in consecutive hauls was observed, probably caused by a too slow reconstruction of supplies exhausted by draughting, which was caused in its turn by the lack of intensive currents transporting animals in the profundal zone. Also the decrease of draughts during the second half of night in the littoral and pelagic zones (Tables II and IV) can be regarded as resulting from exhaustion of supplies near the trap. Probably the rate of

catching the animals by the 1000 lm light trap is higher than the rate of restoration of the fauna. It was only during the intervals between draughts that such restoration was possible. However, this is but a tentative interpretation. The decrease of efficiency of hauls after 10 p.m. could be brought about as well by lower activity of animals, or by still other causes. The data do not allow for unambiguous explanation of this phenomenon, as well as of the increase of the draught efficiency in the second half of the night on September 29-30 in the pelagic zone, and of some other irregularities.

From the results here discussed some practical conclusions can be drawn as to the light trap method of catching invertebrates. Good results can be attained in the littoral and pelagic zones even if many draughts are taken at the same spot; most ample hauls can be expected during the first half of night.

The increase of efficiency with the use of brighter light seems to be accountable by its more extensive range of influence. An analogous phenomenon was found for fish by Nikonorov (1963), thereby proving that invertebrates are not exceptional in this respect in the animal world.

We failed to explain the difference of reaction to the increase of light intensiveness in Cladocera and Copepoda. It is also difficult to account for the observed increase of attraction to light in pelagic Crustacea in the littoral environment alien to them, as well as in the littoral species *Sida cristallina* in the pelagic zone (Table V). However, some consequences can be drawn even from those unexplained observations. It can be seen that the reaction of invertebrates to light depends not only on their taxonomic position, but also on the zone of the lake in which the reaction is studied.

The A:B coefficient values, expressing the animals' attraction to light, were compared with similar data from an earlier work (Szlauer 1969). It turned out that though absolute values of the coefficient differed in the two studies, the rank orders of the species by their attraction to light remained almost identical. Highest and lowest ranks were occupied by the same species. Thus the earlier results were corroborated by the more recent ones.

The 1000 lm light trap outputs, exceeding one million of individuals, and reaching 90 ml of volume, suggest that the device can be used for commercial fishing for Crustacea. Moreover, animals caught by this method are all alive, and there is no phytoplankton mixed with them. Another advantage of the light trap is that it can be used in places where other methods might fail, like in areas with dense plant overgrowth, or in the profundal zone near to bottom.

Discussing the results of a study of invertebrates reaction to bulb light, the nature of such reaction must be explained. It seems that the reaction is connected with the animals' behaviour in natural light; and it ought to be explained by biological factors responsible for it. Natural light helps the animals in their space orientation due to its directional action and its gradient of intensity. Light permits the animals to find their proper environments and to remain there. Reaction to bulb light is caused by the same biological mechanisms.

5. SUMMARY

During the summer of 1968, water Arthropoda, mainly Crustacea, were caught into light traps with 18 lm and 1000 lm bulbs. Draughts into 1000 lm trap reached 90 ml of volume and approached a million of individuals during three hours. Repeated ample draughts at the same spot caused no decrease of the amounts caught in the littoral and pelagic zones, while it did have such effect in the profundal. In the littoral, irregular and large oscillations in the output were observed. Pelagic Crustacea occurring in the littoral showed the least consistent pattern of incidence. Better results were obtained before than after 10 p.m. Brightness of light was decisive for the efficiency of draughts. More bright light caused multiple increase of Cladocera amount in the trap, but only a small increase of Copepoda.

The particular species differed very much in their attraction to light. In the littoral, it was highest for *Eurycercus lamellatus* and *Megacyclops viridis*, and in the pelagic zone, for the *Bythotrephes longimanus, Megacyclops gigas, Heterocope appendiculata, Bosmina coregoni crassicornis, Leptodora kindtii.* Pelagic species showed much higher attraction to light in the littoral environment than in their native pelagic area.

A 1000 lm light trap is a convenient device for hauling large amounts of water Arthropoda. It yields draughts containing animals only. It is particularly useful for catching animals in densely overgrown areas and from over the bottom.

6. STRESZCZENIE

Latem 1968 r. w jeziorze Ińsko poławiano na światło stawonogi wodne, głównie skorupiaki, przy pomocy samołówki z żarówkami 18 lm lub 1000 lm. Połowy na światło. Były one powodowane głównie wahaniami połowów skorupiaków śród- 90 ml objętości oraz liczyły setki tysięcy osobników. Wielokrotne dokonywanie tak obfitych połowów w tym samym miejscu nie powodowało spadku ich obfitości w litoralu i pelagialu, powodowało natomiast taki spadek w strefie profundalowej. W litoralu stwierdzono bardzo nieregularne i wysokie wahania obfitości połowów na światło. Były one powodowane głównie wahaniami połowów skorupiaków śródjeziornych, występujących w tej strefie. Połowy na światło dawały lepsze rezultaty na początku nocy niż po godz. 22^{00}. Decydujący wpływ na obfitość połowów wywierała jasność światła. Zwiększenie jasności światła wywoływało wielokrotny wzrost połowów Cladocera oraz tylko niewielkie zwiększenie połowów Copepoda.

Dążność do światła poszczególnych gatunków była bardzo różna. W litoralu największą dążność do światła wykazały *Eurycercus lamellatus* i *Megacyclops viridis*, a w strefie śródjeziornej — *Bythotrephes longimanus, Megacyclops gigas, Heterocope appendiculata, Bosmina coregoni crassicornis, Leptodora kindtii.* Gatunki śródjeziorne wykazywały w obcym dla nich środowisku litoralowym znacznie większą dążność do światła niż na śródjeziorzu.

Samołówka ze światłem o jasności 1000 lm jest przyrządem nadającym się do odłowu dużych ilości stawonogów wodnych. Daje połów składający się wyłącznie ze zwierząt. Szczególnie przydatna jest do wyławiania zwierząt z gąszczu roślin i znad dna.

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CARTESIAN DIVER MICRORESPIROMETRY FOR AQUATIC ANIMALS [1]

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ABSTRACT

The paper presents respirometric technique of work, with the use of Zeuthen's stoppered divers, mainly for purposes of ecological physiology and ecological bioenergetics. The complete apparatus and accessory equipment are described as well as the manner of making Cartesian divers for measuring of O_2-consumption and CO_2-output, and course of determination of both these processes.

CONTENTS

1. INTRODUCTION

Since the first application of Cartesian divers by Linderstrom-Lang (1937) for manometric gasometry, this device has been used in a great variety of modification (reviews in: Glick 1961, Holter 1961, Holter and Zeuthen 1966). The first, very broad presentation of theoretical backgrounds of Cartesian diver gasometry is given in a paper by Linderstrom-Lang (1943). Published simultaneously, Holter's (1943) paper presents a thorough description of methods how to use so-called "standard divers" (main points of these methods can be also found in Holter 1961). Zeuthen has introduced some essential modifications by: miniaturization (Zeuthen 1943, 1955), construction of stoppered divers (Frydenberg and Zeuthen 1960, Zeuthen 1949, 1950 a, b, 1955) and of ampula divers (Holter and Zeuthen 1966, Lovlie and Zeuthen 1962 a, Zajicek and Zeuthen 1953, Zeuthen 1961 and others).

Cartesian diver gasometer is a constant volume, changing pressure system. It is meant to measure the buoyancy in flotation medium of a gas-containing vessel — the diver. It is container enclosing a gas bubble, connected with surrounding fluid medium in such a way that any changes in pressure on the medium are transferred into the gas space in the diver interior. An increase in pressure on the flotation medium brings about a decrease in gas space volume in the diver, which makes

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it sink. On the other hand, a decrease in pressure brings about an increase in the gas volume, and the diver moves upward.

For a particular diver with a given charge, it is gas volume ("diver constant"), which enables the diver to float at a certain level (the equilibrium mark) in the flotation medium. If gas is absorbed or liberated in the diver the gas volume will tend to contract or expand but changes in gas volume can be compensated by changes in the equilibrium pressure. Full compensation is indicated by flotation of the diver at the equilibrium mark. This "equilibrium pressure" change is controlled and measured manometrically, and from manometer readings the change in the amount of gas can be calculated.

The Cartesian diver gasometer is a sensitive instrument since the equilibrium pressure is unstable. In a rising diver, the gas is affected by decreasing hydrostatic pressure of the flotation medium; the gas volume expands and the diver movement is accelerated. Similar holds for the downward movement of the diver.

Of a number of types and modifications of the Cartesian divers, Zeuthen's stoppered diver seems to be most adequate tool in the hands of a hydrobiologist who deals with problems of production, and specially with bioenergetics.

The construction and calibration of these divers does not need any complicated tools or instruments, the calibration itself being easy and little time-consuming. The divers are charged free-hand and this procedure is simple as compared with that for other types of divers of a similar volume (except for ampulla divers).

These divers make possible the measurements of both O_2 consumption and CO_2 production at one run of observations on the same object (Klekowski et al. 1967, Klekowski and Shushkina 1966 a, b, Frydenberg and Zeuthen 1960, Zeuthen 1949). Using divers of different size, oxygen consumption can be measured over a broad range, starting from ca $1 \cdot 10^{-3}$ µl/hr (then an accuracy of about $1 \cdot 10^{-5}$ µl).

In a typical stoppered diver, the head of the diver chamber is directed upwards. Inactive objects (e.g. eggs) will come to rest on the meniscus between the air and water, reducing to a minimum diffusion distance between the respiring objects and the diver's gas space.

It is relatively easy without any damage to introduce the examined organisms into divers and remove them after the completion of the measurements. Due to this one can run repeatedly the records of metabolism of the same individual within its developmental cycle (Klekowski and Shushkina 1966 a, b), for early development stages, e.g. eggs (Berg and Kutsky 1951, Frydenberg and Zeuthen 1960, Klekowski et al. 1967, Zeuthen 1949, 1950 a, b, 1955), as well as of the individuals undergoing some experimental treatments between the measurements (Duncan and Klekowski 1967, Klekowski and Guttowa 1968, Klekowski and Duncan 1967).

Stoppered divers can be miniaturized to record the O_2 consumption of the order of $1 \cdot 10^{-4}$ µl/hr (Zeuthen 1955). However, such an extreme diminution renders considerable technical difficulties so that for measurements of oxygen consumption lower than $1 \cdot 10^{-3}$ µl/hr, other type of divers seems to be more suitable, namely, Zeuthen's ampulla diver (Lovlie 1964, Lovlie and Zeuthen 1962 a, Zajicek and Zeuthen 1961, Zeuthen 1953).

Further, a more detailed information will be given of the technique of O_2 consumption and CO_2 production measurements in Zeuthen's stoppered divers (basic description in: Zeuthen 1950 a). As we cannot give extensive description of the instruments and their operation, the reader is advised to look for detailed instructions and theoretical explanations in the specific literature on this subject, mainly in: Frydenberg and Zeuthen (1960), Glick (1961), Holter (1943), Holter and Zeuthen (1966), Linderstrom-Lang (1943), Zeuthen (1950 a). If we can make any suggestions, it is advised to commence the reading with: Glick (1961), Holter (1961), and Holter and Zeuthen (1966).

2. GENERAL EQUIPMENT

A large water bath (Fig. 1) with submerged flotation vessels (1) holding divers should have temperature regulation with an accuracy of at least 0.01°C. This can be achieved using a toluen thermostat and an electric lamp (15) of 60-

150 W, the bulb submerged in the bath water. For precision of temperature regulation the relative positions in the bath of the heat source, the temperature sensitive element and the stirrer is important (for details see Lovlie and Zeuthen 1962 b). Temperature regulation can be improved by an outer isolation of the bath with polystyrene foam mats as well as by crude thermostabilization of the room in which the bath is located.

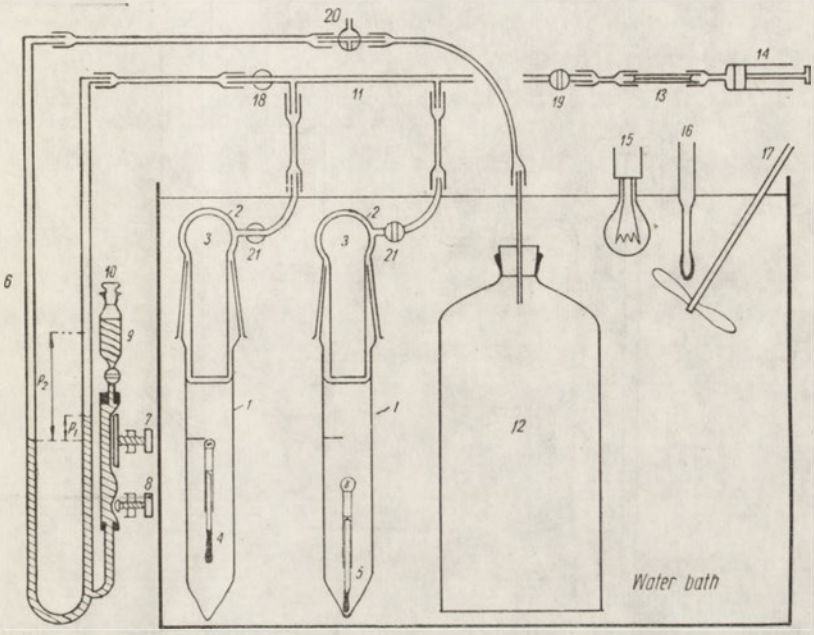

Fig. 1. Schematic drawing of Cartesian diver assembly (scale distorted). 1 — Flotation vessel; 2 — cap; 3 — space occupier; 4 — diver floating at the equilibrium mark; 5 — diver resting at the bottom between measurements; 6 — manometer; 7 — coarse; 8 — fine pressure regulation screws; 9 — the reservoir with manometer fluid; 10 — reservoir stopper; 11 — manifold; 12 — air bottle; 13 — air brake; 14 — syringe (100 ml); 15 — heating bulb; 16 — thermoregulator; 17 — stirrer; 18-21 — taps

The water bath should be protected against vibrations and therefore be placed on a stable base. For the same reason, the stirrer (17) cannot be attached to the bath frame; it would be the best if it could be fastened to the wall, with no connection with the water bath and its base.

The flotation vessels (Fig. 2) can be of different shape and size. The type of vessel presented in Fig. 2 is presently used in our laboratory (unpublished data). In the course of measurements, the entire vessel together with its cap (4) is submerged in the bath water. Such submerging prevents the pressure change in the vessel which would have resulted from the temperature changes in the air surrounding the bath. The vessel air volume is reduced to its minimum by placing a hollow space occupier (5) in the upper part of the vessel. The lower end of the flotation vessel is cone-shaped in order that the diver can be centered along vertical axis of the vessel. There is a horizontal reference line (2) engraved into the glass at about one-third the distance between the flotation liquid meniscus and the bottom. The vessel is suspended on a autrigger (9) which is mounted in the ball joint (10). The latter is movably fastened to a vertical supporting rod (11) at the edge of the water bath frame. Each vessel is suspended on a separate rod, when putting in or removing the diver from the flotation vessel, the latter should

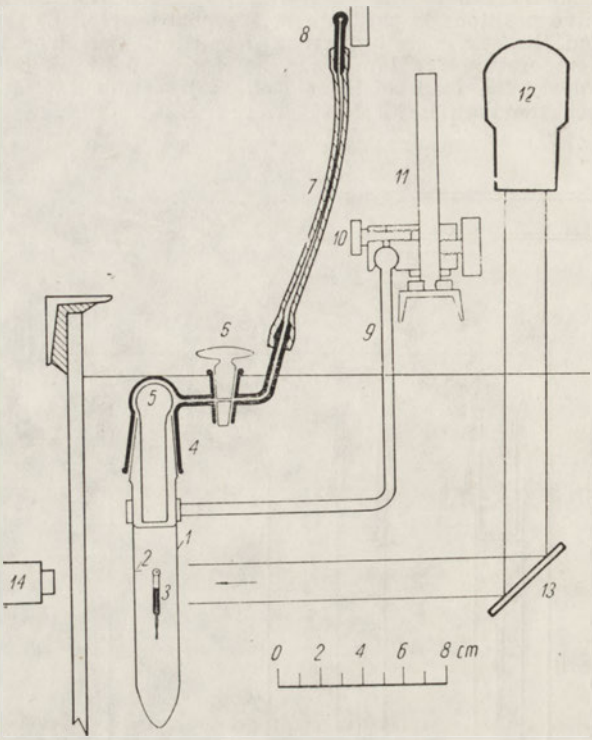

Fig. 2. Flotation vessel with supporting device. 1 — vessel; 2 — mark; 3 — diver; 4 — cap; 5 — space occupier; 6 — tap; 7 — thick-walled rubber tubing; 8 — manifold; 9 — autrigger; 10 — ball-joint; 11 — supporting rod; 12 — lamp; 13 — white screen; 14 — cathetometer

be rised so that the entire ground-glass joint is above the surface of the bath water.

8-10 flotation vessels are connected by a thick-walled, thin-bore rubber tubing (Fig. 2 — 7) with the manifold (Fig. 1 — 11, Fig. 2 — 8) whose one end is connected with the manometer. The manometer (Fig. 1 — 6) of glass capillary, 2-2.5 mm of inner diameter, is 150-180 cm high. Behind both manometer arms there is a mm-scale. The pressure is regulated by coarse and fine adjustment screws (Fig. 1 — 7, 8) and to the system is attached a reservoir with the manometer fluid. The second arm is connected with an air bottle (5-10 l) (Fig. 1 — 12) which is submerged in the water bath. The air bottle makes the whole system independent from changes of the barometric pressure.

Brodie's solution [2] is usually used as the manometer fluid; 10,000 mm of solution is approximately equivalent to 760 mm Hg.

When measuring CO_2 output in the stoppered divers, a more sensitive manometer can be necessary (constructional details in Frydenberg and Zeuthen 1960, a description of other type of sensitive burette manometer in: Zajicek and Zeuthen 1961, Zeuthen 1953).

The position of the diver is checked visually by means of a cathetometer. The flotation vessel is then illuminated by a movable lamp (Fig. 2 — 12). The

[2] In our practice we used: NaBr (desiccated in 160°C) — 44 g, water to 1 l; 1 ml salt-resistant detergent and dye (e.g. Evans blue or fluoresceine) added. Specific gravity ($d_{20} = 1.033$) adjusted with the use of a pycnometer.

light source can be coupled with the cathetometer, and then a mat white screen is placed behind the flotation vessels.

The following tools are necessary to operate the divers:

Forceps for placing the diver into the flotation vessel and for its removal (Fig. 3), (for detailed description see: Glick 1961, Holter 1943). It consists of a metal tube terminated with tweezers (1) at one end, made of springy, thin metal sheets, about 3 mm broad at the distal end. By pushing knob (2) at the other end of the tube, the metal rod (3) moves forwards in between the tweezers, causing their opening. When spring (4) withdraws the rod, the tweezers close again.

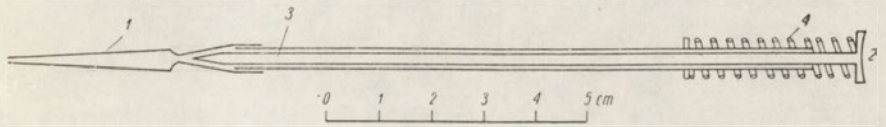

Fig. 3. Forceps. 1 — tweezers; 2 — knob; 3 — rod; 4 — spring

Holter's braking pipette; one of many types, easy to make, is presented in Fig. 4. A capillary (1) of a constant inner diameter throughout its entire length is mounted into a glass jacket tube (2) by means of cement (3). The capillary end (4) within the jacket tube is drawn out to a hair-thin tip. The flow of liquid through the pipette is determined by the rate at which air can pass through this very fine "air-brake". The jacket tube is connected with a mountpiece through a rubber tubing (5). For a given pipette the ratio: length (in mm) per 1 µl of volume is known. The braking pipette allows to measure a required gas volume and introduce it into the diver as well as to determine an unknown gas volume at diver calibration and, also to introduce very fine and delicate, living objects.

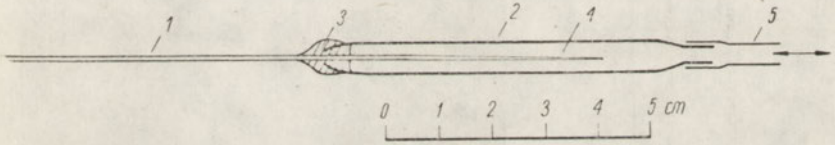

Fig. 4. Braking pipette. 1 — capillary; 2 — jacket; 3 — cement; 4 — air brake; 5 — rubber tubing

A micro-burner (Fig. 5) is used for making the divers and braking pipette. It is made from an injection needle (1). The flame is controlled with a screw clamp. The parts above mentioned can be fixed on a piece of angle iron (3).

Thin capillaries are cut with a diamond mounted in a penlike holder (commercially available), or with a carborundum piece mounted into a pyrex glass rod (in oxygen flame).

3. THE STOPPERED DIVER FOR MEASURING OXYGEN CONSUMPTION

A. DESCRIPTION OF DIVER

The diver (Fig. 6) consists of a diver chamber (1), whose head (2) can be spherical. The diameter of the cylindrical part of the diver chamber can range from somewhat less than 1 mm to about 2 mm in siliconed

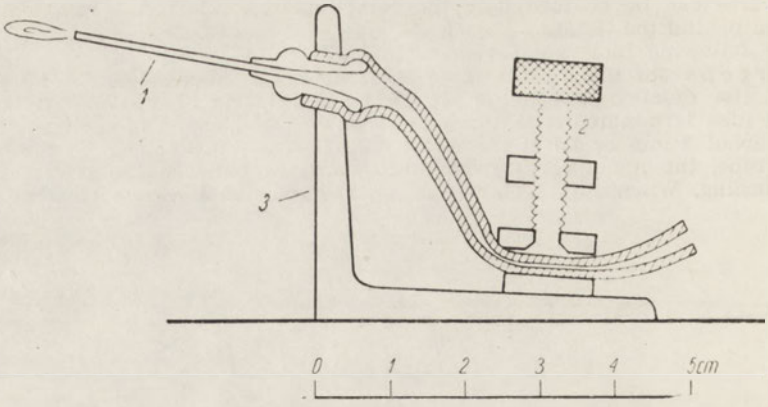

Fig. 5. Microburner. 1 — injection needle; 2 — screw clamp; 3 — angle iron

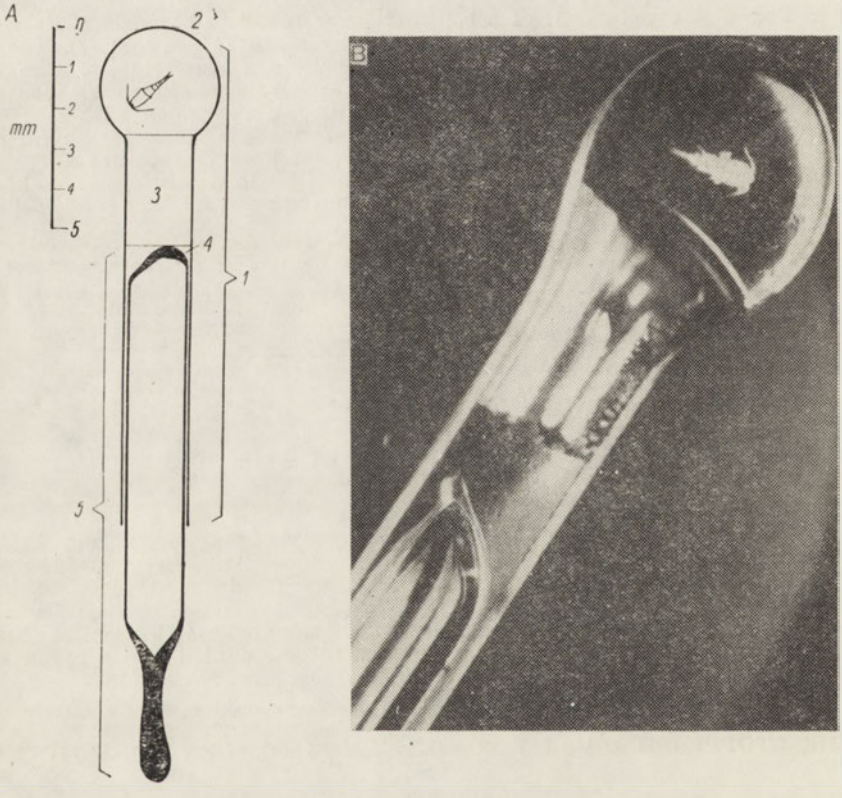

Fig. 6. Stoppered diver. A — Scheme: 1 — diver chamber; 2 — chamber head; 3 — gas bubble; 4 — 0.1 N NaOH solution; 5 — hollow glass stopper; B — Diver with *Macrocyclops albidus* (from Klekowski and Shushkina 1966 b)

divers. In the chamber head, there is water with an organism (or organisms). A gas bubble (3), of a known and specific volume for a given diver, separates the water filling the diver head from a thin layer of CO_2-absorbing 0.1 N NaOH solution (4). Hollow glass stopper (5), inserted in the neck of the diver chamber reduces the leakage of gases from (or to) the interior of the diver chamber. In order to achieve a satisfactory reduction of the diffusion of gases, the contact zone between the top of the stopper and the open end of the chamber must be 8-10 mm long. The stopper has a solid tail at is lower end, stabilizing the diver in vertical position. Zeuthen (1950 a) advises to use stoppers with only 3-4 times the volume of the air bubble in the diver chamber, to avoid an excessive size of the whole diver. The diver chamber and the stopper cross-sections are usually not circular. Accordingly, the stopper needs to be oriented both length- and crosswise before it can be inserted into the chamber's neck. It is inserted so deep that it comes close to the meniscus without making direct contact. The stopper is fixed in this position by gently twisting it untill mechanical resistance is felt. Through the stopper-neck clearance the changes in pressure, affecting the flotation medium (also 0.1 N NaOH), were transferred into the gas bubble in the diver chamber.

B. MAKING THE DIVER

To make divers of 1-2 µl gas volume, it is necessary to draw capillaries from pyrex, rasotherm or other hard glass in a broad oxygen flame. Soft glass is less convenient because of its lower mechanical and chemical resistance. The ratio between inner and outer diameters of glass tubing for drawing the capillaries should be about 0.9. Capillaries can be drawn from such tubes so that the ratio internal/external diameter is essentially maintained. If tubes with thicker walls are only available, it is necessary to blow out slightly the heated section of the tube and immediately draw the capillary. In such capillary the wall will be relatively thinner than in the initial material. It is desirable (1) to have the finished diver float with stopper downwards. This requires a somewhat extended tail. It is also desirable (2) to have mechanically stable divers which requires not too thin glass. On the other hand (3) the ratio between volume of stopper (gas enclosed) and volume of compressible air ("diver constant") should not be excessively high (maximum 3-4), because this will make the diver react sluggishly to pressure changes. The maker of a stoppered diver must make his own compromise considering in each situation the relative importance of the three points named above.

The technical steps in making the diver (Fig. 7) should be as follows: 1. Select two sections from the same tubing, one section fit inside the other. 2. Insert the broader piece of the capillary into a bored hole in a rubber stopper which is fitted into a glass jacket, to the other open

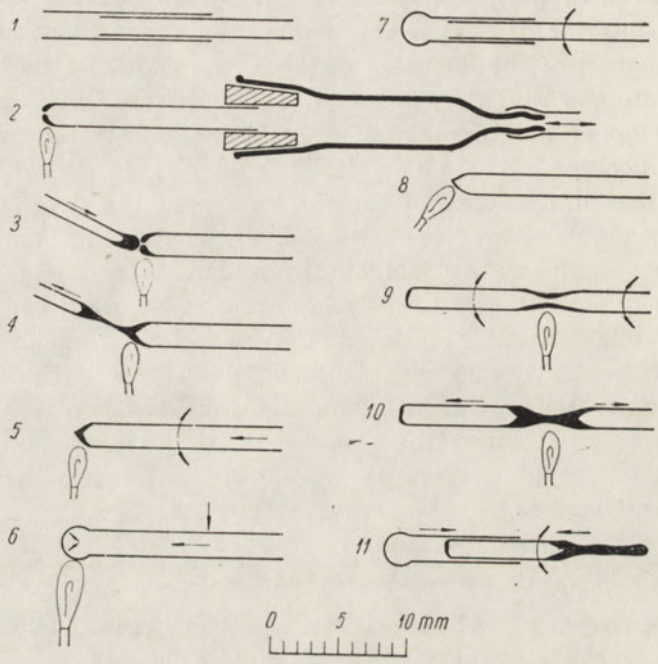

Fig. 7. Making the diver. For explanation see text

end which is attached a rubber tube with a mouthpiece. Using a microflame melt one end of the tube. 3, 4. Close the head of the diver with another piece of glass. 5. Heat the diver head uniformly on all sides. 6. Gently blow out the diver head and cut off excess length. 7. Find suitable length of tubing for stopper. 8. Close stopper end as shown in 2, 3 and 4; collapse by sucking in the closed end to flatten it. At suitable point, melt and thicken wall to form the stopper tail, close the stopper tail. 10. Shape the stopper tail. 11. Insert stopper into the chamber and close firmly by turning delicately the stopper. Before the newly fabricated divers can be used for O_2 measurements, they have to be tested for tightness against this gas. For this, the divers must be charged according to the instruction given below, however with no organisms consuming oxygen put in the diver head. Thus charged divers should be placed into the flotation chambers, filled with flotation medium saturated with air. Then, measurements of the equilibrium pressure should

run for about 2 hours, at 15 min intervals. Later on, the flotation vessels must be opened and the flotation medium well saturated (by bubbling) with nitrogen for half an hour. After closing again the flotation vessels, further measurements of the equilibrium pressure should be carried out for about 2 hours. The difference in the equilibrium pressure between all records as well as between the measurements taken in air- and in N_2-saturated media should not exceed ca 3-5 mm.

C. MAKING AND CALIBRATING THE BRAKING PIPETTE

Capillary tubing for a Holter's braking pipette must have constant cross-section surface at its entire length. The walls should not be too thin if a pipette of a satisfactory mechanical resistance is desired. The making procedure (Fig. 8) is as follows: 1. Heat a capillary tube at one point. 2. Take the capillary off the flame and draw out quickly to

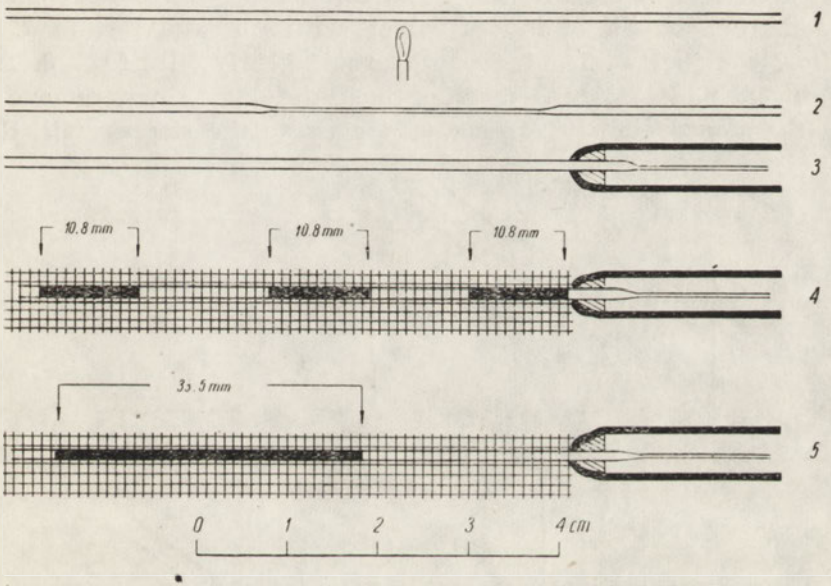

Fig. 8. Making and calibrating a braking pipette. For explanation see text

a very fine bore, cut in two. Place the wide bore end into water and check if about 2-3 mm of water enters in 1 second. If less, then break off short piece of fine bore and test again. 3. Seal capillary with sealing wax (e.g. DeKhotinsky cement [3]) into an open-ended tube, fine bore inside. 4. Draw up a drop of mercury into the capillary and measure its length at different parts of the capilary; it must be of the same length

[3] DeKhotinsky cement: 80 g of shellac is added in small amounts to 30 g of heated pine tar. Stir at frequent intervals for 3-4 hours and maintain at 130°C or slightly lower.

throughout (i.e., the capillary must have a constant bore). Rinse free of Hg. 5. Draw up some water into the capillary, measure its length with millimeter graph paper and blow out into a weighing dish. Weigh the water. Calculate the length (in mm per 1 µl volume) taking the density of water to be 1. Note: mercury is not appropriate fluid for calibration since the pipette is meant to measure the gas volume which is left between the water menisci and water-coated capillary walls.

The braking pipettes can be cleaned with concentrated H_2SO_4; then rinsed several times with distilled water. How to remove the fluid from the fine bore tip — consult Glick 1961.

D. CALIBRATION OF THE DIVER

In order to determine the volume of air needed to float the diver, the following procedure is recommended (Fig. 9): 1. Fill diver chamber with water (e.g., in the way as in Fig. 12). Submerge the diver chamber and add an air bubble of desired volume with a braking pipette. 2. Close the diver chamber with the stopper, turning gently until firm. 3. Try to float the diver in flotation medium. 4. If the diver sinks, remove glass from the stopper tail; if it rises, add glass to the stopper tail. Repeat

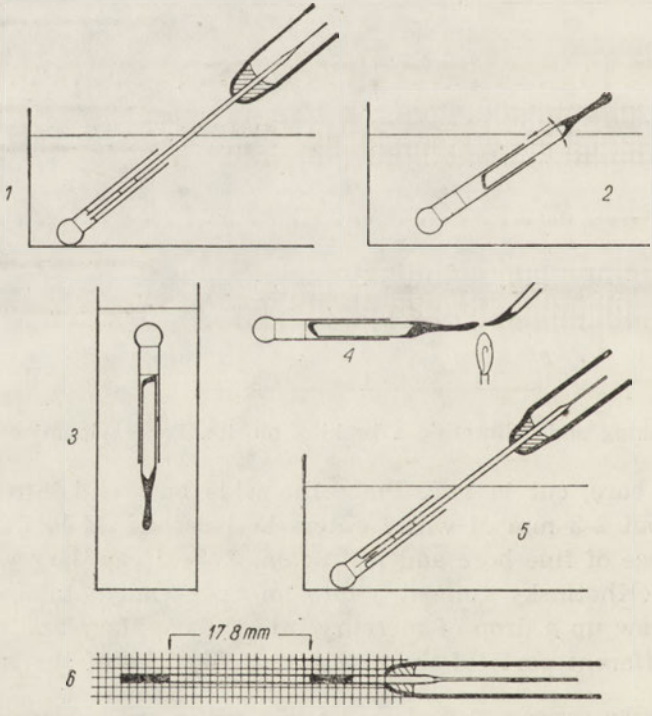

Fig. 9. Calibration of divers. For explanation see text

until the diver floats. 5. Suck up the air bubble into a braking pipette. 6. Measure length of air bubble with mm graph paper. Calculate volume of air bubble. Record the number of the calibrated diver and its bubble volume (i.e., diver constant).

E. FILLING OF THE DIVERS

After preparing the divers as described in the section on "cleaning the divers" (page 107), the sequence of manipulations is as follows (Fig. 10): 1. Rinse the diver chamber many times with water from a pipette connected with a mouthpiece, and leave the diver filled with water.

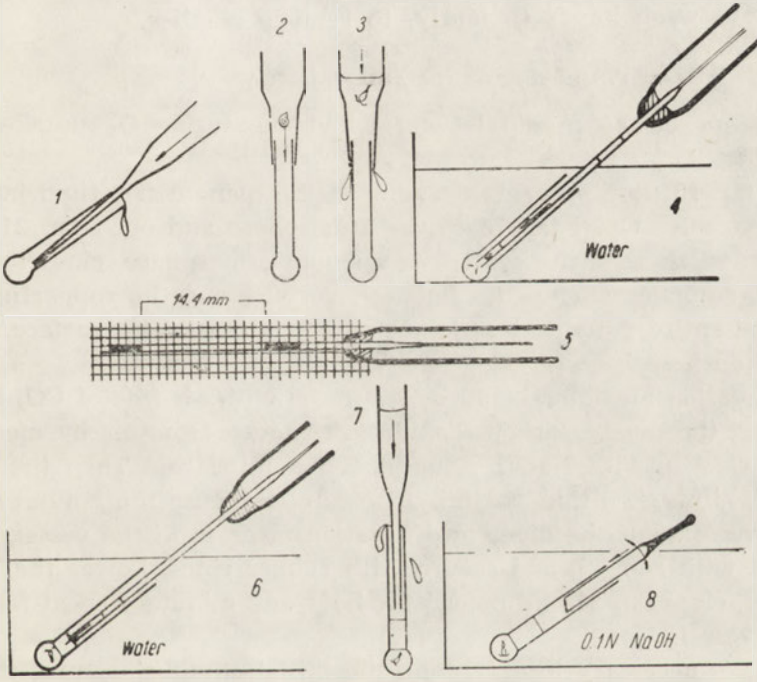

Fig. 10. Filling the diver. For explanation see text

2. Drop from a pipette an immobile experimental organism into the diver chamber so that it sinks to the head bulb. 3. An organism which moves can be pushed into the chamber by means of a water meniscus which moves downwards in the pipette. 4. A small, moving object can be driven into the diver head bulb with a braking pipette. 5. Draw into the braking pipette the volume of air necessary to float this particular diver. 6. Insert air bubble into the neck of diver chamber behind the water with the organism. 7. Introduce a fine bore pipette with 0.1 N NaOH in the neck of the diver. The water behind the air bubble is re-

placed with alkaline solution; take care not to expel the air bubble from the diver neck. 8. Close the diver chamber by inserting the stopper, under 0.1 N NaOH solution in the dish, turning it gently until firm. N.B.: the stopper should be inserted slowly to allow the excess of NaOH solution to flow out through the clearance between the chamber wall and the stopper: avoid an excessive compression of air bubble and shifting the meniscus.

It is suggested to perform all these manipulations with bare hands; operation gloves are not recommended since they hinder the work. A short contact with alkaline solution does not make much damage to the skin. To avoid contamination at charging the next diver, it is necessary to wash hands carefully, to remove alkalies.

F. MEASUREMENTS AND CALCULATIONS

The sequence of manipulations when measuring O_2 uptake is as follows (Fig. 1):

Close tap 18 (this prevents drawing in the manometric fluid into the flotation vessels). Open tap 19 to the atmosphere and open tap 21, turn 3-way tap 20 so that the system is open to atmosphere, close it after, say a few seconds. Lift up the flotation vessel along the supporting rod so that the entire ground-glass joint emerges above water surface. Open the flotation vessel.

The flotation medium should be saturated with air free of CO_2 before introducing the diver. Carbon dioxide is removed from air by means of bubbling washer with NaOH solution and a mist trap. Then the air is introduced through a thin polyethylene capillary onto the bottom of the flotation vessel. Put the diver into flotation medium in the vessel, cover the vessel with cap (2), and secure with rubber rings. Lower the vessel into water, close tap 21 and record the hour and minute. Repeat for each flotation vessel.

Now it is necessary to determine the initial equilibrium pressure for each diver. Open tap 19 and set the manometer fluid half way up both arms. Check the equilibrium pressure for each diver as follows: close tap 19, open tap 21 and with help of pressure regulator (screws 7 and 8) establish the diver at equilibrium level. The position of the diver at equilibrium level is arbitrarily taken as that at which the top of the diver chamber coincides with the mark on the vessel or the cross-hair in the cathetometer. The initial difference between the levels of Brodie's fluid in the both arms of the manometer (P_1 in Fig. 1) should not exceed ca 20 cm; that is, the diver has been filled with air with an accuracy of $\pm 2\%$ of the "diver constant" of this particular diver. After recording the time and initial equilibrium pressure (0.5 mm accuracy),

increase it by about 50 mm (then the diver will sink) and close tap 21. At the measurement intervals the diver should rest at the bottom of the flotation vessel.

N.B. There must be no air bubbles, not even a minute one, on the outer surface of the diver. They must all be removed after the diver has been placed into the flotation vessel, or even during the measurement run, if they then appear.

At time intervals chosen for a given experiment (e.g., every 15 min), repeat measurements of the equilibrium pressure in the following way:

Using screws 7 and 8 (if necessary also a large syringe 14 connected with the system through air-brake 13, and tap 19), set the manometer fluid approximately at the equilibrium pressure obtained for that diver at the previous measurement, plus 50 mm. This, will equalize the pressure over the flotation medium and that in the rest of the system.

Open tap 21. Slowly reduce the pressure until the diver begins to float. If the pressure is already much lower (usually by about 10 cm) than the previous equilibrium pressure, but still the diver lingers at the bottom and does not float, then tap gently the vessel cover until it moves up.

Alter the pressure with screws 7 and 8 so that the diver remains at the equilibrium level for at least 10 seconds without any further control. Record the time and the level of the manometric fluid in both arms to 0.5 mm accuracy. Increase the pressure by about 50 mm and close tap 21. Calculate the measured equilibrium pressure (P_2 in Fig. 1).

When the measurements are finished, close tap 18 (!), open taps 21, 19 and 20. Remove vessel cap 2 and take the diver out. Replace the cover (without the rubber rings).

The organism examined can be removed from the diver without any damage. In order to do this: Remove the stopper under water surface in Petri dish. Rinse with water the NaOH solution from the diver chamber neck (as in Fig. 10 — 7). Drawn out under water gas bubble with a braking pipette. Keep the diver chamber vertically with its opening under water surface; inactive objects drop down onto the dish and active organisms — flow out.

Table I and Figure 11 illustrate the records of the measurements of O_2 consumption by 3 copepodites-I of *Macrocyclopus albidus* (Jur.) (data sheet from: Klekowski and Shushkina 1966 b). For each diver, the following were recorded: time, manometric fluid level in the left and right arm, and the difference between them i.e., equilibrium pressure (P in Fig. 1).

The equilibrium pressure values of the consecutive records for each diver are marked on milimeter graph paper (Fig. 11): abscissa — time, usually 1 min = 1 mm; ordinate — equilibrium pressure, usually 1 mm Brodie = 1 mm). Figure represents the measurements listed in Table 1.

Table I. Example of the records of measurements of O_2-consumption

Macrocyclops albidus (Jur.) copepodite-I										Temp. 21° C 9.I.1965
No. of diver: 130 $V_g = 1.49$ µl (9^{52})				No. of diver: 131 $V_g = 1.43$ µl (9^{58})				No. of diver: 136 $V_g = 1.30$ µl (10^{18})			
Time	Manometer readings (mm)		P	Time	Manometer readings (mm)		P	Time	Manometer readings (mm)		P
	left	right			left	right			left	right	
9^{56}	873	878.5	− 5.5	10^{02}	428	459	− 31	10^{22}	368	226	+142
				10^{32}	440	487	47	10^{40}	387.5	257.5	130
10^{55}	862.5	888	25.5	11^{02}	443.5	501.5	58	11^{10}	455.5	339	116.5
11^{21}	868	905	37	11^{24}	429	498.5	69.5	11^{34}	370	264	106
11^{48}	879	927	48	11^{41}	444	520	76	11^{56}	355.5	259.5	96
12^{10}	845	910	65	12^{13}	427	516	89	12^{22}	356	271.5	84.5
12^{51}	847	929	82	12^{56}	436.5	541.5	105	13^{06}	337.5	272	65.5
13^{12}	843.5	934	90.5	13^{15}	435.5	548	112.5	13^{22}	348	292	56
14^{09}	852	966.5	114.5	14^{13}	424	557.5	133.5	14^{22}	324	291	33
$\Delta P = 25.5$ mm/hr $\Delta V = \frac{1.49 \cdot 25.5}{10.000} \cdot 0.93 =$ $= 3.53 \cdot 10^{-3}$ µl/hr				$\Delta P = 22.0$ mm/hr $\Delta V = \frac{1.43 \cdot 22.0}{10.000} \cdot 0.93 =$ $= 2.93 \cdot 10^{-3}$ µl/hr				$\Delta P = 26.5$ mm/hr $\Delta V = \frac{1.30 \cdot 26.5}{10.000} \cdot 0.93 =$ $= 3.20 \cdot 10^{-3}$ µl/hr			

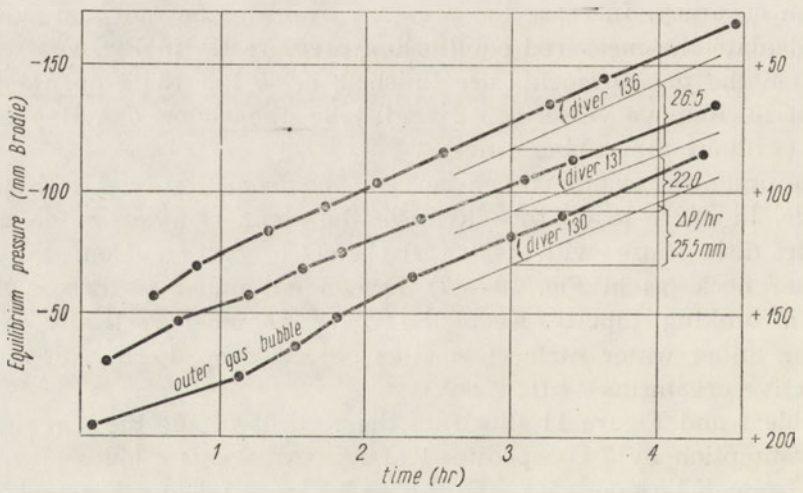

Fig. 11. Respiration of *Macrocyclops albidus*, copepodites-I; 21°C. Left scale for divers 130 and 131, right scale — for diver 136

Using a lucite rule with parallel lines, estimate the slope curves and calculate the average equilibrium pressure difference, Δ P, per hour (Fig. 11). The initial period of measurements, when gas equilibrium in the system gains its steady state, is not taken into account.

When no correction is required for the solubility of the gas in the liquids in the diver, the oxygen consumption per hour is calculated from the following formula:

$$\Delta Vo_2 = \frac{Vg \cdot \Delta P}{P0} \cdot \frac{273}{T}$$

where: V_g — air bubble volume (diver constant), ΔP — the equilibrium pressure change in mm per hour, P_0 — the normal pressure (10,000 mm Brodie sol.), T — temperature in Kelvin's scale (calculations exemplified in the lower part of Table 1).

G. CLEANING AND SILICONING THE DIVER

According to Zeuthen's (1955) advice, the diver stopper and chamber are rinsed in concentrated H_2SO_4, and successively in a number of fresh portions of distilled water. The diver chamber is rinsed always by sucking large amounts of fluid into the pipette which is introduced deep into the submerged chamber (Fig. 12). Later on, the divers are dried up at 105°C, each diver in its labelled Petri dish.

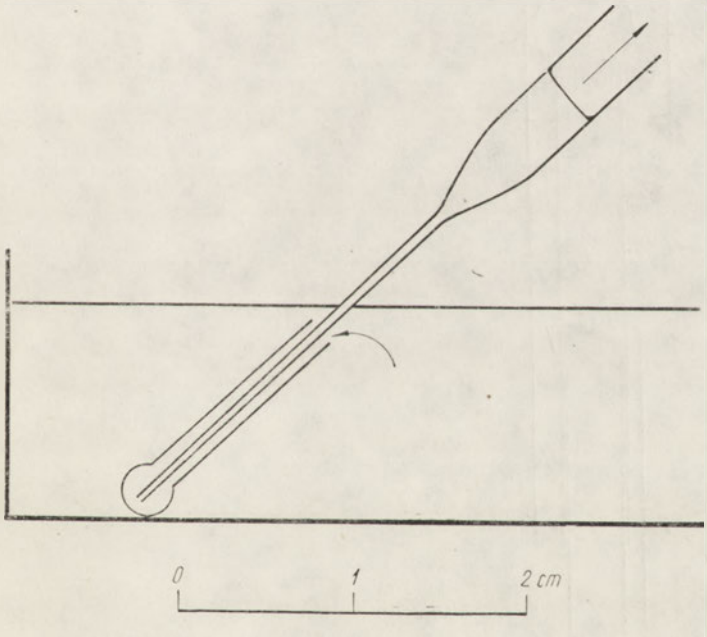

Fig. 12. Cleaning the diver

There is a risk of creeping the fluids, along the chamber walls within the air bubble. This is especially true for divers of wide bore. It can lead to mixing the NaOH solution with water holding an organism.

Coating with silicone has been introduced by Schwartz (1949 and Waterlow and Borrow (1949) (see also: Glick 1961 p. 68; Holter and Zeuthen 1966). In our practice, a weak kind of siliconing was used. After each cleaning the chamber was filled with 5% v/v $(CH_3)_2$ $SiCl_2$ in CCl_4, and heated to 180°C for 1 hr. Before charging, the chamber was rinsed many times with water, as described above.

4. STOPPERED DIVER FOR CO_2-OUTPUT MEASUREMENTS

A. DESCRIPTION OF THE DIVER

This type of divers has been introduced by Zeuthen (1949); full description in: Frydenberg and Zeuthen 1960, theoretical basis in Linderstrom-Lang 1943, Holter 1943. This diver (Fig. 13), allowing to measure O_2 consumption and CO_2-output in the same run of measurements, differs from the one described earlier (Fig. 6) in the following features:

The volume of the pressure sensitive gas bubble must be enlarged so that the ratio of volume of fluids (in diver head and in diver cham-

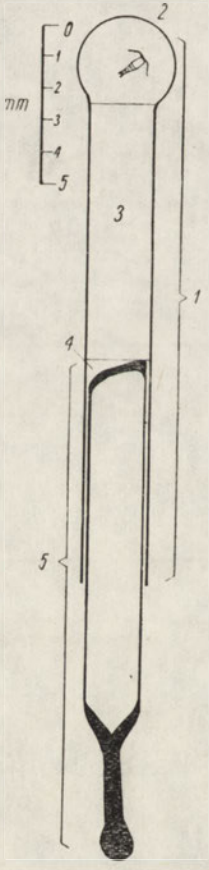

Fig. 13. The diver for CO_2-output measurements. 1 — diver chamber; 2 — chamber head (contains water volume V_{f_1}); 3 — gas bubble (volume V_g); 4 — 0.1 N NaOH solution (volume V_{f_2}); 5 — hollow gas stopper

ber neck over the stopper) to the volume of gas phase is $\leqslant 0.2$. With such ratio, the major part of released CO_2 (when it is not absorbed) appears as gas and is manometrically measurable. Such condition involves the necessity of a possibly highest reduction of liquid phase volume in the diver, i.e., that of the water holding an animal (V_{f1}) and of the fluid over the stopper (V_{f2}). For example, Frydenberg and Zeuthen (1960) used divers with ratio $V_f/V_g = 0.14\text{-}0.20$ ($V_g = 8.5$, 14.1 µl). In our practice (Klekowski and Shushkina 1966 b) on *Macrocyclops albidus:* $V_f/V_g \approx 0.15\text{-}0.17$; $V_g \approx 20\text{-}23$ µl; on the eggs and freshly hatched larvae of Tribolium (Klekowski et al. 1967): $V_f/V_g \approx 0.05$; $V_g \approx 10$ µl.

The relatively large gas phase volume in the diver allows making the diver from capillary tubing with thicker walls; the ratio of inner/ /outer diameter can be ≈ 0.8.

After completing the calibration of the diver, two marks should be made on the menisci of the gas bubble. They should be very thin. Black glass paint can be used; after drying, scratch excess paint and heat the diver chamber in the oven to the temperature appropriate for this paint (usually some 500°C).

Similarly as for the above described divers for O_2-consumption measurements, it is necessary to test each diver for tightness to CO_2 (a very important step, should not be overlooked!). For testing arrangements the following calculation can be useful. Even at a very high rate of O_2-consumption, e.g. in freshly hatched Tribolium larvae: $70 \cdot 10^{-3}$ µl/larva · hr, the amount of CO_2 in the diver, with $V_g \approx 10$ µl, will increase after 3 hr (without CO_2 absorbtion) to ca 2% v/v of CO_2. Thus, it will be satisfactory to fill the diver with gas holding 3% v/v of CO_2. The diver with no organism in it is filled under the surface of water saturated with appropriate mixture of gases. This gas mixture is drawn into the braking pipette from the tube outlet which releases slowly the gas and is submerged under the water surface in the same dish (Zeuthen 1946). The measurements of equilibrium pressure should be carried out with 0.1 N HCl as a flotation medium; the same fluid should be over the stopper in the diver chamber neck (manipulations will be described further in the text). The measurements will last for several hours.

Now it should be evaluated whether the observed CO_2 leakage is permissible for planned measurements. Let us mention two examples (from our experience):

1. *Macrocyclops albidus*, adult female; in the diver with $V_g = 20.8$ µl, $V_f = 3.22$ µl, $V_f/V_g = 0.16$. Measured CO_2-output $= 23 \cdot 10^{-3}$ µl/hr.

2. *Tribolium castaneum*, larva 30 hrs after hatching; in the diver

with $V_g = 11.0$ µl, $V_f = 0.2$ µl, $V_f/V_g = 0.02$. Measured CO_2-output = $65.4 \cdot 10^{-3}$ µl/hr.

Let us assume that the permissible CO_2 leakage from the diver can amount to 1-2% of the rate at which it is being formed, and that, for example, our newly mark and tested diver (with 3% v/v CO_2 in gas phase) leak $1 \cdot 10^{-3}$ µl CO_2/hr, i.e. $\Delta P = 1$ mm Brodie fluid in the ordinary manometer system, if $V_g = 10$ µl. Therefore, the diver will fit for CO_2-output measurements in Tribolium larvae (leakage of $CO_2 \approx 1.5\%$ of its production rate), however at measurements with Macrocyclops adult, the loss of CO_2 will be somewhat too large ($\approx 4\%$ of production). Note: an ordinary manometer (as in Fig. 1) can be used only in the case of objects which both intensively consume O_2 and produce CO_2. In majority of cases, however, more sensitive manometers should be used, of the type described in Frydenberg and Zeuthen's paper (1960, also in Holter and Zeuthen 1966) or a burette-manometer (Zeuthen 1953, Zajicek and Zeuthen 1961, Holter and Zeuthen 1966).

B. FILLING IN THE DIVER AND MEASUREMENTS

The sequence of manipulation should allow to calculate the volume of the liquids filling the diver as well as to run alternately measurements with absorption and without absorption of CO_2, released by an organism. To achieve this, the following procedure are adapted:

1. Fill half of the flotation vessels with 0.1 N NaOH and the other half with 0.1 N HCl. 2. Weigh together the dry diver chamber and stopper, to an accuracy at least 0.05 mg, or higher, W_1. 3. Fill the diver chamber with water, insert an animal, remove excess water by means of a braking pipette so that the water meniscus will drop towards the "upper" mark. Weigh then together the chamber and stopper, W_2. 4. Insert the stopper into the diver chamber neck, fill the clearance between the chamber and stopper with water (put a droplet of water at the rim of the chamber), remove excess water, weigh the diver, W_3. 5. Under water after having removed the stopper, suck out such amount of air from the diver chamber neck that the water meniscus will coincide with the "lower" mark. Insert the stopper, try the diver to float, if the deviation of initial equilibrium pressure from zero level is too high, add or remove a tiny quantity of air with a braking pipette, then weigh again, W_4. 6. Calculate: (a) Volume (1 mg = 1 µl) of water in the diver head: $V_{f1} = W_2 - W_1$; (b) volume of the liquid over the stopper: $V_{f2} = W_4 - W_3$; (c) volume of space between the chamber and stopper $= W_3 - W_2$. The latter value permits (see: Frydenberg and Zeuthen 1960) to evaluate the expected rate of CO_2 leakage from the diver and to

compare with empirical data obtained earlier. Values of V_{f1} and V_{f2} should be determined before each run of measurements. 7. Place diver in a specimen tube with 0.1 N NaOH, close the tube with the rubber stopper. There is a glass tube going through the rubber stopper, connected with a mouthpiece through a rubber tube. By altering the pressure, rinse out (several times) the water from above the stopper and replace with NaOH solution. Note: prevent from allowing some air into the space between the chamber and stopper! 8. Put the diver into the flotation vessel with NaOH solution and carry out the first series of measurement of P (oxygen consumption), e.g. for 2 hours. 9. Remove the diver from the flotation vessel, again place diver into a specimen tube, this time with water, and in the same way replace NaOH solution with water. 10. Put the diver into the flotation vessel with 0.1 N HCl, in the same way, replace water with HCl (use tap 19 and syringe 14 in Fig. 1; tap 18 must be closed!). Carry out the measurements of P (CO_2-output). 11. Remove the diver from the flotation vessel with HCl, put into a specimen tube and replace HCl with water. 12. Return the diver to the flotation vessel with NaOH solution. Carry out the second series of measurement of P (oxygen consumption). Note: NaOH solution should not be replaced directly with HCl and vice versa, since then undesired release of CO_2 takes place (from Na_2CO_3, which can be always found in NaOH solutions which are in contact with atmosphere).

C. CALCULATIONS

Denotations: V_{f1} — volume of the water holding an animal (µl); V_{f2} — volume of the fluid in diver chamber neck over the stopper (µl); V_f — sum of fluids volume (µl); V_g — volume of the gas phase (µl); $\Delta'P$ — the equilibrium pressure change in mm per hr when CO_2 is absorbed (0.1 N NaOH in flotation vessel and in the neck); $\Delta''P$ — the equilibrium pressure change in mm per hr when CO_2 is not absorbed (0.1 N HCl in flotation vessel and in the neck); V_{O2} — volume of consumed O_2 (µl per hour); ΔV_{CO_2} — volume of produced CO_2 (µl per hour); P_0 — normal pressure (10,000 mm Brodie); T — temperature of the system: $\alpha'CO_2$ — absorption coefficient of CO_2 in the liquid phase at the experimental temperature (and not at 0°C), this is volume of gas dissolved per volume of liquid (compare: Linderstrom-Lang 1943, p. 371). $\alpha'CO_2$ for 0.1 N HCl is only negligibly ($< 0.5\%$) different from this for H_2O.

Oxygen consumption is calculated from the formula given on page 107. Two runs of measurements with CO_2-absorption should be averaged for calculation of $\Delta'P$. The CO_2-output is calculated from the formula:

$$\Delta V_{CO_2} = \left(1 + \frac{V_f \cdot \alpha CO_2}{V_g}\right) \cdot \left(\frac{V_g \cdot \Delta' P}{P_0} \cdot \frac{273}{T} - \Delta V_{O_2}\right)$$

The following example can be useful: it is a further description of the Tribolium larva metabolic measurement, given on pages 109-110.

$$\Delta V_{O_2} = \frac{11.0 \cdot 69.0}{10{,}000} \cdot 0.90 = 68.3 \cdot 10^{-3}\ \mu l/hour$$

$$\Delta V_{CO_2} = \left(1 + \frac{0.2 \cdot 0.76}{11.0}\right) \cdot \left(\frac{11.0 \cdot 3.8}{10{,}000} \cdot 0.90 - 0.0683\right) = 65.4 \cdot 10^{-3}\ \mu l/hour$$

Note: The 0.1 N NaOH and HCl solutions have different specific gravities. Thus it is convenient to calibrate the diver in such a way that its equilibrium pressure in NaOH is somewhat above zero. Although the equilibrium pressure in HCl is clearly lower (for the example discussed here, it is about 300 mm Brodie solution) it does not affect significantly the CO_2 output calculations since during the measurements without CO_2 absorption, the change in the gas amount is relatively small.

5. FINAL REMARKS

Cartesian diver microrespirometer, with all its advantages has also some unfavourable properties, which one should bear in mind, when using this technique.

This is respirometer of a "closed vessel" type. In the course of measurement, the concentration of oxygen decreases and that of metabolites increases (compare also: Winberg and Belyatskaya-Potayenko 1963, Zeiss 1963). This should be taken into account while planning the size of divers and the duration of measurements. The O_2 concentration decrease should not bring about a significant (for a particular measurement) decrease in its consumption (for more information on "oxygen content — respiration intensity" problem, see Beadle 1961). In the earlier example (Table 1, Fig. 11), the oxygen reserve in the diver was ca. 0.3 µl. Within 5-hour measurement, 0.015 µl of oxygen was consumed, i.e., about 5% of the initial quantity. From the available data, it can be assumed that such a decrease in oxygen concentration would not cause any significant change in respiration intensity of Macrocyclops.

The amount of water (and therefore the diffusion distance for the gases) cannot be enlarged infinitely. In spite of advantages of Zeuthen's stoppered divers, such enlargement hinders the diffusion of gases and attainment of equilibrium (discussion of this problem in: Holter 1961, Holter and Zeuthen 1966, Linderstrom-Lang 1943).

At investigating metabolic rates of different animals, one should be conscious which ecological value of the metabolism has been measured in the diver for a particular study object. This will depend on the relation of the living space in the diver to the size of the organism tested and the degree of its activity. For example, in the diver with 3-4 µl of water space, the activity of Protozoa will be only slightly restricted, and the measured intensity of their metabolism will be close to the "Active Metabolic Rate" (or "Average Metabolic Rate"). On the other hand, in the same diver, the results for adult Copepoda will correspond rather to the "Resting Metabolic Rate".

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