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Occurrence and Physiological Properties of Bacterioplankton of Lake Chełmżyńskie (Poland)

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Abstract

This paper presents the results of research on the numbers of planktonic bacteria in Lake Chełmżyńskie, their developmental dynamics and their physiological properties. It was found that the total number of planktonic bacteria (TNB) was between 0.01 to 34.90 x 10^7 cells · ml^{-1} while the number of heterotrophic bacteria (TVC 22°C) in the water of the research sites in the lake varied between 0.17 to 42.06 x 10^2 cells · ml^{-1}. The maximum number of bacteria was found in summer, and the minimum in autumn. In the part of the lake near the town (sites I-VI) a distinctly greater number of planktonic bacteria was found in spring and autumn than in the part far from the town (sites VII- IX). Based on Korsh's bacteriological "Q" index (Q > 1000) the water of Lake Chełmżyńskie is rated as clean. Among the planktonic bacteria of Lake Chełmżyńskie, Gram-negative rods were dominant, and the majority were slowly growing strains. The most numerous among planktonic bacteria were strains that hydrolyse fat, protein, deoxyribonucleic acid and starch. The least numerous were chitinolytic, nitrifying and ureolytic bacteria. Bacteria belonged to genera *Flavobacterium, Aeromonas* and *Alcaligenes* dominated in all examined periods.

Keywords: bacterioplankton, heterotrophic bacteria, generic composition, physiological properties

Introduction

Heterotrophic bacteria are one of the most important groups of microorganisms occurring in aquatic environments. On account of their varied physiological properties and ability to biodegrade various organic compounds, they contribute to the reconstruction of organic matter and at the same time the self-cleaning processes of waters. Their numbers in water bodies, their developmental dynamics and changes in the population of different physiological groups are usually a reflection of the current state of the water body and its trophy as well as being the first, most common reaction to anthropogenic contamination.

Bacterioplankton is a group of water microorganisms that are exceedingly sensitive to all disturbances of the homeostasis of the environment in which they live. Changes to the balance of the environment which are natural fluctuations as well as those caused by human activity are reflected in bacterioplankton, in the form of microbiological parameters such as number, morphology, development dynamics and physiological-biochemical properties.

The aim of the present paper was to determine these parameters.

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Materials and Methods

Study Area

Microbiological research was carried out in the northwestern part of Lake Chemżyńskie (Fig. 1) at 9 research sites near the town (sites I-VI) and far from town (sites VII-IX).

The lake lies in the Chełmińsko-Dobrzyński lake district at a distance of about 20 km from Toruń and is part of the catchment area of the Fryba and Vistula rivers. The surface area of the lake is about 271.1 ha, capacity 16451.9 thousand m^3, maximum depth 27.1 m and average depth about 6.0 m. The lake has a dismembered shape. The north-western shore adjoins built-up areas of the town of Chełmża. The remaining lakeshore areas are mainly cultivated fields and meadows. The majority of the shore around the lake is flat, 60% of which is accessible for bathing, making this lake a perfect water body for recreation and water sports for people of the town of Chełmża, the surroundings and nearby Toruń.

Sampling

Samples of the surface water from a depth of 20 cm were taken from 9 sites (Fig. 1) in spring (23.05.01), summer (10.07.01) and autumn (16.10.01). The water was collected in sterile pipettes using an automatic sampler Pipet-Boy (De Ville), poured into sterile glass bottles and taken to the laboratory in a container filled with ice where the temperature did not exceed +4°C. Time from the moment of taking the samples to conducting the analyses did not exceed 6 hours.

Estimating the Number and Development Dynamics of Planktonic Bacteria

The total number of planktonic bacteria (TNB) in 1 ml of water was determined by counting the bacteria directly on membrane filters [1]. The water samples for these tests were fixed immediately after being taken in 40% formaldehyde, whose final concentration was 4%.

The number of heterotrophic bacteria (TVC) was determined by means of spread plates method, using iron-peptone agar as the medium [2]. Inoculation was carried out in 3 parallel repetitions. The bacterial colonies that grew up were counted after 3 and 10 days of incubation at a temperature of 22°C, distinguishing rapidly and slowly growing strains. The result was calculated per 1 ml of lake water.

Isolation of Bacterial Strains

After incubation and counting of the bacterial colonies, 25 colonies from each experiment were randomly picked and transferred into a semi-liquid iron-peptone agar medium (5 g of agar/l) and incubated for 6 days at a temperature of 20ºC. After checking the cleanliness of the bacterial cultures in the slides coloured with the Gram method, the strains were stored at +4ºC. The strains were grafted into a fresh iron-peptone medium every two months.

Morphological Studies

The morphology of planktonic bacteria and their differentiation into Gram negative and Gram positive was determined in preparations coloured using Gram's method [3]. Culture after 48 and 120 hours of incubation at a temperature of 22°C was used for tests in liquid iron-peptone medium.

The Identification of Planktonic Bacteria

The identification of isolated strains was carried out according to the scheme proposed by Allen, et al. [4].

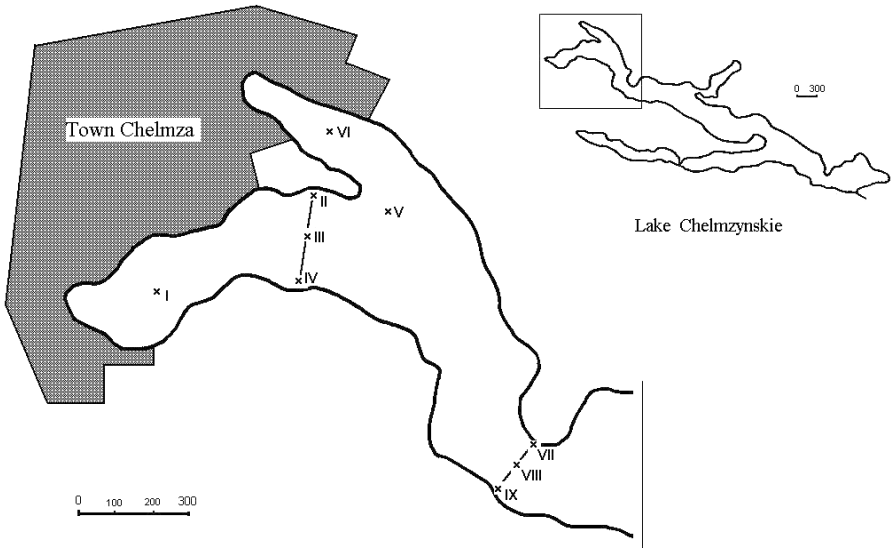

Fig. 1. Outline of Lake Chełmżyńskie. I - IX – standpoints of researches.

Table 1. Total number of planktonic bacteria (TNB) in Lake Chełmżyńskie (TNB x 10^7 cells · ml^{-1}).

Date of Sampling	Sites									
	I	II	III	IV	V	VI	VII	VIII	IX	Average
Spring (23.05.01)	3.90	28.80	15.50	5.02	19.90	27.30	6.10	11.80	19.93	15.36
Summer (10.07.01)	29.75	22.95	34.90	22.86	23.39	29.37	19.46	24.61	22.60	25.50
Autumn (16.10.01)	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.01	1.02	0.01

Table 2. Number of heterotrophic bacteria (TVC 22ºC) in water of Lake Chełmżyńskie (TVC x 10^2 cells · ml^{-1}).

Date of Sampling	Sites									
	I	II	III	IV	V	VI	VII	VIII	IX	Average
Spring (23.05.01)	42.06	8.33	3.15	2.90	5.13	12.83	2.00	7.50	14.23	10.90
Summer (10.07.01)	17.77	16.67	16.73	15.10	23.77	27.87	8.93	7.87	2.10	15.19
Autumn (16.10.01)	0.53	1.07	0.30	0.33	0.47	0.17	3.10	0.80	0.60	0.82

Table 3. Cleanliness index "Q" of Lake Chełmżyńskie (after Korsh [8]).

Date of Sampling	Sites									
	I	II	III	IV	V	VI	VII	VIII	IX	Average
Spring (23.05.01)	9272	345738	492063	172103	387913	212783	305000	157333	139986	246910
Summer (10.07.01)	167417	137672	208607	151391	98401	105534	217917	312706	1076190	275093
Autumn (16.10.01)	1887	935	3333	6061	2128	5882	645	1250	170000	21347

Physiological Properties

The physiological properties of bacteria were determined by seeding individual strains on a range of test media containing the relevant substrate. The ability of the bacteria to degrade protein, deoxyribonucleic acid, urea, starch, fat, pectin, cellulose and chitin and to conduct the processes of ammonification, heterotrophic nitrification and denitrification was taken into account in the tests. The media used for the tests were prepared according to Donderski [5, 6] and Lalke-Porczyk [7].

Results

It follows from the data presented in Table 1 concerning the total number of planktonic bacteria (TNB), and in Table 2 concerning the number of heterotrophic bacteria (TVC 22°C), that these data depended on the site and time at which

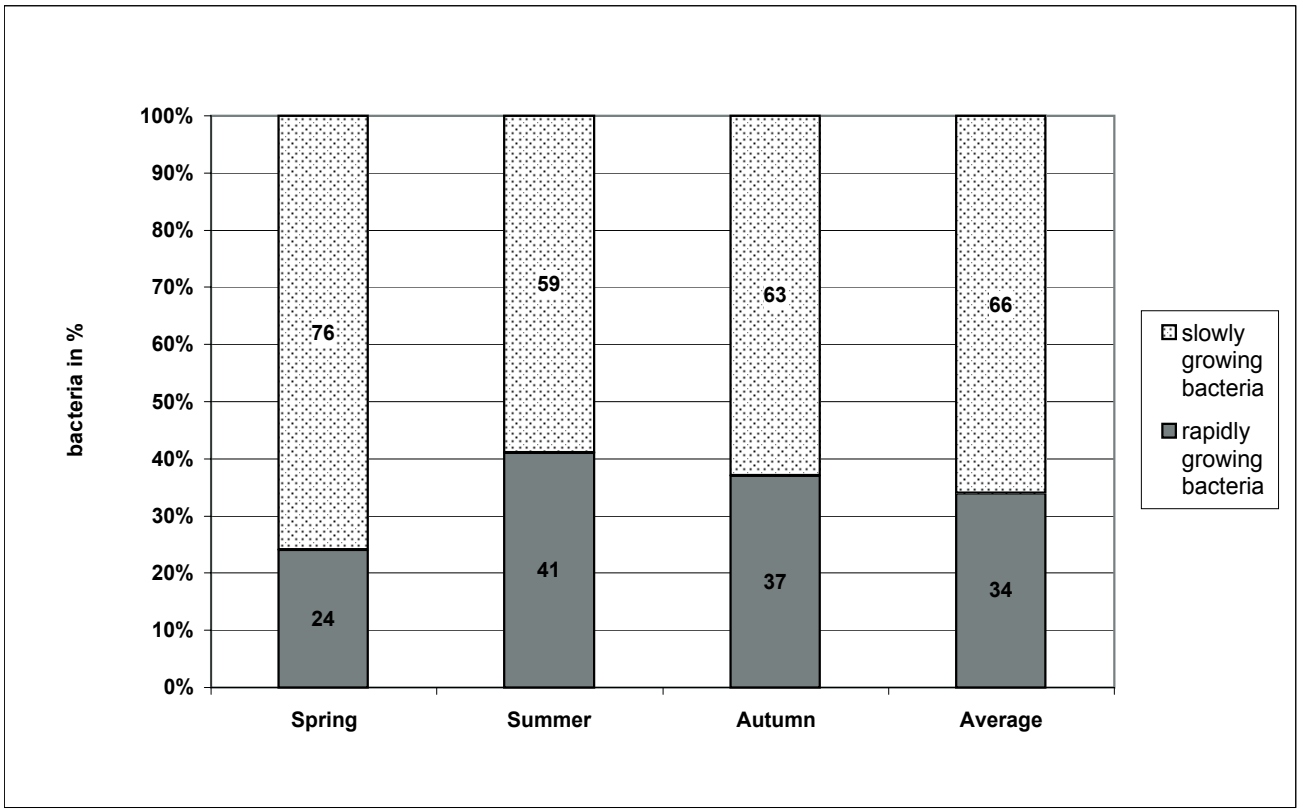

Fig. 2. Development dynamics of bacteria in water of Lake Chełmżyńskie.

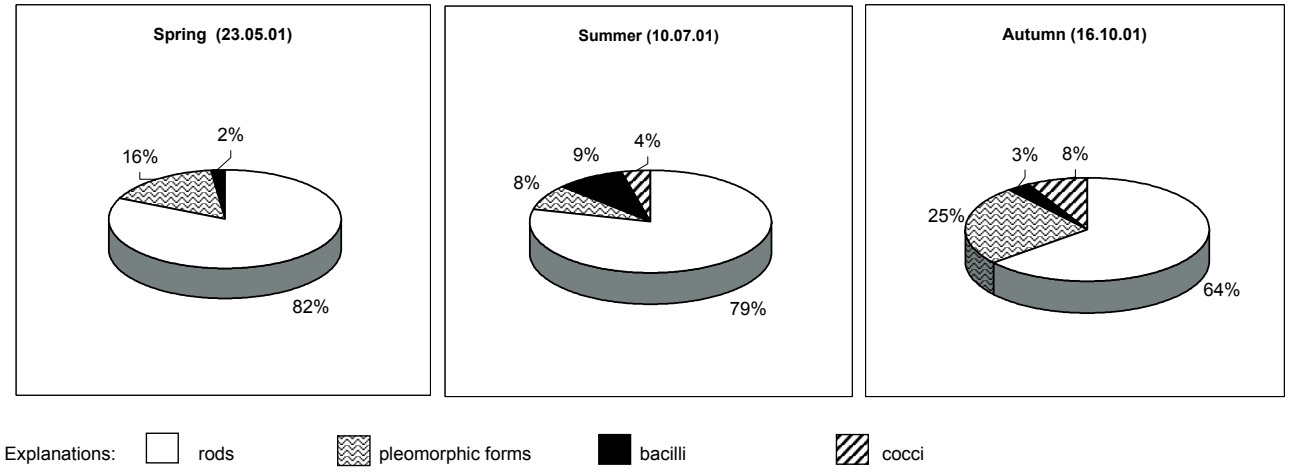

Fig. 3. Morphological types among planktonic bacteria in Lake Chełmżyńskie.

the research was conducted. The maximum number of these bacteria was found in summer, and the minimum in autumn. In the part of the lake near the town (sites I-VI) a distinctly greater number of planktonic bacteria was observed in spring and summer than in the part far from the town (sites VII-IX).

The values of the bacteriological index "Q" after Korsh [8] show that the waters of Lake Chełmżyńskie are clean (Q >1000) (Table 3).

Among the planktonic bacteria of Lake Chełmżyńskie, slowly growing bacteria were dominant (Fig. 2), representing 66% of total bacterioplankton. The proportion of rapidly growing strains in the tested period was 34% on average.

Among the morphological types of bacteria living in the water of the studied lake (Fig. 3) Gram negative rods were decidedly predominant, representing on average 75% of the total bacterioplankton. Pleomorphic forms were also comparatively numerous (average 16%). The least numerous were Gram positive bacilli and cocci (5% and 4% respectively).

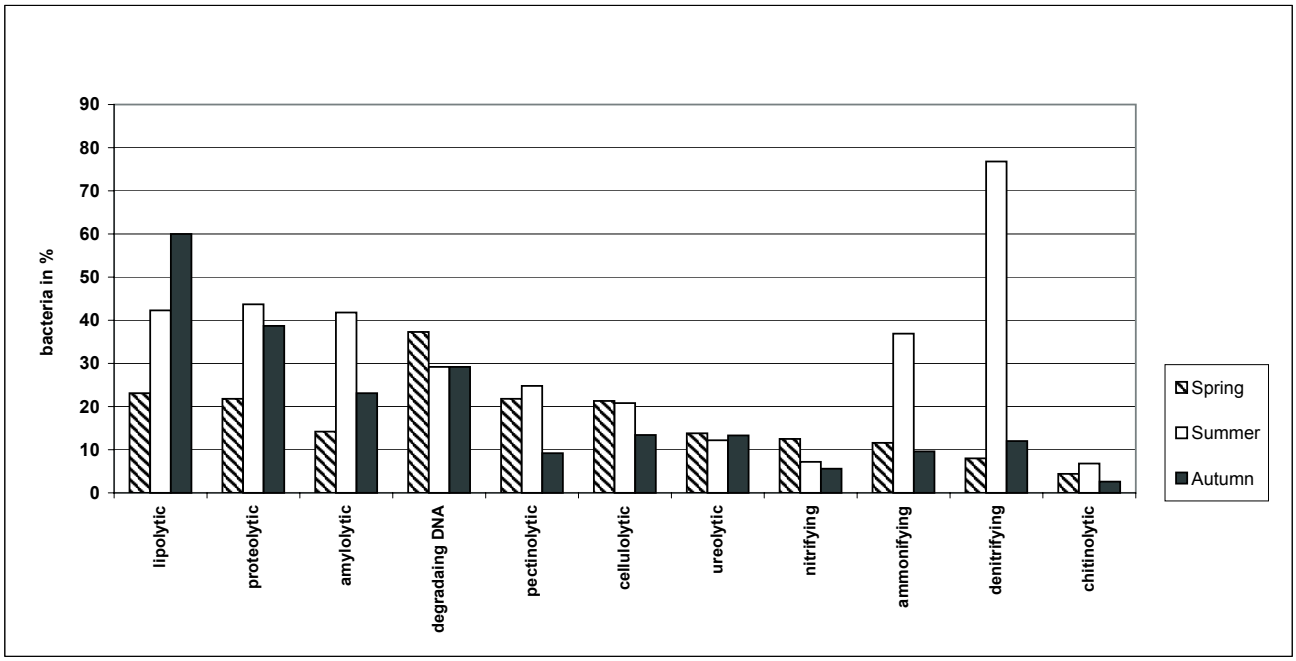

Fig. 4. Physiological groups among planktonic bacteria in Lake Chełmżyńskie (average).

Table 4. Generic composition of planktonic bacteria in Lake Chełmżyńskie (bacteria in %, average)

Genus of bacteria	Spring (23.05.01)	Summer (10.07.01)	Autumn (16.10.01)	Average
Acinetobacter	0	10	0	3.3
Aeromonas	15	21	4	13.3
Alcaligenes	18	23	20	20.3
Bacillus	3	4	28	11.6
Flavobacterium	51	32	32	38.3
Micrococcus	0	5	16	7.0
Staphylococcus	13	5	0	6.0

Among the physiological groups the most numerous were microbes degrading fat (average 42%), protein (average 35%), deoxyribonucleic acid (average 32%) and starch (average 26%). In summer the most numerous group was strains that reduce NO_3^- to NO_2^- (average 77% of strains). The least numerous among planktonic bacteria were chitinolytic bacteria (average 5%), nitrifying bacteria (average 8%) and ureolytic bacteria (average 13%). Bacteria degrading fat, protein, starch and deoxyribonucleic acid were most numerous in summer (Fig. 4).

It follows from research that generic composition of the planktonic bacteria in Lake Chełmżyńskie was similiar in all examined periods. Most of the bacterial strains belonged to the genera *Flavobacterium*, *Alcaligenes* and *Aeromonas* (Table 4). In spring *Flavobacterium*, *Alcaligenes* and *Aeromonas* genera were abundant, in summer the number of bacteria belonged to the genera *Flavobacterium*, and *Staphylococcus* decreased and number of *Alcaligenes* and *Aeromonas* genera increased. In summer *Acinetobacter* and *Micrococcus* genera were noted. In autumn as in spring *Flavobacterium* genus was the most frequent and the higher abundance of *Bacillus* and *Micrococcus* genera was noted in contrast with the genera *Alcaligenes* and *Aeromonas,* which were less abundant.

Discussion

The quantity of bacterioplankton in lake waters depends above all on the trophy of the water body and on contamination flowing into them from the catchment area and domestic waste. In this study, significantly higher numbers of planktonic bacteria were found in the part

of the lake near the town of Chełmża than in the part far from town, which is consequently less exposed to the inflow of contamination of anthropogenic origin. The ratio of the total number of planktonic bacteria (TNB) to the number of heterotrophic planktonic bacteria (TVC 22°C) determining the "Q" index after Korsh [8], where Q>1000, enables the waters of Lake Chełmżyńskie to be rated as clean. The number of heterotrophic bacteria represents but a small fraction of a percent of the total number of bacterioplankton in this lake (about 0.007%), and as many reseaerchers claim [6, 9] the number of heterotrophic bacteria increases with the trophy and contamination of water bodies. Amann et al. [10] and Bernard et al. [11] state that the number of heterotrophic bacteria in fresh water reaches 0.25% total number of bacterioplankton. According to Niewolak [12] the proportion of these bacteria in badly contaminated waters can come to 5% of the total number of bacterioplankton. According to Olah [13] and Niewolak [14] the summer maximum number of bacteria is connected to the increased content of organic substances secreted by plants, and also to the increase in water temperature. According to many researchers [15, 16] algae play an important role in the process of the internal enriching of the water in water bodies in soluble organic material. From 37-70 % of the original production produced by algae can be assimilated by bacterioplankton [17, 18]. The heightened increase in allochtonic contamination as a result of intensive tourist traffic can also have an effect on the increase in the number of bacteria in summer. In turn the cause of a significant reduction in the number of bacteria in the autumn period is probably the low temperature of the water restricting the assimilation by microflora of secretions which degrade macrophytes and zooplankton.

Changing environmental conditions have an undoubted effect on the physical-chemical parameters of lake water and, consequently, on the development of the whole bacterial population. It follows from the research that the dominant group among heterotrophic planktonic bacteria in Lake Chełmżyńskie are slowly growing strains with optimal growth after 10 days of incubation. The fastest developing bacteria were observed in summer. A decline in the number of fast developing strains from summer to spring is probably connected with a reduction in available nutrients and a lowering of the water temperature [19, 20], which in turn reduces the activity of the bacteria and prolongs the time for the generation of individual populations. According to Świątecki [21], temperature is a factor that limits the reproduction rate of bacteria at levels below 10°C. At higher temperatures, and particularly during summer, no dependency is found between this factor and reproduction rate. In such environmental conditions the main factor that regulates the increase in the number of bacteria is access to easily assimilated nutrient substrates [6, 21, 22]. The shortest time for bacterioplankton generation observed in summer is probably connected with the large amount of easily available organic material, including algae blooms. On the other hand, the large proportion of rapidly growing bacteria observed in the autumn period should be linked with the last phyto- and zooplankton blooms and with the dying plants (hydromacrophytes) which are the source of nutrients.

The morphological composition of bacterioplankton is conditioned by many factors: above all the trophy of the water body, physical-chemical factors occurring in lakes and the influence of other organisms. It follows from Fig. 2 that, among the bacterioplankton of Lake Chełmżyńskie, Gram negative rod-shaped bacteria are predominant, making up on average 75% of total bacteria and noted as the dominant morphological type by other authors [5, 23]. Relatively large numbers of pleomorphic forms (average 16%) occurred in the lake under investigation. According to Beveridge and Davis [24] pleomorphism of cells is probably linked to the environmental stress to which they are exposed during their life cycle and which is composed of many factors like temperature, pH or concentration of electrolytes and useless nutrients. Gram positive bacilli and cocci (5% and 4% respectively) were the least numerous in Lake Chełmżyńskie. Many authors have observed that the growth and development of gram positive bacteria is impeded by active biological substances secreted by numerous algae and cyanobacteria [15, 25], particularly during blooming.

Heterotrophic bacteria in water bodies are a population of very different physiological groups with varying levels of biochemical activity [5, 6, 26]. In this paper the ability of planktonic bacteria to degrade certain polymeric substances occurring in natural waters was studied.

Fats produced by plants and animals are one of the most important groups of biopolymers occurring in fresh waters [27]. In the opinion of Arts et al. [28] bacteria can actively cumulate and use fatty compounds, among others, as a source of energy. Lipids can also fulfil the function of mechanical and heat insulators. Organisms like green algae, cyanobacteria and copepoda can significantly contribute to the occurrence of a large number of lipolytic bacteria because they accumulate considerable amounts of fat in their cells [29]. Donderski and Strzelczyk [30] and Lalke-Porczyk [7] also contend that lipolytic bacteria are one of the most numerous groups of bacteria occurring in the waters of Lake Jeziorak.

Apart from fat, one of the most readily degraded large-particle compounds was protein. Planktonic bacteria with proteolytic abilities represented on average 35% of total heterotrophic bacteria studied. Saava [31] and Suigita [32] state that bacteria that degrade protein in water bodies can make up 70 or even 100% of the total number of bacteria. The large numbers of proteolytic bacteria occurring in the water of Lake Jeziorak confirm research conducted by many authors [7, 30]. In the opinion of Litte et al. (quoted in Mudryk [26]), the occurrence of such a large number of bacteria degrading protein is connected with the fact that the main components of organic material in water bodies, after carbohydrates, are proteins, polypeptides and aminoacids. Their source is secretions from phytoplankton, macrophytes and zooplankton and their remains. The

highest proteolytic activity was observed in this study during summer. The low proteolytic activity in autumn was probably the result of low temperatures. The optimal temperature for synthesis of proteases is 18°C, according to Helemake and Weyland [33], while in autumn, the water temperature in the lake did not exceed 14.3°C.

The large numbers of bacteria that hydrolyse deoxyribonucleic acid found in the water of Lake Chełmżyńskie (average 32%) are in accordance with the data obtained by Strzelczyk, Donderski and Lewosz [34] and Donderski [6].

Starch, pectin and cellulose are natural components of aquatic plants. Bacteria actively use them as a source of carbon and energy. It follows from research on the degradation of starch that amylolytic bacteria are a comparatively numerous group of bacteria [23, 35]. The ability to hydrolyse starch was displayed during the study period by, on average, 26% of the bacterial strains and it was most intensively degraded in summer and autumn. The ability to hydrolyse pectin in the water of Lake Chełmżyńskie was displayed by 19% of heterotrophic bacteria. Donderski and Strzelczyk [30] demonstrated that among bacteria isolated from the water of Lake Jeziorak they made up from 4 to 12 % of the studied strains. In this paper we also found a considerable proportion of cellulolytic bacteria. They were on average 19% of the total number of microbes. According to Zdanowski [36] environmental factors like temperature, degree of eutrophication of the water body and the pH of the water influence the degradation of cellulose. In our climatic zone the degradation of cellulose takes place most intensively in higher temperatures from May to October. In this paper the degradation of cellulose was distinctly greater in spring and summer, when the temperature of the water was relatively high, than in autumn, which confirms Zdanowski's observations [35].

It follows from the data obtained in this study that chitin was the least accessible compound for the studied microorganisms, although it is produced in an aquatic environment in large quantities. The pH of the water, which was never lower than 8.3, can affect the process of metabolising chitin in water bodies [6]. The optimal pH for hydrolysing chitin, as follows from papers by Rogers [37] and Donderski [6], is between 5.0-6.0. It is therefore more probable that such a high pH of the water in Lake Chełmżyńskie, reaching as much as 9.2, could cause the inactivation of chitinases or impede their synthesis and thus considerably lower the detectability of chitinolytic bacteria.

Ammonification is a very important process, particularly in water bodies where algae bloom frequently, bringing into the water and bottom deposits considerable quantities of proteins and aminoacids [38]. The activity of ammonifying bacteria in surface waters testifies to the mineralisation of organic nitrogen compounds taking place. In this study the proportion of planktonic bacteria capable of conducting the process of ammonification came to 19% on average of the total number of heterotrophic bacteria. The relatively numerous group of bacteria capable of conducting the process of ammonification suggests that aminoacids are a group of compounds which have great significance for the optimal development of aquatic microorganisms [6]. Another, very important physiological group of bacteria engaged in transforming nitrogen compounds in water bodies is denitrifying bacteria. Their presence in the studied lake indicates on the one hand the content of easily assimilated organic material and the considerable abundance of nitrates, and on the other self-cleaning processes taking place in the water of the water body. In this study the highest percentage of denitrifying bacteria (77%) was observed in the summer period, which is probably connected with the influx into the lake of waters rich in nitrogen substances washed off from the fields and the rise in temperature that stimulates the activity of the bacteria [39, 40] and also with the oxygen deficit that occurs in connection with the development of aerobic heterotrophic bacteria conducting mineralising processes.

Heterotrophic nitrifying bacteria, oxidising ammonia to nitrites or nitrates are a comparatively small group of microorganisms in the water of Lake Chełmżyńskie. Similar numbers of strains capable of conducting this process were found by Donderski [5] and Strzelczyk et al. [41].

It follows from research on the generic composition of heterotrophic planktonic bacteria of Lake Chełmżyńskie that three groups of bacteria dominated in all seasons: *Flavobacterium*, *Aeromonas* and *Alcaligenes*. As follows from reports by many researchers [6, 42, 43], representatives of the group *Flavobacterium-Cytophaga* are microorganisms that are frequently isolated in samples of both fresh and sea waters. The considerable number of microorganisms in this group found in water is probably connected with their ability to produce pigments that protect them against the lethal action of radiation from the sun. In the opinion of Cherry et al. [44], the large number of coloured bacteria in water, including organisms from the genus *Flavobacterium*, may be an indicator of the purity of a given aquatic ecosystem as opposed to the presence of bacteria from the genus *Escherichia*. It follows from research on pure cultures of representatives of the group *Flavobacterium-Cytophaga* that the microorganisms in this category are capable of decomposing a wide spectrum of substrates in oxygenic conditions, ranging from various proteins, carbohydrates, pesticides and insecticides through to complex macromolecules [45]. Ripen – Kirchner [46] notes that microorganisms from the group *Flavobacterium-Cytophaga* are the most active organisms taking part in the process of decomposing dead algae cells.

Bacteria belonging to the genus *Aeromonas* constitute a numerous group of strains isolated from Lake Chełmżyńskie in spring and summer, which is consistent with the observations of Kaper et al. [47]. Bacteria from this group play an important role in the destruction of organic material, including that from dead zooplankton [48]. This suggests that many organisms of this genus are proteolytic and chitinolytic organisms. According to Kaper et al.

[47], bacteria from the genus *Aeromonas* are good indicators of the high trophy of waters. Unusually versatile as regards biochemical properties, they display a wide spectrum of tolerance in relation to biogenic compounds.

Microorganisms from the genus *Alcaligenes*, occurring in soil, water, sewage and the dietary tract of animals, were characterised by their relatively large proportion among the bacterial seedlings identified in Lake Chełmżyńskie. Microorganisms belonging to this genus were isolated from sewage contaminated with arsenite, containing detergents and also from sea waters contaminated with oil substances. At the same time, their ability to oxidise arsenite and to biodegrade superficially active substances and contamination from oil substances was observed [49]. Bacteria belonging to the genus *Alcaligenes* are also involved in the metabolisation of nitrogen compounds in surface waters, taking part in processes so important for the water body as ammonification, denitrification or the decomposition of urea [50].

Heterotrophic planktonic bacteria are one of the most important groups of microorganisms occurring in the aquatic environment because of their varied physiological properties, active participation in processes of degradation and transformation of organic compounds, including some not easily undergoing degradation like aromatic hydrocarbons or pesticides to simple, mineral compounds assimilated by photosynthesising organisms. They contribute to the reconstruction of organic material and cause the self-cleaning of waters and detoxication of the environment. The effect of the process of self-cleaning is a reduction in the load of contamination carried to the lake. The high correlation of microbiological and physical-chemical parameters indicates the active processes of transformation of organic and mineral forms of nutrients.

Conclusions

1. Total number of planktonic bacteria (TNB) in the water of Lake Chełmżyńskie fluctuated between 0.01 to 34.90 x 10^7 cells . ml^{-1}, while the number of heterotrophic bacteria (TVC 22°C) varied between 0.17 to 42.06 x 10^2 cells · ml^{-1} and depended on location of the site and season of the year.
2. The maximum of planktonic bacteria in Lake Chełmżyńskie was found in summer and the minimum in autumn.
3. Bacteriological cleanliness index "Q" after Korsh shows that the waters of Lake Chełmżyńskie are clean (Q > 1000).
4. Among morphological types of bacteria living in the Lake Chełmżyńskie Gram negative nodes were the predominant forms (75%), the least numerous were Gram positive bacilli and cocci (8 and 4%, respectively).
5. Among the physiological groups the most numerous were bacteria degrading fat, protein, deoxyribonucleic acid and starch. The least numerous were chitinolytic, nitrifying and ureolytic bacteria.
6. Generic composition of the planktonic bacteria was similar in all examined periods. Among the bacterial strains the most numerous were strains belonging to the genera *Flavobacterium, Alcaligenes* and *Aeromonas*.

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