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## **PHYSIOLOGICAL PROPERTIES OF BACTERIA INHABITING COASTAL LAKE SURFACE AND SUBSURFACE WATER LAYERS**

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### **Abstract**

Results of the studies on the occurrence of bacteria displaying particular physiological properties in coastal Lake Gardno are presented. Most numerous groups among neustonic and planktonic bacteria studied were ammonifying and producing hydrogen sulphide from organic compounds strains. Nitrifying bacteria were not numerous among isolated strains. It was demonstrated that significant differences in the abundance of individual physiological groups bacteria existed in water layers, stations and seasons.

**Key words:** coastal lake, bacterineuston, bakterioplankton, physiological properties

### **INTRODUCTION**

Bacterial microflora plays the key role in the process of biological balance in the water ecosystems (Heinänen 1992, Berman et al. 1994). Those organisms, heterotrophic bacteria populations displaying high level of metabolic activity in particular, highly contribute to the process of organic matter decomposition and transformation (Goosen et al. 1995, Murrell 2003). This process is also aided by the participation of physiologic heterogenic groups of bacteria showing differentiated levels of biochemical activity (Krstulović and Solić 1988). Owing to the dynamic autoinduction of their systems, those bacteria have the facility of fast metabolic reaction to every chemical compound newly introduced into the water ecosystem (Kerstein 1991). As a result, the bacteriocenosis actively assimilates the organic matter transforming it into its own biomass or uses it for the respiratory purposes as a source of energy (Donderski et al. 1998). Hence the key role of the bacteria in the process of organic matter decomposition and its flow through the microbiological loop which makes the necessary condition of homeostasis keeping in every water ecosystem.

This paper presents the results of the study of physiological properties of neustonic and planktonic bacteria inhabiting coastal Lake Gardno. Such studies may provide information for better understanding of the role of bacteria in controlling the biological balance in estuaries.

## MATERIAL AND METHODS

### Description of the studied area

The studies were carried out in estuarine Lake Gardno situated in the World Biosphere Reserve – Słowiński National Park (Poland). The lake is very shallow (1.3 m average depth) but covers a large area (2 500 ha). Lake Gardno is characterized by intermediate conditions between marine and inland environments. On one hand it is supplied by the water of the River Łupawa, on the other hand via a 1.3 km long channel it is connected to the Baltic Sea (Fig. 1) whose large volumes of sea water abundantly penetrate into the lake. Therefore, the water of the lake, or its part acquires seawater properties, resulting in 2-5 PSU salinity. Consistently with the Venetian system, Lake Gardno can be classified as mixo-oligohaline type (0.5-5.0 PSU) (Dethier 1992).

The studied estuary is a polymictic water basin with no thermal or oxygen stratification of a considerable level eutrophication. This high trophic level together with a high concentration of nutrients (Mudryk et al. 1993) and the penetration of light to the bottom of the lake create perfect conditions for the development of phytoplankton, whose bloom lasts practically from spring to autumn. The phototrophic com-

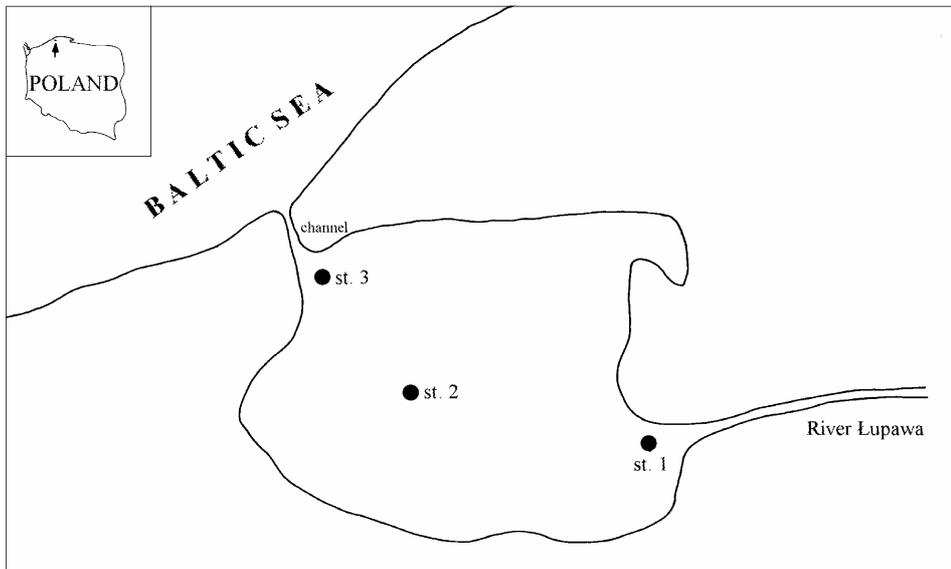


Fig. 1. Lake Gardno with location of sampling sites

munity is dominated by aggregate-forming cyanobacterium *Anabena flos-aquae*, *Aphanizomemon flos-aquae* and *Microcystis aeruginosa* (Strzelecki and Piótorak 1971).

The shallow and productive studied estuarine lake is characterized by extensive growth of macrophytes. The emergent macroflora covers 4% of the Lake Gardno surface forming a 20-100 m wide offshore belt, which constitutes home to many bird species. The main species of macrophytes are: *Typha angustifolia*, *Phragmites australis*, *Scirpus lacustris* and *Schoenoplectus lacustris*.

### Sampling

Water samples were taken in 1998 in a quarterly cycle system (spring, summer, autumn) from three stations (Fig. 1): one near the River Łupawa inflow (freshwater zone) (station 1), one in near-sea part (seawater zone) (station 3) and at one station in mid-lake (mixed zone) (station 2). Water samples for bacteriological analyses were taken from three layers. Film layer (FL) samples (thickness of  $90 \pm 17 \mu\text{m}$ ) were taken with a  $30 \times 30$  glass plate (Harvey and Burzell, 1972), surface layer (SL) samples (thickness of  $242 \pm 40 \mu\text{m}$ ) were collected with a  $40 \times 50$  cm Garrett net (24 mesh net of 2.54 cm length) (Garrett 1965). Glass plate and polyethylene net were rinsed with ethyl alcohol and distilled with sterile water prior to sampling. The water from subsurface layer (SUB) was sampled from the depth of about 10-15 cm. All the water samples were collected into sterile glass bottles and stored in an ice-box, where the temperature did not exceed  $7^\circ\text{C}$  until they were taken for analysis. The time between sample collection and performance of the analyses usually did not exceed 6-8 h.

### Isolation of bacterial strains

Plate techniques were used in order to isolate neustonic (FL and SL) and planktonic bacteria (SUB). Water samples were vortex mixed, and then serial tenfold dilutions were prepared with sterile buffered water (Daubner 1967) to reach final concentrations ranging from  $10^{-1}$  to  $10^{-4}$ . Diluted samples were inoculated by the spread method in three parallel replicates on iron-peptone agar medium (IPA) prepared according to Ferrer et al. (1963). Incubation was carried out at  $20^\circ\text{C}$  for 10 days. Then, from the whole surface of the plates or from selected sectors, 30 bacterial colonies from each station and water layer were picked out and transferred onto a semiliquid ( $5.0 \text{ g agar per dm}^{-3}$ ) IPA medium. The cultures maintained on this medium after purity control were kept at  $4^\circ\text{C}$  and used for further investigation on their physiological properties.

### Determination of physiological properties of the isolated bacteria

The following physiological properties of bacteria were considered:

1. The ability to ammonification was examined in a liquid medium prepared according to Rodina (1968).  $\text{NH}_3$  was detected with Nessler's reagent.

2. The ability to produce hydrogen sulphide from organic compounds was tested in a liquid medium prepared according to Rodina (1968). The presence of hydrogen sulphide was detected with paper strips saturated with 10% lead acetate and placed inside test tubes above the medium.
3. The ability to carry out heterotrophic nitrification was tested in a liquid medium prepared according to Donderski (1971). Nitrates were detected with  $\alpha$ -naphthylamine and sulphanic acid.
4. The ability to reduce nitrates to nitrites (partial denitrification) was tested in a liquid Katznelson's medium (Donderski 1971). Nitrates were determined as described above.
5. The ability to reduce methylene blue was tested in a liquid medium containing culture medium B (Rodina 1968) with 0.02% aqueous solution of methylene blue. Medium decolouring gave a positive result.
6. Uric acid hydrolysis was tested on a solid medium prepared according to Stienmann (1976). Clear zones around the colonies denoted a positive result.

The pH of all media was adjusted to 7.0-7.4 and sterilised at 117°C. All media were inoculated with one loop of bacterial suspension obtained from a 48 h culture in a liquid IPA. Inoculated media were incubated for 7 days at 20°C. Only media for indicating nitrification and denitrification processes were incubated for 10 days.

## RESULTS

The data presented in Fig. 2 shows that the most abundant physiological group inhabiting the Lake Gardno water were bacteria responsible for the occurrence of the ammonifying process and H<sub>2</sub>S forming out of organic compounds. Those organisms

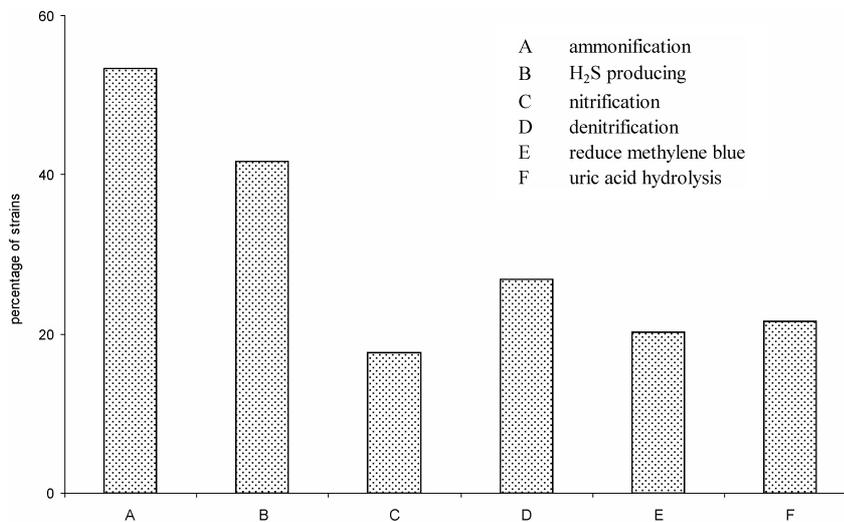


Fig. 2. Occurrence of physiological groups among bacteria isolated from water of Lake Gardno (percentages derived from the pooled data of all sites, seasons and water layers)

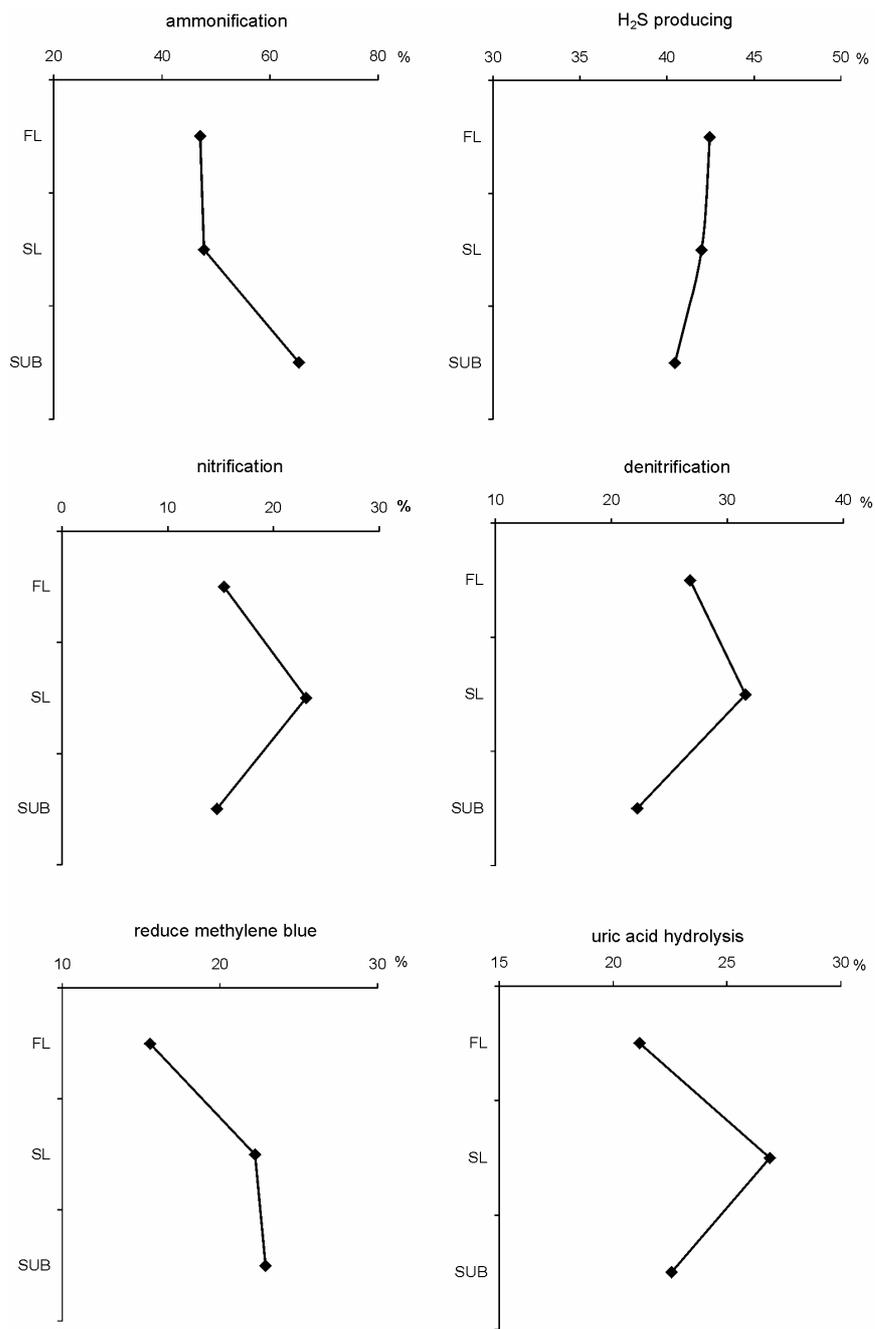


Fig. 3. Vertical distribution of different physiological groups of bacteria inhabiting three surfaces of the studied water layer (percentages derived from the pooled data of all sites and seasons)

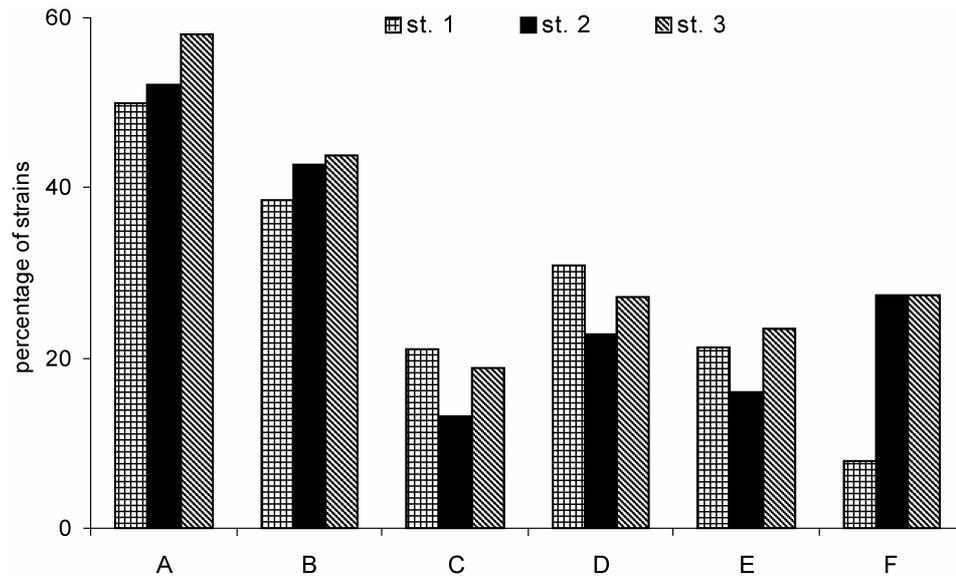


Fig. 4. Spatial distribution of the studied bacterial physiological groups (percentages derived from the pooled data of all water layers and seasons) (A-F see fig. 2)

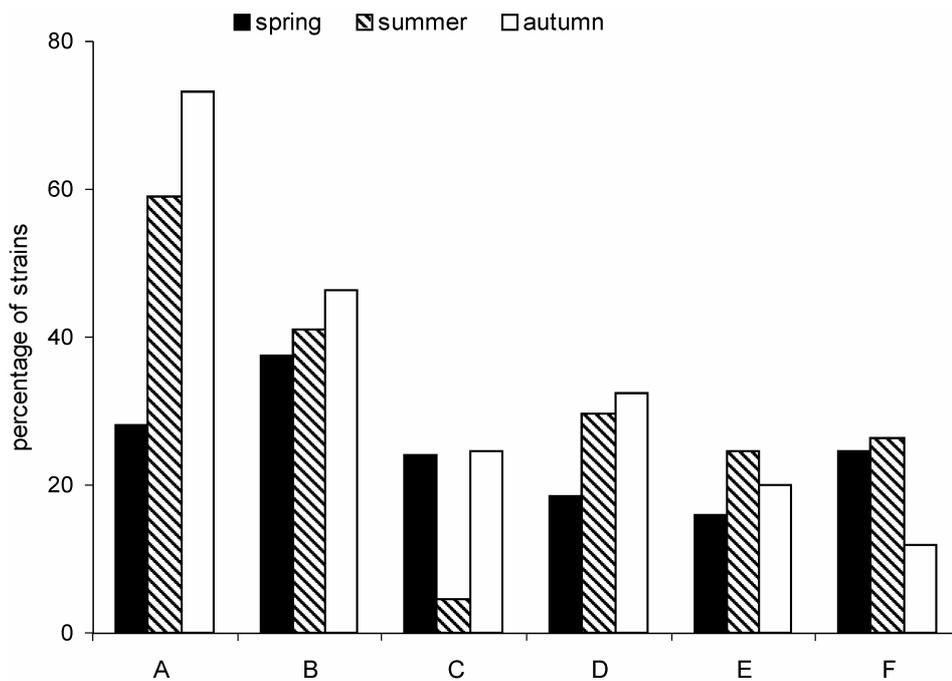


Fig. 5. Seasonal variation in occurrence of different physiological groups of bacteria (percentages derived from the pooled data of all water layers and sites) (A-F see fig. 2)

made over 40% of the total isolated bacterial strains. Bacteria carrying on with heterotrophic nitrification process (17%) were the least numerous.

It can be clearly seen with Fig. 3 that distribution of the physiological groups under study was stratified and there were differences between surface and subsurface water layers. In surface layer (SL) the nitrifying, denitrifying and uric acid decomposing bacteria were the most abundant. Ammonifiers and methylene blue reducing bacteria were most numerous in subsurface water (SUB) while bacteria producing H<sub>2</sub>S out of organic compounds were equally distributed across the studied layers of water.

Fig. 4 presents the horizontal distribution of the studied bacterial groups in Lake Gardno. The highest percentage of ammonifying, H<sub>2</sub>S producing out of organic compounds and methylene blue reducing bacteria were encountered in the offshore zone (station 3). Nitrifying and denitrifying bacteria dominated the Łupawa River mouth (station 1) while the uric acid hydrolyzing ones were mostly found in mid-lake water (station 2) and the offshore zone (station 3).

Data concerning the seasonal changeability of the studied physiological groups occurrence in Lake Gardno is presented in Fig. 5. The ammonifying, denitrifying bacteria and those producing H<sub>2</sub>S out of organic compounds were most numerous in autumn while spring saw their minimum amounts. The greatest percentage of nitrifying bacteria was noted in spring and autumn while in summer they were least abundant. Uric acid decomposing and methylene blue reducing bacteria dominated in summer while autumn saw their minimum numbers.

## DISCUSSION

The results of the study proved ammonifying bacteria were the most abundant physiological group of the bacterial microflora in Lake Gardno. Those were microorganisms which are responsible for amino acids deamination due to which they play the key role in the processes of organic nitrogen mineralisation (Koike et al. 1986). Thus, the intensity of the ammonification process in water bodies depends on the presence and concentration of various forms of organic nitrogen, which primarily includes proteins, polypeptides, peptides and amino acids. Studies carried out by many scientists (Donderski 1983, Owens and Stewart 1984, Petrycka et al. 1990, Mudryk et al. 1991) proved that ammonifying bacteria, regardless whether in freshwater, estuarine or marine basins, made one of the most numerous physiological groups of microorganisms. Billen and Fontigny (1987) point to the fact that ammonification is a very important process, particularly in those water bodies where algae blooms occur frequently bringing significant amounts of proteins and amino acids. Facing nearly continuous algae blooms in Lake Gardno, ammonification must be then the basic physiological process there ensuring its homeostasis.

Apart from ammonifying bacteria, other abundantly occurring ones in Lake Gardno were bacteria producing H<sub>2</sub>S out of organic compounds. Previous scientific studies have demonstrated a wide range of the quantity of this bacterial physiological group. Prieur (1989) found out that in the West Pacific as many as 82-96% of bacteria pro-

duced hydrogen sulphide using organic connections. At the same time, studies carried out in offshore areas of the Gulf of Finland (Väätänen 1976) and South Baltic (Mudryk et al. 1991) displayed the amounts of that physiological group less than 10% of the total of isolated bacteria. Those discrepancies between particular water bodies concerning numbers of bacteria releasing  $H_2S$  out of organic compounds must have resulted from differences in concentrations of protein and sulphur containing amino acids, such as cystine, cysteine and methionine (Morra and Dick 1991).

In Lake Gardno, about 30% of the total of the isolated strains were the denitrifying bacteria. The above mentioned Prieur (1989) found out that over 50% of the total of the bacteria in the Pacific were  $N-NO_3$  to  $N-NO_2$  reducing organisms. Denitrifying bacteria develop abundantly in heavily eutrophicated water basins with high concentrations of nitrate ions (Kaspar 1985) and this is the case with the eutrophic Lake Gardno. According to Seitzinger et al. (1984) nitrogen loss due to the denitrification process going on in the eutrophicated water ecosystems may reach as much as 30-50%. That is why intensive denitrifying bacteria growth is able to significantly reduce the nitrate resources easily accessible for plants and lower the water body trophy level at the same time.

Although denitrification is said to be an anaerobic process, Ellis-Evans (1985) and Giese (1988) were able to detect nitrate reducing bacteria also in surface water. In Lake Gardno similarly, the greatest number of bacterial strains carrying out the denitrification process inhabited the surface layers of the water. Sharp et al. (1986) explain this phenomenon by the fact that denitrifying bacteria colonize particles of organic matter suspended in water around which anaerobic microniches are formed which stimulate the development of this physiological group of bacteria.

In estuarine Lake Gardno, heterotrophic nitrification bacteria made the least abundant physiologic group. It was also Donderski (1983) and Petrycka et al. (1990) who pointed to the fact that nitrifying bacteria made one of the least numerous physiological groups in water basins. Nitrifying bacteria compete with phytoplankton and phytobentos for ammonia which is the most sought source of nitrogen by algae (Kiel and Kirchman 1991). As a rule, phytoplankton  $NH_4^+$  assimilation is faster than its oxidising by bacteria (Ward et al. 1984). For this reason the losing nitrifying bacteria in that competition for ammonia ions must have been responsible for the reduced numbers of those organisms. In Lake Gardno the minimum amount of nitrifying bacteria occurred in summer when the development of the competing phytoplankton for ammonia compounds with bacteria was most intense.

## CONCLUSIONS

1. The most abundant physiological group inhabiting the Lake Gardno water were ammonifying bacteria and  $H_2S$  forming out of organic compounds. Bacteria carrying on with heterotrophic nitrification process were the least numerous.
2. Distribution of the physiological groups under study was stratified and there were differences between surface and subsurface water layers.

3. It was demonstrated that significant differences in the abundance of individual physiological groups bacteria existed on stations and seasons.

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## WŁAŚCIWOŚCI FIZJOLOGICZNE BAKTERII ZASIEDLAJĄCYCH POWIERZCHNIOWE I PODPOWIERZCHNIOWE WARSTWY WODY JEZIORA PRZYMORSKIEGO

### Streszczenie

W pracy przedstawiono wyniki badań dotyczących określenia właściwości fizjologicznych bakterii neustonowych i planktonowych zasiedlających przymorskie estuariowe jezioro Gardno. Wśród wyizolowanych szczepów bakterii najliczniej występowały organizmy przeprowadzające proces amonifikacji oraz wytwarzania siarkowodoru z połączeń organicznych. Najmniej liczną grupę fizjologiczną stanowiły bakterie nitryfikacyjne. Stwierdzono wyraźne zróżnicowanie w występowaniu poszczególnych grup fizjologicznych bakterii w powierzchniowych i podpowierzchniowych warstwach wody. Zanotowano także różnice w horyzontalnym rozmieszczeniu badanych grup fizjologicznych bakterii w jeziorze Gardno. Wykazano również sezonową zmienność występowania poszczególnych grup fizjologicznych bakterii.