VERTICAL AND HORIZONTAL CHANGES IN THE ABUNDANCE AND ACTIVITY OF BACTERIA IN LAKE HAŃCZA, NE POLAND

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Abstract

A bacteriological study was conducted in late summer (early September 2018) in the deepest postglacial reservoir in the central lowland depression – Lake Hańcza (northeastern Poland). The aim of the study was to determine the spatial variation in the density of planktonic bacteria, the level of their hydrolytic activity and the ratio of live to dead bacterial cells. During the fieldwork 92 water samples were collected from thirteen stratified sites located in different parts of the lake. The bacterial number ranged from 0.57 to 3.97 (10^6 cells ml^{-1}). No horizontal or vertical changes in bacterial number were observed in a uniform pattern. The highest bacterial abundance was recorded at two coastal sampling points, both located in the southern part of the lake at the direct catchment area with commercial development ("agroturism" guesthouses). There was little difference in bacterial number across the horizontal gradient ($F = 1.865$; $p = 0.170$). Many water quality parameters appeared to be significant factors correlating with bacterial abundance in Lake Hańcza. There were no statistically significant differences in hydrolitical bacterial activity and live/dead ratios between investigated sites and across horizontal gradient. Our results indicate that bacterial activity in deep lakes is affected by many water quality parameters, such as temperature ($r=0.667$), Ca^{2+} ($r=-0.4510$), Mg^{2+}($r=0.511$), NO_3^- ($r=-0.587$) and oxygen saturation ($r=0.582$).). Taking into account previous hydrobiological studies, Lake Hańcza was found to be very patchy in terms of bacterioplankton abundance.

Key words: bacterioplankton, hydrolytic activity, spatial variation

Introduction

Lake Hańcza (LH) is considered to be a homogeneous natural water reservoir due to its structure – the shape of the lake basin and shoreline of little variety. Despite the increasing anthropopressure in the catchment area, the lake still maintains its water quality level. It retains a fairly natural character and low trophy. Differences in environmental conditions in the presence of human activity in various parts of the lake can lead to significant changes of water quality across the reservoir. It is usually assumed that those differences are negligible for small, shallow lakes with a more compact shape and greater stability of water masses (Łopata et al. 2014). In the case of large lakes with an extensive shoreline, the differences in water quality in individual parts of the reservoir are very well documented, e.g. as recorded for Lake Wigry (Górniak and Zieliński 2006). However, even lakes such as LH, with poorly differentiated lake basin morphology, may also have significant spatial variation in water quality. This may be influenced by the hydrological character of the lake, through which the Czarna Hańcza River flows. The distribution and activity of bacteria may be a good indicator of spatial variation in water microbiological quality. According to Bergström and Jansson (2000) the contribution of allochthonous bacteria can account for up to 70% of the internal bacterioplankton production in the lake's epilimnion. Since there is such a high proportion of these allochthonous bacteria, there is a differential distribution of bacterioplankton in the epilimnion related to organic matter availability (Lindström and Bergström 2004).

Bacteria play a substantial role in shaping water quality through cycling of organic and inorganic matter of freshwater ecosystems (Chróst and Siuda 2006). The rate of this circulation depends on chemical, physical and biological factors. Recent studies show that bacterioplankton can also be an indicator of the trophic state of a lake in relation to anthropogenic factors and their influence on water quality (Zieliński et al. 2006). LH is well investigated in terms of zooplankton (Jekatierynczuk-Rudczyk et. al 2014; Karpowicz and Ejsmont-Karabin 2017; Ejsmont-Karabin et al. 2020), phytoplankton (Napiórkowska-Krzebietke and Hutorowicz 2013; Karpowicz et al. 2016) and bacterioplankton (Niewolak and Gotkowska-Płachta 1999; Gotkowska-Płachta et al. 2003; Zieliński et al. 2006; Gotkowska-Płachta 2008), hydrochemistry (Hillbricht-Ilkowska and Wiśniewski 1993; Pyka et al. 2007), macrophytes (RIEP 2017) and fish (Brylińska et al. 2002, Kotusz et al. 2004; Dziekońska-Rynko et al. 2018).

The aim of the study was to determine the spatial variation in the occurrence of planktonic bacteria density, the level of their hydrolytic activity and the ratio of live to dead bacterial cells, which was crucial for establishing whether LH is homogeneous in terms of microbiological and hydrochemical water quality on the vertical and horizontal scale.

Materials and methods

LH, commonly regarded as a model mesotrophic lake, is the deepest water reservoir not only in Poland, but in the European Lowlands, with depth reaching 105.6 m (Borowiak et al. 2020). The surface area of the lake is 303.6 ha and the volume of the lake is 0.1186 km^3 (Popielarczyk and Templin 2014). LH is a part of the East Suwalskie Lake District and it is a flow-through lake, with the Czarna Hańcza River (CHR) flowing through it. CHR is a tributary of the Nemen River and is characterised in its upper section by a very natural, meandering riverbed (Kołodziejczyk 1999). The main factor in the exchange of lake water, which takes about 144 years, is rainwater and water from surface runoff (Mitręga et al. 1993). The lake lies within the Suwałki Landscape Park. It has been protected in its entirety, along with its shores, as the Lake Hańcza Natural Reserve, since 1963. It is a unique body of water, located in a post-glacial trough, the catchment area of which is mostly occupied by agricultural and agrotourism areas. The rest of the catchment area consists mainly of forests (Figure 1).

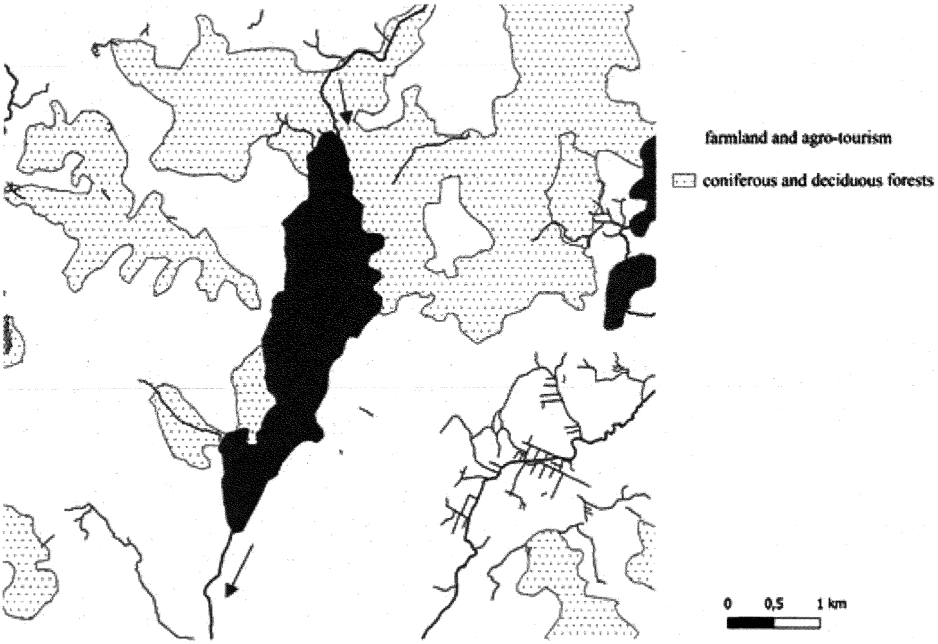

Figure 1. Management map of the Lake Hańcza and its direct catchment.

Considered one of the cleanest lakes in Poland (Gotkowska-Płachta et al. 2003), due to its unique structure and depth, it has been listed as a UNESCO Aqua Project since 1974. It is estimated that LH, with a maximum length of 4.5 km, a width of 1.2 km and a total shoreline length of 12.6 km, was created during the Late Weichselianglacier outburst floods in North-Eastern Poland around 15-17 thousands years ago (Weckwerth et al. 2019).

It is assumed that the oldest rocks forming the deep bedrock surrounding LH are the highly fractured and fault-cut Precambrian anorthosite and norite rocks of the so-called Suwałki Anorthosite Massif. The sediments shaping the surface of LH's surroundings are Quaternary formations reaching the thickness of up to 281 m and differentiated into glacial, fluvioglacial and limnoglacial sediments and form an organic sediment of the Eemian interglacial. The surface formations are represented mainly by peats, silts and sands of river terraces and bottoms, lacustrine organic and mineral sediments (gyttjas), as well as sands and deluvial clays. Along the lake shores there are accumulations of boulders constituting a layer often exceeding 2 meters (Pochocka-Szwarc et al. 2013).

In our study, all water samples were collected on one day during the summer stagnation period (September 2018), using a Ruttner apparatus, from 13 sites designated for the deep lake sites (no 1-3) and the coastal sites (no 4-13), according to the bathymetric map (Figure 2). In this study, sampling points were delineated taking into account the lake's direct catchment area, including the community beach (station 4).

A total of 92 samples was obtained, 62 of which were collected at deep lake sites, every meter from a depth of one meter to 15 meters and then at 10 meter intervals to the bottom. Water transparency level was measured at these sites using a Secchi disk. The remaining 30 samples were collected at coastal sites from the three stratification layers of the studied lake. Water for microbiological analysis from each site was collected into sterile containers.

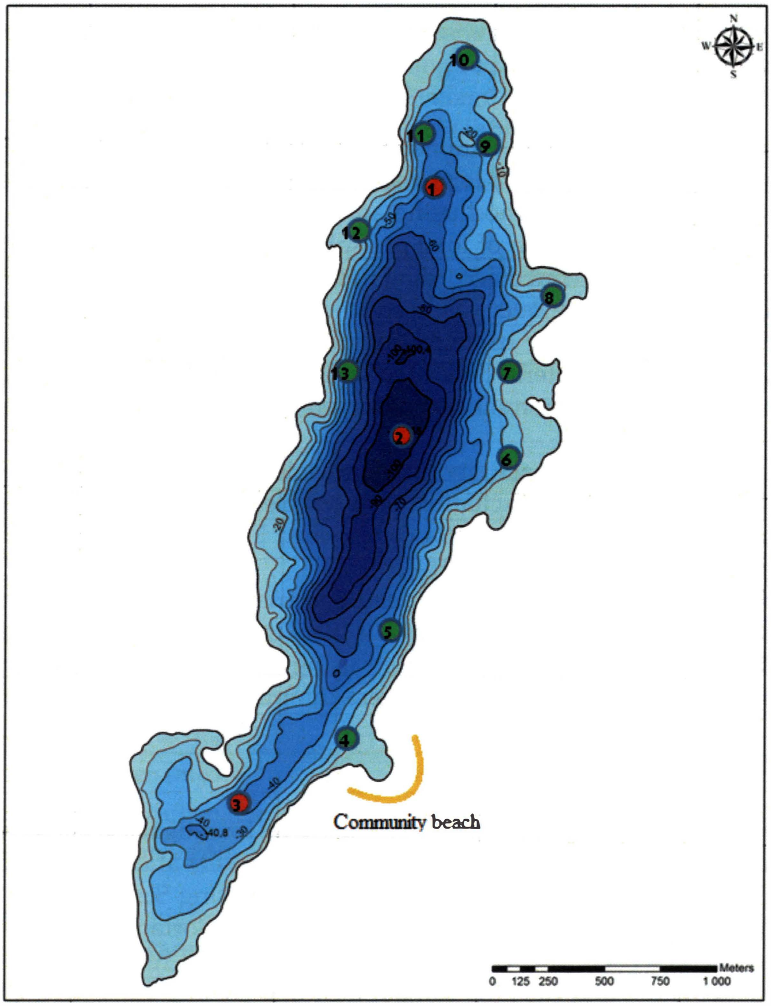

Figure 2. Map of the location of research sites (red circles – *deep lake sites*; green circles – *coastal sites*) on the background of the bathymetric map of Lake Hańcza (*based on:* Popielarczyk and Templin 2014).

Directly in the field, in-situ measurements were performed with the Hach-Lange multi-parameter probe (HQ-40) taking into account: water temperature, electrolytic conductivity, pH and dissolved oxygen concentration and oxygen saturation. The main hydrochemical parameters were quantified using standard procedures as described by WEF and APHA (2005) approximately 24 hours after collection to ensure that the sample did not deteriorate significantly during storage. Dissolved organic carbon (DOC) and total nitrogen (TN) were determined in the laboratory using a high temperature combustion method with the Shimadzu TOC-L analyser. Specific UV absorbance (SUVA) was used to evaluate the aromaticity of dissolved organic matter. UV absorbance normalized to the DOC concentration of the sample was computed using the formula:

$$SUVA = \frac{Abs_{254} \times 1000}{DOC}$$

(Chin et al., 1994, modified). Parameters such as different nitrogen (NH_4^+ and NO_3^-) and phosphorus (PO_4^{3-}) ions and content of Ca^{2+} and Mg^{2+} were determined using the HPLC method using an ion chromatograph Thermo Dionex ICS-1100. The determination of total phosphorus has been made with the molybdenum method measured according to standard methods (APHA 1998).

Epifluorescence technique was used to determine the total bacterial number (BN) after staining the bacterial cells with 4',6-diamidino-2-phenylindole (DAPI). To count the bacteria deposited on 0.2 μm filters, they were placed on basal slides with immersion oil and observed using an epifluorescence microscope (Olympus BX43).

The bacteria hydrolytic activity (BHA) was determined using fluorescein diacetate and analysed at an excitation light wavelength of 480 nm and emission of 505 nm with the modified method described by Adam and Duncan (2001) following Burkowska-But et al. (2014).

To determine the ratio of live to dead bacteria (LD), samples were stained with LIVE/DEAD® Bacterial Viability Kit (BacLight™) according to the manufacturer's instructions and then counted using an epifluorescence microscope (Olympus BX43). The final LD values were computed using the formula:

$$Ratio_{G/R} = \frac{F\ cell,em1}{F\ cell,em2}$$

Various statistical models have been used to investigate relationships between BN, BHA, LD and the hydrochemical parameters at each site and at different depths. Statistical analyses were performed using Statistica ver. 13.3 for analysing the level of statistical significance with importance level $p < 0.005$. The standardised coefficients of variation (CV = [SD / m] 100%, where SD is the standard deviation and m is the sample mean), were also calculated and used for comparison of bacterial and hydrochemical parameters from all collected samples. To determine if there was a significant difference between studied parameters, the Kruskal-Wallis test was used. Spearman's rank correlation analysis, with the level of statistical significance of $p < 0.05$ and $p < 0.01$, was used to show the relationship between hydrochemical and bacteriological parameters.

Results

The highest visibility level measured with a Secchi disk for LH during the sampling period was 4.2 m. The average temperature of the lake was 12 °C, the average temperature of surface water was 17.7 °C, electrical conductivity 266 μS and pH 7.5. The nitrate extreme values ranged from 0.001 mgN l^{-1} to 0.27 mgN l^{-1} and both of them were detected at sampling site No 3. The ammonium value was the lowest at 6 meters of the first sampling site – 0.0002 mgN l^{-1}, the highest in the thermocline of site No 9 – 0.31 mgN l^{-1}. Soluble reactive phosphorus (PO_4^{3-}) ranged from 0.0002 mgP l^{-1} in metalimnion at No 7 sampling site to 0.02 mg l^{-1} in surface water from the first sampling site – where the dissolved organic carbon was also the highest – 7.14 mg l^{-1}. Average total phosphorus was 0.05 mg l^{-1} and the average total nitrogen – 0.88 mg l^{-1} (Table 1).

Table 1. The hydrochemical characteristic of Lake Hańcza – mean values for all samples (September 2018).

Parameter	Mean value	Standard deviation	Min -Max
	n=92		
Temperature (°C)	12	5.9	4 – 20
Electrical conductivity (μS cm^{-1})	266	5.4	254 – 279
pH	7.5	0.42	6.45 – 8.37
Oxygen saturation	102.8	13.8	67.7 – 119.8
Ca^{2+} (mg l^{-1})	42.49	3.38	28.17 – 47.55
Mg^{2+} (mg l^{-1})	8.55	0.35	8.54 – 8.66
NO_3^- (mgN l^{-1})	0.1	0.11	0.001 – 0.27
NH_4^+ (mgN l^{-1})	0.02	0.05	0.00002 – 0.31
PO_4^{3-} (mgP l^{-1})	0.003	0.003	0.0002 – 0.02
Total phosphorus (mg l^{-1})	0.05	0.04	0.0001 – 0.17
Total nitrogen (mg l^{-1})	0.88	0.15	0.64 – 1.24
Dissolved organic carbon (mg l^{-1})	5.69	0.42	4.98 – 7.14
Specific UV absorbance (Abs_{254} gC^{-1})	20.4	3.02	14.8 – 28.8

On average, the highest BN was recorded in the epilimnion layer – 2.38 10^6 cells ml^{-1} in coastal sampling sites and 2.34 10^6 cells ml^{-1} in deep lake ones. The lowest average BN value was recorded at site 1 where it was 1.33 10^6 cells ml^{-1}, and the highest at site 4 with the count of 3.08 10^6 cells ml^{-1} (Figure 3).

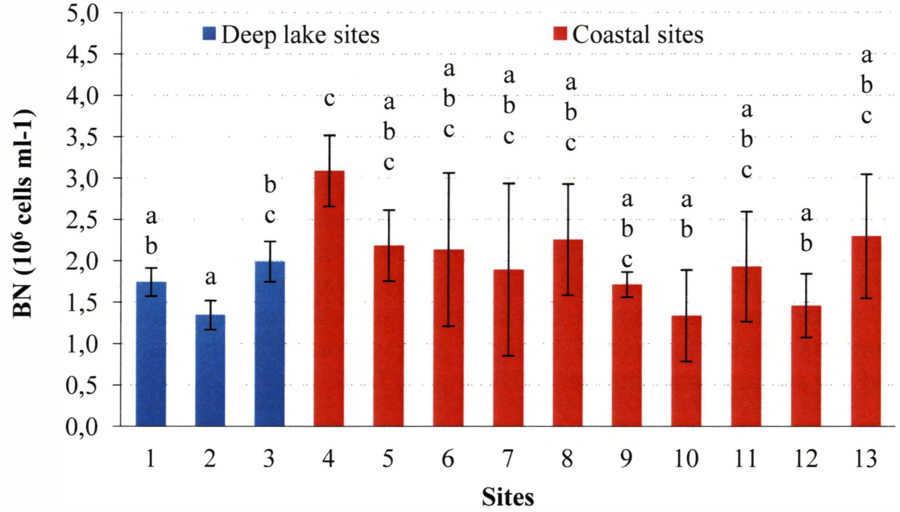

Figure 3. The average bacterial number (BN) (±SD) of 13 sampling sites in Lake Hańcza. Letters indicate statistically significant differences.

The vertical and horizontal changes of BN in LH ranged from 0.57 10^6 cells ml^{-1} at a depth of 14 meters at one of the central sites to 3.97 10^6 cells ml^{-1} at depths of 1 and 15 meters of the nearshore site designated as site 6 (Figure 4).

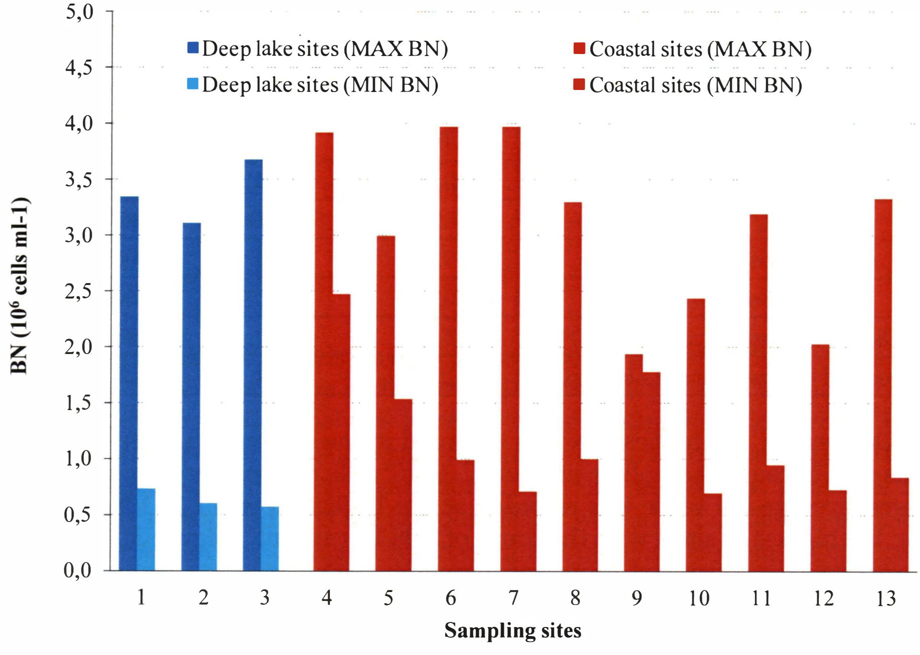

Figure 4. The extreme values (Min, Max) of bacterial number (BN) of all investigated sampling sites in Lake Hańcza.

The average abundance of bacteria in LH was 1.66 10^6 cells ml^{-1} for deep lake sites and 2.02 10^6 cells ml^{-1} for the coastal sites at the end of the growing season (September 2018) (Figure 5).

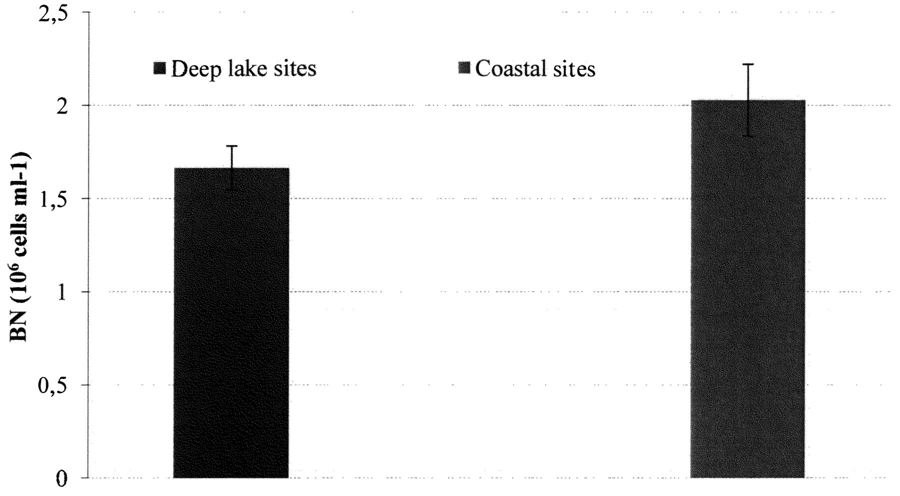

Figure 5. Average bacterial number (± SD) on deep lake sites and coastal sites in Lake Hańcza.

The most statistically significant differences in BN were observed between shore sampling point No 4 and deep lake sites: 1 and 2 as well as north eastern sites, near the forested shore: 10 and 12 ($p<0.005$). Mean bacterial abundance varied considerably among the strata of the deep lake stations. The coastal sites were less diverse in mean BN values. In both cases, the highest BN was recorded in the epilimnion layer, but the lowest number of bacteria of deep lake stations was in the hypolimnion layer, in contrast to coastal stations – metalimnion. The greatest difference in mean BN was noted in the hypolimnion at 1.05 10^6 cells ml^{-1} for deep lake stations and 1.9 10^6 cells ml^{-1} for coastal stations ($t = 3.8036$ $p=0.0005$) (Figure 6).

The highest bacterial number at the deep lake sites was recorded at site No 3 in the epilimnion – 3.7 10^6 cells ml^{-1}. Each deep site was characterized by large BN fluctuations both in the epilimnion and in the metalimnion. Only after the thermocline passes, in the hypolimnion, the bacterial abundance decreased significantly, fluctuating between 0.7 and 1.8 10^6 cells ml^{-1} (Figure 7).

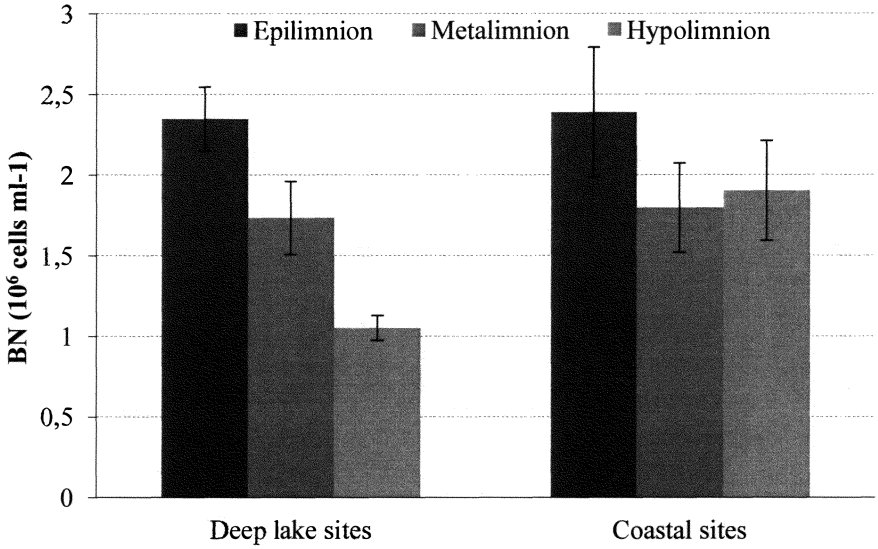

Figure 6. Average bacterial number (BN; ± SD) in different stratification layers on deep lake and coastal sites of Lake Hańcza.

The BN reduces in hypolimnion by up to three times, compared to epi- and metalimnion. The biggest differences are between site 1, closest to the inflow, and site 3, closest to the outflow. The greatest rise of BN at each of the deep lake sites was recorded in the metalimnion (Figure 7).

One of the most varied factors examined in this study was BHA, averaging 813 μgFl. $ml^{-1} h^{-1}$ for the entire lake (CV=31.2%). The lowest average BHA in LH was found at site No 2 – the deepest sampling point – 648 μgFl. $ml^{-1}h^{-1}$ and that was also the station with the lowest hydrolytic activity in all samples tested, with a value of 239 μgFl. $ml^{-1} h^{-1}$. The highest average value of all the coastal sampling sites was in the north-eastern part of the lake (No 9) with a rate of 1004 μgFl. $ml^{-1} h^{-1}$. The most significant differences were found between the deepest sampling site and coastal sites numbered 8, 9, 11 and 12 (Figure 8), located on both sides of the northern part of the lake.

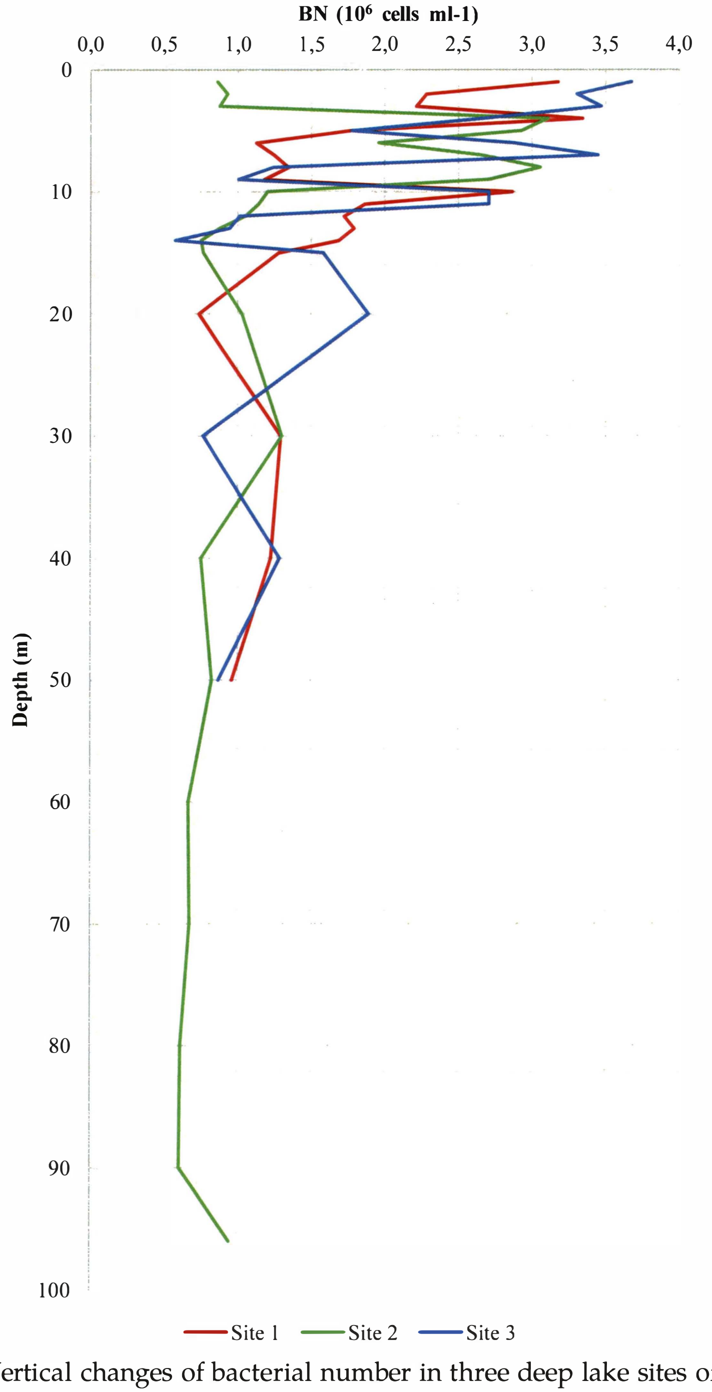

Figure 7. Vertical changes of bacterial number in three deep lake sites of Lake Hańcza.

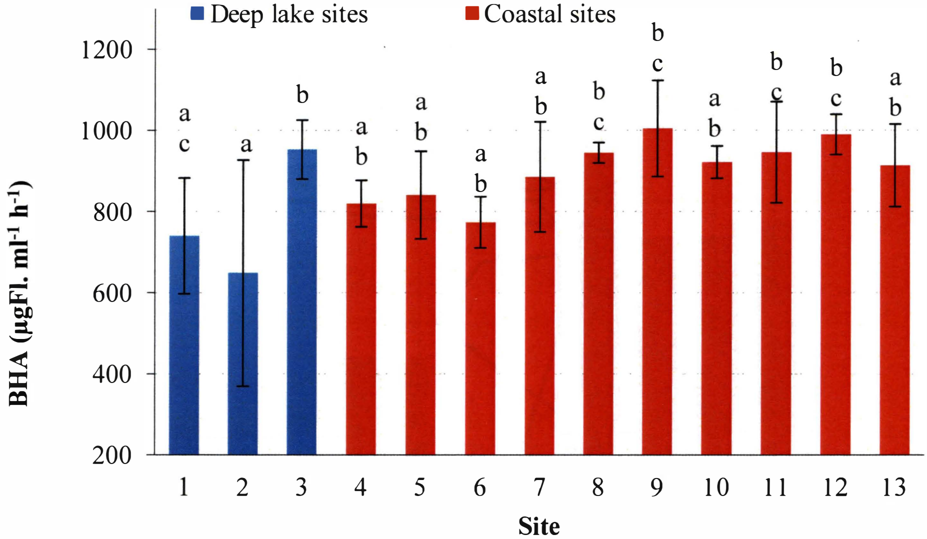

Figure 8. The average bacterial hydrolytic activity (± SD) in Lake Hańcza at each of 13 investigated sites.

There was also a significant difference in BHA between the most southern deep lake site located nearest to the lake outflow and both other deep lake sampling sites. The two deep lake sites furthest from the lake centre (No 1, 3) also displayed the most significant differences in BHA between stratification layers of the lake (Figure 9).

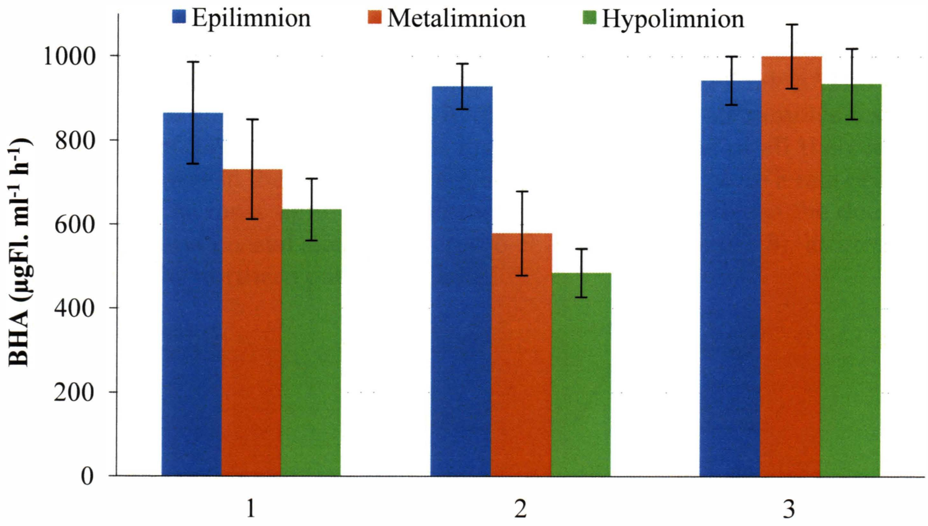

Figure 9. The mean bacterial hydrolytic activity (± SD) in the different thermal layers of Lake Hańcza measured in deep lake sites 1, 2 and 3.

The highest average live and dead bacterial cells ratio was noticed at north-eastern forested coastal sites (No 6) with a value of 0.081, while the lowest at station No 1, which is the deep lake sampling site closest to the inflow of river Czarna Hańcza, with a value of 0.064. There was also a substantial difference be een the two furthest deep lake si es numbered 1 and 3. The study showed no statistically significant differences in the ratio of L/D within the studied lake.

Discussion

The hydrochemical studies carried out in this work (Tab. 1) confirm the low trophic state of the lake. LH, being a flow-through lake of the greatest depth in Europe (105.6 m) (Borowiak et al. 2020), should be characterised by a low trophic level – currently mesotrophic (Karpowicz and Kalinowska 2018). In comparison with the results conducted by Jekatierynczuk-Rudczyk et al. (2014), the TSI_{SD} value was very similar at 39.2, while the TSI_{TP} value was more than slightly lower and reached a value of 60.6. LH is often considered to be very homogeneous due to its depth, the shape of the lake and the little diversified shoreline, which is confirmed by earlier studies on zooplankton (Jekatierynczuk-Rudczyk et. al 2014; Ejsmont-Karabin et al. 2020), phytoplankton (Napiórkowska-Krzebietke et al. 2012; Karpowicz and Ejsmont-Karabin 2017) and bacteria (Niewolak and Gotkowska-Płachta 1999; Zieliński et al. 2006; Gotkowska-Płachta 2008;).

However even lake Hańcza, protected as a reserve and part of the Suwałki Landscape Park, has noticeable human influence. Studies conducted by Gotkowska-Płachta et al. (2005) show that the amount of bacteria in LH is increasing, which may indicate intensification of allochtonic factors, eg. enrichment with organic matter or biogenic substances. Even though our studies clearly classify LH as mesotrophic, even if phosphorus level is relatively high, which is common in lakes of NE Poland (Hillbricht-Ilkowska 1993; Hillbricht-Ilkowska and Kostrzewska-Szlakowska 1996; Zdanowski 2003).

Bacterioplankton can be considered a useful factor showing the horizontal and vertical variation of microbiological water quality. The discussed studies indicate that LH has a diverse distribution of bacteria both vertically and horizontally, ranging from 0.57 10^6 cells ml^{-1} to 3.97 10^6 cells ml^{-1} for all the lake samples with coefficient of variation (CV = 54.67%). The average BN in LH was 1,66 10^6 cells ml^{-1} for deep lake sites and 2.02 10^6 cells ml^{-1} for coastal ones, which is respectively 53.4% and 43.2% less than in studies conducted ea lier (Zieliński et al. 2006). This may possibly be due to seasonality – sampling took place in a significantly colder period of the summer, but still shows the same pattern of decreasing the amount of bacterioplankton with depth. Compared to other oligo-mesotrophic lakes like Mamry or Przystań of the Masurian Lake District, Lake Hańcza has noticeably lower average BN (Górniak and Świątecki 2007).

The most of the statistically significant differences in horizontal distribution of BN were observed between deep lake sites (1, 2, 3) and shore sites located in the north eastern part of the lake ($p<0.005$) (Figure 3). While the maximum BN values do not differ much, the values in deep lake sites were less variable.

The minimum values are significantly lower on deep lake sites and much less variable than coastal sites, as confirmed by the CVs, for which the values are 12.2% and 49.8% respectively. It is possible that smaller values of BN in the northern part of LH are due to the inflow of the Czarna Hańcza River. This may be due to the character of the direct catchment area of the investigated lake, which is covered with wetlands. The statistically significant differences in average BN occur at the forested shoreline and the station located near the community beach (Figure 2). Statistically significant differences in BN were found between deep and coastal sites (Figure 4), which confirms the spatial patchiness of bacterioplankton.

The average abundance of bacteria in LH was lower at deep lake sites with an abundance of 1.66 10^6 cells ml^{-1} and 2.02 10^6 cells ml^{-1} for the coastal sites (Figure 5). In comparison of stratification layers of both deep and coastal lake sites, the highest value was recorded in the epilimnion layer. The difference of BN between types of sites was visible in comparison of metalimnion – lowest for coastal sites – and hypolimnion – lowest for deep lake ones (Figure 6). Differences in bacterial abundance in stratification layers between different types of sites show that Lake Hańcza is not homogenous in terms of spatial distribution of bacterioplankton.

The thermocline depth is known to be an important factor for bacterioplankton structuring in aquatic ecosystems and it is natural for the BN to be highest there (Mieczan 2008, Gotkowska – Płachta et al. 2003). The overall distribution of bacterioplankton differs a lot between station 1 and 3 (Figure 7), the epilimnion layer has a higher BN in epilimnion, probably due to the nearer outflow. Vertical changes in BN at the deep sites of LH are comparable to those in other lakes of similar trophy (Mieczan 2008). It is likely that the hypolimnion BN difference effect is due to the influence of bottom sediments in shore sites with relatively higher temperature. Research by Bajkiewicz-Grabowska (2004) shows that LH is heavily fed by groundwater and it is possible that the movement of groundwater may influence the bacterial number derived from the external environment, or from bottom sediments located near the water supplying sites.

Differences were also found in the hydrolytic activity of bacteria in LH. The most significant differences in BHA were found between the deepest sampling site No 2 and coastal sites located on both sides of the north part of the lake (nr 8, 9, 11 and 12), where the BHA was the highest (Figure 9). All mentioned sites are located in the part of the lake with river inflow (Figure 1). It can be assumed that wetlands are an important source of metabolically active bacteria from the direct Czarna Hańcza River catchment during the full growing season, although the study was conducted at the end of the growing season. Unfortunately, no correlations were found between the type of management of the direct catchment area and the BHA. Perhaps this type of analysis requires further study in full summer and with denser shoreline sampling sites. We assume that the forested catchment may be the main source of active bacteria for the northern part of the lake. Statistically significant differences in BHA indicate vertical differentiation of the BHA even between deep lake sites (Figure 8) and may be caused by the close outflow location of the third sampling station, which showed very little BHA differences between the stratification layers, as opposed to the other two deep lake sites. The BHA is a very important indicator and is used to assess the condition of bacterioplankton and distinguish water types (Zieliński et al. 2020).

The dependence of investigated bacterial parameters on water quality was confirmed in the study. The development of bacteria in LH is influenced by different hydrochemical factors at deep and coastal lake sites. For the first type, the most important parameters influencing BN were temperature (r=0.623), oxygen saturation (r=0.588) and concentration of Ca^{2+} (r=-0.607), NO_3^- (r=-0.539) as well as dissolved organic carbon (r=0.534). There were no significant correlations at the coastal sites between BN and hydrochemical parameters, as confirmed by the highly variable habitat of bacteria assemblages in coastal zones, while the deep lake sites are more stable. Some other correlations were also noted for BHA at deep lake stations: temperature (r=0.667), oxygen saturation (r=0.582) Ca^{2+} (r=-0.451), Mg^{2+}(r=0.511), NO_3^- (r=-0.587). BHA at coastal sites showed correlations with temperature (r=0.545), oxygen saturation (r=0.520) and specific UV absorbance (r=-0.556). The negative correlation found between BHA and SUVA confirms the great importance of organic matter quality in the rapid growth of bacteria in the aquatic environment. Both BN and BHA at deep lake sites were correlating negatively with Ca^{2+}, which indicates that the hardness of the water is not conducive to bacterial growth. Maybe it is the precipitate – bacteria may sink to the bottom with it. We assume that the alkalising effect may be caused by the groundwater phase of water circulation.

Most of the bacteria were metabolically inactive and the proportion of live bacteria did not exceed 8%, averaging 6.79%. That might be caused by nitrogen and phosphorus deficits, which may result in a low proportion of viable bacteria (Chróst et al. 2009). There were no significant differences in spatial L/D ratio distribution in LH.

Although the lake is considered to be very homogeneous, the study does not confirm this and indicates that the water quality, including the microbiological one, varies considerably – especially between the central part of the lake and its shores, but also between the northern and southern parts. The conducted study shows many differences in both vertical and horizontal changes of BN as well as BHA. As a result of the study it was established that LH is heterogeneous in terms of hydrochemical water quality and microbiological quality.

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References

1. Adam G., Duncan H. (2001) Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology and Biochemistry*, *33*(7-8), 943-951.
2. APHA. (1998) Standard methods for the examination of water and wastewater (19th ed.). Washington, D.C.: American Public Health Association.
3. Bajkiewicz-Grabowska E. (2004) The role of lakes in natural groundwater drainage. *Miscellanea Geographica*, *11*(1), 175-184.
4. Bergström A.K., Jansson M. (2000) Bacterioplankton production in humic Lake Örträsket in relation to input of bacterial cells and input of allochthonous organic carbon. *Microbial Ecology*, *39*(2), 101-115.
5. Borowiak M., Borowiak D., Nowiński K. (2020) Spatial Differentiation and Multiannual Dynamics of Water Conductivity in Lakes of the Suwałki Landscape Park. *Water*, *12*(5), 1277.
6. Bylińska M., Boguszewski A., Chybowski L. (2002) Reproductive biology of burbot, Lota lota lota, in Lake Hancza, Poland. *Folia Zoologica (Czech Republic)*.
7. Burkowska-But A., Sionkowski G., Walczak M. (2014) Influence of stabilizers on the antimicrobial properties of silver nanoparticles introduced into natural water. *Journal of Environmental Sciences*, *26*(3), 542-549.
8. Chin Y.P., Aiken G., O'Loughlin E. (1994) Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environmental Science & Technology* 28, 1853–1858.
9. Chróst R.J., Adamczewski T., Kalinowska K., Skowrońska A. (2009) Inorganic phosphorus and nitrogen modify composition and diversity of microbial communities in water of mesotrophic lake. *Polish Journal of Microbiology*, *58*(1), 77-90.
10. Chróst R.J., Siuda W. (2006) Microbial production, utilization, and enzymatic degradation of organic matter in the upper trophogenic layer in the pelagial zone of lakes along a eutrophication gradient. *Limnology and Oceanography*, *51*(1part2), 749-762.
11. Dziekońska-Rynko J., Mierzejewska K., Kubiak K., Rydzewska M., Hliwa P. (2018) Helminths of European smelt Osmerus eperlanus (Linnaeus, 1758) in Lake Hańcza and the Vistula Lagoon, with special regard to their zoonotic threats. *Acta Veterinaria Hungarica*, *66*(1), 96-106.
12. Ejsmont-Karabin J., Kalinowska K., Karpowicz M. (2020) Structure of ciliate, rotifer, and crustacean communities in lake systems of Northeastern Poland. In *Polish River Basins and Lakes–Part II* (pp. 77-101). Springer, Cham.
13. Federation W.E., APH Association (2005) Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA.*
14. Gotkowska-Płachta A., Niewolak S., Korzeniewska E. (2003) Vertical distribution and seasonal changes in the number of bacterioplankton in the water of Lake Hańcza, particularly in the period of reservoir summer stratification. Wyd. 2. *EJPAU Ser. Environ. Dev*, *6*.
15. Gotkowska-Płachta A., Korzeniewska E., Niewolak S. (2005) Pollution degree and sanitary state indicator bacteria as the indicators of the purity of Lake Hańcza waters. *Archiwum Ochrony Środowiska*, *31*(2), 53-68.
16. Gotkowska-Płachta A. (2008) Psychrophilic and psychrotrophic bacteria in the water of Lake Hańcza. *Polish Journal of Natural Science*, *23*(2), 428-439.

17. Górniak D., Świątecki A. (2007) Temporal and spatial diversity of virioplankton in the water of the Great Mazurian Lake complex. *Limnological Papers*, *2*, 111-120.
18. Górniak A., Zieliński P. (2006) Jeziora Wigierskiego Parku Narodowego. *Aktualna jakość i trofia wód. Wyd. UwB, Białystok*, 54-70.
19. Hillbricht-Ilkowska A. (1993) The dynamics and retention of phosphorus in lentic and lotic patches of two river-lake systems. *Hydrobiologia*, *251*(1), 257-268.
20. Hillbricht-Ilkowska A., Kostrzewska-Szlakowska I. (1996).Ocena ladunku fosforu i stanu zagrozenia jezior rzeki Krutyni [Pojezierze Mazurskie] oraz zaleznosc pomiedzy ladunkiem a stezeniem fosforu w jeziorach. *Zeszyty Naukowe. Polska Akademia Nauk. Komitet Naukowy przy Prezydium PAN Człowiek i Środowisko*, *13*, 97-123.
21. Hillbricht-Ilkowska A., Wiśniewski R.J. (1993) Trophic differentiation of lakes of the Suwalki Landscape Park(north-eastern Poland) and its buffer zone-- present state, changes over years, position in trophic classification of lakes. *Ekologia polska*, *41*(1), 195-219.
22. Jekatierynczuk-Rudczyk E., Zieliński P., Grabowska M., Ejsmont-Karabin J., Karpowicz M., Więcko A. (2014) The trophic status of Suwałki Landscape Park lakes based on selected parameters (NE Poland). *Environmental monitoring and assessment*, *186*(8), 5101-5121.
23. Karpowicz M., Górniak A., Więcko A., Cudowski A. (2016) The variability of summer phytoplankton in different types of lakes in North East Poland (Suwałki Landscape Park). *Limnological Review*, *16*(4), 229-236.
24. Karpowicz M., Ejsmont-Karabin J. (2017) Effect of metalimnetic gradient on phytoplankton and zooplankton (Rotifera, Crustacea) communities in different trophic conditions. *Environmental Monitoring and Assessment*, *189*(8), 1-13.
25. Karpowicz M., Kalinowska K. (2018) Vertical distribution of the relic species Eurytemora lacustris (Copepoda, Calanoida) in stratified mesotrophic lakes. *Biologia*, *73*(12), 1197-1204.
26. Kołodziejczyk A. (1999) Molluscs on Characeae in an oligotrophic Hancza Lake (NE Poland). *Folia Malacologica*, *7*(1).
27. Kotusz J., Krappe M., Kusznierz J., Propiołek M., Riel P., Waterstraat A., Witkowski A. (2004) Distribution, density and habitat of Cottus poecilopus (Heckel, 1836) in Lake Hancza (North East Poland) as compared with the situation in the Luzin lakes (North East Germany). *Verh Ges Ichthyol Bd*, *4*, 91-105.
28. Lindström E.S., Bergström A.K. (2004) Influence of inlet bacteria on bacterioplankton assemblage composition in lakes of different hydraulic retention time. *Limnology and Oceanography*, *49*(1), 125-136.
29. Łopata M., Popielarczyk D., Templin T., Dunalska J., Wiśniewski G., Bigaj I., Szymański D. (2014) Spatial variability of nutrients (N, P) in a deep, temperate lake with a low trophic level supported by global navigation satellite systems, geographic information system and geostatistics. *Water science and technology*, *69*(9), 1834-1845.
30. Mieczan T. (2008) Diversity and vertical distribution of planktonic ciliates in a stratified mesotrophic lake: relationship to environmental conditions. *Oceanological and Hydrobiological Studies*, *37*(1), 83-95.
31. Mitręga J., Paczyński B., Płochniewski Z. (1993) Wody podziemne Suwalszczyzny. *Przegląd Geologiczny*, *41*(8), 569.
32. Napiórkowska-Krzebietke A., Hutorowicz A. (2013) A comparison of epilimnetic versus metalimnetic phytoplankton assemblages in two mesotrophic lakes. *Oceanological and Hydrobiological Studies*, *42*(1), 89-98.

33. Napiórkowska-Krzebietke A., Pasztaleniec A., Hutorowicz A. (2012) Phytoplankton metrics response to the increasing phosphorus and nitrogen gradient in shallow lakes. *Journal of Elementology, 17*(2).
34. Niewolak S., Gotkowska-Płachta A. (1999) Ocena stopnia zanieczyszczenia i stanu sanitarno-bakteriologicznego jeziora Hańcza. *Inżynieria i Ochrona Środowiska, 2*(3/4), 265-278.
35. Pochocka-Szwarc K., Ber A., Szpygiel J., Szpygiel P. (2013) Jezioro Hańcza– wstępne wyniki geologicznej interpretacji obrazów sonarowych. *Przegląd Geologiczny, 61*(3), 187-194+.
36. Popielarczyk D., Templin T. (2014) Application of integrated GNSS/hydroacoustic measurements and GIS geodatabase models for bottom analysis of Lake Hancza: the deepest inland reservoir in Poland. *Pure and Applied Geophysics, 171*(6), 997-1011.
37. Pyka J.P., Zdanowski B., Stawecki K., Prusik S. (2007) Trends in environmental changes in the selected lakes of the Mazury and Suwałki Lakelands. *Limnol. Rev, 7*(2), 101-109.
38. Regional Inspectorate of Environmental Protection (RIEP) in Białystok announcement No. 2 / 2017 / SUW of June 2017 on research report and preliminary assessment of the status of Lake Hancza in 2016.
39. Weckwerth P., Wysota W., Piotrowski J.A., Adamczyk A., Krawiec A., Dąbrowski M. (2019) Late Weichselian glacier outburst floods in North-Eastern Poland: Landform evidence and palaeohydraulic significance. *Earth-Science Reviews, 194*, 216-233.
40. Zdanowski B. (2003) Precipitation of phosphorus in the zone of river and lake water mixing: R. Hańcza and Lake Wigry (North-East Poland). *Polish Journal of Ecology, 51*(2), 143-154.
41. Zieliński P., Jekatierynczuk-Rudczyk E., Puczko K. (2020) Factors affecting the abundance and activity of the bacterioplankton in lowland forest springs in north-eastern Poland. *Ecohydrology & Hydrobiology, 20*(4), 675-686.
42. Zieliński P., Kłoczewska A., Górniak A. (2006) Summer bacterioplankton abundance and biomass in lakes of Suwałki Landscape Park. *Polish Journal of Environmental Studies, 15*, 543-547.