

# Trophic State, Eutrophication, and the Threats for Water Quality of the Great Mazurian Lake System



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**Abstract** One of the greatest threats to water quality is accelerated eutrophication, resulting from human activity, like the high intensity of tourism, surface runoffs from fertilized fields, and municipal pollution. Water eutrophication manifests as excessive growth of phytoplankton caused by overabundant nitrogen, phosphorus, and other nutrient supply which causes deterioration of water quality related to the amount of bacterial biomass in eutrophicated water reservoirs. The Great Mazurian Lake System (GMLS) is a chain of lakes located in mesoregion of the Great Mazurian Lakes in the Northeastern Poland. All lakes of the GMLS are connected by natural or artificial channels built in the eighteenth and nineteenth centuries and nowadays create widely spilled, long (the easiest route from northern to southern edge is about 110 km) gutter unique on the scale of the continent. The lakes of GMLS are of glacial origin. During the last five decades, all lakes of the GMLS passed different levels of eutrophication, thus significantly changing their trophic states. This report describes past and present trophic conditions of lakes of GMLS and analyzes environmental factors responsible for eutrophication of their waters.

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Eutrophication processes are not only responsible for high nutrients levels in lakes, extensive growth of phytoplankton biomass and productivity, cyanobacterial predominance, etc., but eutrophication is also responsible and connected to several threats for water quality. Presence of pathogenic bacteria, as well as the potential presence of many antibiotic-resistant bacteria in lakes of the GMLS, is discussed.

**Keywords** Antibiotic resistance · Eutrophication · Lakes · Pathogenic bacteria

## 1 Introduction

### 1.1 *The Great Mazurian Lake System*

The Great Mazurian Lake System (GMLS) is a chain of lakes located in mesoregion of the Great Mazurian Lakes in the Northeastern Poland, stretched between mesoregions: Sępopolska Lowland, Mrągowo Lake District, Mazury Forest, Elckie Lakeland, and Borecka Forest (Fig. 1). All lakes of the GMLS are connected by natural or artificial channels built in the eighteenth and nineteenth centuries and nowadays create widely spilled, long (the easiest route from northern to southern edge is about 110 km) gutter unique on the scale of the continent. The lakes of the GMLS are of glacial origin. Of these, several basic types are distinguished. In addition to mostly round lakes of head moraines and bottom moraine, the system consists of numerous similar to them, but incomparably shallower, floodplain lakes and deep gutter lakes. From the morphological point of view, the GMLS can be roughly divided into three subsystems – complex of Lake Mamry located at the northern outskirts of the system connected through melt-type Lake Niegocin with deep gutter lakes (Jagodne, Tałtowisko, Ryńskie, Tały, Mikołajskie, and Beldany) and finally with complex of Lake Śniardwy. From the hydrological point of view, the whole GMLS, which comprises about 20% of surface water resources of Poland, consists of various trophic types of lakes belonging to two different river basins. The northern meso-eutrophic part of the system including lakes Przyszań, Mamry, Dargin, Łabap, and Kisajno drains water to the Pregoła River, whereas the southern eutrophic one comprising lakes Niegocin, Boczne, Jagodne Szymoneckie, Szymon, Kotek, Tałtowisko, Ryńskie, Tały, Mikołajskie, Beldany, and Śniardwy channels water to Vistula River (Fig. 1).

The total catchment area of the GMLS covers about 3,645 km<sup>2</sup>. Although the water content in the southern part of GMLS is only a little more than that in the northern one (1,258 and 1,024 million m<sup>3</sup>, respectively), their catchment areas differ substantially (3,030 and 615 km<sup>2</sup>, respectively). This results in a relative stability of trophic conditions in northern lakes and a much greater susceptibility of southern lakes to eutrophication processes [1]. The basic limnological parameters of the GMLS lakes are included in Table 1.



**Fig. 1** The Great Mazurian Lakes System, Northeastern Poland

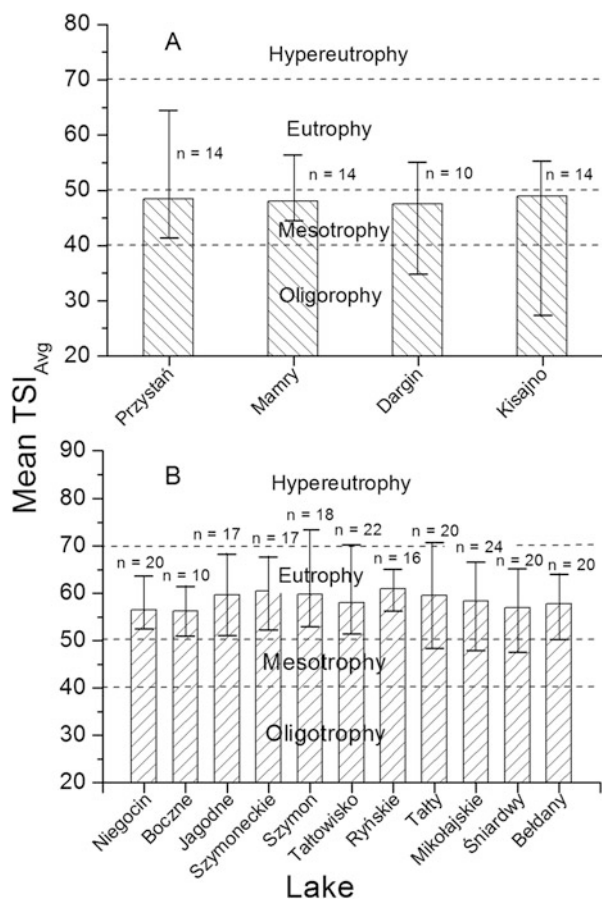
**Table 1** Basic limnological parameters of lakes of the Great Mazurian Lake System

Lake	Area (km <sup>2</sup> )	Volume (10 <sup>6</sup> m <sup>3</sup> )	Depth (m)		Average	Shoreline length (km)	Catchment area (km <sup>2</sup> )	Schindler's coefficient
			Maximum	Average				
Mamry <sup>a</sup>	25.04	298.300	43.8	11.7	34.00	31.3	0.192	
Dargin	30.3	322.100	37.6	10.6	32.80	87.1	0.354	
Kisajno	18.96	159.264	25.0	8.4	50.10	nd	nd	
Niegocin	26.00	258.522	39.7	10.0	35.40	51.7	0.302	
Jagodne	9.43	82.705	37.4	8.7	35.41	89.5	1.206	
Tałowisko	3.27	45.831	39.5	14.0	11.50	72.1	1.647	
Ryńskie	6.71	67.406	50.8	10.0	27.7	53.4 <sup>b</sup>	0.289 <sup>b</sup>	
Tały	11.6	180.856	44.7	15.6	31.0			
Mikolajskie	4.98	55.740	25.9	11.2	15.10	14.1	0.342	
Śniardwy	113.4	660.212	23.4	5.8	97.15	15.0	0.195	
Beldany	9.41	94.848	46.0	10.0	34.4	46.1	0.590	

<sup>a</sup>Together with Lake Przystań<sup>b</sup>Summarized for lakes Tały and Ryńskie

The destination of the border of the watershed, which separates both parts of the system, was a serious problem. From the mid-nineteenth century, its location was changed twice. For over 60 years, it ran through the Kula Channel at the southern end of Lake Niegocin. The studies of Skibniewski and Mikulski [2] as well as hydrometric measurements and field studies of Mikulski [3] determined its location at the southern end of Lake Kisajno. This location confirms the Hydrographic Division of Poland in force since 1980 [4]. The analysis of current research supplemented with current observational materials now allows the separation of the bifurcation area within the GMLS (placed between Lake Kisajno and Lake Niegocin), which, depending on the method of water management, variously supplies the systems of both river basins.

The Council Directive 75/440/EEC of 16 June 1975 (<http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31975L0440&from=en>) defines surface water quality in terms of 46 biological and chemical parameters. It should be pointed out that data concerning their concentrations in majority of Mazurian lakes are scarce or not available. However, one can expect that in the GMLS lakes, which are not exposed to direct chemical pollution generated by heavy industry or to waste imported from waters flowing from other parts of Poland, concentrations of most of them (such as heavy metals, surfactants, phenols, polycyclic aromatic hydrocarbons, and many others) are probably below dangerous level and thus practically have no diagnostic significance. In contrast to chemical threats, the GMLS is primarily responsible for biological hazards such as raw and only partially purified domestic sewages, which contain biogenic substances, pathogenic bacteria, and viruses, overfertilization by artificial fertilizer residues, overproduction of organic matter, and related disturbances of matter and energy flow through lake ecosystems. Because of that to characterize water quality of the GMLS in this study, only basic physicochemical and biological parameters were chosen. The most important among them were water transparency (Secchi disc visibility – SD), pH, specific conductivity, oxygen concentrations ( $O_2$ ) and its spatial distribution in depth profiles, concentrations of chlorophyll<sub>a</sub> (Chl<sub>a</sub>), total phosphorus (TP), total nitrogen (TN), and dissolved organic carbon (DOC). The trophic conditions in lakes of GMLS were quantitatively expressed as average Carlson's trophic state index (TSI<sub>Avg</sub>) [5] calculated as a mean value of TSI based on chlorophyll<sub>a</sub> (TSI<sub>Chl</sub>), Secchi disc visibility (TSI<sub>SD</sub>), and total phosphorus concentration (TSI<sub>TP</sub>), which is directly related to intensity of many biological processes such as gross primary production (GPP), bacterial number (BN), bacterial production (BP), enzymatic activity [6], and planktonic respiratory activity (dark oxygen consumption – RSP) [7]. The uniqueness of GMLS as an experimental object for research of eutrophication and evolution of lakes of Central and Eastern Europe lies in the diversity of the lakes that make up the system. Lakes of each part of the system share a comparable agricultural, wetland, and urban areas. Therefore, they are, to some extent, similar in terms of external input of mineral and organic matter and finally in their trophic conditions (Fig. 2). On the other hand, both parts of the GMLS substantially differ in respect to their trophic status, the size of catchment area, and the level of anthropopressure [8]. In contrast to the catchment area of the southern lakes, where



**Fig. 2** Mean trophic state index of lakes of the northern (a) and southern (b) part of GMLS in summer seasons (July–August) of years 2005–2017.  $TSI_{Avg} = (TSI_{Chl} + TSI_{TP} + TSI_{SD})/3$ ,  $n$  – the number of determinations, thin bars – the range of variability

the largest cities in the region are located, in a much less populated drainage basin of the northern lakes, there are no large urban centers, villages, and tourist centers. Moreover, in opposite to southern part of the GMLS, the shores of the northern lakes are low and in 80% overgrown with reeds. Their littoral zones that, with areas of reeds and bulrushes, create an effective barrier protecting them from the external input of biogenic substances are much wider and richer with submerged vegetation. Because of that northern lakes still represent various stages of meso- and meso-eutrophy, whereas the present trophic status of southern ones varies from low to advanced eutrophy. The only exception together to this rule is Lake Śniardwy, the largest Polish lake located on the southern edge of the system. In respect to trophic conditions, its northern part is similar to meso-eutrophic northern lakes, whereas the trophic of southern area resembles that of the southern lakes of the GMLS.

## ***1.2 Eutrophication of the Great Mazurian Lake System: An Overview***

According to the OECD definition from 1982, eutrophication is “increasing water content in food substances, which stimulates a series of symptomatic changes, among which an increase in the production of algae and macrophytes and lowering (dropping) of water quality are considered harmful and unfavorable for a human economy.” The nutrients that particularly strongly stimulate the growth of fertility of aquatic ecosystems are mineral and organic compounds of phosphorus and nitrogen [9]. Apart from primary production or phosphorus and nitrogen concentrations, good determinants of trophic status of lakes are also water turbidity, concentration of dissolved organic carbon, secondary production, bacterial organic carbon demand (defined as the sum of bacterial production and respiration), bacterial number, and activity of some extracellular enzymes, such as aminopeptidase, esterase, and summer alkaline phosphatase [6].

According to Odum [10], it is necessary to distinguish natural eutrophication as a manifestation of the natural evolution of water reservoirs, and anthropogenic eutrophication evoked and stimulated by human activity. Human influence on lakes of GMLS (anthropogenic eutrophication) has started in the fifteenth century. At the time at least, some of GMLS lakes were oligo- or mesotrophic [11, 12]. Further colonization of Mazuria region resulting from human settlement, deforestation, and agriculture development changed the GMLS catchment area, intensified external input of biogenic substances to the lakes, and finally led to continuous but slow rising in their productivity [13]. Since the beginning of the 1960s of the last centuries, the impact of civilization on Mazuria region has significantly increased and has become more diverse and complex. Therefore nowadays, natural component of evolution of trophic status of the GMLS lakes has only of purely theoretic significance because anthropogenic eutrophication processes became incomparably faster and more important than the natural ones. This is particularly true for southern lakes, whose drainage area has changed much more drastically than drainage area of the northern lakes.

Present trophic status of the GMLS was shaped by four basic factors: (1) geographical location, which determines bifurcation nature of the system and its division into two separate parts carrying waters into two watersheds and evolving in a diverse way; (2) relatively low anthropogenic impact on catchment areas of northern lakes and strong anthropopressure exerted simultaneously on the catchment areas of southern lakes, which resulted in accumulation of substantial amounts of phosphorus in their waters and sediments; (3) political economic and social changes in Poland in the years 1980–1990, which initiated a drastic decline in the external input of nutrients caused by the collapse of nonrational agriculture and tourism in the region; and (4) climate changes occurring in the last few decades, resulting in an increase in mean daily temperatures in winter, shorter ice cover [14], and also shortening of autumn and spring homothermal periods.

In theory, lake eutrophication should be directly related to rates of phosphorus and nitrogen loading from drainage area [15]. In practice however, this relationship can be significantly modified by a variety of other factors such as weather conditions, morphometric characteristics of the lake (i.e., depth, extent of the shoreline, and time of water retention), human activity within the lake (i.e., fishery and degradation of ecotone zones), and mechanisms of carbon and nitrogen cycling specific for a given lake [16–18].

In order to understand the mechanisms that influenced the current trophic status of individual lakes of the GMLS and to predict the direction of their evolution in the future, it is useful to analyze changes in phosphorus concentration in Lake Niegocin which is the key element of the system. Due to the location of border of the watershed and direction of the water flow through both parts of the GMLS, fluctuations in the trophic conditions and water quality of this lake primarily affected the present trophic status of the southern part of the GMLS. However, they did not affect, or influenced only to a small extent, trophic conditions of northern lakes.

Lake Niegocin is one of the largest lakes of the GMLS. The surface of the lake is 2,600 ha, average depth 10 m, and the maximum depth 39.7 m. The bottom has numerous depressions and shallows. Water from Lake Niegocin flows through Kula Channel to the south supplying directly Lake Jagodne and indirectly all other lakes of the southern part of the GMLS. About 25% of its direct catchment area covers arable land; similar part is overgrown with forests. Urban areas occupy about 20%. On the northern shore of the lake is situated the city of Giżycko – the largest urban agglomeration in the region. In year 2016, the city was inhabited by 29,642 permanent residents but during the 4 months of the tourist season (July–September) visited by 19,849 tourists. Until 1995, in which in Giżycko the first sewage treatment plant was established, Lake Niegocin was a receiver of untreated municipal sewage and pollutants from galvanizing dairy and fish canning factory. As a result of that, in the 1980s of the last century, this lake became one of the most polluted and degraded water reservoirs in Europe.

Analysis of total phosphorus in surface and bottom zones of this lake (Fig. 3) showed that after dramatic changes in the years 1975–2005 [1], during the last decade, concentrations of this biogen stabilized on acceptable level. Such trends were not observed in the waters of the northern lakes, where the total concentration of phosphorus as well as other determinants of the trophic state remained almost unchanged for 30 years.

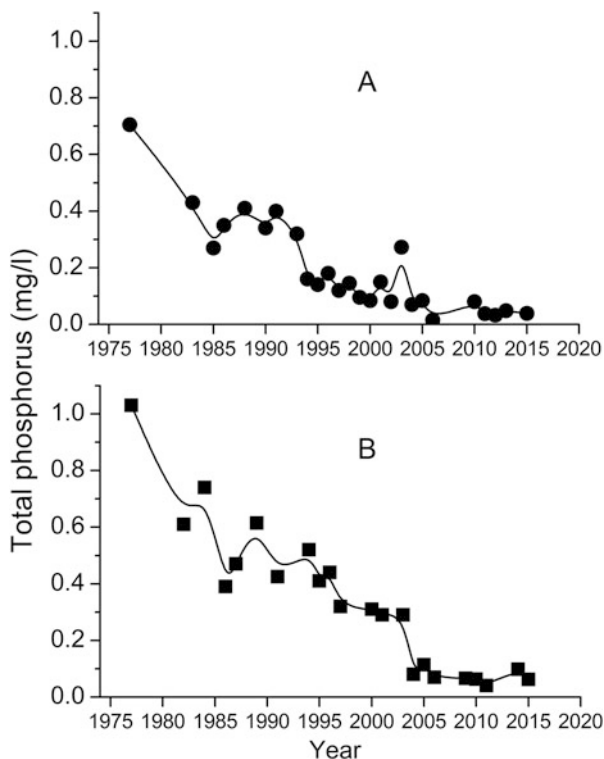
## 2 Trophic State of the Great Mazurian Lake System

### 2.1 Northern Lakes

Similarly, as in the case of the southern part of the GMLS, present trophic conditions of northern lakes were affected, to some extent by external input of biogenic substances from northwestern suburbs of Giżycko City, which affected directly

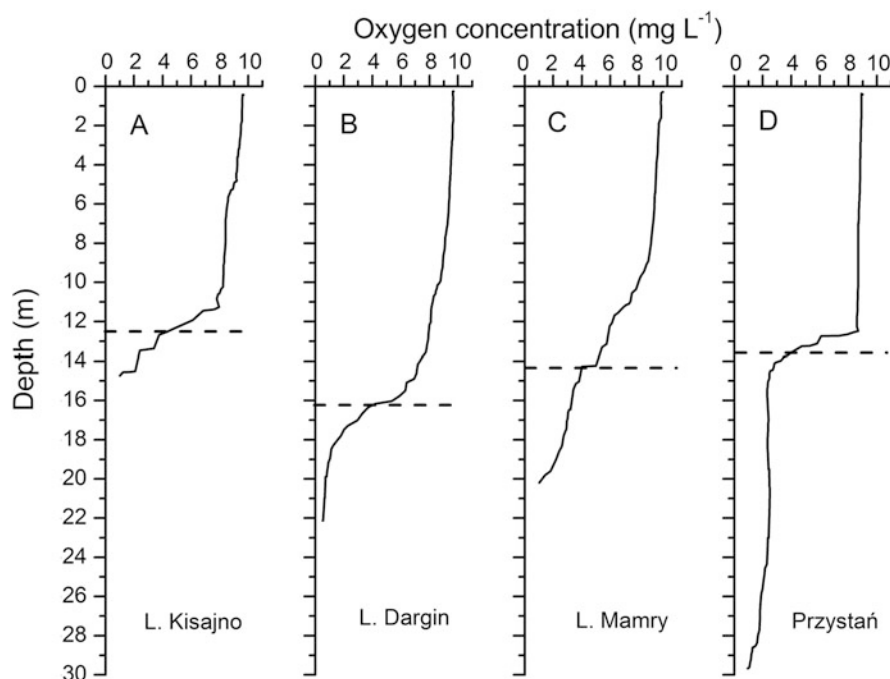


**Fig. 3** Changes in total phosphorus concentration in surface (a) and profundal (b) waters of Lake Niegocin in summer periods (July–August) 1977–2015. Based on published data [19–22] and data collected by the Department of Microbial Ecology and Environmental Biotechnology, University of Warsaw, in unpublished GMLS database



relatively deep Lake Kisajno and indirectly all other northern lakes. However, anthropogenic impact on the trophic status of Lake Kisajno was, in comparison to Lake Niegocin, much smaller and less visible because the majority of wastes produced by Giżycko City were discharged to the southern part of the GMLS. Although there is no available data concerning phosphorus load collected in bottom sediments of Lake Kisajno, one can presume that it is relatively low. Additionally, because water column of this lake is relatively well oxygenated (Fig. 4a), insoluble phosphates accumulated in its bottom sediments seem to be at least partially protected against re-solubilization and penetration into the profundal zone, as in lakes in which deep anoxia is developed. Consequently, during mixing periods only small amounts of phosphorus compounds liberated from bottom sediments of Lake Kisajno are exported to Lake Dargin and further to other lakes of the northern part of the GMLS.

Variations in concentrations of P but also N forms available for phytoplankton in the photic zone of lakes of the northern part of the GMLS are primarily dependent on the rate of their regeneration from organic compounds in water column and to a lesser extent on external input determined by weather conditions or poor efficiency of their translocation from profundal waters during spring water overturn [7]. The characteristics of the northern part of the GMLS in terms of selected physicochemical and biological factors are presented in Table 2.



**Fig. 4** Depth profiles of dissolved oxygen concentrations in lakes of the northern part of the GMLS in August 2009. Lakes are arranged according to the direction of water flow from the south (**a**) to the north (**d**). Dashed line – the depth below which oxygen concentration is critical for fish (<4 mg/L)

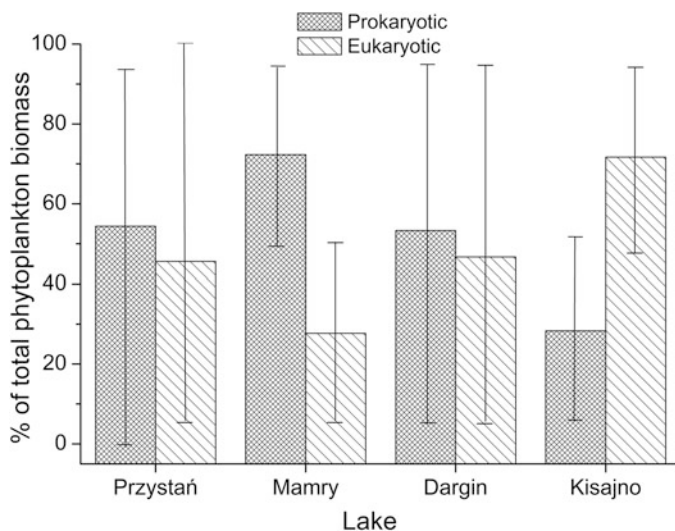
**Table 2** Mean values ( $n = 10\text{--}14$ ) of basic physicochemical and biological parameters of surface waters of lakes of the northern part of the GMLS during summer months (July–August) of years 2005–2015

Parameter	Lake			
	Przystań	Mamry	Dargin	Kisajno
Trophic state index	49.7	50.7	49.3	50.6
Secchi disc (m)	3.2	2.6	2.8	2.7
pH	8.2	8.2	8.3	8.2
Specific conductivity ( $\mu\text{S}/\text{cm}$ )	326	308	345	329
Total phosphorus (mg P/L)	0.021	0.025	0.025	0.027
Total nitrogen (mg N/L)	0.655	0.708	0.762	0.807
DOC (mg C/L)	9.7	9.9	10.3	10.1
Chlorophyll <sub>a</sub> ( $\mu\text{g}/\text{L}$ )	17.2	12.8	21.9	13.8
Bacterial number ( $\times 10^6$ cells/ml)	4.0	3.5	4.8	4.2
Primary production <sup>a</sup> (mg C/L/day)	0.621	0.770	0.631	0.790
Respiration <sup>a</sup> (mg C/L/day)	0.331	0.462	0.252	0.460

<sup>a</sup>Mean values ( $n = 6$ ) for summer seasons 2009–2011

The ratio between nitrogen and phosphorus concentrations is one of most important factors, which determines species composition and phytoplankton production of freshwater environments. The productivity of majority of oligo- and mesotrophic lakes, in which TN/TP ratio is relatively high, is mainly limited by phosphorus concentrations, whereas in eutrophic or hyper-eutrophic environments with much higher TN/TP ratio, planktonic primary production is commonly limited by nitrogen concentrations [23–25]. Moreover, low TN/TP ratio is commonly used as indicator of cyanobacterial dominance [26]. It has been the topic of many studies, but their results are equivocal. The molar TN/TP ratios, which were reported as promoting cyanobacterial growth, varied from 11 [27] to 64–66 noticed by Smith [28] in 17 lakes throughout the world and by Nöges et al. [29] in two Estonian lakes. According to Kauppinen [30] and Siuda et al. [7], during summer period (July–August) years 2010–2012 in northern lakes, mean TN/TP ratio (molar) was 46 and varied from 11 in Lake Przystań to 113 in Lake Mamry. Therefore, although in lakes of the northern part of the GMLS phytoplankton was probably limited alternately by dynamically changing availability of P and N resources, P limitation was more frequent. Another consequence of variations in N/P ratio in all lakes of the northern part of the GMLS was only weak dominance of cyanobacterial over eukaryotic phytoplankton biomass (Fig. 5).

One of the most evident symptoms of eutrophication of lakes is the increase in concentration of dissolved organic matter (DOM) in their waters caused by growing difference between net primary production summarized with external DOM input and DOM respiration rates. Such a difference defines the trophic type of the lake as net autotrophic or net heterotrophic [31] and allows drawing conclusions concerning its ecological stability and eutrophication/deterioration rate. Although there is no



**Fig. 5** Average ( $n = 5$ ) percentage of prokaryotic (cyanobacteria) and eukaryotic phytoplankton (algae) in the total phytoplankton biomass in lakes of the northern part of the Great Mazurian Lake System during summer periods of 2009–2011. Thin bars represent standard deviations

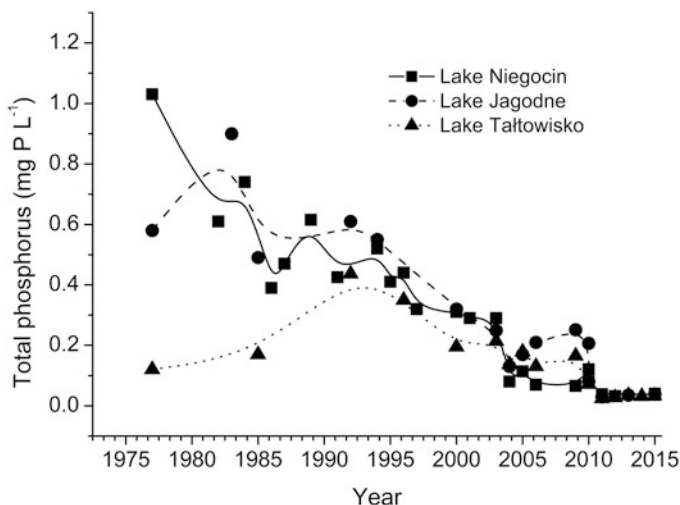
information on the scale of external DOM input to lakes of the northern part of GMLS, one can assume that it is probably small, compared to the DOM produced by phytoplankton.

During investigations carried out in years 1994–2002, Chróst and Siuda [6] found that phytoplankton primary production in northern lakes (Przystań and Mamry) reached 0.65 mg C/L/day. Later research done in years 2009–2011 by Siuda et al. [7] showed that gross primary production calculated for whole photic zone of northern lakes was about two times lower than in lakes of the southern one and varied from 0.24 mg C/L/h in Lake Dargin to 1.172 mg C/L/h in Lake Mamry. They also speculated that this apparent increase in primary production rates was rather an effect of differences in methodology used than proof of the increase in the trophic conditions of the studied lakes. An additional evidence of stability and still relatively low trophic status of northern lakes is the fact that in contrast to the southern part of the GMLS, where cyanobacterial production dominated, in lakes of the northern part of the GMLS, also contribution of eukaryotic phytoplankton to overall primary production rates was quantitatively significant [7].

## 2.2 *Southern Lakes*

The present trophic conditions of lakes, which form the southern part of the GMLS were strongly affected by four major factors: (1) intensive fertilization of Lake Niegocin in years 1960–1995; (2) the collapse of agriculture and tourism in Mazuria Region caused by economic crisis in Poland, which lasted until the end of the twentieth century; (3) translocation of biogenic substances (mainly phosphorus nitrogen and dissolved organic matter) down the system in years 1995–2005 and now; and (4) mainly by internal loading of phosphorus from bottom sediments of deep gutter lakes and rapidly increasing touristic movement. Until the early 1990s of the last century, Lake Niegocin was a main receiver of raw or insufficiently cleaned domestic sewage from Giżycko City and pollution of agricultural origin, mainly the rests of lavishly used artificial fertilizers and manure from animal farms. After year 1995, large amounts of phosphorus and organic matter collected in this lake overflowed first to relatively deep Lake Jagodne which, as a kind of natural “settler” for Lake Niegocin, received about 30 mg P/m<sup>3</sup>/year [1] and then to the other lakes placed down the system (Fig. 6), where “wave of the pollution” underwent gradual dilution.

A distinct improvement in water quality in Lake Niegocin has been observed since 2002, after the extension of the sewerage network and significant modernization of the municipal sewage treatment plant in Giżycko City. At present, it operates in accordance with the highest standards of Polish and EU environmental law. In years 2005–2016, during summer months the mean trophic state index of Lake Niegocin oscillated around 56.2 and changed from 52.6 to 63.7. Analysis of other physicochemical and biological parameters showed that at the same time rapid de-eutrophication of this lake observed in years 1997–2005 slowed down or even stopped.



**Fig. 6** Changes in total phosphorus concentrations in surface waters of lakes: Niegocin, Jagodne, and Tałtowisko in summer seasons in 1977–2015. Based on published data [19–22], Kufel (unpubl.), and results collected by Department of Microbial Ecology and Environmental Biotechnology, University of Warsaw, in unpublished GMLS database

Lake Jagodne, and its southwestern bay treated sometimes as a separate reservoir known as Lake Szymoneckie, is very diverse, and the whole lake is rather deep (max. depth 37.5 m, mean depth 8.7 m) and deprived of larger areas less than 2 m in depth. Poor afforestation of the larger part (about 30%) of its shoreline and northwestern shores strongly transformed by man activity made it susceptible to degradation processes. Long-term input of phosphorus from Lake Niegocin and supplementation with dissolved organic matter, nitrogen, and other biogenic substances throughout numerous drainage ditches draining low-lying meadows and wetlands covering the southeastern part of lake catchment area caused its rapid hyper-eutrophication. During the last years, water quality in Lake Jagodne has been noticeably improved. However, analysis of physicochemical and biological parameters of its surface waters collected in Table 3 proves that it still remains one of the most eutrophicated and degraded lakes of the GMLS.

The impact of the moving “pollution wave” was particularly evident in Lake Tałtowisko, the deepest gutter lake located in the mainstream of water flow (max. depth 45.5 m, mean depth 14 m). Despite the relatively high phosphorus content in water of this lake, until the early 1970s, it was still mesotrophic, whereas in the mid-1990s, substantial phosphorus input (about 32.4 mg P/m<sup>3</sup>/year [1]) caused its rapid eutrophication leading to hypertrophy (trophic state index = 92).

The trophic evolution of Lake Tałtowisko was tightly associated with lakes Szymon and Kotek. These small, shallow lakes are another key element of the southern part of the GMLS and work as a kind of natural sewage treatment plants, which protects directly Lake Tałtowisko, and indirectly the rest of the GMLS from

**Table 3** Average values ( $n = 10-20$ ) and the range of variability (in brackets) of basic physicochemical and biological parameters of surface waters of lakes of the southern part of the Great Mazurian Lake System during summer months (July–August) of years 2005–2015

Parameter	Lake <sup>a</sup>									
	N	J	Ta	R	T	M	S <sup>b</sup>	B		
Trophic state index	56.2	59.4	57.7	60.9	59.5	59.0	58.9	57.3		
Secchi disc (m)	1.9	1.3	1.5	1.1	1.3	1.5	1.9	1.6		
pH	8.2	8.2	8.2	8.4	8.4	8.2	8.2	8.1		
Specific conductivity ( $\mu\text{S}/\text{cm}$ )	383	380	385	377	376	344	334	334		
Total phosphorus (mg P/L)	0.050	0.079	0.037	0.041	0.046	0.042	0.045	0.043		
Total nitrogen (mg N/L)	0.912	1.107	1.092	1.140	1.203	0.884	0.857	0.810		
DOC (mg C/L)	11.4	12.5	13.9	12.4	12.3	10.9	10.4	10.3		
Chlorophyll <sub>a</sub> ( $\mu\text{g}/\text{L}$ )	19.0	38.6	22.2	43.3	39.4	28.5	22.3	25.1		
Bacterial number ( $\times 10^6$ cells/ml)	9.1	7.8	7.2	10.7	9.8	9.1	7.1	9.1		
Primary production <sup>c</sup> (mg C/L/day)	0.904	1.592	2.383	1.938	2.017	1.335	1.804	1.252		
Respiration <sup>c</sup> (mg C/L/day)	0.379	0.706	1.292	1.329	0.949	0.479	0.563	0.653		

<sup>a</sup>Lake: *N* Niegocin, *J* Jagodne, *Ta* Taltowisko, *R* Rynskie, *T* Taity, *M* Mikołajskie, *Ś* Śniardwy, *B* Beldany

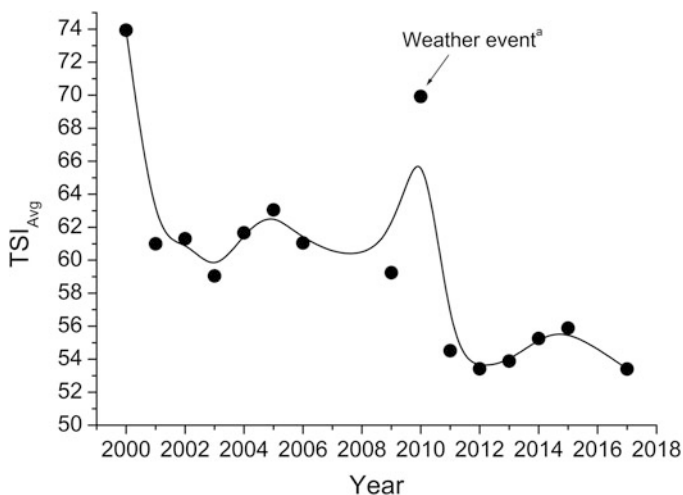
<sup>b</sup>Sampling site was located about 1 km east of the tributary from Lake Mikołajskie

<sup>c</sup>Mean values ( $n = 6$ ) for summer seasons 2009–2011

excessive biogenic load flowing from lakes located above. Due to “bottleneck effect,” lakes Szymon and Kotek act as a kind of natural trap for phosphorus compounds and organic suspension flowing down Szymoński Channel from Lake Szymoneckie. Capture of phosphorus and mineralization of organic matter in these lakes is favored by rich submerged vegetation and especially location on the navigable route. Permanent mixing of their water body and surface layers of bottom sediments by motorboats and ships of Mazurian Shipping Company increase their oxygenation and thus stimulate phosphorus trapping and organic matter mineralization and affect nitrogen cycling.

It was not a coincidence that during period of economic collapse (tourism and water transport, agriculture) two decades ago, water quality of Lake Niegocin improved very quickly, whereas at the same time, Lake Tałtowisko underwent rapid eutrophication. When, after 2000, shipping and water tourism were reestablished and intensified, the protective action of both of these lakes was restored – the trophic state index (TSI) of Lake Tałtowisko began to rapidly decrease (Fig. 7), and presently parameters of its surface waters are characteristic for typically eutrophic lakes (Table 3).

Lake Ryńskie creates a lake water complex with Lake Tały, separated by a narrow and shallow neck, which because of the significant rise of the lake bottom in this place does not allow for direct unrestricted flow of waters from Lake Ryńskie to Lake Tały. Therefore, although whole complex exchanges about 60% of their waters per year, water exchange in Lake Ryńskie alone is probably very slow. At the northern end of the lake lies the touristic town Ryn (about 3,000 inhabitants) with large yacht port and tourist center hosting several thousand tourists during the summer holidays. Over 50% of the direct catchment area of the lake is covered by arable land, about 30% by forests and the rest (about 21%) by urbanized areas.



**Fig. 7** Changes in the trophic status of Lake Tałtowisko in years 2000–2017 (Weather event<sup>a</sup> – very hot and dry summer period 2010)

The characteristic features of this lake are the large depth (up to 51 m), relatively high and stable TSI, heavy phytoplankton blooms, and physicochemical parameters characteristic for strongly eutrophicated lakes (Table 3). The relatively low production/respiration ratio (1:1) suggests that the lake can be substantially enriched with allochthonous organic matter [7].

In addition to surface runoff, the main source of biogenic substances reaching Lake Ryńskie is an effluent of purified sewage (581 m<sup>3</sup>/year) from the municipal sewage treatment plant in Ryn. Monitoring carried out by Polish environmental protection services in 2016 showed a bad ecological potential of the lake. The elements decisive for low assessment were phytoplankton, low water transparency, and anoxic hypolimnetic zone. According to unpublished data (collected by the Department of Microbial Ecology and Environmental Biotechnology, University of Warsaw) summer concentrations of total nitrogen (TN) and total phosphorus (TP) in surface waters of Lake Ryńskie varied from 0.880 to 1.349 mg N/L and from 0.029 to 0.055 mg P/L, whereas in deep anoxic zone, they exceeded 1.351 mg N/L and 0.265 mg P/L, respectively.

Lake Tały is the one of the deepest lakes of the GMLS (max. depth 44.7 m). In terms of chemical regime and trophic conditions, one could divide it into two different parts: a northern edge situated above Tałcki Channel and connected by narrow and shallow neck with Lake Ryńskie and a southern edge located along the line of water flow through the GMLS. To the end of the twentieth century, the northern part of deep gutter Lake Tały was supplemented with substantial amounts of biogenic substances by three main sources: system of Jorka River and outputs from Lake Tałtowisko and Lake Ryńskie. During summer months of years 1990–1995 total phosphorus concentrations in surface waters of this lake varied from 0.10 to 0.13 mg P/L, whereas in profundal zone they reached 0.470 mg P/L [7].

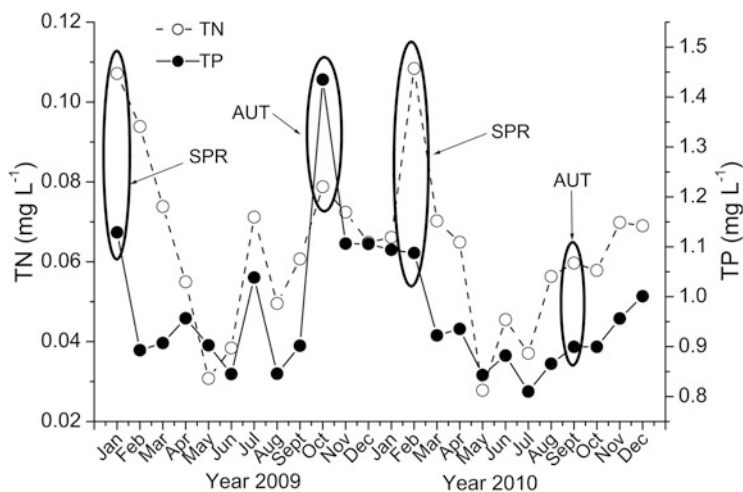
The pronounced decline in phosphorus content in the waters of the northern part of Lake Tały, observed in the beginning of the twenty-first century, was probably an effect of substantial reduction of the import of this element through Jorka River. At the turn of the years 1970–1998, the waters of this river enriched Lake Tały with significant quantities of P and N carried out from Głębokie Lake which was heavily degraded by rainbow trout aquaculture, quitted in the second half of the 1980s [32]. Phosphorus and nitrogen loads received by the northern part of Lake Tały from Lake Ryńskie were probably low. The high depth of Ryńskie Lake, high primary production rates [7] binding large quantities of biogenic elements during summer months and relatively slow (probably more than 2 years), water exchange [3, 33] allows to think that the phosphorus retention time in this lake was rather long.

While at present the trophic conditions of the northern part of Lake Tały seem to be relatively stabilized, its southern part still undergoes substantial but decreasing gradually human impact. The load of biogenic substances leached from Lake Tałtowisko, which reached waters of the northern part of Lake Tały in the years 1995–2000, was considerably increased by the point input of nitrogen and phosphorus from the inefficiently operating wastewater treatment plant localized in town Mikołajki. According to the data obtained in the municipality of Mikołajki, average capacity of the treatment plant in 2012 was 1,226 m<sup>3</sup>/day, and the amount of



*N* and *P* introduced to the deep waters of the lake was, respectively, 5.4 and 0.11 tons per year. This effect was best seen in depression of the bottom at the southern end of the lake. During summer months (July, August) the greater part of vertical profile (below 6 m depth) was strongly deoxygenated ( $O_2$  conc. < 3–4 mg/L), and the concentrations of DOC and mineral forms of *P* and *N* were reaching occasionally even 16 mg C/L, 0.504 mg P/L, and 0.70 mg N/L, respectively [34]. The averaged values of basic physicochemical and biological parameters characterizing surface waters of Tałty Lake during the years 2005–2016 are presented in Table 3.

Lake Mikołajskie elongated from the north to the south and connects Lake Tałty with Lake Śniardwy through shallow narrowing bay. Although it was one of the best-studied lakes in the GMLS, however, the majority of systematic investigations on water chemistry and biology of this lake were carried out in the 1970s and 1980s of the twentieth century [35, 36]. Later studies are relatively few and often occasional [7, 30, 33, 36–38]. Although its northern and northeastern shores along which the city of Mikołajki extends are severed, waters of this lake are still subject to strong fertilization. Two times a year, during mixing periods it is exposed on large amounts of biogenic substances carried out from the bottom depression in the southern edge of the Lake Tałty (Fig. 8). At the peaks of the tourist seasons, when several thousand tourists visit town Mikołajki, substantial amount of pollutants is generated by the yacht port and flows down with storm waters from the streets contaminated more than usual. The shore vegetation is being destroyed by long-term inflow of pollutants generated by ineffective wastewater management, unordered tourism, and agricultural activity in the southeastern edges of its direct catchment area. Additionally, the relatively narrow littoral zone of Lake Mikołajskie constitutes



**Fig. 8** Delayed effects of translocation of total nitrogen (TN) and total phosphorus (TP) load from the bottom depression in the southern edge of Tałty Lake to surface waters of Lake Mikołajskie. Ellipses marked SPR – translocation of TN and TP after spring mixing periods, AUT – translocation of TN and TP after autumn mixing periods

poor barrier for inflowing wastes. Only southwestern shore of the lake is covered with forest and well protected against inflow of biogenic substances.

All lake waters of the southern part of the Great Mazurian Lake System finally are discharged to Lake Śniardwy. Lake Śniardwy constitutes the largest freshwater lake in Poland (11,340 ha), which is shallow (average 5.8 m depth), and localized in a relatively flat area, which results in high wind water mixing by waves. Large water area and very long diverse shoreline (covered by wetlands, meadows, forests, and agriculture areas) create various water trophic conditions of the different parts of Lake Śniardwy. Generally, the southeastern bay, which receives waters directly from Lake Beldany and Lake Mikołajskie, is highly eutrophicated contrary to the northern part of Lake Śniardwy (Fig. 9, Table 4).

Selected data of water properties of Lake Śniardwy in summer 2012 (shown in Table 4) indicate that depending on lake area and sampling location (Fig. 9) the lake is generally eutrophic; however, the southeastern part in late summer reached hyper-eutrophic conditions. The clear symptoms of highly eutrophicated conditions (hyper-eutrophy) of this part of Lake Śniardwy were coupled with predominance of unicellular blue-green algae in phytoplankton community. One of the most important factors preventing Lake Śniardwy from heavy eutrophication is high microbial respiratory activity [7] together with sufficient oxygenation due to shallow water body and wind mixing of water column from the surface to bottom sediments.



**Fig. 9** Lake Śniardwy sampling sites (S1–S8) (July–August 2012)

**Table 4** Selected characteristics of the surface water of Lake Śniardwy

Parameter	Range of values of sampling sites S1–S8	Mean and $\pm$ standard deviation <sup>a</sup>
Secchi disc (m)	1.12–1.38	1.25 $\pm$ 0.11
Chlorophyll <sub>a</sub> ( $\mu\text{g/L}$ )	21.4–35.8	30.1 $\pm$ 6.8
Total P (mg P/L)	0.032–0.045	0.038 $\pm$ 0.005
Total N (mg N/L)	0.502–0.846	0.721 $\pm$ 0.065
DOC (mg C/L)	12.4–16.2	13.8 $\pm$ 1.3
pH	7.9–8.5	13.8 $\pm$ 1.3
Bacterial number ( $\times 10^6$ cells/mL)	4.3–5.7	4.9 $\pm$ 0.2
Bacterial production ( $\mu\text{g C/L/day}$ )	30.1–57.6	41.8 $\pm$ 0.9
TSI <sup>b</sup>	60.6–65.7	62.6 $\pm$ 1.7

Range and mean values in July–August 2012 of the sampling sites shown in Fig. 9

<sup>a</sup>Mean value for surface water calculated from sampling sites S1–S8

<sup>b</sup>Trophic state index (TSI) calculated from chlorophyll<sub>a</sub> data according to Carlson [5]

### 3 Trophic State Evolution of the Great Mazurian Lakes System

Depending on the human impact and usage, and climatic conditions of watershed of the Great Mazurian Lakes System, every particular lake of the system and the total GMLS have significantly changed during the last five decades (Fig. 10).

Very drastic changes of lake water trophic conditions of the GMLS have happened in the 1980s, when its southern part, between Lake Niegocin and Lake Tały, reached hyper-eutrophic conditions. Since the beginning of the twenty-first century, these hyper-eutrophic lakes have recovered and improved their water quality, and at the present, they show different stages of eutrophication (from moderate eutrophic to high eutrophic state). One of the most important factors responsible for this positive change in water quality were sewage treatment plants built at the end of the twentieth century and changes in regional water and sewage management policy.

Higher rates of eutrophication have been also observed in the northern part of the GMLS (Fig. 10). Unfortunately, the most northern lakes, which were for a long period of time oligotrophic, became mesotrophic and eutrophic during the last decade. This was mostly caused by intensive touristic and recreational activities developed in close vicinity of lakes.



#### 4 Potential Microbial Threats of the Great Mazurian Lake System: Presence of *Legionella* spp. and *Aeromonas* spp. in Lake Water

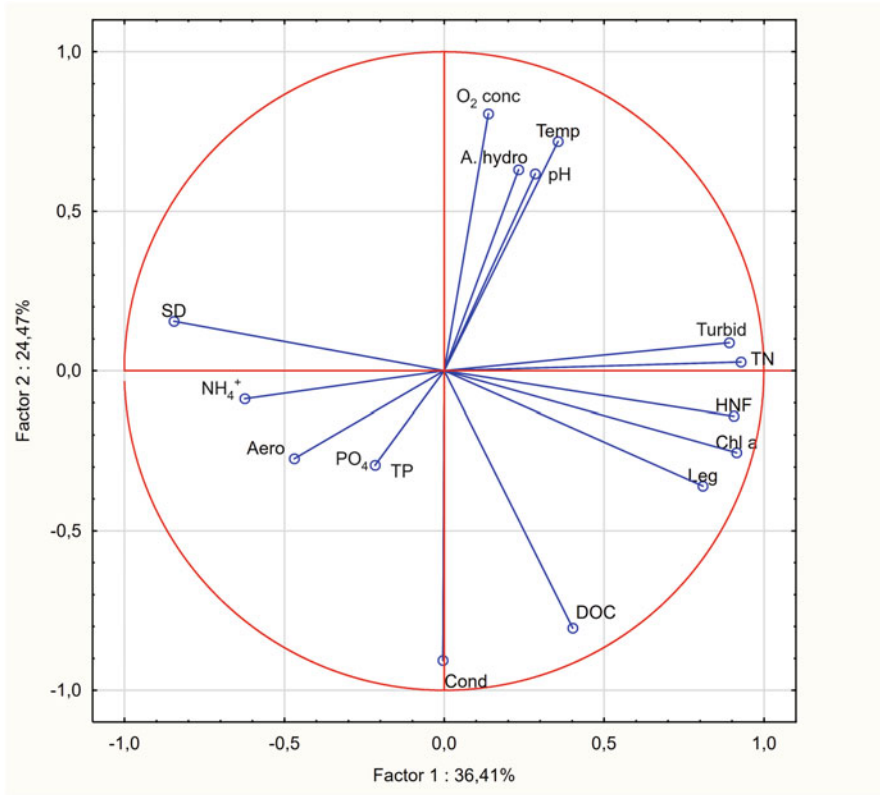
One of the greatest threats to water quality is accelerated eutrophication, resulting from human activity, like the high intensity of tourism, surface runoffs from fertilized fields, and municipal pollution. Water eutrophication manifests as excessive growth of phytoplankton caused by overabundant nitrogen, phosphorus, and other nutrient supply, which causes deterioration of water quality. In consequence, increased content of organic matter and the amount of microplankton is positively related to the amount of bacterial biomass in eutrophicated water reservoirs [39]. Moreover, in the face of global climate warming, the presence of warming conditions favoring the development of pathogenic microflora and their presence is becoming more likely, at least temporary during periods of warm summers.

There are reports that climate changes related to global warming may affect occurrence and survival of pathogens in the water environments [40]. Manifestations of global warming have been already noticed in Europe in summer 2010 and 2018. We were dealing with heat wave which contributed to the increase of the Great Mazurian Lakes System water temperature above 25–27°C. It was proven that this incident would not have occurred without climate warming [41]. The aforementioned circumstances prompted to assess the impact of trophic state and physicochemical parameters of water of the GMLS on the occurrence and amount of *Legionella* and *Aeromonas* spp.

Both *Legionella* and *Aeromonas* are closely related to environmental water quality, and their presence, especially in large numbers, poses a serious threat to human and animal health [42]. *Legionella* spp. cover more than 61 species, among them 22 are responsible for human diseases. Pathogenic strains of *Legionella* sp. contribute to severe pneumonia called Legionnaires' disease or less-serious Pontiac fever. Inhalation of *Legionella*-contaminated aerosols is the route of infection [43]. This microorganism strictly associated with man-made water systems is often reported in natural water reservoirs [44]. The studies prove that it is able to survive at temperatures from 0 to 63°C and multiplies at temperature ranging from 20 to 45°C [45]. In turn *Aeromonas* sp. initially associated with fishes and cold-blooded animal pathogen is currently described also as emerging human pathogen. Among all *Aeromonas* species, *Aeromonas hydrophila* is at the forefront of causing diseases in human. The main illnesses caused by contagion of *Aeromonas* species are gastrointestinal, skin, soft tissue, and urinary tract infections [46, 47].

Figure 11 presents the results of principal component analysis, aimed to display the impact of various physicochemical and biological parameters on the number of *Legionella* spp. and *Aeromonas* spp. in lakes with different trophic states.

The studies were conducted based on samples collected during summer season in 2016. For the analysis, 16 lakes belonging to the system of the Great Mazurian Lakes System covering the entire geographical location were selected. These lakes differed in the trophic status calculated on the basis of the phosphorus and chlorophyll<sub>a</sub> concentration and Secchi disc visibility according with Carlson [5]. Therefore, the studied lakes included water reservoirs from mesotrophic, with



**Fig. 11** Principal component analysis (PCA) results showing the impact of physicochemical and biological water parameters of the Great Mazurian Lakes System on *Legionella* spp. and *Aeromonas* spp. abundance. *Cond* conductivity ( $\mu\text{s}/\text{cm}$ ), *DOC* dissolved organic carbon ( $\text{mg C}/\text{L}$ ), *Leg* *Legionella* spp. number (copies/L), *Chl a* chlorophyll *a* concentration ( $\mu\text{g}/\text{L}$ ), *HNF* heterotrophic nanoflagellates (cells/mL), *TN* total nitrogen amount ( $\mu\text{g}/\text{L}$ ), *Turbid* turbidity (NTU), *Temp* temperature ( $^{\circ}\text{C}$ ), *A. hydro* *A. hydrophila* number (copies/L), *O<sub>2</sub> conc* oxygen concentration ( $\text{mg O}_2/\text{L}$ ), *SD* Secchi disc visibility (m), *NH<sub>4</sub><sup>+</sup>* ammonium concentration ( $\text{mg N}/\text{L}$ ), *TP* total phosphorus concentration ( $\mu\text{g P}/\text{L}$ ), *PO<sub>4</sub>* orthophosphate concentration ( $\mu\text{g P}/\text{L}$ ), *Aero* *Aeromonas* spp. number (copies/L)

mean TSI value equals 42.2 in the case of the least eutrophicated lake, to hyper-eutrophic ones (mean TSI = 70). The principal component analysis indicated that the amount of chlorophyll<sub>a</sub>, total nitrogen content, water turbidity, and presence of heterotrophic nanoflagellates (HNF) mainly promoted the occurrence of *Legionella* spp. The amount of dissolved organic matter also slightly promoted the presence of *Legionella* spp.

In turn, the presence of *Aeromonas* spp. was promoted by concentrations of total phosphorus and orthophosphates, as well as, ammonium concentration (Fig. 11). Furthermore, the number of *Aeromonas hydrophila* was included in the analysis, and as it turned out, inter alia temperature is a factor promoting its occurrence. All these factors affecting the number of potentially pathogenic bacteria are closely related to



eutrophication, and the influence of temperature on *Aeromonas hydrophila* presence can be a sign of the threat posed by climate warming. Particularly noteworthy is the fact that increased amount of nutrients, which is the main reason of eutrophication resulting from excessive anthropogenic pressure, affects the number of *Legionella* spp. and *Aeromonas* spp. Also, chlorophyll<sub>a</sub> concentration, which is a measure of the phytoplankton amount and one of the main indicators of water body's trophic state, was positively correlated with the *Legionella* spp. number [48]. Water turbidity, which is a parameter of general amount of suspension in water consisting living microorganisms and dead organic and mineral matter, was also correlated with *Legionella* spp. number. It is important due to the fact that as the turbidity increases, the trophic state of water also rises [49].

## 5 Antibiotic Resistance of Bacterial Community in the Great Mazurian Lake System

Antibiotics are among one of most often used antimicrobial drugs in modern pharmacology. Because of their irresponsible, excessive use, antibiotic resistance has become an important issue of current microbiology. There are more than 200 different antibiotics registered for use in medicine and veterinary [50]. They belong to many different groups, wherein beta-lactams account for approximately 55–70% of total antibiotic use. Antibiotic consumption, expressed in doses (DDD), increased by 65% (from 21.1 to 34.8 billion DDDs) between 2000 and 2015 [51], and the global consumption of antimicrobials will increase by 67%, from  $63.51 \pm 1.560$  tons to  $105.596 \pm 3.605$  tons between 2010 and 2030 [52].

Several antibiotics, such as certain beta-lactams or streptomycins (up to 80%), are naturally produced by microorganisms, and the soil environment is the largest reservoir of bacteria and fungi capable of producing natural antibiotics [53, 54]. Flow from the catchment may play some role in antibiotic input into the aquatic environment. Nevertheless, the largest source of antibiotics and antibiotic-resistant bacteria is human medicine and the food production sector, including animal and plant production and/or aquacultures. The presence of antibiotic resistance genes in natural water is often associated with their transfer from wastewater treatment plants, which receive municipal and hospital wastewater. In this type of sewage, resistant microorganisms are often present and can be transferred into the environment along with the outflow. For example, Korzeniewska et al. [55] have demonstrated the presence of extended-spectrum beta-lactamases (ESBL)-producing *Escherichia coli* from the hospital and the municipal sewage and water treatment area of wastewater treatment plants located in Olsztyn (Poland). The presence of relatively high concentration of antibiotics in Polish sewage treatment plant has also been confirmed [56]. It is no surprise that antibiotic-resistant strain of bacteria is found in many aquatic habitats in Poland as in the Baltic Sea, rivers, and many lakes [57–59].

Though there are many studies concerning antibiotic resistance in the environment, up-to-date information about antibiotic concentration and occurrence of

antibiotic-resistant bacteria in GMLS, the biggest lake complex in Northern Poland, are strongly limited. Hereby, we present the preliminary results of an assessment of antibiotic resistance in the bacterial community inhabiting GMLS. Water samples were taken from littoral zone (1–3 m depth) during summer 2016 from the selected lakes of the GMLS.

For the assessment of the expressed antimicrobial drug resistance at the community level, the Biolog PM MicroArrays plate's tests (PM11C i PM12B, Biolog Inc., USA) were used [60]. The analyses were conducted according to the Biolog (USA) manufacturer procedure with modifications. Before measurement, the samples were preincubated for 12 h in 24°C with 0.1 × Biolog IF-10b GN/GP Base for GN and GP bacteria (Biolog, Inc.) to avoid potential carbon limitation. For fungi inhibition, 5-fluorocytosine was added (final conc. 0.3 mg/ml). After preincubation, the optical densities of samples were checked; the initial differences were lower than 5%. For respiration intensity detection, the ×1 Biolog Redox Dye Mix D and samples were transferred to the plate wells. The absorbance (ABS) of reduced Redox Dye Mix D was measured every 2 h for 12 h in Synergy H1 (590 nm, BioTek Corporation). Only the wells with maximum concentration of every antibiotic were included in the analysis. Wells without any antimicrobial drugs treated in the same way as wells containing antibiotics or other antimicrobial drugs were used as control wells.

The relative respiration intensity was calculated as the difference between ABS measured in time 0 and time 12 h. The potential antibiotic influence on the bacterial community was calculated as a percentage of respiration in wells containing specific antibiotic or antimicrobial drug compared to respiration in control wells (separately for every lake sample). Table 1 contains groups (first column) and names (second column) of all tested antimicrobial drugs.

We used the following scale to define the relative susceptibility of bacterial communities to tested antimicrobial drugs. When the respiration rates in antibiotic-containing well exceeded 90% of the respective rates in the control well, we assumed that the bacterial community was resistant to tested antibiotics and belongs to class IV. When the respiration rates were lower than 10% of the control, we assumed the bacterial community to be highly susceptible to tested antibiotics and belong to class 1. Between these values we defined the bacterial community as moderately susceptible (class 2, from 10% to 50% of the control respiration rates) and moderately resistant (class 3, from 50% to 90% of the control respiration rates).

Table 5 shows the susceptibility profile of bacterial communities from nine lakes belonging to the GMLS. The highest susceptibility of bacteria in all lakes was recorded with regard to a wide spectrum of macrolides, aminoglycosides, and tetracyclines impairing protein synthesis (like rifampicin, spiramycin, chlortetracycline, minocycline) or DNA replication (like novobiocin). We found the highest resistance of bacteria to beta-lactams, which may be caused by their high occurrence in natural environments. The high resistance of aquatic bacteria to beta-lactams was well documented [61].

Higher antibiotic resistance was demonstrated for the microorganisms present in less eutrophicated, northern lakes (Fig. 12). For example, bacteria communities from Lake Przystań were resistant (class 4) or moderately resistant (class 3) to up to



**Table 5** Relative impact of the various antibiotics and other antimicrobial compounds influence on bacterial communities inhabiting lakes of the GMLS

Group	Name	Lake <sup>a</sup>									
		P	M	K	N	J	T <sub>a</sub>	T	M <sub>i</sub>	Ś	
Amino-coumarins	<b>Novobiocin</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	
Aminocyclitol antibiotics	Spectinomycin	3	3	3	2	3	3	2	2	2	
Aminoglycosides	Amikacin	2	2	2	2	2	2	1	1	2	
	Capreomycin	2	2	2	2	1	2	1	1	1	
	Gentamicin	2	2	2	3	2	2	1	2	2	
	Kanamycin	2	2	2	2	2	2	1	2	2	
	<b>Neomycin</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>2</b>	
	Paromomycin	2	2	2	2	1	2	1	2	1	
	Sisomicin	2	2	2	3	2	2	1	2	2	
	Tobramycin	2	2	2	2	1	1	1	1	1	
Chloramphenicol	Chloramphenicol	2	2	2	2	1	1	1	2	1	
Cyclic peptides	Colistin	4	4	4	4	3	3	2	3	2	
Fluoroquinolones	Enoxacin	2	2	2	1	1	2	2	2	1	
	Lomefloxacin	2	2	2	2	2	3	2	2	1	
	Ofloxacin	3	3	2	2	2	3	2	2	2	
Glycopeptides	Bleomycin	3	3	3	3	3	4	2	3	3	
	Vancomycin	2	2	2	2	2	2	2	2	2	
Macrolides	<b>Spiramycin</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	
	Erythromycin	2	2	2	2	1	1	2	2	1	
	Lincomycin	2	2	2	2	2	2	2	2	2	
	<b>Rifampicin<sup>b</sup></b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	
Polypeptides	Polymyxin B	3	4	3	3	3	4	2	4	4	
Quinolones	Nalidixic acid	2	2	2	2	2	3	2	2	1	
Sulfanilamides	Sulfamethazine	3	4	3	3	3	3	2	2	2	
	Sulfadiazine	3	3	3	3	2	3	2	2	2	
	Sulfathiazole	3	3	3	3	3	3	2	2	2	
	Sulfamethoxazole	3	4	3	3	3	3	3	2	2	
Tetracyclines	<b>Chlortetracycline</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	
	Demeclocycline	2	2	2	2	2	2	2	2	2	
	Tetracycline	2	2	2	2	2	2	2	2	2	
	Penimepicycline	3	2	3	2	1	2	2	2	1	
	<b>Minocycline<sup>c</sup></b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	
Beta-lactams	Amoxicillin	2	3	3	2	2	2	2	2	2	
	<i>Cefazolin</i>	3	3	3	3	4	4	4	4	4	
	Ceftriaxone	3	3	3	2	3	2	3	3	3	
	<i>Cephalothin</i>	3	4	4	3	4	3	4	4	3	
	Cloxacillin	2	2	2	2	2	2	2	2	2	
	Nafcillin	2	2	2	2	2	2	2	2	2	
	<i>Penicillin</i>	4	4	4	3	3	3	3	3	4	
	Oxacillin	2	2	2	2	2	2	2	2	2	
	<i>Carbenicillin</i>	3	4	4	3	3	3	3	3	3	

(continued)

**Table 5** (continued)

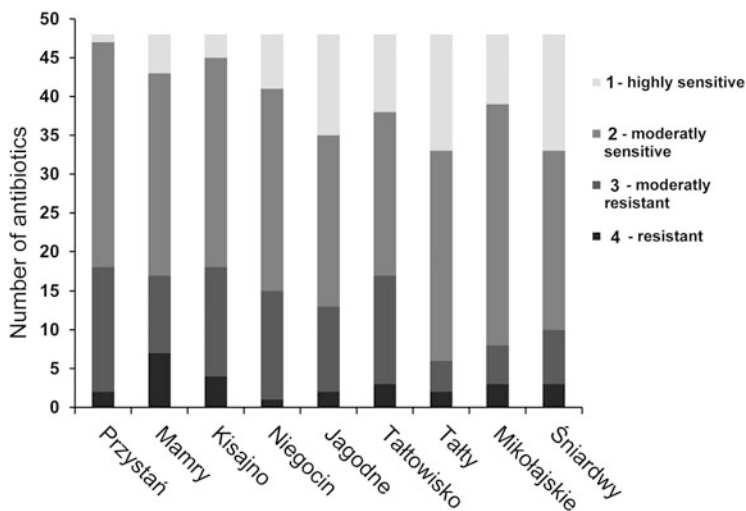
Group	Name	Lake <sup>a</sup>									
		P	M	K	N	J	T <sub>a</sub>	T	M <sub>i</sub>	Ś	
Other	Potassium tellurite	3	3	3	3	3	3	2	2	3	
	2,4-Diamino-6,7-diisopropyl- pteridine	2	3	3	2	2	3	2	2	3	
	D,L-Serine hydroxamate	2	2	2	1	1	1	1	2	2	
	Benzethonium chloride	3	2	3	3	2	2	2	2	2	
	5-Fluoroorotic acid	2	2	2	2	2	2	2	2	2	
	L-Aspartic-B-hydroxamate	2	1	2	2	2	2	2	2	2	
	Dodecyltrimethylammonium bromide	3	2	2	2	2	2	2	2	3	

Numbers correspond to the susceptibility of bacteria to tested chemicals: 1, highly sensitive (less than 10% of control respiration); 2, moderately sensitive (10–50% of control respiration); 3, moderately resistant (50–90% of control respiration); and 4, resistant (more than 90% of control respiration). The italic characters indicate antibiotics that are characterized by low effectiveness against aquatic bacteria living in the studied lakes (only 3 or 4 for all studied lakes). The bold characters indicate the most effective antibiotics (mostly in class 1)

<sup>a</sup>Lake: *P* Przystań, *M* Mamry, *K* Kisajno, *N* Niegocin, *J* Jagodne, *T<sub>a</sub>* Tałtowisko, *T* Tały, *M<sub>i</sub>* Mikołajskie, *Ś* Śniardwy

<sup>b</sup>Macrolide analogue

<sup>c</sup>Tetracycline analogue



**Fig. 12** Summarized numbers of antibiotic and antimicrobial compounds belonging to four classes of effectiveness in microbial respiration inhibition

18 tested chemicals and highly sensitive to only 1 tested antibiotic minocycline. In the southern lakes, generally more eutrophicated, except from Lake Śniardwy, bacteria were highly susceptible (class 1) to 9–15 tested chemicals. However, this result does not necessarily mean that the southern lakes were poorer in potentially antibiotic-resistant bacteria species.

The test used by us is based on the measurement of bacterial respiratory activity. Thus, we did not test the potential antibiotic resistance of bacterial community (the presence of antibiotic resistance genes) but the expressed antibiotic resistance by active microorganisms. The activity of individual bacteria in aquatic ecosystems, also GMLS, is a complex phenomenon and not yet fully explained [61–63]. It is affected by many factors such as the bacterivorous pressure, the competition between bacteria of different species, the quality and quantity of available organic carbon, as well as the physicochemical conditions, including oxygen saturation [64]. Favorable living conditions in a given lake increase the respiratory activity of bacteria, allowing them to cope more easily with the threat of antibiotics. Bacteria living in sub-optimal conditions are exposed to many stress factors, which may handicap the mechanism of active antibiotic detoxification, for instance, energy dependence efflux of tetracyclines. Therefore, due to difficult conditions in eutrophicated environments (strong nutrient competition, potential toxic metabolites of e.g., cyanobacteria, bacterivorous pressure, less carbon source liability), the potential antibiotic resistance may not be fully expressed. This could also explain the lack of the expected influence of sewage treatment plants on bacterial resistance in Lake Tały and Lake Niegocin (where two sewage treatment plants are located). The relatively long distance (few hundred meters) of the sampling area from the outflow of effluent wastewater and the potentially insufficient survival rate of sewage-derived bacteria in natural lake water may further explain these results. Additionally, using Illumine sequencing we found (data not yet published) that the phylogenetic composition of the bacteria community from the northern and southern parts of the GMLS differs significantly, which may also influence the antibiotic resistance pattern.

**Acknowledgments** These studies were financially supported by the National Science Centre, Poland, grant OPUS 2015/17/B/NZ9/01552 awarded to R.J. Chróst and grant NN304 080135 awarded to W. Siuda. Field studies were performed in the Research Station in Mikołajki of Nencki Institute of Experimental Biology of Polish Academy of Sciences.

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