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Citation style: Halabowski Dariusz, Bąk Małgorzata, Lewin Iga. (2021). Distribution and ecology of two interesting diatom species Navicula flandriae Van de Vijver et Mertens and Planothidium nanum Bąk, Kryk et Halabowski in rivers of Southern Poland and their spring areas. "Oceanological and Hydrobiological Studies" (Vol. 50, iss. 2 (2021), s. 137-149), doi 10.2478/oandhs-2021-0013

Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 50, No. 2, June 2021
pages (137-149)

sciendo

ISSN 1730-413X
eISSN 1897-3191

Distribution and ecology of two interesting diatom species *Navicula flandriae* Van de Vijver et Mertens and *Planothidium nanum* Bąk, Kryk et Halabowski in rivers of Southern Poland and their spring areas

by

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DOI: **10.2478/oandhs-2021-0013**
Category: **Original research paper**
Received: **September 21, 2020**
Accepted: **December 15, 2020**

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Abstract

In this paper, we report new records of rare diatoms that have recently been found in Southern Poland. *Planothidium nanum* was found only in the upper reaches of the Centuria River, which is not exposed to human influence, while *Navicula flandriae* was found in two rivers (the Bolina and the Mleczna) affected by salt mine water from hard coal mines. The impact of anthropogenic salinity on diatom communities is as yet poorly documented. Therefore, we conducted a survey on this phenomenon. We surveyed seven sampling sites for diatom assemblages and habitat characteristics, including three sampling sites impacted by mine water. *Navicula flandriae* was recorded at both sampling sites affected by salt mine water and in two other rivers (the Centuria and the Mitręga), indicating its wide preference for salinity. We confirmed the occurrence of *P. nanum* at the same site where this species was found and described in our previous paper. In addition, we recorded the occurrence of this species at two other sites in habitats with similar characteristics (lower reaches of the Centuria and Wiercica rivers). Our results indicate a more common occurrence of *N. flandriae* and *P. nanum*, which means that further monitoring of diatoms is necessary.

Key words: Bacillariophyta, salinization, Upper Silesia, Kraków-Częstochowa Upland, anthropopressure, phytobenthos, water quality, taxonomy

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1. Introduction

Diatoms are widespread microorganisms associated with aquatic environments. They can be found in any type of water environment such as canals, rivers, ponds, reservoirs, seas and oceans, as well as in wet soil, on submerged plants and on anthropogenic structures. Furthermore, diatoms can successfully inhabit both marine and freshwater environments, and are the dominant group of aquatic organisms in phytoplankton and periphyton (Furnas 1990; Ács et al. 2004). Even though they constitute only about 1% of Earth's photosynthetic biomass, they are an important group of aquatic organisms. They account for about 45% of the total primary production in the oceans and, in addition, they are of great importance in oxygen production and carbon dioxide removal (Field et al. 1998; Yool & Tyrrell 2003; Ács et al. 2004). Diatoms are considered good indicators of the state of the environment, because the ecological preferences of many diatom species are known. Although their preferences pertain mainly to organic pollutants, pH, hardness, salinity, trophic conditions etc., they also affect their distribution. Both the high productivity and short life cycle of diatoms enable them to detect short-term contaminants or other environmental changes, because of their ability to respond very quickly. Therefore, diatoms are important in surface water monitoring and are considered early warning indicators (Johnston et al. 2006; Bis 2008; Zgrundo et al. 2018). The use of diatoms in biomonitoring is particularly important because, unlike other groups of aquatic organisms, they occur in a wide range of waters from clean waters to those that are highly polluted (Kelly 2002; Bąk et al. 2004; Vilbaste et al. 2004; Bąk & Szlauer-Łukaszewska 2012). Therefore, diatoms appear to be good indicators for monitoring secondary saline waters where anthropogenic factors may limit the occurrence of aquatic fauna and vascular flora (Williams 1987). For example, some fish species can tolerate increased salinity provided it increases slowly. However, a rapid increase, which often occurs in waters impacted by salt mine waters, and an increase in blood salt concentration in individuals of these species within the range of 7000–13 000 mg dm^{-3} results in osmoregulation disturbances in some freshwater fish species (Bacher & Garnham 1992; James et al. 2003). Moreover, the commonly used method of monitoring fish fauna in Poland, compliant with the EU Water Framework Directive (Directive 2000; Prus et al. 2016), fails in highly saline rivers (D. Halabowski, unpublished observations).

One of the areas where intensive underground coal mining is still carried out is Upper Silesia. Currently, about 35 mines still operate in this area, and their operation requires pumping salt mine water into surface waters, quite often directly into rivers. Despite the fact that more and more mines are being closed, salt water continues to be discharged into surface waters (Strozik 2017). Water resulting from leaching processes of coal mining waste discharged from coal mine dewatering systems is usually characterized by very high values of electrical conductivity, total dissolved solids as well as very high concentrations of chlorides, sulfates, nitrates, phosphates, heavy metals and even radioactive elements (Helios-Rybicka 1996; Jankowski & Rzętała 2000; Harat & Grmela 2008; Cañedo-Argüelles et al. 2013; Lewin et al. 2018; Halabowski & Lewin 2020; Halabowski et al. 2020; Sowa et al. 2020).

Research on diatom assemblages in Upper Silesia and adjacent areas is limited, and most of the published studies are not available to international readers. Only a few of them are indexed in international JCR databases and among those only a few papers discuss the Polish part of Upper Silesia. Some of the papers pertaining to Upper Silesia and adjacent areas deal with the occurrence of diatoms in springs (e.g. Kwandrans 1986; Wojtal 2004; Wojtal & Sobczyk 2012; Wojtal 2013) and in rivers (e.g. Wasylik 1985; Kwandrans 1989, 1993, 1998; Kwandrans et al. 1998, 1999; Kwandrans 2000, 2002; Kawecka & Sanecki 2003; Wojtal & Kwandrans 2006; Cichoń 2016). Recently, the first major paper on the distribution and species composition of diatom assemblages in this region was published by Bąk et al. (2020). However, it does not satisfactorily cover some important aspects and the authors suggest that further research is necessary, especially on secondary saline habitats (Bąk et al. 2020). The conducted research showed that a large proportion of diatom assemblages in highly saline rivers can be represented by brackish and marine diatom species. For example, brackish *Pleurosira laevis* var. *laevis* (Ehrenberg) Compère and var. *polymorpha* Compère were included in a new method for assessing the ecological condition of flowing waters in Poland (Zgrundo et al. 2018). Therefore, more research is required on these habitats as well as on the ecology and distribution of diatom species that have recently been found in Poland – *Navicula flandriae* Van de Vijver et Mertens (Beauger et al. 2015) and *Planothidium nanum* Bąk, Kryk et Halabowski (Bąk et al. 2020), especially the distribution of the former one. To date, they have only been reported from Belgium, the Netherlands, Poland and Egypt from environments with different levels of salinity, indicating a relatively wide geographic and ecological distribution. It is likely that the species is expanding its biogeographic range, but according to current knowledge it should be considered rare, despite the fact that locally it can form quite large populations, as it has been identified in only three fairly remote locations in five years since its description (Beauger et al. 2015; Cantonati et al. 2016; Bąk et al. 2020). Given that *N. flandriae* has a fairly wide range of ecological tolerance (this study), it may not to be a rare species in the near future. We may even be currently witnessing the beginning of an invasion of this species.

Planothidium nanum, which is undoubtedly closely related to *Planothidium dubium*, is an interesting example of sympatric speciation as both species were found in the same samples. Explaining the causes of this interesting phenomenon involves the collection of much more data on the distribution and autecology of the recently described species *P. nanum*.

The objective of this survey was to present the current distribution and ecological characteristics of the two diatom species – *Navicula flandriae* and *Planothidium nanum* – that have recently been recorded for the first time in Poland by Bąk et al. (2020). In addition, in this paper we propose a course of further research on diatom assemblages in the study area.

2. Materials and methods

The research was carried out at seven sampling sites (five rivers) that are located in Southern Poland (Upper Silesia and adjacent areas) in September 2018 (Fig. 1).

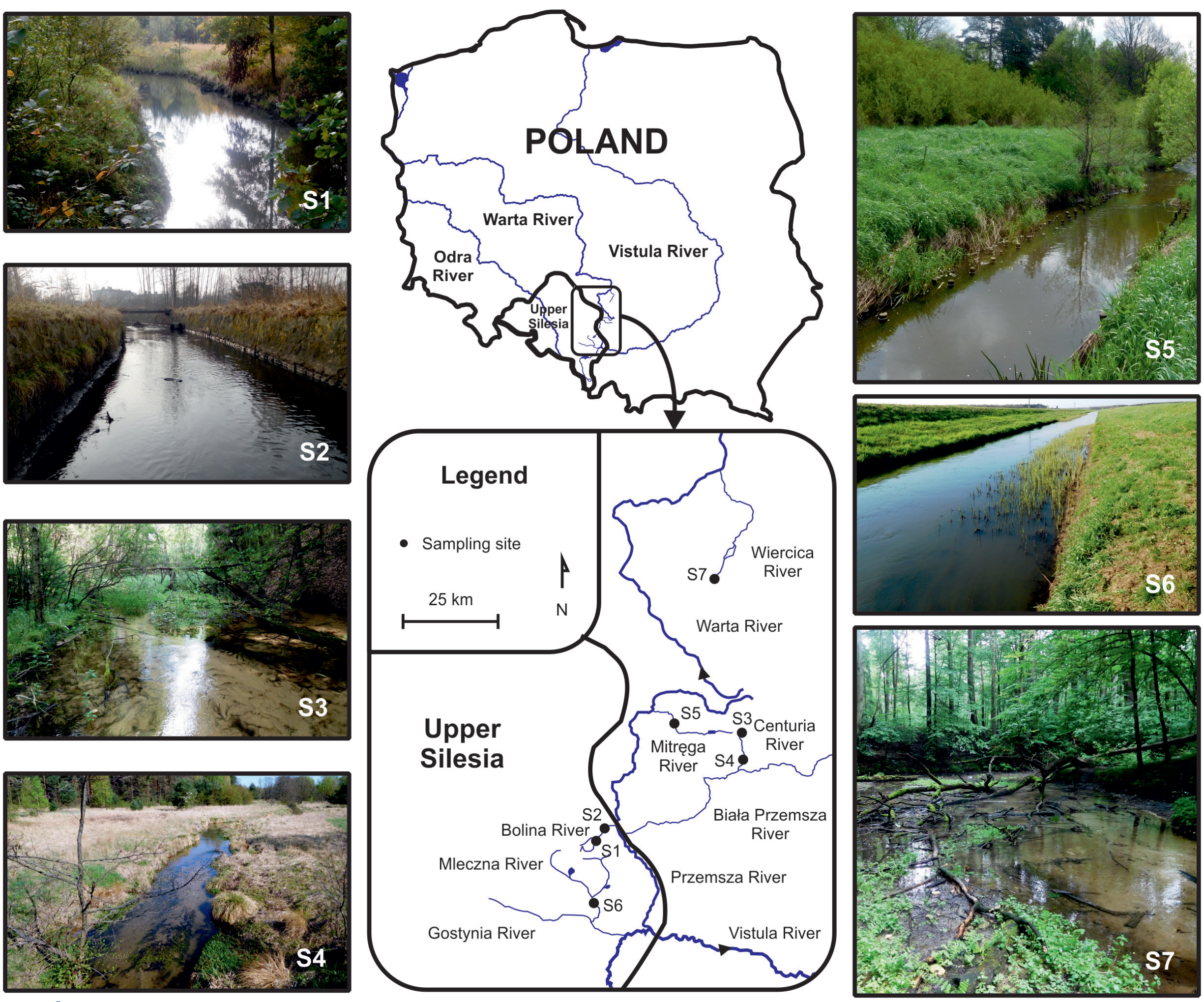

Figure 1

Location of the study area and the diatom sampling sites. Abbreviations: S1 – the upper reaches of the Bolina River, S2 – the lower reaches of the Bolina River, S3 – the upper reaches of the Centuria River, S4 – the lower reaches of the Centuria River, S5 – the lower reaches of the Mitręga River, S6 – the lower reaches of the Mleczna River, S7 – the upper reaches of the Wiercica River

The upper reaches of the Centuria and Wiercica rivers are located in protected areas, namely the "Źródła Centurii" (Centuria springs) natural monument and the "Parkowe" nature reserve, respectively. The abiotic types of the studied rivers were defined according to the Directive (2000) and together with the geographic coordinates and altitude of the rivers are listed in Table 1.

Table 1

General characteristics of the studied rivers (sampling sites)

Geographic coordinates/ Characteristic features	the Bolina River, upper reaches	the Bolina River, lower reaches	the Centuria River, upper reaches	the Centuria River, lower reaches	the Mitręga River, lower reaches	the Mleczna River, lower reaches	the Wiercica River, upper reaches
Coordinate N	50°13′47.6″	50°14′44.5″	50°24′52.7″	50°21′55.2″	50°26′04.2″	50°07′01.1″	50°41′18.0″
Coordinate E	19°05′08.5″	19°06′04.7″	19°29′11.4″	19°29′40.9″	19°17′57.4″	19°04′29.2″	19°24′42.0″
Altitude (m a.s.l.)	262	257	343	311	300	236	309
Abiotic type	(type 5) mid-altitude siliceous streams with a fine particulate substrate				(type 6) mid-altitude calcareous streams with a fine particulate substrate on loess		(type 17) lowland sandy streams

rivers was assessed using a multimetric diatom index (IO) calculated using Excel forms commissioned by the Chief Inspectorate of Environmental Protection in Poland (Zgrundo et al. 2018). The EU WFD uses abiotic parameters to classify streams and rivers into types. For rivers, a fixed typology, i.e. "System A" typology, is defined in the Directive according to ecoregions and size based on catchment area, catchment geology and altitude. Ecoregions are based on the fauna living in European inland waters. Therefore, based on the "System A" typology, homogenous water bodies were selected for the sampling sites in accordance with the above requirements of the Directive (2000), including ecoregions. According to the Regulation (2019) and based on the obtained IO values, the ecological condition classes are discussed using the following class limit values:

Water samples for physical and chemical analyses were collected from each sampling site at the same time as biological samples. Physical and chemical parameters of water, i.e. electrical conductivity, total dissolved solids, oxygen concentration, temperature and pH, were measured in the field using HI-9811-5 Hanna Instruments and CO-401 Elmetron portable meters. Other parameters of water (concentrations of nitrates, nitrites, ammonium, phosphates, chlorides, sulfates, iron, alkalinity, calcium, magnesium and total hardness) were analyzed in the laboratory according to the standard methods of Hermanowicz et al. (1999). Morphometric features of the riverbeds and velocity were measured according to Hauer & Lamberti (2007).

Diatoms for taxonomic analyses were collected from each sampling site by cutting submerged macrophytes and scraping diatoms from a surface area of 10 cm^2 with a soft brush (Zgrundo et al. 2018). Permanent slides for light microscopy were prepared following the standard protocol according to Battarbee (1986) and Bodén (1991). The slides were examined using Zeiss Axio Scope A1 and Nikon Eclipse E600 light microscopes. SEM micrographs were created using a Hitachi SU 8010 SEM at the Podkarpacie Innovative Research Center of the Environment (PIRCE) at the University of Rzeszów, Rzeszów, Poland. Measurements and photographic documentation were made using AxioVision Rel. 4.8 software. For all analyzed samples, a minimum of 400 valves were identified to the species or variety level and their relative abundance was determined. Based on the identified diatom taxa and the number of valves in the studied samples, the ecological condition of the

For type 5 of rivers – mid-altitude siliceous streams with a fine particulate substrate: very good condition (> 0.69), good condition (≥ 0.50), moderate condition (≥ 0.30), poor condition (≥ 0.15), bad condition (< 0.15);

For type 6 of rivers – mid-altitude calcareous streams with a fine particulate substrate on loess: very good condition (> 0.69), good condition (≥ 0.48), moderate condition (≥ 0.30), poor condition (≥ 0.15), bad condition (< 0.15);

For type 17 of rivers – lowland sandy streams: very good condition (> 0.54), good condition (≥ 0.39), moderate condition (≥ 0.30), poor condition (≥ 0.15), bad condition (< 0.15).

3. Results

Conductivity, total dissolved solids, chlorides and total hardness were extremely high at the sampling sites located in Upper Silesia and affected by underground salt mine water, i.e. in the upper and lower reaches of the Bolina River and the lower reaches of the Mleczna River (sampling sites S1, S2 and S6). Such results are a consequence of the discharge of mine water containing high concentrations of salts from Carboniferous rocks by underground hard coal mines (Strozik 2017). This is in contrast to other sampling sites located in the Kraków-Częstochowa Upland, where rivers originating from calcium carbonate rocks do not have similarly elevated conductivity (Kondracki 2011). Flow velocity of the studied rivers ranged from 0.023 to 0.722 m s^{-1}. Organic pollution of water was also relatively high as evidenced by the values of biochemical oxygen demand (BOD) and the concentration of sulfates (sampling sites S1, S2 and S6). The concentrations of ammonium, nitrites and nitrates were up to 1.81, 2.702 and 21.26 mg dm^{-3} at sampling sites S2 and S6, respectively (Table 2). Although water parameters at the sampling sites located in areas adjacent to Upper Silesia are determined primarily by the geology of a river, they are also affected by varying degrees of anthropopressure resulting from hard coal mining, agriculture, urbanization and domestic sewage. The studied rivers impacted by saline coal mine drainage water, located in a highly urbanized area, were characterized by increased conductivity (9290–14 630 μS cm^{-1}), increased total hardness (850–3700 mg $CaCO_3$ dm^{-3}), increased concentration of sulfates (212–660 mg dm^{-3}) and chlorides (3960–16 180 mg dm^{-3}) in water as well as other related water parameters in contrast to other sampling sites where water parameters are generally affected by geology. The studied rivers flowing in the upland are characterized by conductivity values of 280–400 μS cm^{-1}, total hardness of 145–320 mg $CaCO_3$ dm^{-3} and concentrations of sulfates and chlorides in water of 12–42 mg dm^{-3} and 10–29 mg dm^{-3}, respectively (Table 2).

A total of 141 diatom taxa from 54 genera were identified in the surveyed rivers. The highest number of diatom taxa was recorded for two genera – *Navicula* (26 taxa) and *Nitzschia* (19 taxa). The most common diatom taxa were *Achnanthidium minutissimum* (Kützing) Czarnecki (86% of samples: S1, S2, S3, S4, S6 and S7), *Navicula flandriae* (71% of samples: S1, S2, S4, S5 and S6) and *Cocconeis pseudothumensis* Reichardt (57% of samples: S2, S3, S4 and S7), *Gomphonema parvulum* (Kützing) Kützing (57% of samples: S2, S3, S4 and S6), *Halamphora coffeiformis* (C.Agardh) Levkov

Table 2

Physical and chemical parameters of water, velocity, morphometric features of the studied rivers (sampling sites).

Abbreviations: S1 – the upper reaches of the Bolina River, S2 – the lower reaches of the Bolina River, S3 – the upper reaches of the Centuria River, S4 – the lower reaches of the Centuria River, S5 – the lower reaches of the Mitręga River, S6 – the lower reaches of the Mleczna River, S7 – the upper reaches of the Wiercica River

Parameter	Unit	S1	S2	S3	S4	S5	S6	S7
Riverbed width	m	7.10	4.30	4.07	3.60	2.70	8.10	7.00
Riverbed depth	cm	27.0	21.0	14.0	58.5	30.0	105.0	26.5
Flow velocity	m s^{-1}	0.055	0.451	0.105	0.722	0.023	0.170	0.143
Temperature	°C	19.3	19.4	10.4	11.8	17.5	16.5	11.0
pH		8.3	8.2	8.7	8.6	6.9	7.8	6.8
Conductivity	μS cm^{-1}	14 630	35 700	280	320	400	9290	330
Total dissolved solids	mg dm^{-3}	7320	17 840	140	150	200	4640	160
Chlorides		6160	16 180	10	12	29	3960	14
Dissolved oxygen		4.54	5.29	4.07	4.35	3.24	2.24	4.21
Sulfates		660	480	40	41	42	212	12
Iron		0.36	0.91	0.06	0.25	0.86	0.30	0.01
Ammonium		0.58	1.83	0.00	0.12	0.38	1.03	0.01
Nitrites		0.532	2.687	0.000	0.143	0.069	2.702	0.054
Nitrates		0.00	0.00	2.66	6.65	1.33	21.26	11.08
Total nitrogen		1.9	4.1	2.3	2.5	5.9	2.5	4.1
Phosphates		0.06	0.06	0.01	0.04	0.25	0.44	0.11
Total phosphorus		0.210	0.240	0.130	0.100	0.065	0.380	< 0.050
Biochemical Oxygen Demand (BOD)		7	23	< 3	< 3	< 3	4	< 3
Total Organic Carbon (TOC)		2.5	3.5	< 2.0	< 2.0	9.8	8.7	< 2.0
Calcium		412	696	57	60	70	190	78
Magnesium		75.57	476.64	0.73	0.12	35.36	91.25	19.56
Total hardness	mg $CaCO_3$ dm^{-3}	1340	3700	145	150	320	850	275
Alkalinity		360	280	95	130	150	235	150

(57% of samples: S1, S2, S5 and S6), *Nitzschia palea* var. *palea* (Kützing) Smith (57% of samples: S1, S2, S3 and S4), *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot (57% of samples: S3, S4, S5 and S6), *Pleurosigma salinarum* (Grunow) Grunow (57% of samples: S1, S2, S6 and S6) and *Staurosirella pinnata* (Ehrenberg) Williams & Round (57% of samples: S2, S3, S4 and S7). Although none of the identified species was present at all sampling sites, as many as 80 taxa were recorded in one sample. The number of identified taxa for each sampling site is presented in Table 3. The percentage of the identified taxa (for taxa with relative abundance above 5% at least at one site) is presented in Table 4.

The lowest values of the IO index were recorded in the rivers impacted by salt mine water (the Bolina and Mleczna rivers). A similar value of the IO index was recorded for the Mitręga River, whose TOC value was the highest of all the rivers studied (Tables 2 and 3). However, about twice as high values of the IO index were recorded for other rivers. In addition, only the upper reaches of the Wiercica River (S7) were characterized by very good ecological condition. Both S3 and S4 were characterized by good ecological condition, while the remaining sampling sites (S1, S2, S5 and S6) – by poor ecological condition (Table 3).

Navicula flandriae (Fig. 2) was primarily recorded in the rivers located in Upper Silesia, which are affected

Table 3

Number of diatom taxa, values of the diatom index (IO) for the studied rivers (sampling sites)

Characteristic	the Bolina River, upper reaches	the Bolina River, lower reaches	the Centuria River, upper reaches	the Centuria River, lower reaches	the Mitręga River, lower reaches	the Mleczna River, lower reaches	the Wiercica River, upper reaches
Number of taxa	39	45	43	48	15	19	24
Diatom index (IO)	0.254	0.294	0.582	0.557	0.285	0.236	0.632

Table 4

Percentage of taxa (%) in diatom assemblages at the sampling sites (relative abundance >5% at least at one site).

Abbreviations: S1 – the upper reaches of the Bolina River, S2 – the lower reaches of the Bolina River, S3 – the upper reaches of the Centuria River, S4 – the lower reaches of the Centuria River, S5 – the lower reaches of the Mitręga River, S6 – the lower reaches of the Mleczna River, S7 – the upper reaches of the Wiercica River

Taxa	S1	S2	S3	S4	S5	S6	S7
Achnanthidium minutissimum (Kützing) Czarnecki	1.0	0.5	16.9	16.4	-	0.9	0.9
Amphora inariensis Krammer	-	-	1.4	3.7	-	-	5.5
Amphora pediculus (Kützing) Grunow	-	0.5	-	1.5	-	-	9.1
Cocconeis euglypta Ehrenberg	-	-	0.7	5.2	-	-	2.7
Cocconeis placentula var. placentula Ehrenberg	-	-	0.7	18.7	-	-	0.9
Cocconeis pseudolineata (Geitler) Lange-Bertalot	-	-	-	5.2	-	-	-
Encyonema ventricosum (C.Agardh) Grunow	-	-	9.2	-	-	-	-
Gyrosigma attenuatum (Kützing) Rabenhorst	0.5	5.4	-	-	-	-	-
Gyrosigma peisonis (Grunow) Hustedt	-	10.3	-	-	-	-	-
Halamphora coffeiformis (C.Agardh) Levkov	1.5	7.6	-	-	10.2	15.1	-
Karayevia clevei (Grunow) Round	-	-	2.1	1.5	-	-	9.1
Navicula cryptotenella Lange-Bertalot	-	-	19.7	-	-	-	-
Navicula erifuga Lange-Bertalot	3.4	8.7	-	-	-	-	-
Navicula flandriae Van de Vijver & A.Mertens	28.2	6.0	-	0.8	75.2	74.9	-
Navicula lacuum Lange-Bertalot, Hofmann, Werum & Van de Vijver	0.5	8.2	-	-	-	-	-
Navicula salinarum var. *salinarum* Grunow	-	5.4	-	-	4.4	0.5	-
Navicula supergregaria Rumrich & Lange-Bertalot	-	6.0	-	-	-	-	-
Nitzschia frustulum (Kützing) Grunow	10.2	6.5	-	-	0.7	-	-
Planothidium dubium (Grunow) Round & Bukhtiyarova	-	-	0.7	0.8	-	-	5.5
Sellaphora raederae (Lange-Bertalot) Wetzel	-	-	-	-	-	-	6.4
Seminavis sp.	17.0	0.5	-	-	-	-	-
Staurosirella martyi (Héribaud-Joseph) Morales & Manoylov	-	-	5.6	3.0	-	-	-
Staurosirella pinnata (Ehrenberg) Williams & Round	-	0.5	2.8	1.5	-	-	33.6

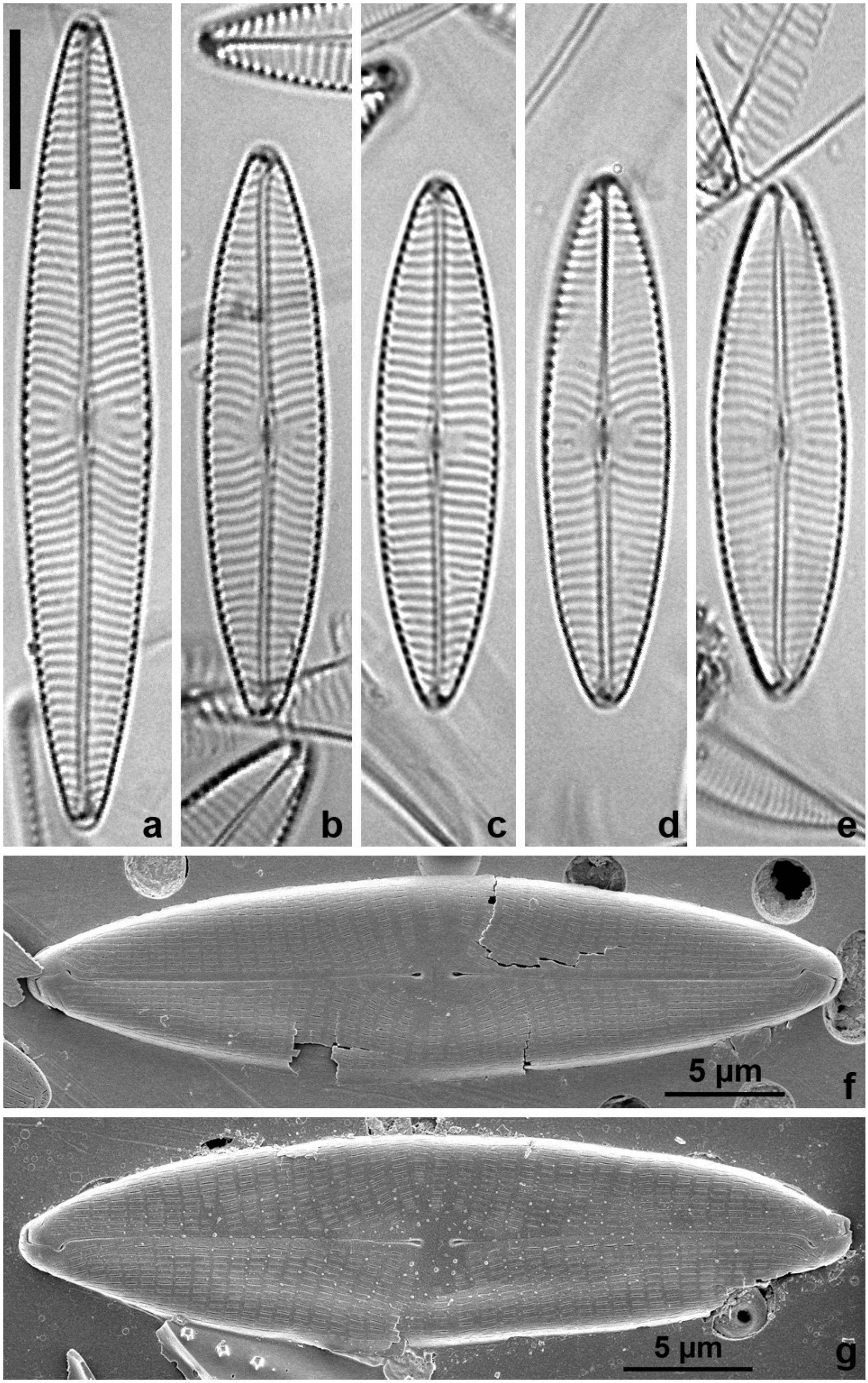

Figure 2

LM (a–e) and SEM (f, g) micrographs of *Navicula flandriae* Van de Vijver & A. Mertens

Scale bars = 10 µm in a–e; 5 µm in f, g

by salt water discharge from coal mines, the upper and lower reaches of the Bolina River and the lower reaches of the Mleczna River as well as in the lower reaches of the Mitręga River and the lower reaches of the Centuria River. The percentage of *N. flandriae* in diatom assemblages ranged from 0.8% (S4) to 75.2% (S5; Table 4). *Navicula flandriae* occurred in the Bolina, Mitręga and Mleczna rivers, which were characterized by poor ecological condition. These were the rivers with the lowest IO values (Table 3). However, *N. flandriae* was also found in the lower reaches of the Centuria River characterized by good ecological condition (much higher IO value than that determined for the Bolina, Mleczna and Mitręga rivers, but similar to the upper reaches of the Centuria River; Tables 3 and 4).

Planothidium nanum (Fig. 3) was recorded at three sampling sites: the upper and lower reaches of the Centuria River (S3, S4) and the upper reaches of the Wiercica River (S7). The percentage of *P. nanum* in diatom assemblages was generally low (upper reaches of the Centuria River – 0.70%, lower reaches of the Centuria River – 1.49% and upper reaches of the Wiercica River – 1.82%). *Planothidium nanum* was recorded in the rivers with the best ecological

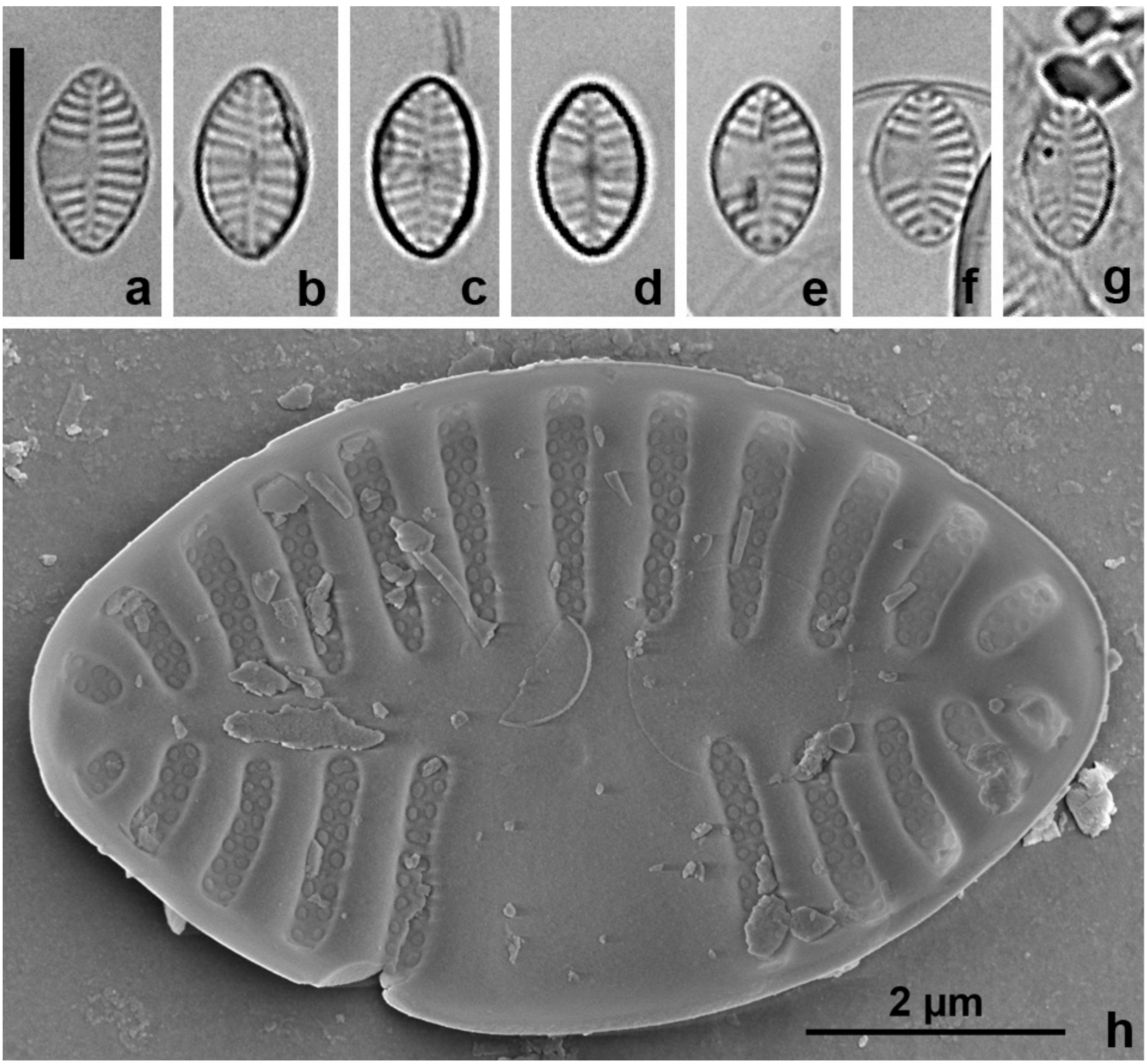

Figure 3

LM (a–g) and SEM (h) micrographs of *Planothidium nanum* Bąk, Kryk & Halabowski

Scale bars = 10 µm in a–g; 2 µm in h

condition: the Wiercica River (very good condition) and the Centuria River (good condition). These were the rivers with the highest IO value (Table 3).

Navicula flandriae valves from samples collected in 2018 were 32–50 µm long, 7.2–8.0 µm wide and had 13–14 striae per 10 µm (Fig. 2) and ca. 32 lineolae per 10 µm. In samples from 2017, *N. flandriae* was less variable in length – 35–46 µm. According to Van de Vijver and Mertens in Beauger et al. (2015), valves are 35–65 µm long, 7.0–9.5 µm wide and have 12–13 striae per 10 µm and ca. 32 lineolae per 10 µm. This comparison means that the length range for *N. flandriae* should be extended with respect to the lower size, as the specimens found were only 32 µm long. Although most specimens found in Poland had 13 striae per 10 µm, some specimens were characterized by a higher density of striation (14/10 µm).

The dimensions of *Planothidium nanum* valves from samples analyzed in this study were generally within the nominal ranges for this species (length 5.5–11.5 µm, width 4.0–5.5 µm, striae 12–14/10 µm; Bąk et al. 2020) except for width as a few specimens were 3.8 µm wide, and therefore the range should be extended (3.8–5.5 µm).

4. Discussion

The rivers flowing through Upper Silesia, which is one of the most industrialized and urbanized regions in Europe, are exposed to strong anthropopressure. The strongest pressure is associated with discharge of salt mine water from underground hard coal mines into the rivers. In addition, in northern regions, including Poland, salt is used for de-icing of roads, which also gets into aquatic environments. This phenomenon leads to the formation of saline lotic and lentic habitats with salinities sometimes similar to those in the Baltic Sea or the North Sea. Both climate change and the growing human population will exacerbate the problem of global salinization of inland waters (Neubauer & Craft 2009). Because of climate change, less de-icing salt is needed each winter in northern areas (such as Poland). As a result, only anthropogenically salinated rivers (e.g. by underground hard coal mines) are a permanent environment with such properties. Examples of such secondary saline lotic waters are the Bolina and Mleczna rivers, which flow through the study area.

Anthropogenic salinity of inland waters leads to the disappearance of freshwater diatom taxa and their replacement by brackish and marine species, which is contributed by, among other things, osmotic stress caused by excess salt in water (e.g. Fritz et al. 1991; Saros & Fritz 2000; Čecháková et al. 2014; Herbert et al. 2015; Bąk et al. 2020). Our results confirm these observations. We recorded a similar number of taxa in the rivers of one abiotic type (type 5 – mid-altitude siliceous streams with a fine particulate substrate) in both the Bolina and Centuria rivers. However, the Bolina River is exposed to strong pressure of salt mine water, while the Centuria River receives no saline water inflow. When comparing rivers of the same abiotic type (type 6 – mid-altitude calcareous streams with a fine particulate substrate on loess), we recorded more taxa in the Mleczna River (under the pressure of salt mine water) than in the Mitręga River (with no inflow of salt water). This phenomenon can be explained by the high proportion of eurytopic species as well as salt-tolerant and marine species. However, it is difficult to conclude which of the phenomena we observe in the studied secondary saline rivers – an invasion of marine species, or periodic occurrence of these organisms under conditions unfavorable for freshwater species (Bąk et al. 2020). On the other hand, the dominance of *Navicula flandriae* was observed in the Mitręga River, which can limit the expansion of other diatom species. We confirmed the occurrence of *N. flandriae* in both the Bolina River and the Mleczna River. This species was discovered in Poland in 2017 by Bąk et al. (2020). In addition, we recorded the occurrence of *N. flandriae* in the lower reaches of the Mitręga and Centuria rivers, which are not impacted by salt mine water. The sampling site on the Mitręga River is located within wastelands and domestic sewage is sometimes introduced into the river. There is also a dam reservoir upstream of the site. For comparison, although the sampling site in the lower reaches of the Centuria River was also located within wastelands, the concentration of nitrates in water was much lower than in the Mleczna River. Currently, in addition to several rivers in Southern Poland (the Bolina, Centuria, Mitręga and Mleczna rivers), the global occurrence of *N. flandriae* also includes several rivers and canals (i.e. Leopoldkanaal, Oostpolderkreek) in Flanders – the northern region of Belgium, where this species was described in 2015 (Beauger et al. 2015). *Navicula flandriae* was also found at several other sites in the Netherlands (the province of Zuid–Holland and the province of Zeeland) and in inland waters in Egypt (the Damietta Branch of the Nile River; Cantonati et al. 2016; Bąk et al. 2020). To date, *N. flandriae* has only been recorded in highly saline habitats with nutrient-rich waters, such as the Bolina and Mleczna rivers, and in several canals and rivers in Flanders with a conductivity ranging from 5780 (in the Oostpolderkreek River) to 46 600 µS cm^{-1} (in the Bolina River). However, the largest population of this species was recorded in the type locality – Leopoldkanaal, with a conductivity of 7270 μS cm^{-1} (Beauger et al. 2015). Bąk et al. (2020) observed numerous populations of *N. flandriae* in secondary saline tributaries that had higher conductivity. The discovery of a large population of *N. flandriae* (over 75% contribution to diatom assemblages) in the Mitręga River indicates that this species is a euryhaline species with a broad salinity tolerance and can survive in lower salinity (conductivity up to 330 μS cm^{-1}). Our results and other studies (Beauger et al. 2015; Cantonati et al. 2016; Bąk et al. 2020) indicate that this species is associated with waters moderately rich or rich in nutrients, especially nitrates, ammonium and nitrites. The low percentage of *N. flandriae* in the diatom assemblage (less than 1%) in the Centuria River, where values of nutrients were low, further confirmed its broad trophic preferences. In such conditions, *N. flandriae* probably does not form large populations. However, according to Wojtal (2013), species that are known to occur in habitats with high trophic status can also successfully occur in waters with low or very low nutrient content. Although we confirmed the suggestion by Bąk et al. (2020) that this species may be widespread in nutrient-rich waters, to date it may have been confused with other similar species from the genus *Navicula*. However, the combination of a relatively low density of striae, more distally placed central striae, valve outline and non-protracted apices, makes it possible to distinguish *N. flandriae* from all common *Navicula* taxa such as, e.g. *N. tripunctata* (O.F.Müller) Bory or *N. recens* (Lange-Bertalot) Lange-Bertalot (see Beauger et al. 2015; Bąk et al. 2020). The taxonomic composition in the studied rivers is different from that studied by Beauger et al. (2015), because *Nitzschia liebetruthii* Rabenhorst, *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot, *Tabularia fasciculata* (Agardh) Williams & Round and *Cyclotella meneghiniana* Kützing were dominant in diatom assemblages in several canals and rivers in Flanders. During the 2017 survey in Poland, *Navicula flandriae* was accompanied by *Pleurosira laevis* var. *laevis* (Ehrenberg) Compère, *Gomphonema parvulum*, *Cyclotella meneghiniana*, *Halamphora coffeiformis*, *Cocconeis placentula* var. *placentula* Ehrenberg, *Navicula perminuta* Grunow in Van Heurck, *Planothidium delicatulum* (Kützing) Round & Bukhtiyarova, *Conticribra weissflogii* (Grunow) Stachura-Suchoples et D.M.Williams, *Navicula gregaria* Donkin, *Navicula veneta* Kützing, *Ctenophora pulchella* (Ralfs ex Kützing) D.M.Williams & Round, *Haslea spicula* (Hickie) Bukhtiyarova and *Nitzschia palea* var. *palea* (Bąk et al. 2020). In samples from 2018, *Halamphora coffeiformis*, *Navicula salinarum* Grunow, *Nitzschia frustulum* (Kützing) Grunow and *Seminavis* sp. were the most dominant taxa accompanying *N. flandriae*. The differences in the composition of taxa accompanying *N. flandriae* show that *N. flandriae* tolerates unstable environmental conditions and does not occur in any typical species composition, but rather with randomly gathered species that happened to be present in the studied ecosystem at a given time. A common feature of most of these species is their high tolerance to changes in salinity and their ability to thrive in electrolyte-rich flowing waters. Some of these species are also tolerant of increased trophic status and organic pollution, while others are associated with clear or moderately polluted waters. Based on the analysis of the autecological features of the accompanying taxa, it can be concluded that *N. flandriae* is a species with wide ranges of tolerance to trophic and saprobic conditions.

A recent paper by Bąk et al. (2020) has demonstrated the necessity to include *Pleurosira laevis* var. *laevis* and var. *polymorpha* Compère (dominant species in diatom assemblages recorded in highly salinized rivers) in flowing water monitoring. Therefore, we suggest first of all additional research on the occurrence of diatoms in highly anthropogenically transformed surface waters (especially in highly saline rivers). At the same time, based on our results, we suggest including *Navicula flandriae* in the monitoring of flowing waters. According to the results obtained by Bąk et al. (2020), after the inclusion of *P. laevis* in the ecological status assessment, the final classification of rivers improved by one level. Therefore, the IO value obtained for the Mitręga and Mleczna rivers, where relative abundance of *N. flandriae* was about 75%, is probably inaccurate. However, the proposed research may identify new species that could be used as indicators of salinity.

Planothidium nanum was recently described by Bąk et al. (2020) from only one location, the unimpacted part of the upper reaches of the Centuria River (spring area). Our research confirmed the occurrence of *P. nanum* in this part of the Centuria River. In addition, we recorded *P. nanun* in the lower reaches of the Centuria River as well as in the unimpacted part of the upper reaches of the Wiercica River (spring area). At each of these sites, *P. nanum* occurred in low abundance in diatom assemblages (< 2%). Our survey also revealed the co-occurrence of *P. nanum* with *Karayevia clevei* (Grunow) Round, *Cocconeis pseudothumensis*, *P. dubium* (Grunow) Round & Bukhtiyarova, *C. neothumensis* Krammer and *Amphora inariensis* Krammer, similar to the results obtained by Bąk et al. (2020), although they occurred in different dominance patterns. The most similar composition of dominant species was recorded in the Wiercica River.

Achnanthidium minutissimum and *Staurosirella pinnata* were the dominant species in diatom assemblages in the Centuria River and in the upper reaches of the Wiercica River. This result corroborates the results of the survey conducted by Wojtal (2013) who showed the same dominance pattern in diatom assemblages in similar aquatic habitats. We found that *P. nanum*, which co-occurred with *A. minutissimum*, *P. lanceolatum* (Brébisson ex Kützing) Lange-Bertalot and *Amphora pediculus* (Kützing) Grunow in the selected rivers of Southern Poland, was recorded in both habitats – unpolluted (Kawecka 2012; Wojtal 2013) and polluted waters (Żelazna-Wieczorek 2011; Wojtal & Sobczyk 2012). According to Bąk et al. (2020), the habitat preferences of *P. nanum* include oligotrophic and oligosaprobic and slightly alkaline freshwater with low conductivity. Although our results generally confirmed these observations, at the same time they showed that *P. nanum* can also occur in slightly acid water. Despite previous surveys carried out in the same or similar habitats by other researchers (e.g. Wojtal 2013), *P. nanum* was not discovered or was identified as a different species probably due to its low percentage in samples. However, Bąk et al. (2020) remarked that *P. nanum* can be easily distinguished from other species of this genus by the small size of *Planothidium nanum* and its elliptical outline combined with very widely spaced central striae on the sternum valve (sinus) and widely spaced central striae on the raphe valve. The reported occurrence of *P. nanum* outside the spring areas of the Centuria and Wiercica rivers indicates that this species may be more widespread in other rivers with similar habitat characteristics. Protected areas with habitats of this species play an important role in the conservation of *P. nanum*. Thus, this species is expected to survive and further research on the diatom flora in the rivers of Southern Poland could identify new sites of *P. nanum* in the future.

5. Conclusions

Considering the current occurrence of *Navicula flandriae* and *Planothidium nanum*, it can be expected that new sites of these species will be found in the future. Protected areas where *P. nanum* has been recorded play an important role in the conservation of this species. In addition, a new type of protected area in the form of a nature reserve should be considered in order to protect the entire Centuria River or at least those parts of the river that are still close to natural conditions. However, the occurrence of *N. flandriae* both in the highly polluted rivers and in the rivers with minimal human impact indicates a widespread distribution of this species in the flowing waters of Southern Poland. Our results reveal a paucity of research on diatoms in flowing waters, especially in Southern Poland, and we therefore call for its intensification in these types of habitats. In addition, continuous monitoring of rare diatom species and their habitats is necessary.

Acknowledgements

This research was partially supported by the University of Silesia in Katowice (Young Scientists 2018 grant and Fast Track Grants).

The authors are grateful to Dr. Łukasz Peszek for his assistance with SEM micrographs. The authors are also grateful to the Head of the Regional Directorate for Environmental Protection in Katowice and the Major of the town of Łazy for their cooperation and permission to carry out the fieldwork in the "Parkowe" nature reserve (permission No. WNP.6205.8.2017. MM) and the natural monument "Zespół Źródeł rzeki Centurii zlokalizowany w miejscowości Hutki Kanki, Gmina Łazy" ("Complex of Centuria River springs located in the village of Hutki Kanki, the commune of Łazy"; permission No. WK-OŚ.604.21.2017, Resolution No. XXXIII/256/13). The authors would also like to thank the anonymous reviewers for their very valuable substantive comments that helped to improve the final version of this paper.

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