Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 44, Issue 1, March 2015
pages (51-67)

ISSN 1730-413X
eISSN 1897-3191

Half a century of research on diatoms in athalassic habitats in central Poland

by

Joanna Żelazna-Wieczorek
Rafał M. Olszyński*
Paulina Nowicka-Krawczyk

DOI: **10.1515/ohs-2015-0006**
Category: **Original research paper**
Received: **October 11, 2014**
Accepted: **December 22, 2014**

Department of Algology and Mycology, Faculty of Biology and Environmental Protection, University of Łódź, ul. Banacha 12/16, 90-237 Łódź, Poland

Abstract

Part of the geology in the Łódź province was formed during the Upper Permian period when rich Zechstein salt was deposited. Groundwater drains the deposits and flows out in the village of Pełczyska, creating a unique hydrogeological site in Central Poland. An inland, athalassic ecosystem can be a reference site for halophile microflora. The outflow with surrounding marshes has been an algological research site since 1964.

The research reveals changes recorded in diatom assemblages from athalassic habitats, characterized by a wide range of salinity levels, and verifies the tolerance of taxa to salinity. The comparative analysis was based on the diatom material sampled in 1964-1965, 1992-1994 and on recently collected samples.

The analysis revealed the temporal change in assemblages caused by a change in the chloride concentration, and the spatial change from one to another habitat type, characterized by varying salinity levels. The halophilic species in the studied habitats included e.g. *Halamphora dominici*, *H. tenerrima*, *Navicula digitoconvergens*, *N. meulemansii*, *Staurophora salina*. The analysis of changes allowed the verification of the species' requirements and tolerance range to the salinity factor. Therefore, in the case of *Fragilaria famelica* and *Halamphora sydowii*, we propose a change in the halobion system classification.

Key words: Bacillariophyta, halobion system, salinity, temporal and spatial changes

* Corresponding author: *ra.ols@biol.uni.lodz.pl*

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at oandhs.ocean.ug.edu.pl

Introduction

The salt marshes near Łęczyca (Central Poland) are, on a global scale, unique natural brackish water habitats, where diatoms, typical of saline waters, are present. Inland habitats characterized by a high concentration of chloride ions have hardly been investigated. There are only a few locations in the world where diatom assemblages occur in inland salt waters. Saltwater inland ecosystems (athalassic ecosystems) are vulnerable to climate change, therefore the presence of halophilic diatom taxa at these sites may be exposed to dynamic changes (Veres et al. 1995). The hot springs in Kenya and New Zealand are an example of habitats also characterized by an increased concentration of chloride ions, however, a very high temperature was the factor that had the greatest influence on the dynamics of diatom assemblages in these habitats (Owen et al. 2008). Saltwater ecosystems, such as lakes and springs, located in coastal zones are significantly different from those located inland, because the water chemistry at these sites is strongly associated with the sea or ocean (Starmach 1969; Aboal et al. 1998; Novelo et al. 2007; Lutyńska 2008; Antón-Garrido et al. 2013).

The first study of macro- and microflora at the salt marshes near Łęczyca was performed by Olaczek, and the results of these observations were presented in Olaczek (unpublished PhD thesis, 1963). However, the data on the algal communities, and in particular the diatoms, were very scanty. A few years later, Pliński presented (unpublished MS thesis, 1966) an analysis of algological material from a spring and a pond located in the Pełczyska village. Part of the analysis concerning the diatom assemblages was published (Pliński 1969).

The algological material collected by Pliński, deposited at the University of Łódź, Department of Algology and Mycology, and the data from Pliński (1969) were used for the verification and comparative analysis of the recently collected material in 2013 and 2014.

In the 1970s, a few papers concerning algal communities from the salt marshes near Łęczyca were published (Pliński 1971a, Pliński 1971b, Pliński 1971c; Pliński 1973). Twenty years later, the outflow of salt waters in the village of Pełczyska became a study site of Żelazna-Wieczorek who presented the results of algological analysis in Żelazna-Wieczorek (unpublished PhD thesis, 1996). Żelazna-Wieczorek focused on the *Vaucheria* De Candolle (2002) genus, however, diatoms simultaneously collected with the *Vaucheria* samples have been recently verified according to modern taxonomy. The studies of diatoms from the outflow and the pond in Pełczyska were also performed by Rakowska (1997) in 1972 and 1995. The results of Rakowska (1997) provide comparative material for the observation of changes in diatom assemblages occurring over 50 years in salt water habitats of Central Poland.

Long-term research on the diatom microflora from the athalassic habitats allowed the determination of qualitative and quantitative changes in diatom assemblages, on both temporal and spatial scales. The changes were associated with various levels of salinity, as well as anthropogenic transformations.

This paper presents the long-term changes which have been recorded in diatom assemblages from the athalassic habitats characterized by various levels of salinity, and verifies the tolerance of particular diatom taxa to salinity.

Study area

The study area is located in Pełczyska, a village next to the town of Łęczyca in the Łódź province (51°58'35.68"N, 19°14'17.02"E) (Fig. 1). This area is located on the border of two large geological structures: the Kujawy anticlinorium and the Łódz synclinorium. The Kłodawsko-Łęczycka anticline – part of the Kujawy anticlinorium, extends from the village of Solec Wielki, through the towns of Łęczyca and Błonie, towards the town of Kłodawa.

The Zechstein salt occurs in the core of the Kłodawsko-Łęczycka anticline. The structure of the anticline is asymmetric. Its eastern part has a gentle decline, while the western part is steep and

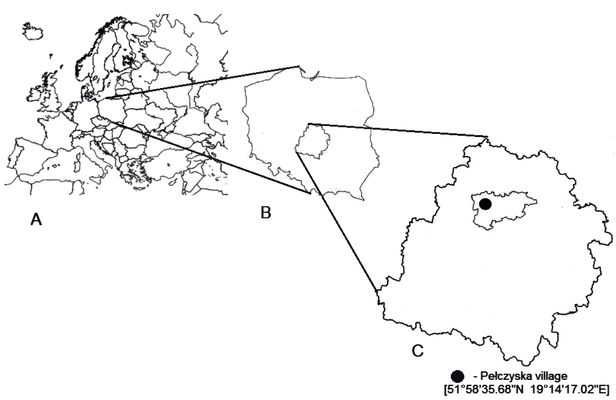

Fig. 1

Location of Pełczyska. A. Location of Poland. B. Location of Łódź province. C. Location of Pełczyska intersected by faults. The Zechstein salt near Łęczyca is deposited at a depth of 1500 m, however, due to its location at the crossing of the Pełczyska faults, the water may drain the deposits and flow out in the Pełczyska area (Olaczek, unpublished PhD thesis, 1963).

The genesis of the salt water outflows and the salt water marshes, which were the study area of many algological research projects, dates back to the late 18th century. During this period, many boreholes were made in search of brine, and consequently a spontaneous salt water outflow in Pełczyska can be observed to this day. Next to the outflow, a pond was made, which accumulated the water discharged from the drilling (Olaczek, unpublished PhD thesis, 1963). The morphology of the outflow in Pełczyska evolved over the decades. Since the 1960s, when the first study of the microflora of this habitat was carried out, the outflow was surrounded by a concrete slabs (Fig. 2). In the 1990s, the concrete gradually degraded (Fig. 3) until the first decade of the 21st century when the outflow was backfilled with a material of unknown composition (Fig. 4) (Pliński, unpublished MS thesis, 1966; Żelazna-Wieczorek, unpublished PhD thesis, 1996).

The study area included three sampling sites. The first site was located at the backfilled outflow, the second one at the ditch through which the water flows from the outflow to the third sampling site, i.e. the pond (Fig. 5).

Data on physical and chemical water analysis of the study area were obtained from Olaczek (unpublished PhD thesis, 1963), and Żelazna-Wieczorek (unpublished PhD thesis, 1996). In recent studies (2013/2014), the physical and chemical conditions of the water were analyzed at all sampling sites. All data are compiled in Table 1.

Fig. 2

Sampling performed by Marcin Pliński in the outflow in 1964/1965

Fig. 3

Degraded concrete slabs surrounding the outflow in 1992/1994

Fig. 4

Backfilled outflow in 2013/2014

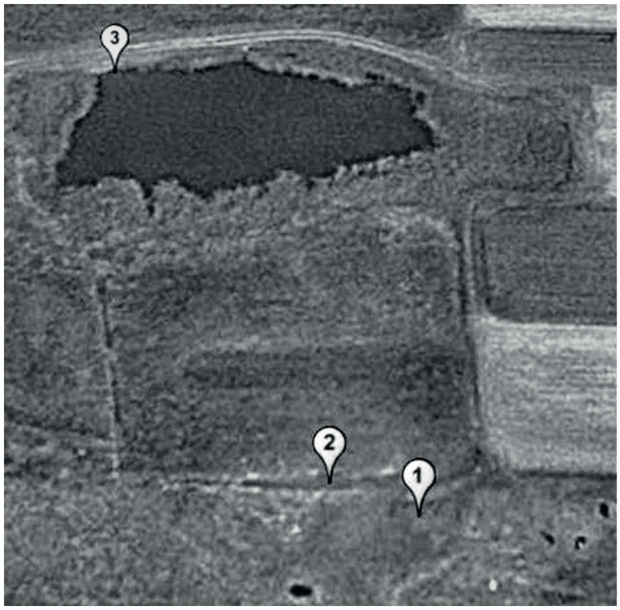

Fig. 5

Location of the sampling sites: 1 – the outflow; 2 – the ditch; 3 – the pond (source: maps.google.com)

DE GRUYTER

Table 1

Physical and chemical parameters of water at the studied sites analyzed in: 1962 (Olaczek, unpublished PhD thesis, 1963), 1994 (Żelazna-Wieczorek 2002) and 2014

Unit		1962	1994		2014		
		X - OUTFLOW	IV - OUTFLOW	X - OUTFLOW	III - OUTFLOW	III - DITCH	III - POND
T	(°C)	7.00	7.00	8.00	6.70	7.80	8.20
pH		7.40	7.20	7.22	7.99	6.40	7.82
Cond	(μS)	n/d	7764.00	7681.00	4450.00	5170.00	2930.00
T. Hard	(mval l^{-1})	37.60	10.84	11.85	n/d	n/d	n/d
Cl^-	(mg l^{-1})	2560.00	2910.86	3116.19	1585.00	1006.00	685.00
SO_4^{2-}	(mg l^{-1})	225.00	154.44	214.44	187.60	165.00	166.20
NH_4^+	(mg l^{-1})	2.00	2.38	2.20	2.09	0.19	0.46
NO_2^-	(mg l^{-1})	n/d	0.02	0.01	n/d	n/d	n/d
NO_3^-	(mg l^{-1})	n/d	0.51	1.50	n/d	n/d	n/d
PO_4^{3-}	(mg l^{-1})	n/d	0.18	0.05	12.46	8.57	9.10
Na^+	(mg l^{-1})	n/d	1528.00	1413.00	500.70	453.30	277.30
K^+	(mg l^{-1})	n/d	19.65	22.60	n/d	n/d	n/d
Ca^{2+}	(mg l^{-1})	480.00	132.10	144.80	171.30	165.00	75.80
Mg^{2+}	(mg l^{-1})	195.00	51.68	56.16	43.8	35.90	25.6

Materials and methods

A total of 15 samples collected by Pliński in 1964/1965, 3 samples collected by Żelazna-Wieczorek in 1992/1994, and 12 samples collected in 2013/2014 were used in the diatomological analysis (Table 2). Diatoms from 1964/1965 were sampled from the outflow (4 samples), the area surrounding the outflow (6 samples), the ditch (2 samples) and the pond (3 samples). The material from 1992/1994 was sampled only from the outflow, while the recent samples, i.e. from 2013/2014, were collected from the outflow, the ditch and the pond (4 samples were collected from each site).

Benthic samples were collected by a pipette from the surface layer of the sediment. Samples were transferred into 125 ml containers and transported to a laboratory. Every sample was preserved with 4% formaldehyde solution. To obtain pure diatom frustules, the collected material was exposed to chemical purification according to Żelazna-Wieczorek (2011). The purified diatom suspension was pipetted onto cover slips and left overnight at room temperature. Permanent microscopic slides were mounted in Naphrax® synthetic resin (refractive index 1.73). All samples and slides are deposited in the collection of the Department of Algology and Mycology, the University of Łódź.

Taxonomical analysis of the benthic diatoms was made by examining permanent slides under a light microscope (Nikon YS 100 and Nikon Eclipse E400) with 1000× magnification (planachromatic oil-immersion objective 100×/1.25). Five hundred valves were identified and counted in consecutive visible areas over half the width of the 20 mm × 20 mm cover glass. In addition, the slides were re-examined and taxa previously unidentified were added to the list. The percentage of taxa in each sample was calculated. Dominant taxa, with relative abundance above 10%, were determined.

The tolerance of particular taxa to salinity was determined according to the data obtained from OMNIDIA 5.3 computer software, and the following references: Krammer, Lange-Bertalot 1986; Krammer, Lange-Bertalot 1991a; Krammer, Lange-Bertalot 1991b; Krammer, Lange-Bertalot 1997; Krammer 1997a; Krammer 1997b; Krammer 2000; Witkowski et al. 2000; Lange-Bertalot 2001; Krammer 2003; Lange-Bertalot et al. 2003; Levkov 2009, Hofmann et al. 2011. Diatom taxa tolerance to salinity was classified on the basis of the seven-grade halobion system, introduced by Van der Werff and Hulls 1957-1974 (Denys et al. 1983). This system was later used by Van Dam et al. 1994 to establish a reduced, four-grade system. However, the reduced halobion system does not include brackish-marine, marine-brackish and marine species (Denys et al. 1983, Van Dam et al. 1994) (Table 3).

Mathematical methods were used to compare the diatom assemblages, and to analyze both the diatom diversity and the relationship between diatom taxa distribution. The Bray-Curtis similarity index was used to determine the similarity between samples from different study periods and sampling sites (Żelazna-Wieczorek 2011). Hierarchical Cluster analysis based on the Bray-Curtis similarity was

Table 2

The total number of samples and the number of samples for each study period and study site; in addition, data from references are provided

	Samples	Taxa	Taxa outflow	Taxa around outflow	Taxa ditch	Taxa pond
Total	30	179	132	87	94	103
1964/1965	15	129	47	87	65	68
1992/1994	3	79	79	n/d	n/d	n/d
2013/2014	12	141	125	n/d	64	75
Pliński 1969	76	62	39	37	20	27
Rakowska 1997	n/d	63	40	n/d	n/d	59

Table 3

The halobion system according to Van Dam et al. 1994 and Van der Werff and Huls 1957-1974 (Denys et al. 1983) and corresponding ranges of chloride ions and salinity

Van Dam et al. 1994		Van der Werff and Huls 1957-1974		Cl^- (mg l^{-1})	Salinity (mg l^{-1})
1	fresh	1	fresh	< 100	<180
2	fresh brackish	2	fresh-brackish	100-500	180-900
3	brackish fresh	3	brackish-fresh	500-1000	900-1800
4	brackish	4	brackish	1000-5000	1800-9000
		5	brackish-marine	5000-10000	9000-18000
		6	marine brackish	10000-17000	18000-30000
		7	marine	>17000	>30000

used to determine whether the groupings are more dependent on the temporal relationships between diatom taxa in particular periods of time (Żelazna-Wieczorek 2011). To analyze the spatial and temporal relationships between diatom taxa in particular habitats, the multidimensional scaling procedure (Multi-Dimensional-Scaling – MDS) based on the Bray-Curtis similarity (Żelazna-Wieczorek 2011) was used. The PRIMER 6.1.10 software was used for the calculations.

Results

The qualitative analysis of 30 samples revealed the presence of 179 diatom taxa from 49 genera. The most numerous genera were *Navicula* (30 taxa) and *Nitzschia* (26 taxa). Species characteristic of saline waters were identified within *Navicula*: *Navicula cincta* (Ehrenberg) Ralfs (Fig. 6 D1-D5), *N. digitoconvergens* Lange-Bertalot (Fig. 6 C1-C5), *N. meulemansii* Mertens, Witkowski & Lange-Bertalot (Fig. 6 E1-E5), *N. peregrina* (Ehrenberg) Kützing, *N. rhynchotella* Lange-Bertalot (Fig. 6 G1-G2), *N. salinarum* Grunow (Fig. 6 H1-H3), *N. salinicola* Hustedt (Fig. 6 B1-B4). *Nitzschia* was represented by: *Nitzschia commutata* Grunow, *N. constricta* (Kützing) Ralfs, *N. liebetruthii* Rabenhorst, *N. nana* Grunow (Fig. 7 G), *N. vitrea* Norman, *N. vitrea* var. *salinarum* Grunow (Fig. 7 F). *Halamphora* was also taxonomically rich and represented by 10 taxa, including: *Halamphora acutiuscula* (Kützing) Levkov (Fig. 8 B1-B3), *H. borealis* (Kützing) Levkov (Fig. 8 F1-F7), *H. dominici* Ács & Levkov (Fig. 8 E1-E5), *H. paraveneta* (Lange-Bertalot, Cvacini, Tagliaventi & Alfinito) Levkov (Fig. 8 C1-C5), *H. sydowii* (Cholnoky) Levkov (Fig. 8 A1-A7), *H. tenerrima* (Aleem & Hustedt) Levkov (Fig. 8 G1-G6), *H. veneta* (Kützing) Levkov (Fig. 8 D1-D4).

Of the nine *Fragilaria* taxa, *Fragilaria famelica* (Kützing) Lange-Bertalot (Fig. 9 B1-B9), and *F. sopotensis* Witkowski & Lange-Bertalot were most abundant (Fig. 9 A1-A7). The *Gomphonema* was represented by 8 taxa, including *Gomphonema italicum* Kützing and *G. utae* Lange-Bertalot & Reichardt. *Surirella* was represented by *Surirella ovalis* Brébisson (Fig. 7 M), *S. striatula* Turpin, and *S. venusta* Østrup (Fig. 7 I1-I2). *Planothidium* was represented by 5 species, including e.g. *Planothidium delicatulum* (Kützing) Round & Bukhtiyarova (Fig. 9 G1-G4), *P. engelbrechtii* (Cholnoky) Round & Bukhtiyarova and *P. pericavum* (Carter) Lange-Bertalot (Fig. 9 F1-F6). Five *Rhopalodia* taxa were present i.a. *Rhopalodia constricta* (W. Smith) Krammer in Lange-Bertalot & Krammer and *Rhopalodia gibberula* (Ehrenberg) Müller (Fig. 7

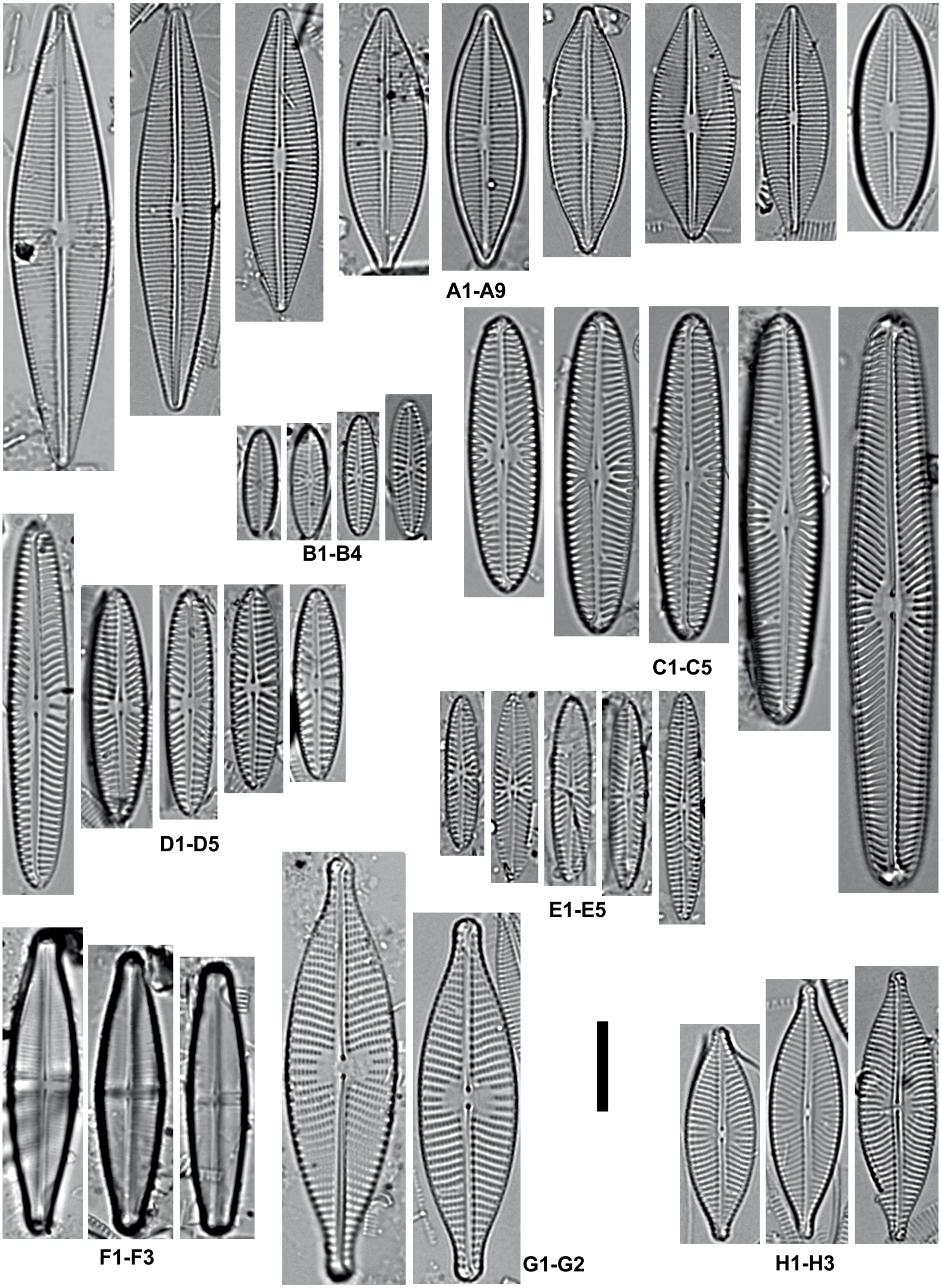

Fig. 6

A1-A9. *Craticula halophila*, B1-B4. *Navicula salinicola*, C1- C5. *Navicula digitoconvergens*, D1-D5. *Navicula cincta*, E1-E5. *Navicula meulemansii*, F1-F3. *Staurophora salina*, G1-G2. *Navicula rhynchotella*, H1-H3. *Navicula salinarum*. Scale bar = 10 μm

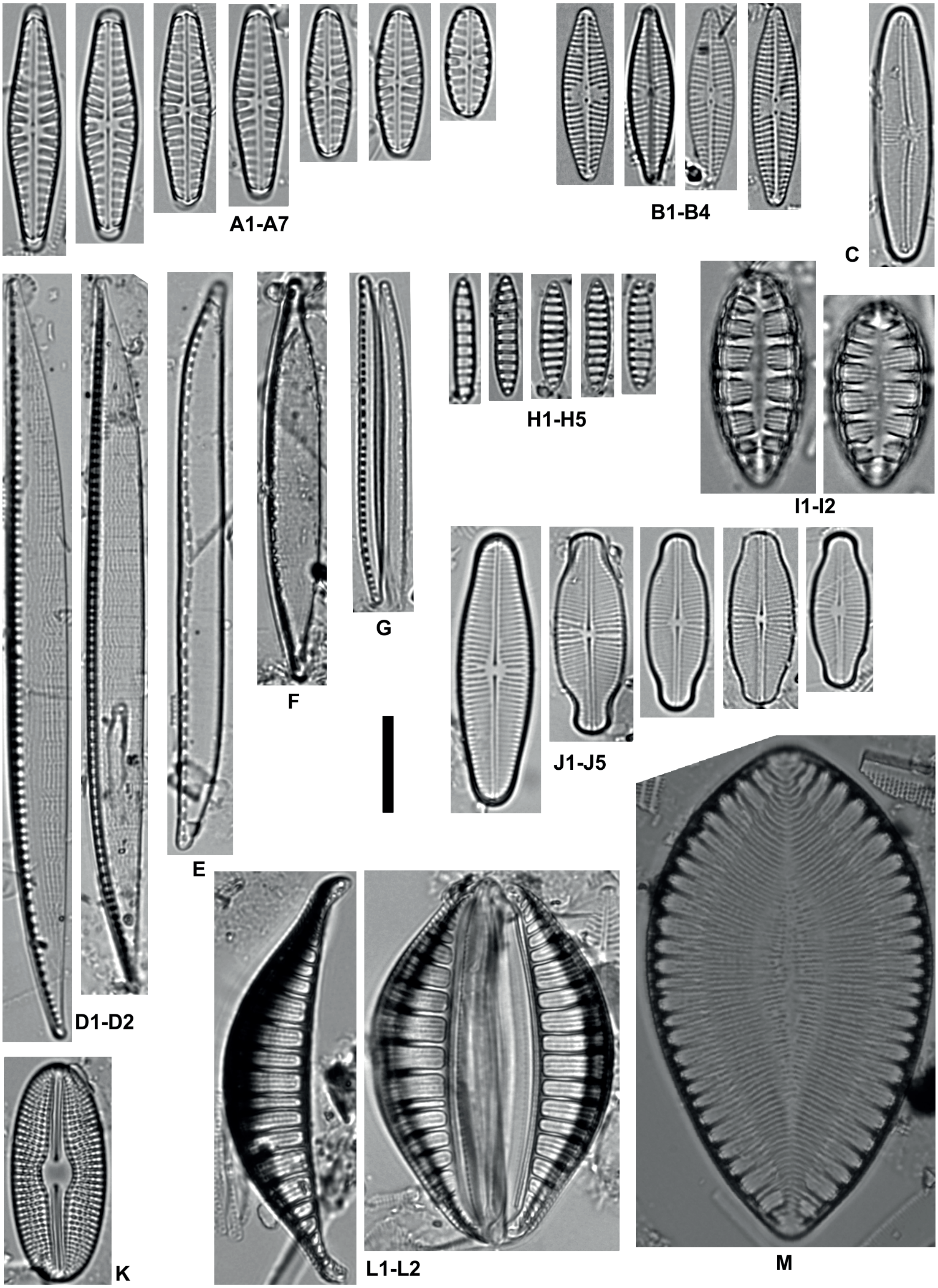

Fig. 7

A1-A7. *Hippodonta hungarica*, B1-B4. *Navicula veneta*, C. *Frustulia creuzburgensis*, D1-D2. *Nitzschia sigma*, E. *Nitzschia scalpelliformis*, F. *Nitzschia vitrea* var. *salinarum*, G. *Nitzschia nana*, H1-H5. *Denticula subtilis*, I1-I2. *Surirella venusta*, J1-J5. *Parlibellus protracta*, K. *Diploneis* cf. *krammeri*, L1-L2. *Rhopalodia gibberula*, M. *Surirella ovalis*. Scale bar = 10 µm

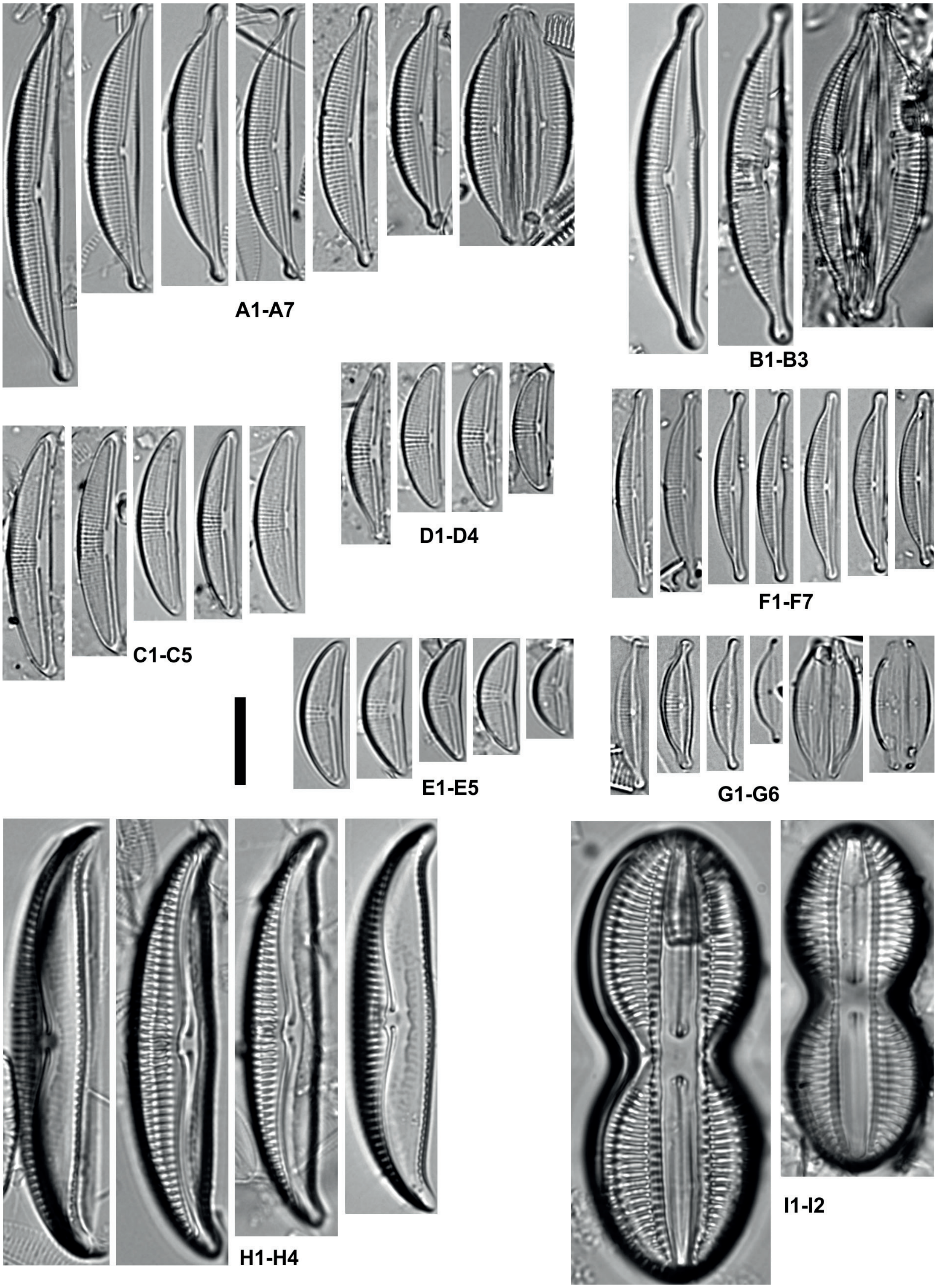

Fig. 8

A1-A7. *Halamphora sydowii*, B1-B3. *Halamphora acutiuscula*, C1-C5. *Halamphora paraveneta*, D1-D4. *Halamphora veneta*, E1-E5. *Halamphora dominici*, F1-F7. *Halamphora borealis*, G1-G6. *Halamphora tenerrima*, H1-H4. *Amphora commutata*, I1-I2. *Diploneis interrupta*. Scale bar = 10 µm

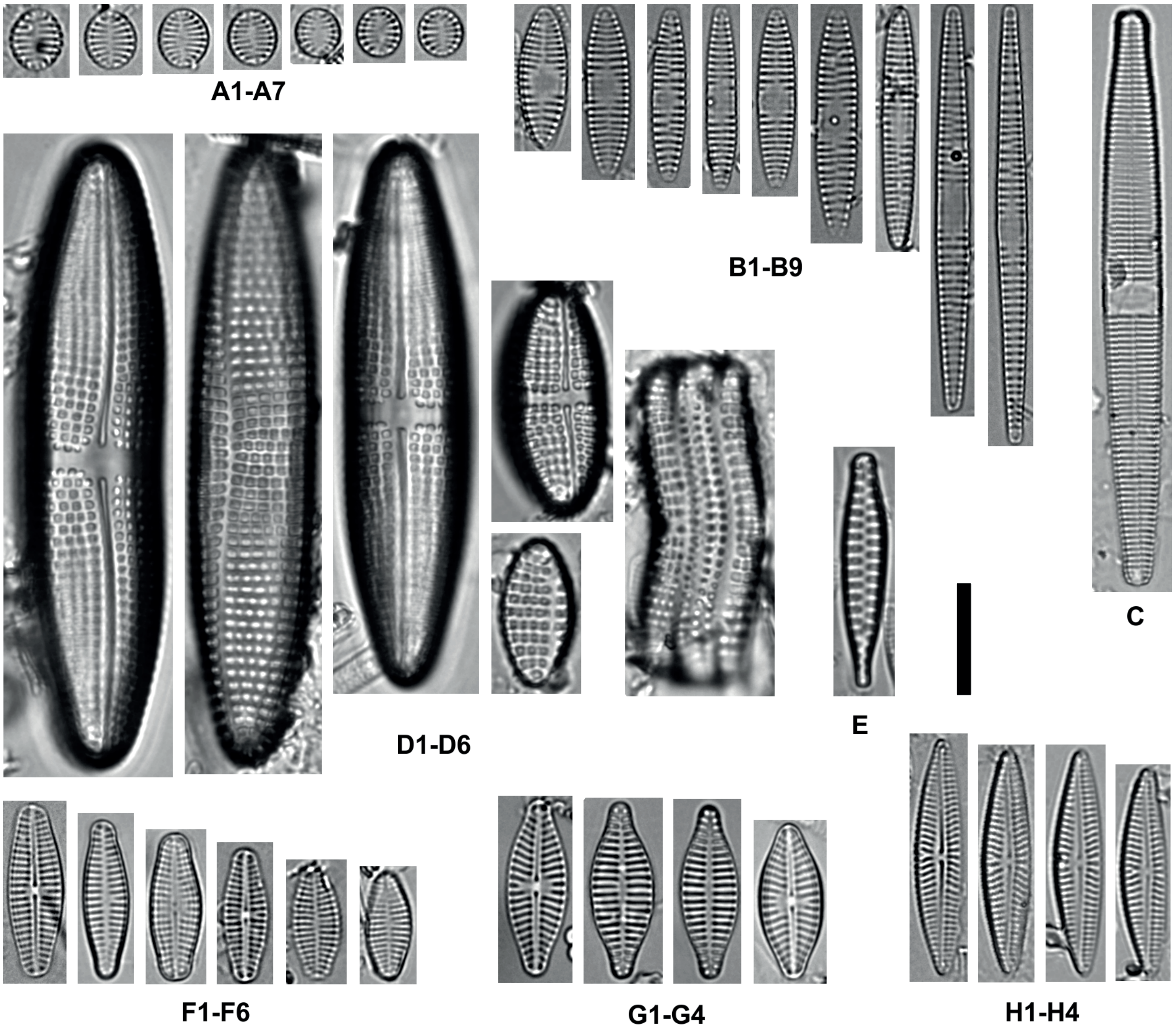

Fig. 9

A1-A7. *Fragilaria sopotensis*, B1-B9. *Fragilaria famelica*, C. *Ctenophora pulchella*, D1-D6. *Achnanthes brevipes*, E. *Opephora mutabilis*, F1-F6. *Planothidium pericavum*, G1-G4. *Planothidium delicatulum*, H1-H4. *Navicymbula pusilla*. Scale bar = 10 µm

L1-L2). Among those identified ones, some taxa apparently represented brackish water forms, e.g. *Achnanthes brevipes* Agardh (Fig. 9 D1-D6), *Amphora commutata* Grunow (Fig. 8 H1-H4), *Anomoeoneis sphaerophora* f. *sculpta* (Ehrenberg) A. Schmidt, *Denticula subtilis* Grunow (Fig. 7 H1-H5), *Diploneis interrupta* (Kützing) Cleve (Fig. 8 I1-I2), *D.* cf. *krammeri* (Fig. 7 K), *Entomoneis paludosa* (W. Smith) Reimer, *Frustulia creuzburgensis* (Krasske) Hustedt (Fig. 7 C), *Mastogloia elliptica* (Agardh) Cleve, *M. smithii* Thwaites ex W. Smith, *Navicymbula pusilla* (Grunow) Krammer (Fig. 9 H1-H4), *Navicula kefvingensis* (Ehrenberg) Kützing, *Nitzschia scalpelliformis* Grunow (Fig. 7 E), *N. sigma* (Kützing) W. Smith (Fig. 7 D1-D2), *Opephora mutabilis* (Grunow) Sabbe & Wyverman (Fig. 9 E), *Parlibellus protracta* (Grunow) Witkowski, Lange-Bertalot & Metzeltin (Fig. 7 J1-J5) and *Staurophora salina* (W. Smith) Mereschkowsky (Fig. 6 F1-F3).

A total of 129 taxa were identified in the samples collected in 1964/1965, only 79 taxa in 1992/1994, and 141 taxa in the samples collected recently (in 2013/2014) (Table 2).

Over the 50 years, the number of species at the studied sites increased from 129 to 141, and the biggest change was observed in the outflow area. The ditch was the least dynamic site in terms of the number of diatom taxa (Table 2).

The contribution of certain diatom species in assemblages significantly changed over the years. Among the dominant species, the percentage of *Craticula halophila* (Grunow) D.G. Mann (Fig. 6 A1-A9), i.e. 50% in the outflow in 1964/1965, clearly decreased in 1992/1994 and 2013/2014 (Fig. 10 A). *Ctenophora pulchella* (Ralfs ex Kützing) Williams & Round (Fig. 9 C) was present in only a few samples, however, the percentage of this species exceeded 20% (Fig. 10 B). *Fragilaria famelica* was a typical species of the outflow and the area surrounding the outflow. This species was recorded at the study sites, both in 1964/1965 (85% contribution) and in 2013/2014 (Fig. 10 C). *Fragilaria sopotensis* (33% relative abundance) was the characteristic species of the pond. It also occurred in the ditch, but with a low percentage – below 5% (Fig. 10 D). In the case of *Navicula veneta* Kützing (Fig. 7 B1-B4), the percentage of this species in the outflow and in the ditch samples gradually increased from 1964/1965 to 2013/2014 (Fig. 10 E). *Planothidium delicatulum* occurred mainly in the outflow in 1992/1994 with a relative abundance exceeding 40% (Fig. 10 F). *Chamaepinnularia krookiformis* (Krammer) Lange-Bertalot & Krammer occurred mainly in the ditch. In the samples from 1964/1965, its abundance was low, while in the samples from 2013/2014, the percentage of this species clearly increased (Fig. 11 A). *Halamphora sydowii* was recorded mainly in the outflow and in the area surrounding the outflow. During the study period, the percentage of the species gradually decreased. In 1964/1965, the contribution exceeded 10%, while in 1992/1994 it decreased below 3%. Recently the species was not recorded (Fig. 11 B). *Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin & Witkowski (Fig. 7 A1-A7) was recorded in the samples from 1964/1965 at all sites except the ditch. In 2013/2014, the percentage of the species decreased in the outflow, and increased in the pond (Fig. 11 C). *Navicula cincta* was recorded mainly in the area surrounding the outflow in the samples from 1964/1965, with the relative abundance not exceeding 10%. In 2013/2014, this species was recorded only in the pond, with relative abundance exceeding 10% (Fig. 11 D). The change in the percentage at the study sites was also evident in the case of *Navicula salinarum*. This species was recorded mainly in the outflow and in the area surrounding the outflow in 1964/1965. In 2013/2014, the percentage of *N. salinarum* decreased in the outflow and increased in the pond (Fig. 11 E). *Tabularia fasciculata* (Agardh) Williams & Round was recorded mainly in the outflow and in the area surrounding the outflow. The highest abundance of the species (28%) was recorded in the outflow samples from 1994 (Fig. 11 F).

The Bray-Curtis similarity index was used to determine the similarity between diatom assemblages in samples from different study periods, and the results are presented in Figure 12. The recently collected samples (2013/2014) were all grouped as a separate cluster (Cl. II). The samples from 1964/1965 and 1992/1994 did not reveal a clear diversification (Cl. III), with the exception of the samples collected from the pond in 1964/1965 (Cl. I). The diatom assemblages from the pond samples collected in 1964/1965 were very specific; the similarity of these samples to others was less than 15%. The separate position of samples from the pond was also confirmed by the MDS analysis, which was conducted to determine the similarity of samples based on the study sites (Fig. 13). The samples collected from the pond were a group separated from the others, while samples collected from the outflow and the ditch in 2013/2014 were very similar.

On the basis of the diatom classification with respect to the salinity, according to the seven-grade halobion system of Van der Werff and Hulls 1957-1974 (Table 3), the following taxa were most abundant in terms of the number of taxa: 2 – fresh-brackish, 3 – brackish-fresh and 4 – brackish. The least numerous were: 5 – brackish-marine, 6 – marine brackish, and 7 – marine (Fig. 14). However, taking into consideration the percentage of particular taxa at each study site, taxa from the 4th category were most abundant in the outflow, and taxa from the 3rd and the 2nd category were less abundant (Fig. 15 A); in the pond – taxa from the 2nd category were most abundant and taxa from the 3rd category were less abundant (Fig. 15 B).

Discussion

The spatial and temporal variability of diatom assemblages observed in the athalassic habitats is associated with different concentrations of chlorides and direct/indirect human impact, such as agricultural use of the surrounding area and backfilling of the depression. The differences in the similarity of diatom assemblages, observed in samples collected from the pond in all study periods,

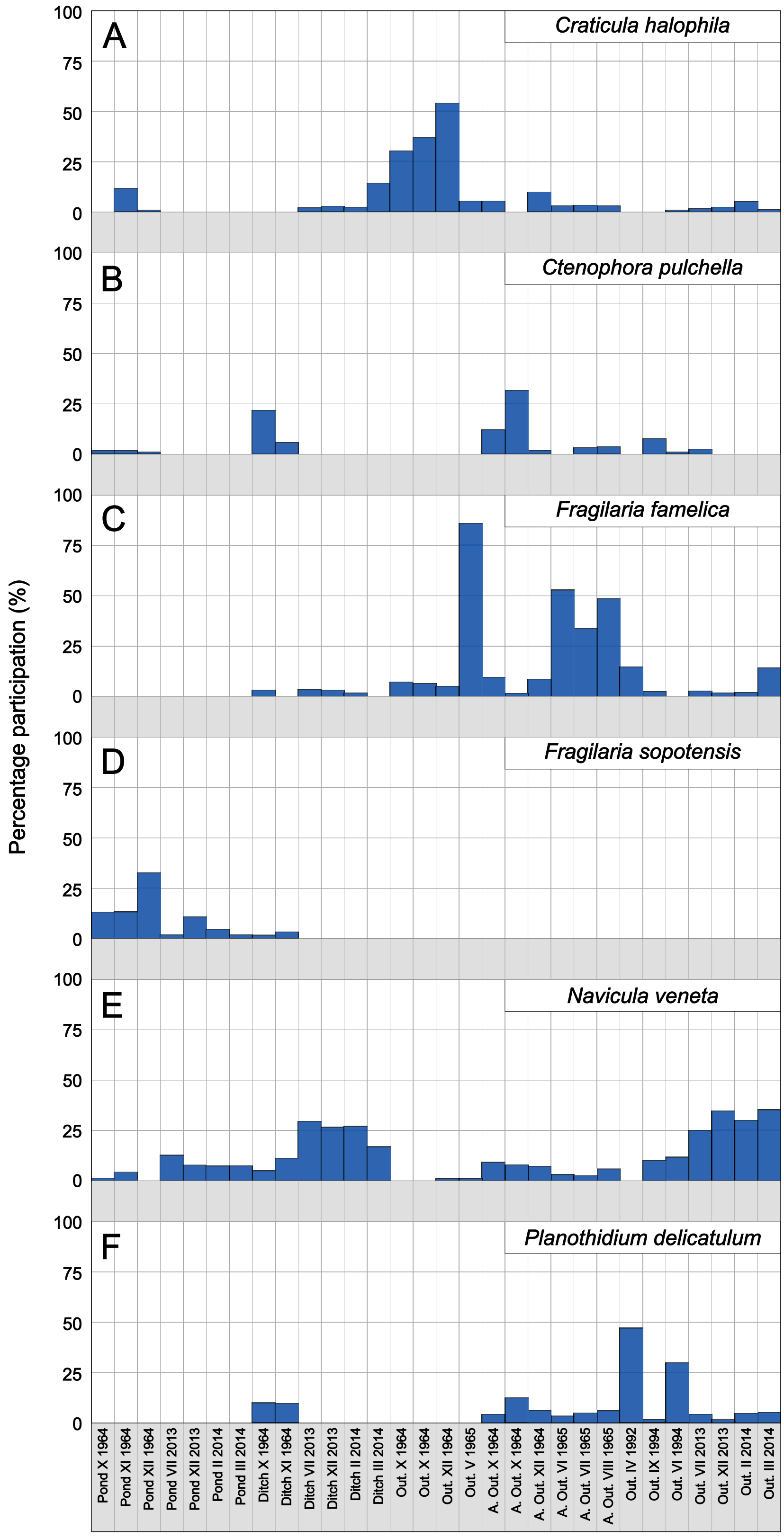

Fig. 10

The relative abundance (%) of diatom species in the studied samples

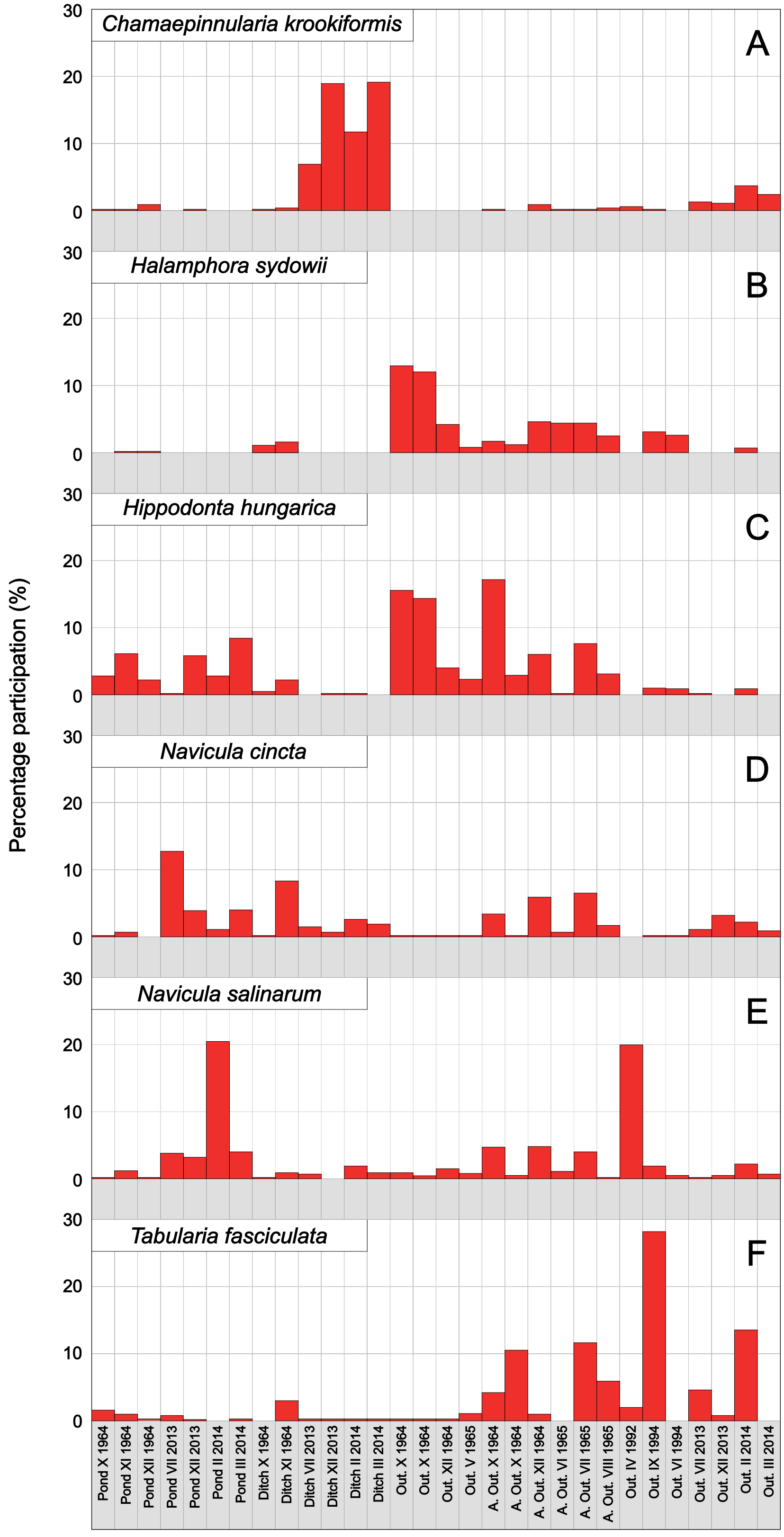

Fig. 11

The relative abundance (%) of diatom species in the studied samples

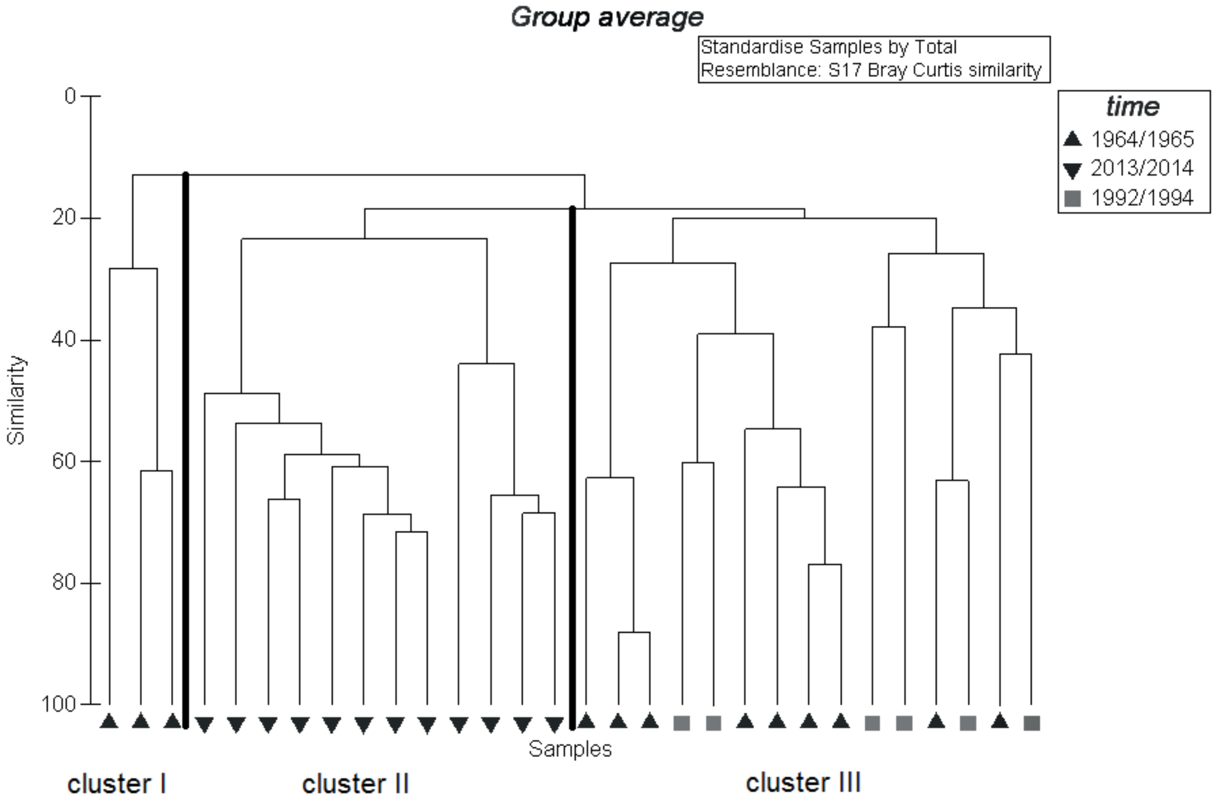

Fig. 12

Cluster analysis based on the Bray-Curtis similarity index for all diatom samples according to the study period

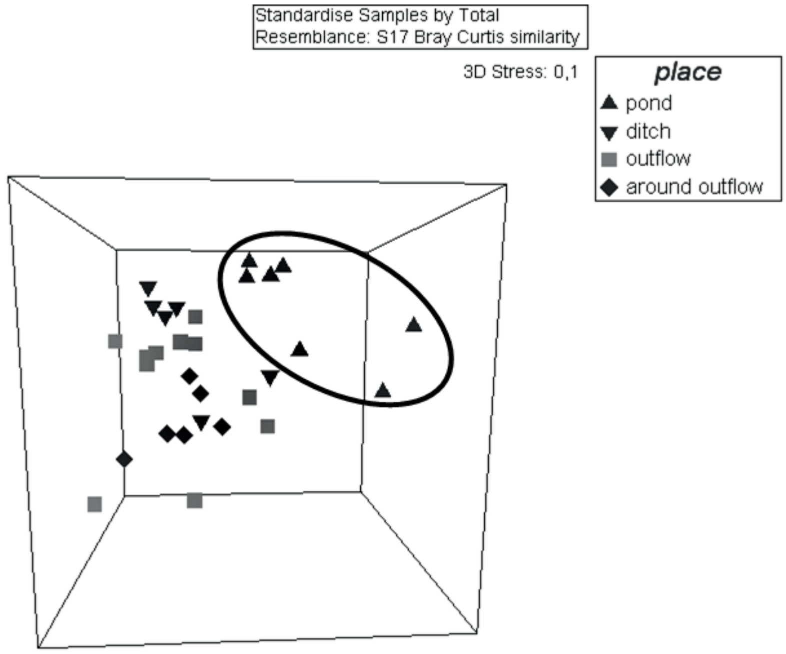

Fig. 13

MDS analysis of diatom samples according to the study sites; marked samples from the pond

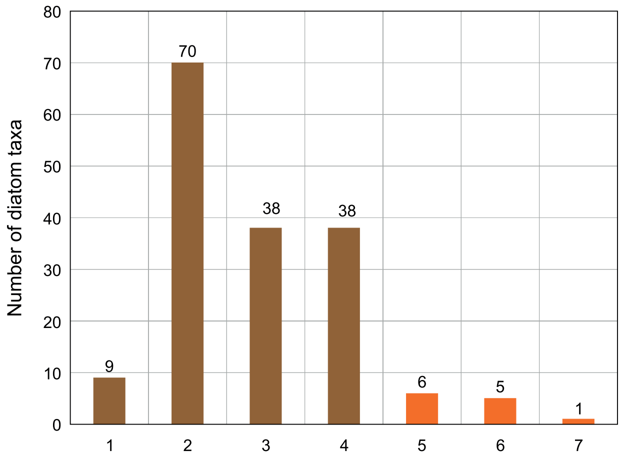

Fig. 14

The number of diatom taxa in each category of the seven-grade halobion system of Van der Werff and Huls (1957-1974) (Denys et al. 1983), with the system of Van Dam et al. 1994 taken into account (dark bars). The classification was based on the OMNIDIA 5.3 software and the references

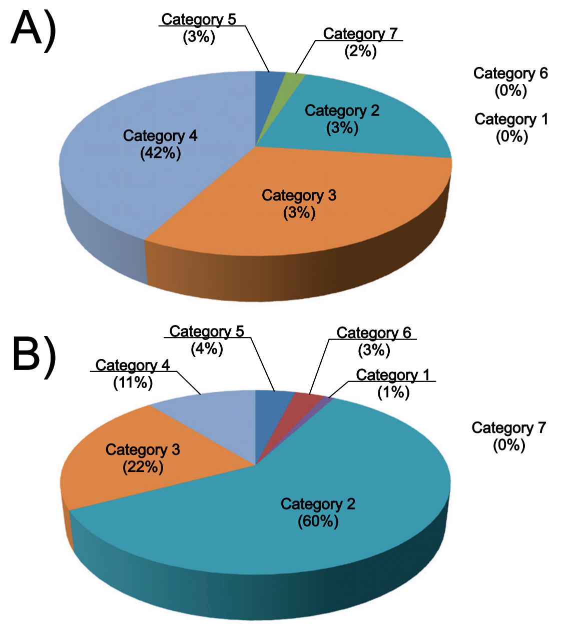

Fig. 15

Relative abundance (%) of diatom taxa in categories of the seven-grade halobion system of Van der Werff and Huls 1957-1974, A. taxa from the outflow; B. taxa from the pond

result from the lower concentration of chlorides in the pond compared with other study sites. In 2013/2014, when the outflow was backfilled with a substrate, the environmental conditions became similar in relation to salinity in the outflow and in the ditch. This caused the change in the structure of diatom assemblages, which in 2013/2014 were characterized by a high degree of similarity.

The identified diatom taxa were classified according to their tolerance to salinity, taking into consideration the isolation of the study sites from marine habitats. The halobion system of Van Dam et al. (1994), which comprises four categories of diatom taxa sensitive to salinity, and occurring in waters with the maximum concentration of chlorides – around 5000 mg l^{-1}, is commonly used for freshwater inland ecosystems. However, it was necessary to apply the halobion system of Van der Werff and Huls 1957-1974, which also includes marine species. This system includes seven categories. The 7th category contains diatom taxa which occur in waters where the concentration of chlorides exceeds 17000 mg l^{-1}. These species are defined as typical marine forms (Table 3) (Denys et al. 1983). Based on the halobion system of Van der Werff and Huls 1957-1974, *Halamphora tenerrima* belongs to the 7th category and may be classified as a marine species (Clavero et al. 2000; Levkov 2009).

Two species were classified as marine-brackish (the 6th category): *Parlibellus crucicula* (W. Smith) Witkowski, Lange-Bertalot & Metzeltin and *Staurophora salina*. Those species are widespread in the North Sea, and they also occur in the Baltic Sea (Witkowski et al. 2000). Both species rarely occurred in the outflow (samples from 1992/1994) and in the area near the outflow (samples from 1964/1965), where the concentration of chlorides ranged between 2500 and 3000 mg l^{-1}. According to the references, three additional taxa may be classified as marine-brackish: *Halamphora dominici*, *H. subsalina* (Cleve) Levkov (Levkov 2009) and *Tabularia tabulata* (Agardh) Snoeijs (Krammer, Lange-Bertalot 1991a).

Three species were classified as brackish-marine (5th category): *Achnanthes brevipes*, *Navicula perminuta* Grunow, and *Opephora mutabilis*. The first species occurred mainly in the ditch and in the pond in the samples from 1964/1965, while in the recent studies, it occurred mainly in the outflow. *N. perminuta* and *O. mutabilis* were present mainly in the samples from 1992/1994. These species occurred in habitats which, according to the concentration of chloride, may be classified as brackish (the 4th category). Moreover, three species according to the references may also be classified as brackish-marine: *Navicula digitoconvergens*, *N. hanseatica* Lange-Bert. & Stachura (Lange-Bertalot 2001) and

N. meulemansii (Mertens et al. 2014).

At the studied sites, 20 species were classified as brackish (the 4th category) and over 30 species as brackish-freshwater (the 3rd category). The changes in the concentration of chloride ions over the 50 years at the study sites could be the cause of the qualitative and the quantitative changes in the diatom assemblages (Table 1). In 1962, the chlorides in the outflow amounted to 2560 mg l^{-1} (Olaczek, unpublished PhD thesis, 1963), while in 1992/1994, it increased to 3000 mg l^{-1} (Żelazna-Wieczorek 2002), and in 2014, it greatly decreased to 1585 mg l^{-1}. In the outflow, an increase in the concentration of orthophosphate ions was also observed. Along with changes in the water chemistry, significant changes in the percentage abundance of some species were observed.

Craticula halophila, considered as a brackish species according to the halobion system of Van der Werff and Huls 1957-1974, was observed in large numbers in the outflow in the period of 1964/1965, while in 2013/2014, it occurred in small numbers. A similar pattern was observed in the case of *Fragilaria famelica* (fresh-brackish) and *Halamphora sydowii*. These species were observed mainly in the area near the outflow (1964/1965) and recently they have been sporadically recorded in the outflow. Due to the apparent disappearance of the above species from the described habitats where the concentration of chloride ions decreased, we propose a change in the classification of the halobion system of Van Dam et al. 1994 concerning these species. For *Fragilaria famelica*, we suggest a transfer from the 2nd to the 3rd category. This species was recorded *inter alia* in alkaline waters with high mineralization in Mexico (Novelo et al. 2007) and fresh to brackish waters of Spanish lakes (Antón-Garrido et al. 2013). For *Halamphora sydowii*, we recommend the 4th category. This species were also recorded in the brackish waters (Levkov 2009) and in the coastal Spanish lakes (Antón-Garrido et al. 2013).

Hippodonta hungarica is a widespread species, however, it prefers waters with high conductivity, which is typical of the study sites. The tolerance range to the chloride concentration of *H. hungarica* is rather broad (Lange-Bertalot 2001; Pavlov et al. 2013). This species was recorded in the outflow and in the area surrounding the outflow in 1964/1965. Recently, it has been recorded mainly in the pond. According to the halobion system of Van der Werff and Huls 1957-1974, this species belongs to the 2nd category, which corresponds to diatoms preferring water with a chloride concentration between 100 and 500 mg l^{-1}. In the samples from 1964/1965, however, the chloride concentration was five times higher than the upper limit of the 2nd category.

Pliński (1969) and Rakowska (1997) published information about the number of taxa identified at each study site in different periods of the study conducted in Pełczyska (Table 2). Part of the samples, collected and published by Pliński (1969), were re-analyzed. Pliński (1969) provides information about 62 taxa identified in all samples, with the highest taxonomic diversity recorded in the outflow. During the re-examination of Piliński's material, 129 taxa were identified, with the highest diversity in the area surrounding the outflow. These differences result from the methods of taxonomic investigation. Presently, the examination is based on the scanning electron microscopy. SEM allows the verification and the description of new taxonomic units among the diatom taxa.

Pliński (1969) and Rakowska (1997) identified the *Amphora coffeaeformis* Agardh (syn. *Halamphora coffeaeformis* (Agardh) Levkov), however, the current taxonomic analysis with the use of SEM did not confirm the presence of this species. Instead, *Halamphora sydowii* was recorded, which might have been previously identified as *Halamphora coffeaeformis* (Archibald, Schoeman 1984). Because of the similarity of *Halamphora tenerrima* to other taxa from the *Halamphora* genus, it could be misidentified (Clavero et al. 2000; Levkov 2009). This species was recorded mainly in marine waters (Clavero et al. 2000; Levkov 2009), inter alia in the Gulf of Mexico (Felder, Camp 2009).

In the pond, three species of the *Halamphora* genus were recorded: *Halamphora veneta*, *Halamphora paraveneta* and *Halamphora dominici*. In the previous studies, they were all identified as *Amphora veneta* Kützing (Lange-Bertalot et al. 2003; Levkov 2009). *Halamphora veneta* is widespread and is noted in many locations around the world (Sala et al. 1998; Żelazna-Wieczorek 2011; Lange-Bertalot et al. 2003; Potapova et al. 2004; Wachnicka, Gaiser 2007; Levkov 2009). Most likely in previous publications by Pliński (1969) and Rakowska (1997), the species recorded as *Amphora veneta* included also *Halamphora veneta, H. paraventa,* and *H. dominici*. *H. veneta* and *H. paraveneta*, which occur in fresh and slightly brackish waters, can be distinguished based on the valve size range (Lange-Bertalot et al. 2003). *Halamphora dominici* prefers inland saline and marine waters, and its valves are characterized by a higher density of striae (Levkov 2009)

In the studied material, *Navicula meulemansi* was recorded in the periods of 1964/1965 and 2013/2014. This species was described in 2014 by Mertens et al. 2014, and it might have been previously identified as the *Navicula* species.

Fragilaria sopotensis has a similar morphology to *Pseudostaurosira cataractarum* (Hustedt) Wetzel, Morales & Ector, and both species can be confused with each other. *P. cataractarum* has shorter striae and a wider central area (Wetzel et al. 2013). *Fragilaria sopotensis* was recorded in the Mediterranean Sea (Witkowski et al. 2000), the Baltic Sea, and in the costal lakes and marshes of the Baltic region (Witkowski, Lange-Bertalot 1993; Witkowski et al. 2000; Lutyńska 2008). During the presented research, the species occurred mainly in the pond in the period of 1964/1965, which may suggest that the species prefers low concentrations of chlorides (to 2500 mg l^{-1}). However, due to lack of data concerning the chloride ion concentration in the pond, we are not able to assess the upper limit of this factor, which would represent the maximum for the species' tolerance and occurrence in the studied habitats.

The long-term research conducted in the athalassic habitats in the village of Pełczyska, varied in terms of hydromorphology (various types of habitats) and hydrochemistry (varying range of salinity), allowed the accurate analysis of spatial and temporal changes in diatom assemblages under different salinity levels. The analysis of changes occurring over time in different types of habitats whose conditions are determined by one predominant factor, is essential for the determination of the species' requirements and tolerance range towards the environmental factor.

Acknowledgments

The authors would like to dedicate this paper to Prof. Marcin Pliński who started the phycological research in these unique habitats of Central Poland.

References

Aboal, M., Puig, M.A. & Prefasi, M. (1998). Diatom assemblages in Springs in Castellón Provence, Eastern Spain. *Algological Studies* 90: 79-95.

Antón-Garrido, B., Romo, S. & José Villena, M. (2013). Diatom species composition and indices for determining the ecological status of coastal Mediterranean Spanish lakes. *Anales del Jardín Botánico de Madrid* 70(2): 122-135. doi: 10.3989/ajbm. 2373.

Archibald, R.E.M. & Schoeman, F.R. (1984). *Amphora coffeaeformis* (Agardh) Kützing: a revision of the species under light and electron microscopy. *S.-Afr. Tydskr. Plankt.* 3(2): 83-102.

Clavero, E., Grimalt, J.O. & Hernández-Mariné, M. (2000). The fine structure of two small *Amphora* species. *A. tenerrima* Aleem & Hustedt and *A. tenuissima* Hustedt. *Diatom Research* 15(2): 195-208. DOI: 10.1080/0269249X.2000.9705495.

Denys, L., Lebbe, L., Sliggers, B. C., Spaink, G., van Strijdonck, M. & Verbruggen, C. (1983). Litho- and biostratigraphical study of quaternary deep Marine deposits of the Western Belgian coastal plain. *Bulletin de la Sociětě belge de Gĕologie* 92(2): 125-154.

Felder, D.L. & Camp, D.K. (2009). Biodiversity. In: J. W. Tunnell, D.L. Felder & A. Earle (Eds.), *Gulf of Mexico origin, Walters, and biota. Vol. 1* (pp. 1-1392). Texas: Texas A&M University Press.

Hofmann, G., Werum, M. & Lange-Bertalot, H. (2011). *Diatomeen im Süβwasser*-Benthos von *Mitteleuropa.* Ruggell: A.R.G. Gantner Verlag K.G.

Krammer, K. (1997a). Die cymbelloiden Diatomeen. Teil 1. Allgemeines und *Encyonema* Part. In: K. Krammer (Ed.), *Bibliotheca Diatomologica. Vol. 36* (pp. 1-382). Berlin – Stuttgart: J. Cramer.

Krammer, K. (1997b). Die cymbelloiden Diatomeen. Teil 2. *Encyonema* part., *Encyonopsis* and *Cymbellopsis.* In: K. Krammer (Ed), *Bibliotheca Diatomologica. Vol. 37* (pp. 1-469). Berlin – Stuttgart: J. Cramer.

Krammer, K. (2000). The genus Pinnularia. In: H. Lange-Bertalot (Ed.), *Diatoms of Europe. Vol. 1* (pp. 1-703). Ruggell: A.R.G. Gantner Verlag K.G.

Krammer, K. (2003). Cymbopleura, Delicata, Navicymbula, Gomphocymbellopsis, Afrocymbella. In: H. Lange-Bertalot (Ed.), *Diatoms of Europe. Vol. 4* (pp. 1-530). Ruggell: A.R.G. Gantner Verlag K.G.

Krammer, K. & Lange-Bertalot, H. (1986). Bacillariophyceae. Naviculaceae. In: G. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), *Süβ*wasserflora von *Mitteleuropa 2/1* (pp. 1-876). Jena: VEB Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1991a). Bacillariophyceae. Centrales, Fragilariaceae, Eunotiaceae. In: G. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), *Süβ*wasserflora von *Mitteleuropa 2/3* (pp. 1-576). Stuttgart – Jena: Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1991b). Achnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis Teil 1-4. In: G. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), *Süβ*wasserflora von *Mitteleuropa 2/4* (pp. 1-436). Stuttgart – Jena: Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1997). Bacillariophyceae. Bacillariaceae, Epithemiaceae, Surirellaceae. In: G. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), *Süβ*wasserflora von *Mitteleuropa 2/2* (pp. 1-611). Jena – Stuttgart – Lübeck – Ulm: Gustav Fischer Verlag.

Lange-Bertalot, H. (2001). Navicula sensu stricto 10 Genera Separated from Navicula sensu lato Frustulia. In: H. Lange-Bertalot (Ed.), *Diatoms of Europe. Vol. 2* (pp. 1-526). Ruggell: A.R.G. Gantner Verlag K.G.

Lange-Bertalot, H., Cavacini, P., Tagliaventi, N. & Alfinito, S. (2003). Diatoms of Sardinia. In: H. Lange-Bertalot (Ed.), *Iconographia Diatomologica. Vol. 12* (pp. 1-438). A.R.G. Königstein: Gantner Verlag K.G.

Levkov, Z. (2009). *Amphora* sensu lato. In: H. Lange-Bertalot (Ed.), *Diatoms of Europe*. *Vol. 5* (pp. 1-916). Ruggell: A.R.G. Gantner Verlag K.G.

Lutyńska, M. (2008). Environmental changes in the Dołgie Wielkie Lake in the light of diatom analysis. *Quaestiones Geographicae* 7A(1): 63-68.

Mertens, A., Witkowski, A., Lange-Bertalot ,H., Riberio, L. & Rhiel, E. (2014). *Navicula meulemansii* sp. nov., (Bacillariophyaceae) from brackish waters in Europe and the U.S.A. *Nova Hedwigia* 98(1-2): 201-212. DOI: 10.1127/0029-5035/2013/0152.

Novelo, E., Tavera, R. & Ibarra, C. (2007). Bacillariophyceae from Karstic Wetlands in México. In: H. Lange-Bertalot & P. Kociolek (Eds.), *Bibliotheca Diatomologica*. *Vol. 54* (pp. 1-136). Berlin, Stuttgart: J. CRAMER.

Olaczek, R. (1963). Zbiorowiska roślinne torfowisk niskich okolic Łęczycy. Unpublished doctoral dissertation, University of Lodz, Poland.

Owen, R.B., Renaut, R.W. & Jones, B. (2008). Geothermal diatoms: a comparative study of floras in hot spring systems of Iceland, New Zealand, and Kenya. *Hydrobiologia* 610: 175-192. DOI: 10.1007/s10750-008-9432-y.

Pavlov, A., Levkov, Z., Williams, D.M. & Edlund, M.B. (2013). Observations on *Hippodonta* (Bacillariophyceae) in selected ancient lakes. *Phytotaxa* 90(1): 1-53. DOI: 10.11646/phytotaxa.90.1.1.

Pliński, M. (1966). Glony słonego źródła w Pełczyskach pod Ozorkowem. Unpublished master dissertation, University of Lodz, Poland.

Pliński, M. (1969). Okrzemki słonego źródła w Pełczyskach pod Ozorkowem. *Zesz. Nauk. UŁ* 2(31): 123-136.

Pliński, M. (1971a). Rzadkie i nowe dla flory Polski gatunki glonów z terenów solnisk w okolicach Łęczycy. *Zesz. Nauk. UŁ* 2(41): 171-184.

Pliński, M. (1971b). Gatunki z rodzaju *Enteromorpha* (Link.) Agardh z terenu solnisk podłęczyckich. *Zesz. Nauk. UŁ.* 2(41): 159-169.

Pliński, M. (1971c). System halobów w świetle współczesnych poglądów. *Wiad. Ekol.* 17(1): 18-29.

Pliński, M. (1973). Glony solnisk podłęczyckich. The algae of salt marches near Łęczyca, *Central Poland. Mon. Botanic.39.*

Potapova, M.G., Charles, D.F., Ponder, K.C. & Winter, D.M. (2004). Quantifying species indicator values for trophic diatoms indices: a comparison of approaches. *Hydrobiologia* 517: 25-41.

Rakowska, B. (1997). Diatom communities in a salt spring at Pełczyska (Central Poland). *Biologia* 52(4): 489-493.

Sala, S.E., Sar, E.A. & Ferrario, M.E. (1998). Review of materials reported as containing *Amphora coffeaeformis* (Agardh) Kützing in Argentina. *Diatoms Research* 13(2): 323-336.

Starmach, K. (1969). Glony źródeł na wybrzeżu morskim w Chłapowie – Algae of springs on the sea coast at Chłapowo (Northern Poland). *Fragmenta Floristica et Geobotanica* 15(4): 503-511.

Van Dam, H., Mertens, A. & Sinkeldam, J. (1994). A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherlands Jurnal of Aquatic Ecology* 28(1): 117-133.

Veres, A.J., Pienitz, R. & Smol, J.P. (1995). Lake Water Salinity and Periphytic Diatom Succession in Three Subarctic Lakes, Yukon Territory, Canada. *Arctic* 48(1): 63-70.

Wachnicka, A.H. & Gaiser, E.E. (2007). Characterization of *Amphora* and *Seminavis* from south Florida, U.S.A. *Diatom Research* 22(2): 387-455. DOI: 10.1080/0269249X.2007.9705722.

Wetzel, C.E., Morales, E.A., Blanco, S. & Ector, L. (2013). *Pseudostaurosira cataractarum* com. nov. (Bacillariophyta): type analysis, ecology and Word distribution of former „centric" diatom. *Acta Nova* 6(1-2): 53-63.

Witkowski, A. & Lange-Bertalot, H. (1993). Established and New Diatom Taxa Related to *Fragilaria schulzii* Brockmann. *Limnologica* 23(1): 59-70.

Witkowski, A., Lange-Bertalot, H. & Metzeltin, D. (2000). Diatom flora of Marine costs I. In: H. Lange-Brtalot (Ed.), *Iconographia Ditomologica. Vol 7* (pp. 1-925). Ruggell: A.R.G. Gantner Verlag K.G.

Żelazna-Wieczorek, J. (1996). Studium taksonomiczne rodzaju Vaucheria De Candolle oraz uwagi o występowaniu zidentyfikowanych gatunków tego rodzaju. Unpublished doctoral dissertation, University of Lodz, Poland.

Żelazna-Wieczorek, J. (2002). *Vaucheria* species in selected regions in Poland. *Acta Societatis Botanicorum Poloniae* 71 (2): 129-139.

Żelazna-Wieczorek, J. (2011). Diatom flora in springs of Łódź Hills (Central Poland). Biodiversity, taxonomy, and temporal changes of epipsammic diatom assemblages in springs affected by human impact. In: A. Witkowski (Ed.), *Diatom Monographs*. *Vol. 13* (pp. 1-419). Ruggell, Liechtenstein: A.R.G. Gantner Verlag K.G.