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PALAEOLIMNOLOGY

An approach to the recent environmental history of Pilica Piaski spring (southern Poland) using diatoms

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Abstract Diatom floras from springs have received little attention until recently, despite the fact that springs provide specific conditions that cannot be found in any other aquatic system, and remain of great importance in terms of general environmental changes. Due to pollution of the Kraków-Częstochowska upland aquifers, the chemical composition of spring waters in the area is anthropogenically modified, and far from the natural state. In order to generate a baseline diatom flora for springs, a paleolimnological study was applied as one of the best recognized and applied methods used to track long-term environmental changes worldwide. Little is known, however, about the direction and nature of such changes in aquatic environments fed by springs. The present article focuses on shifts in diatom assemblages preserved in sediments collected from a small pond, situated close to several spring outlets. They were interpreted as a record of environmental changes that had taken place during the last century. For most of the history of the pond—as recorded in this 84 cm long core—the diatom assemblage was dominated by small *Fragilaria* spp. The major shifts in species composition began in a core depth of 65 cm with a decline in *Fragilaria construens* var. *venter* (Ehrenberg) Grunow and *Fragilaria pinnata* Ehrenberg complexes, and a concurrent increase in *Achnanthidium minutissimum* (Kützing) Czarnecki. The second change was recorded at the depth of 45 cm with a sharp decline of *A. minutissimum*, which again was replaced by the small *Fragilaria* spp. In recent years, further changes in the diatom assemblage occurred, with a notable increase in *Cyclotella delicatula* Hustedt. The assemblage shifts recorded at this site appear to be consistent with environmental changes triggered by land use (e.g. agriculture intensity) and/or possible changes in spring water discharge.

Keywords Springs · Bacillariophyta · Palaeoenvironmental research · Bioindication · Eutrophication

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Palaeolimnological Proxies as Tools of Environmental Reconstruction in Fresh Water

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Introduction

Diatoms are known to be excellent environmental indicators as they are common in aquatic environments, respond rapidly to changing conditions, and different species often have distinct environmental optima (Stoermer & Smol, 1999; Keatley et al., 2006). The composition of siliceous frustules in sediments can directly reflect the floristic composition in the past, and also indirectly reflects water characteristics such as pH, nutrient status, salinity (Battarbee, 1986), and flow velocity. Unlike many other freshwater (fossil or sub-fossil) records from lakes, mires, and ponds, data from spring sediments are very scarce (e.g. Williams & Williams, 1997; Filippi et al., 2008). Moreover, while numerous studies of freshwater environments have focused on the use of diatoms for assessing water quality parameters in streams, rivers, and lakes, these kinds of data are relatively sparse from springs (Cantonati, 2001; Battegazzore et al., 2004; Wojtal, 2006). However, interest in European diatom floras of springs has increased considerably during the last decades (e.g., Reichardt, 1981, 1994; Kadłubowska, 1985; Sabater & Roca, 1990, 1992; Rakowska, 1996; Cantonati, 1998; Werum, 2001a; Werum & Lange-Bertalot, 2004; Poulíčková et al., 2005; Cantonati et al., 2006; Żelazna-Wieczorek & Mamińska, 2006) for several reasons. Thermal stability and continuous water flow in springs are one of the most essential characteristics. Fluctuations of chemical and physical conditions in springs are minimized, thus allowing the study of interactions between limited numbers of species and environmental factors (Round, 1981; Filippi, 2007). Additionally, they remain as biodiversity refuges in anthropogenically influenced areas (Cantonati, 2001; Werum, 2001b). Their biodiversity patterns, therefore, can be applied to understand diatoms distribution, in response to geology (e.g. Cantonati, 1998; Werum, 2001a; Werum & Lange-Bertalot, 2004), water chemistry, temperature, altitude (e.g., Sabater & Roca, 1990; Cantonati et al., 2006), flow velocity (e.g., Sabater & Roca, 1990), and human impact (Wojtal, 2006). The sedimentary records from spring-fed areas are also expected to be highly sensitive indicators of environmental changes (Smol, 2002).

The springs of the Krakowsko-Częstochowska upland are threatened habitats because of significant human pressures such as agriculture and urbanization. They are very vulnerable to pollution because of the karst-fissured geological character of the area, which allows rapid flow of surface water into groundwater systems through natural conduits. Moreover, once an aquifer is contaminated, it may remain polluted for decades (Smol, 2002). One of the most severe environmental problems in the area is eutrophication of these systems caused by high levels of nitrates derived from agricultural run-off and wastes. A steady rise in nitrate levels was observed in most springs in the whole area over the period of 1974–1999 (Baścik et al., 2001). This trend has continued into the twenty-first century (Wojtal, 2006). The total water mineralization (amount of total dissolved solids) in the Pilica Piaski spring increased over 40% within last 25 years (Baścik et al., 2001).

The evaluation of anthropogenic impacts on these springs and developing an effective conservation management strategy require calibration studies and knowledge of the natural state of these freshwater ecosystems. Samples of the bottom sediments from a spring-fed pond in the Pilica Piaski were retrieved, in order to evaluate the diatom assemblages which may represent baseline conditions for springs of the Krakowsko-Częstochowska upland. This article describes changes in the diatom assemblages of the Pilica Piaski spring-fed pond, and attempts to reconstruct changes in the spring water characteristics over a period of at least one century using the bioindication potential of diatoms.

Materials and methods

The Pilica Piaski spring is situated in the Krakowsko-Częstochowska upland, in southern Poland (50°28′N and 19°41′E), at an elevation of about 264 m a.s.l. The water-bearing layer consists of Jurassic (Malm) marl and platy limestone which yields over 40 l per second (Baścik et al., 2001). Most of the Krakowsko-Częstochowska upland springs belong to the HCO_3 -Ca category. Water temperature, conductivity, and pH were measured using an ELMETRON CC-101 and CP-103 conductivity and pH meters, respectively. Other parameters were determined using ion chromatography (Dionex 100), atomic absorption spectrophotometry, and flame AAS techniques. The physical and chemical characteristics of the water in the Pilica Piaski spring-fed pond is given in the analysis of July 2005, as (outflow): temperature—9.5°C, conductivity—372 μS cm^{-1}; pH—7.2; oxygen—9.2 mg l^{-1}, Cl^{-}—13.1 mg l^{-1}; NO_3^{-}—28.4 mg l^{-1}, PO_4^{3-}—<0.0,000 mg l^{-1}; SO_4^{2-}—21.4 mg l^{-1}; alkalinity—3.2 mval l^{-1}; Ca^{2+}—61.3 mg l^{-1}; Na^{+}—5.1 mg l^{-1};

K^+—2.4 mg l^{-1}, whereas in the spring-fed pond temperature was c.a. 20°C, and conductivity amounted to 540 μS cm^{-1}.

The material was retrieved from a shallow pond (40–80 cm in depth) which is fed by several, small outlets, situated at the foot of a steep 6–8 m high scarp, and a few meters from the sampling site. The bottom of several small outlets was imbricated with limestone gravel, while the pond bottom was covered by thick layer of mud, due to the low-flow velocity. The sampling site is isolated from the surrounding area by the scarp and swampy zone, of a few meters in width.

In July 2005, during a very hot summer period, when daytime air temperature exceeded 30°C, an 84 cm sediment core was taken at depth ca. 0.8 m, a few meters from the outflows of the spring using a Mondsee-gravity corer with a diameter of 60 mm. The core was divided lengthwise into two halves, one of which was used for diatom analysis, while the other was stored. The 1 cm^3 samples for diatom analysis were taken at 1-cm intervals immediately after retrieval. The core was predominantly homogenous brown-grey clayey mud, turning dark brown in the uppermost part due to a large amount of organic matter. The remains of terrestrial vascular plants (leaves and stem fragments) observed from the depth of 56 cm can originate from vegetation surrounding the pond. A small addition of fine grained sand was observed at 64–62 cm and 45–44 cm. Coarse sand occurred at 58–46 cm.

Diatom samples were analyzed every 5 cm. In order to reconstruct the environmental conditions during the deposition of the sediments studied, diatoms were grouped according to their environmental requirements. The diatom assemblage zones (DAZ) were identified using TILIA (software written by E.C. Grimm, Illinois State Museum, 1995) dendrograms which utilized the sum of least squares after being transformed with square root. Shannon-Weaver diversity was calculated as follows:

$$H' = -\sum_{i=1}^{s} \frac{n_i}{n} \log_2 \frac{n_i}{n},$$

where n_i = number of individual diatoms of the species i, n = total number of individual counts, $\frac{n_i}{n}$ = relative abundance of the species i.

The ^{14}C dating was performed at the Poznań Radiocarbon Laboratory. The samples for diatom analysis were treated with 10% HCl, washed several times with distilled water, and thereafter boiled in concentrated acids (H_2SO_4, HNO_3), in order to remove organic matter. After washing several times with distilled water, the cleaned diatom material was air-dried on coverslips and mounted in Naphrax®. Observations of the diatoms were performed with a Nikon Optiphot microscope equipped with a Plan ×100 oil-immersion lens and differential interference contrast (DIC) optics. The identification of diatoms was based mainly on the following literature: Krammer & Lange-Bertalot, 1986, 1988, 1991a, 1991b; Lange-Bertalot & Metzeltin, 1996; Krammer, 1997a, 1997b, 2000, 2002, 2003; Lange-Bertalot, 1993, 2001; Lange-Bertalot et al., 2003, and numerous other more specific taxonomic publications. The relative abundance of small *Fragilaria* spp., mainly *Fragilaria construens* var. *venter* and *Fragilaria pinnata* was aggregated in diatom analysis, to avoid possible identification errors in LM, particularly during counting, when frustules in girdle view and of very small valve size made precise identification very difficult if not impossible.

Results

The accumulation of sediments, based on ^{14}C dating of the remains of terrestrial vascular plants, from a depth of 38 and 10 cm, was estimated as covering approximately 50 years (107.85 ± 0.35 and 106.58 ± 0.34 pMC, respectively). The lower part of the core consisted of more compact and pale sediments, whereas more recent sediments were darker, with the inclusion of remains of vascular plants. No laminations were observed.

The diatom analysis of the PIL1 core was based on 18 samples. A total of 136 diatom taxa were identified (11 Centrales and 125 Pennales), but only seven were considered common (i.e., present with a relative abundance exceeding 10% in at least one core depth interval). The following taxa were included in this group: *Fragilaria construens* var. *venter*, *F. pinnata*, *F. capucina* var. *capucina* Desmazières, *F. gracilis* Østrup, *F. capucina* var. *rumpens* (Kützing) Lange-Bertalot, *F. capucina* var. *vaucheriae* (Kützing) Lange-Bertalot, and *Achnanthidium minutissimum*. Other taxa with an abundance of ca. 5% were *Planothidium lanceolatum* (Brébisson) Round and

Bukhtiyarova, *Planothidium frequentissimum* (Lange-Bertalot) Round and Bukhtiyarova, *Cyclotella delicatula* Hustedt, *Gomphonema pumilum* (Grunow) Reichardt and Lange-Bertalot (sensu lato), and *Fragilaria ulna* var. *acus* (Kützing) Lange-Bertalot.

The dominant taxa were considered as tychoplanktonic of periphytic origin with respect to habitat, including e.g., *Fragilaria capucina* var. *capucina*. However, at the depth interval of 84–50 cm, the dominant taxa were periphytic forms including *A. minutissimum*. The flora was dominated by alkaliphilous and circumneutral diatoms. The analysis of salinity tolerances showed a strong dominance of fresh–brackishwater taxa including *F. construens* var. *venter* and *F. pinnata* complexes (Figs. 1, 2). It is notable that there are very few freshwater forms, and that those that are present prefer high levels of dissolved minerals in the water. The analysis of trophic tolerances showed a dominance by meso-eutraphentic taxa in the core depth of 85–65 cm. Their content decreases in successive interval as they are replaced by oligo- to eutraphentic (eurytraphentic) taxa. Finally, their relative abundance increases again, up to 97.4% from the core depth of 45 cm.

Changes in diatom species composition, and hence in proportions between particular diatom ecological groups indicate the presence of two local diatom assemblage zones (DAZ I–DAZ II) in the core. Additional division of DAZ I into two sub-zones, namely DAZ I A–B and DAZ II into three subzones, namely DAZ II A–C is possible. Particular zones and sub-zones were distinguished based on the following environmental variables: changes in habitat categories, water mineralization (salinity), pH, and trophic level. The dominants in the core included *F. capucina*, *A. minutissimum*, *P. frequentissimum*, *P. lanceolatum*, and unresolved small *Fragilaria* spp., mainly *F. construens* var. *venter* and *F. pinnata* complexes.

DAZ I A

Two subzones were distinguished within DAZ I. Sub zone DAZ I-A includes the depth interval of 85–65 cm. Tychoplanktonic forms which are taxa of periphytic origin, with respect to habitat were dominant in this subzone. Their abundance decreases from 76.6% at 84 cm to 16.4% at 65 cm. The only exception in this case is sample at a depth of 75 cm, in which they reach 72.9%. On the contrary, the abundance of periphytic taxa is increasing in this interval, however, at depth of 75 cm, they decrease to 17%. Alkaliphilous forms are dominant. However, their relative abundance decreases upward in the core as they are replaced by circumneutral forms. Analysis of trophic preferences showed an increasing abundance of taxa with a very broad tolerance spectrum covering oligo- to eutrophic waters. Their content increases from 25% to 67.2% upward the core. A strong decrease is observed in meso-eutraphentic taxa at a depth of 65 cm (from 71.5% at 84 cm to 9.5% at 65 cm).

DAZ I B

Subzone DAZ I B spans a depth range of 65 to 45 cm. The habitat category is dominated by periphytic taxa. Their content (ca. 50%) does not show any distinct changes in the whole sub-zone. However, an increase is observed in tychoplankton of periphytic origin. These reach 16.4% at the lower limit of DAZ I B and increase to 95.7% in the upper limit of the sub-zone. In terms of pH characteristics, the circumneutral taxa are dominant, and their content ranges between 50% and 72%.

A strong decrease in the abundance of meso-eutraphentic taxa was observed compared with the preceding subzone. These reach a maximum relative abundance of 23.8% at a core depth of 50 cm. Taxa with very broad tolerance spectra, inhabiting oligo-up to eutrophic waters are dominant. However, their content decreases towards the sub-zone upper limit. Mesotraphentic and eutraphentic taxa reach higher abundances in this sub-zone than in the preceding one.

DAZ II

In DAZ II, three sub-zones were distinguished.

DAZ II A

Sub-zone DAZ II A includes the sediment interval from 45 to 25 cm. There is a strong increase in tychoplaktonic taxa of periphytic origin compared to DAZ I. Their content decreases, however, towards the sub-zone upper limit from 97.7% at 45 cm to

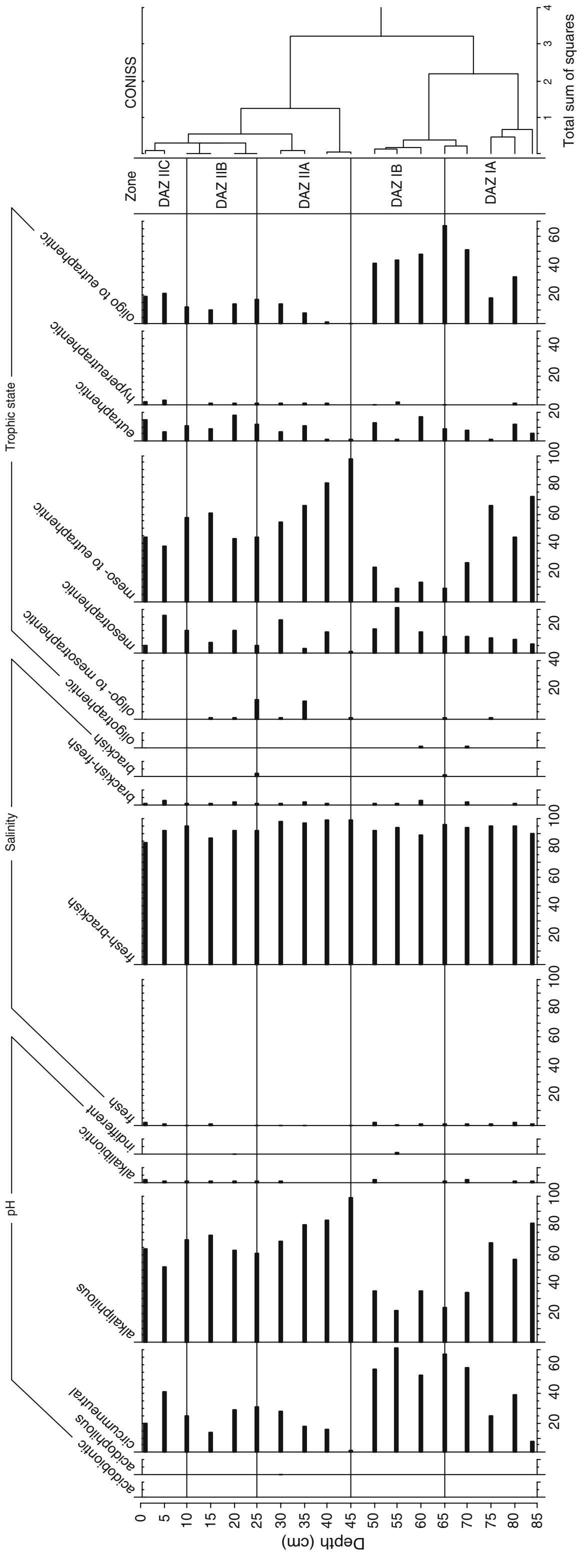

Fig. 1 Stratigraphic distribution of diatom ecological groups in core PIL-1

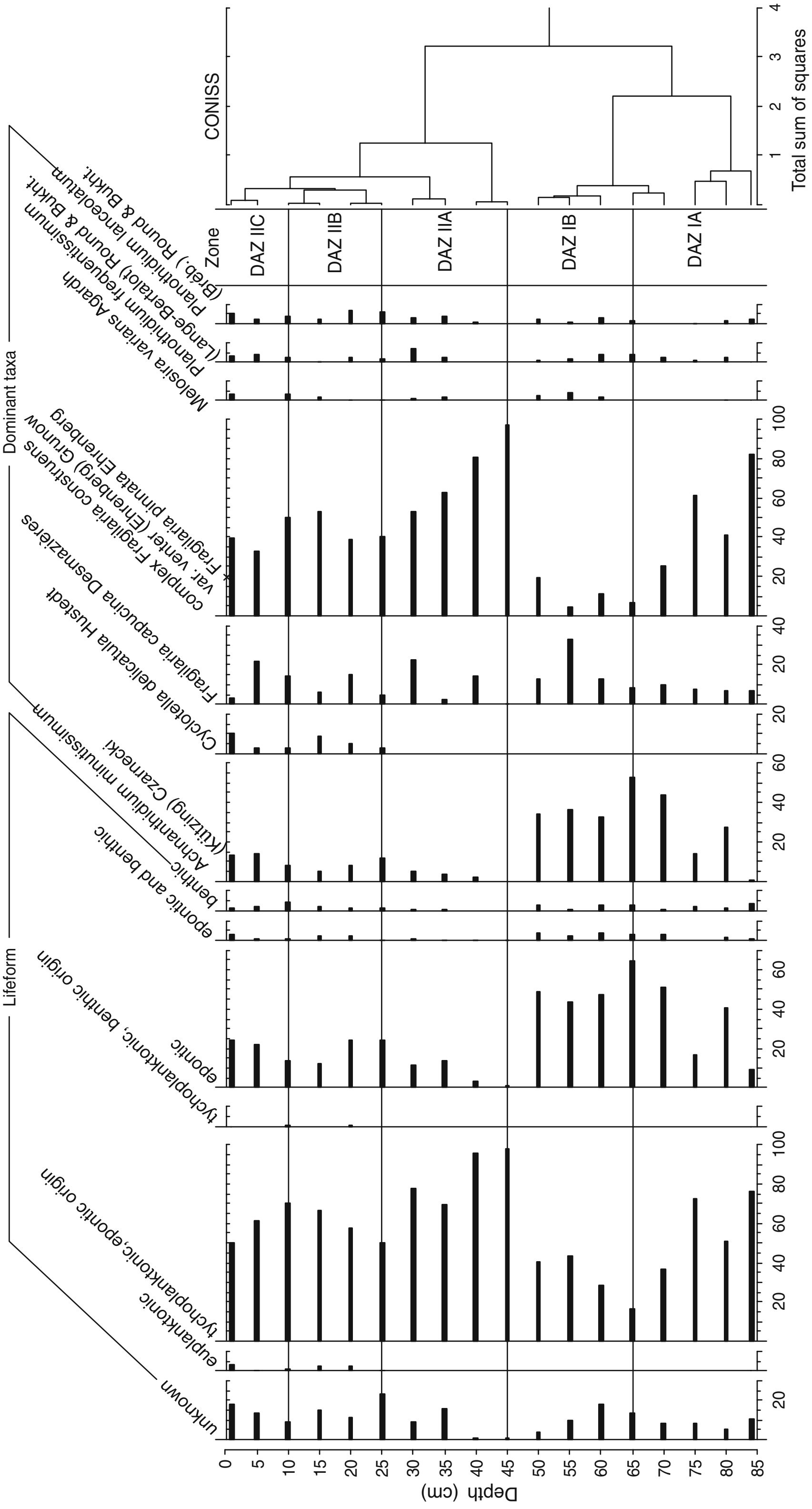

Fig. 2 Stratigraphic distribution of the most abundant taxa and of some ecological groups in core PIL-1

50.3% at 25 cm. The analysis of pH preferences showed strong differences in abundance of particular groups in comparison to DAZ I. Alkaliphilous taxa are dominant. These reach relative abundance of 98.8% at 45 cm and show gradual decrease to 60.9% at 25 cm. The minimum (0.9%) abundance of circumneutral forms occurs in this zone, however, their content increases to 31.6% at 25 cm. Despite this increase, their content is much lower than in DAZ I A and DAZ I B. Meso-eutraphentic forms are dominant among trophic groups. They reach the highest relative abundance for the whole core in this sub-zone. Their abundance decreases, however, gradually from 97.4% at 45 cm to 44.2% at 25 cm. Meso- and eutraphentic taxa remain relatively stable between 10% and 20% in this interval. The relative abundance of taxa with a very broad range of trophic tolerance (oligo- to eutraphentic) increases from 1.7% at 45 cm to 16.7% at 25 cm.

DAZ II B

Sub-zone DAZ II B encompasses a core depth range of 25–10 cm. The habitat category is dominated by the increasing abundance of tychoplanktonic taxa of periphytic origin, whereas the abundance of periphytic (epontic) taxa decreases. Truly planktonic diatoms (e.g., *Asterionella formosa* Hassall and *Aulacoseira ambigua* (Grunow) Simonsen) were represented by single valves).

The relative abundance of alkaliphilous diatoms was stable and reached ca. 70%, when compared with circumneutral forms which decrease. Diatom assemblages are dominated by freshwater–brackish forms, while solitary valves of brackish water species appear at a core depth of 25 cm.

Meso-eutraphentic forms increase in abundance from 44.2% at 25 cm to 57.1% at 15 cm. In addition, mesotraphentic, eutraphentic, and oligo-eutraphentic forms appear in low numbers (maximum up to 16%).

DAZ II C

Sub-zone DAZ II C includes the uppermost part (10–1 cm) of the core. Tychoplanktonic forms of periphytic origin are present in smaller numbers. They decrease from 70.6% at 10 cm to 50% at 1 cm. Periphytic forms increase, however, towards their maximum abundance in the core. True planktonic taxa appear in this sub-zone, and these are primarily represented by *C. delicatula* Hustedt.

Alkaliphilous forms increase in abundance compared to DAZ II B. The number of *A. minutissimum* increases. Fresh-brackish water forms are dominant amongst halobous groups, while brackish–freshwater forms (ca. 3%) appear at a core depth of 5 cm. Meso-eutraphentic species are the dominant trophic form. Their content decreases from 57.1% in the core lower limit to 43.9% in the core uppermost sample. In addition, mesotraphentic, eutraphentic, and oligo- to eutraphentic forms also occur and all reach rather similar abundance.

Discussion

The Krakowsko-Częstochowska upland springs are fed by the largest aquifers in Poland. Extensive human modification (e.g., pollution, increasing water consumption, coal mining, melioration works, and agricultural practices) have diminished the water resources of this system and indirectly damaged and reduced the number of springs supplied by them. The major threat to the springs of the Krakowsko-Częstochowska upland is eutrophication originating from unsustainable agricultural practices and improper wastewater management. The most threatened springs are situated in the area covered by sands, where the pollutants can easily percolate into the groundwater. The most important threat to water quality in the area are nitrates, as their concentration exceeds maximal allowable limits for drinking waters in Poland (Dojlido, 1987).

The absence of orthophosphate ions at detectable concentrations, by means of applied methods, can be related to biological consumption and long-term burial of phosphorus (Dodds, 2003), as precipitation of insoluble Ca-phosphates (Ca–P) is the dominant phosphorous transformation at pH greater than 7.0 (Vymazal, 1995). Fluctuations of the groundwater table influence the total ion content, including that of calcium, due to the retention time in conduits connecting the aquifer and its springs. Additional anthropogenic impact (e.g., agricultural modification, pollution) can enhance the rate of the changes in hydrochemical background.

Despite the fact that springs are the places where groundwater outflows, rarely, as in the case of Pilica

Piaski, there are spring-fed ponds which allow for sediments accumulation. The results of ^{14}C dating of terrestrial vascular plants from the depth of 38 and 10 cm did not provide precise chronologies for that part of the core, whose age could be estimated for approximately 50 years. Supposing that even high sedimentation rates the age of "bottom", samples could represent at least 100-year old diatom assemblage remains. The application of more precise dating methods might furnish with better data.

Sediment samples represent an integrated sample from all habitats within the pond and outlets supplying it, as well as an integration of several seasons of sediment accumulation. The lack of lamination within the core is presumably related to very few seasonal changes in springs due to their thermal stability. The absence of laminae may also be due to bioturbation or sediment resuspension by the wind.

The diatom flora from the Pilica Piaski presents an assemblage with relatively high diversity (H' up to 4.06), which is dominated by circumneutral and/or alkaliphilous, fresh–brackishwater, meso-eutraphentic, and oligo- to eutraphentic taxa that are common constituents of the area limnocrens (Rakowska, 1996; Wojtal, 2006). Calculated H' values were generally similar to the reported from springs of the southern, calcareous part of Pyrenees, with slow-moving to sometimes fast waters at the outflows, and high ionic strength (Sabater & Roca, 1990), but higher than in epilithic diatom assemblages in springs of the Krakowsko-Częstochowska upland (Wojtal, 2006). The occurrence of several diatom species clearly corresponds to diatom assemblages in springs of carbonate-rich water (e.g., Sabater & Roca, 1992; Cantonati, 1998; Werum, 2001a; Werum & Lange-Bertalot, 2004; Cantonati et al., 2006), on the other hand, the lack of such common diatoms as *Diatoma mesodon* (Ehrenberg) Kützing, *Karayevia clevei* (Grunow) Round and Bukhtiyarova, or very scarce species such as *Achnanthidium pyrenaicum* Hustedt is probably related to their preferences of fast flowing water. The composition of diatom assemblages from the Pilica Piaski spring-fed pond is the most similar to the one reported from Madonina Val Lomasona in Italy (Filippi et al., 2008), probably due to most analogous environmental conditions.

The composition of diatom assemblages changed dramatically at depths of 65 and 45 cm (Figs. 1, 2). A marked shift in species composition was recorded at this time, but it is likely that the diatom assemblage began to change even earlier (70 cm and 50 cm). Potential causes contributing to the recent shift in diatom assemblages include changes in water discharge, anthropogenic eutrophication, artificial nitrogen deposition, and/or changes of alkalinity. The relatively stable abundance of fresh–brackishwater diatoms for most of the spring-fed pond's history could be related to the natural ion status of the spring. Changes in diatom tolerances from circumneutral to alkaliphilous may suggest increased natural calcium leaching from the bedrock, but is more likely related to or accompanied by changes in the general nutrient status. The most distinctive shift in diatom composition, from diatoms tolerating broad range of trophic levels (DAZ I) towards meso-eutraphentic (DAZ II) suggests an increase of nutrient concentration. The changes in the composition of the diatom assemblages between DAZ IA and DAZ IB can be related to an increase in discharge as an addition of sand was observed in samples at 64–46 cm core depth, and was accompanied by a shift of alkaliphilous, benthic diatoms, e.g., *F. construens* var. *venter* and *F. pinnata* into circumneutral tychoplanktonic taxa of periphytic (epontic) origin, e.g., *A. minutissimum*.

Anthropogenic nutrient deposition (Baścik et al., 2001) may be a factor driving diatom assemblage change in the most recent Pilica Piaski sediments (beginning from the depth of 25 cm). The increase of centric diatoms [*C. delicatula* Hustedt, *Puncticulata radiosa* (Lemmermann) Håkansson, *Discostella pseudostelligera* (Hustedt) Houk & Klee, *Skeletonema* sp., *Stephanodiscus hantzschii* Grunow, and *S. neoastraea* Håkansson and Hickel] in the topmost part of the core could reflect increasing eutrophication.

Unfortunately, the most abundant diatoms, *F. construens* var. *venter* and *F. pinnata* complexes are also an important component of diatom assemblages found in a wide range of aquatic environments including freshwater (e.g., Bennion et al., 2001; Rühland et al., 2003; Schmidt et al., 2004, brackish–fresh and brackish waters (e.g., Witkowski, 1994; Witkowski et al., 2000); remain understudied. According to Lowe (1996), *F. construens* var. *venter* is one of the most important diatoms in waters of low light, low turbulence, and narrow temperature range, conditions that correspond well with those in Pilica Piaski locality. According to Tuchman (1996), *F. construens* possess the ability to resume photosynthetic activity upon illumination, after being buried below the photic zone in shallow marine sediments for an extended period of time. Small *Fragilaria* spp. were also regarded as poor indicators for the trophic status as they are distributed along the whole length of TP gradient in the northwest European training set (Bennion et al., 2001). Their distribution in a wide range of aquatic environments can be, however, caused by difficulties in precise identification due to the need to resolve their diagnostic characters with a scanning electron microscope (Morales, 2002), and consequently the autecology of this group is only superficially understood. Another important constituent, *A. minutissimum*, is one of the most important species in the calcareous part of Pyrenees where it is found in slow moving to sometimes fast water at spring sources (Sabater & Roca, 1990). It is identified as a resource specialist, that is, a species capable of securing resources that are present at concentrations limited to sympatric species within lentic periphyton communities, and as a phosphorus specialist (Lowe, 1996). Also autecology of other important species, such as *C. delicatula* is very poorly known (Wojtal & Kwandrans, 2006).

Conclusions

Diatom assemblages have changed markedly within twentieth century in the Pilica Piaski spring-fed pond, indicating possible changes in pH, conductivity, and nutrient levels. Changes in current velocity could be regarded as one of the most important factors driving shifts in the diatom assemblage structure. The direction of environmental change would be confirmed by more detailed diatom analysis, especially of small *Fragilaria* representatives, ^{210}Pb dating, and chemical sediments analyses. Additional paleolimnological studies in this region will help to refine and improve future interpretations.

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