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Investigations on benthic diatoms in the Danube River in relation to environmental variables

– Doctoral thesis –

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I. Introduction and objectives

The Danube provides drinking water for millions of people. It is also of major importance for economic (hydroelectric power generation, shipping), flood protection, food (fisheries), tourism, nature conservation (e.g. biodiversity protection) (Sommerwerk et al. 2022). The importance of the Danube is reflected in the fact that a number of international conventions, research and monitoring programmes have been established to protect the river and its catchment area (e.g. Joint Danube Survey, AquaTerra Danube Survey, TransNational Monitoring Network, Modelling Nutrient Emissions in River Systems), which are coordinated by the ICPDR (International Commission for the Protection of the Danube River), which has been operational since 1998 (https://www.icpdr.org/main/).

Due to the complex processes that take place in large rivers (Stojković 2017), understanding and quantifying the relationships between their biota, including the periphytic diatoms, and the river environment can be difficult and requires multifaceted studies (Segurado et al. 2018).

Diatoms are important members of aquatic ecosystems (Kelly et al. 2008, Burliga and Kociolek 2016). They are important for biomass production (Ertl and Tomajka 1973, Rosemarin 1975, Falkowski et al. 1998, Field et al. 1998, Poulíčková 2008) and are thus key components of food webs (Allan and Castillo 2007, Turner and Edwards 2012).

Their resistant, silica-rich frustules are well-structured, which forms the basis of their morphological segregation. Benthic diatoms are widespread, often achieving strong dominance in the biofilm. Due to their fixed life history, they are exposed to environmental stress over a long period of time; they respond to changes in environmental factors and stress by changing their species composition and abundance, and are thus able to provide long-term indicators of changes in the ecological status of water bodies, i.e. they are good bioindicators. Furthermore, they provide a well-defined concept of the individual and are easy to make long-lasting preparations. For these reasons, coated diatoms are suitable organisms for water qualification (Economou-Amilli 1980, Heinonen 1984, Stevenson 1984, Watanabe et al. 1988, Makovinská and Hlúbiková 2014). As a result, in 2000, the European Union, as part of the Water Framework Directive, included benthic diatoms, along with four other groups of organisms, in the list of organisms that are required to be monitored regularly in surface waters, with the aim of achieving good ecological status/potential for surface waters (European Union 2000, Cüneyt and Ács 2011, Ács et al. 2015a).

Various indicators were developed to describe the water quality of surface waters during the status assessment. These indices characterise the sample in terms of different factors (e.g. organic pollution, plant nutrient supply, salinity, pH) based on the species composition of diatom assemblages and the relative abundance of taxa (Ács et al. 2015b). In practice, this type of water quality assessment (i.e. when a water quality indicator is calculated to characterise the ecological status of surface waters based on the pollution sensitivity of individual taxa and their relative abundance) has been widely used in surface water status assessment (Ector et al. 2002, Kelly et al. 2008, Makovinská and Hlúbiková 2014, Ács et al. 2015b). Benthic diatoms are almost continuously present in the Danube with variable abundance, which provides an opportunity for systematic monitoring of the river. The water quality indicator used for the Danube, the so-called 'Pollution Sensitivity Index', the IPS (Indice de Pollusensibilité Spécifique) (Cemagref 1982, Lecointe et al. 1993, Makovinská and Hlúbiková 2014, Ács et al. 2015b), whose hydromorphological stressor dependence was not confirmed when I start of my investigations (the IPS index was primarily developed to detect nutrient loading).

Diatom species can be characterized not only by their sensitivity to pollution, but also by their functional traits, which are also stressor- and disturbance-dependent (Tapolczai et al. 2016). Traits are morphological, physiological or phenological traits that can be measured at the individual, cellular and organismal level and do not require knowledge of environmental conditions or other levels of organization (Violle et al. 2007). Over the last decade or more,

increasing attention has been focused on traits as possible future (complementary) tools for ecological status assessment (Berthon et al. 2011, Rimet and Bouchez 2012). Among them, diatom ecological groups (formerly called 'guilds') have been the focus of this work (Passy 2007, Rimet and Bouchez 2012), and therefore my fellow researchers and I have focused more on the analysis of the applicability of this trait in ecological status assessment in my PhD thesis. The determination of diatom species by light microscopy is sometimes challenging for algae scientists. According to the literature, the usefulness of this method would be increased by the fact that it would not require much taxonomic knowledge, it would be sufficient to classify diatoms only at the genus level (Berthon et al. 2011). Later on, we extended these studies to the investigation of several traits that are mainly related to physiology (e.g. those proposed by van Dam (1994), but manifested at the species level), the comparison of which with the IPS-based method is also presented in my thesis. Furthermore, our studies over several years, mainly on the benthic diatoms of the Danube River, have allowed us to observe the invasion phenomenon of diatoms, which may ultimately be linked to human activities (Richardson 2008) or to global warming (Duleba et al. 2014, Spaulding et al. 2010).
Later on, we extended these studies to the investigation of several traits that are mainly related to physiology (e.g. those proposed by van Dam (1994), but manifested at the species level), the comparison of which with the IPS-based method is also presented in my thesis. Furthermore, our studies over several years, mainly on the benthic diatoms of the Danube River, have allowed us to observe the invasion phenomenon of diatoms, which may ultimately be linked to human activities (Richardson 2008) or to global warming (Duleba et al. 2014, Spaulding et al. 2010).

I have built my work around the following three broad questions, with the following objectives:

I.1 Comparison of the commonly used diatom index (IPS) in ecological status assessment for the Danube River and diatom ecological groups (mid-term collection from the drift line, cross-section sampling, surface sampling of mussel shells)
Exploring the relationships between diatom ecological groups and nutrient content in the Danube.
Exploring the relationships between diatom ecological groups and hydromorphological parameters in the Danube.
Developing our own position for large rivers on the idea, repeatedly put forward in the literature, that for the phytobenthos group of organisms, trait-based approach can be a substitute for, or at least an important complement to, species-based ecological status assessment.

Accordingly, the following hypotheses were tested:
(i) we expected a significant positive correlation between the motile and high profile diatom ecological group and nutrients, however we expected a negative correlation with the low profile group, as described in the literature;
ii) a significant negative correlation between the motility, high profile and IPS, but a significant positive correlation between the low profile and IPS;
iii) a significant negative correlation between high profile group and water discharge and a significant positive correlation between water discharge and low profile.

I.2 Impact of Budapest on the Danube benthic diatom community
To show the differences between upstream and downstream to Budapest in terms of ecological status and physico-chemical variables that can be estimated by studying the benthic diatom community.
Detect changes in the species composition and functional traits of the diatom community (including diatom ecological groups) as a result of urban pressures.

Accordingly, the following hypotheses were tested:
i) a significant difference in the values of physico-chemical variables was expected below and above Budapest;
ii) based on the benthic diatom community, the water quality of the Danube upstream to Budapest is worse than downstream and this is reflected in the functional traits in addition to the species composition.

I.3 Rapid spread of an invasive diatom species, *Achnanthidium delmontii* Pérès, Le Cohu & Barthès 2012 in the Danube and its tributaries

We have endeavoured to provide a detailed taxonomic description of the species in question in order to facilitate as accurate a determination as possible.
The occurrence and distribution of the species in the Danube basin (especially in the Danube and in Hungarian watercourses) will be investigated.
By analysing 18 environmental variables and taking into account associated species, we characterise the autecological properties of the species, and provide the species' optimum and tolerance value limits.
The Site-specific Biological Contamination invasion index (SBC index) is used to estimate the risk of invasive species introduction and spread and to assess the impact of the invasive species in question on the invasive community.

Accordingly, the following hypotheses were tested:
i) *A. delmontii* is an invasive species whose expansion is related to global warming;
ii) the presence of an invasive species in the biofilm has a significant impact on community composition.

II. Material and method

II.1 Comparison of the commonly used diatom index (IPS) in ecological status assessment for the Danube River and diatom ecological groups (mid-term sampling from the drift line, cross-section sampling, surface sampling of mussel shells)

I grouped my research around three major questions. 1) I investigated to what extent the species-based ecological status assessment could be replaced by trait-based approach in case of phytobenthos. For this, I chose the methods in such a way that I could study the effects of the two most important stressors (nutrient and hydromorphology) present in the Danube.
For three years (January 2010 – December 2012), I took benthic diatom samples from the Danube at Göd every month (mid-term sampling), during which the hydrological conditions and nutrient supply also changed. In parallel with the benthic diatom sampling, the physico-chemical variables of the water were also measured.
Furthermore, I also carried out investigations along cross section of the Danube, also at Göd (May 2012, 2013 and 2014), where I was able to study the effect of hydromorphological differences (in terms of chemical variables, the section was considered chemically homogeneous based on the results of the cross-sectional probe investigation carried out by analysers of the Danube Research Institute).
I was also involved in a month-long series of experiments on benthic diatom assemblage grown on mussel shells in Budapest (at an artificial floating island called Green Island, opposite the main building of the Budapest University of Technology and Economics), where the conditions were identical in terms of both hydromorphological conditions and physico-chemical variables, the only difference being the size and surface roughness of the substrates.

II.2 Impact Budapest on benthic diatom community of the Danube River

We chose the right and left banks of two sections of the Danube for our study. The upper section was located about 20 km north of Budapest, and the lower section about 12 km south of Budapest. Szentendre Island is located on the right bank of the upper reaches, which is sparsely populated and not subject to intensive agricultural activity; it is therefore an area with low anthropogenic impact. It also includes a large protected area that supplies drinking water to about 90% of Budapest's population. The other three areas are highly exposed to thuman influences. The town of Dunakeszi is located on the left bank of the upper section. Two waterworks and a sewage treatment plant belong to the town. Alagimajor is located in the peripheral area of the city, where agricultural activities are carried out. The downstream area is influenced by the capital. In parallel to the phytobenthos sampling, the physico-chemical variables of the water and the concentrations of metals, metalloids and nonmetals were also measured.

II.3 Rapid spread of an invasive diatom species, Achnanthidium delmontii Pérès, Le Cohu & Barthès 2012 in the Danube and its tributaries

A total of 142 phytobenthos samples from different locations were used to create an 'ADMO' database (ADMO is the abbreviation used by OMNIDIA software for diatom species *Achnanthidium delmontii*). Samples were collected between 2013 and 2020 (1) from sampling sites of the 3rd and 4th Joint Danube Survey (JDS3 and JDS4), between 2013 and 2019 from the upper, middle and lower Danube in Hungary (Liška et al. 2021), (2) from the entire Hungarian Danube section, and (3) in 2018 and 2019 from surface waters of Hungary in a national survey. ADMO is present in 79 samples in the database, of which 65 samples are from the Danube and 14 from Hungarian Danube tributaries. 128 samples were selected from the initial database, which were collected from the Danube. To assess the extent of biological contamination, we used the 'Site-specific Biological Contamination (SBS) index (Liška et al. 2008, Csányi et al. 2021). Furthermore, we used the results of the JDS4 metabarcoding test to provide evidence on the occurrence and distribution of ADMO in the Danube (Zimmermann et al. 2021).

To answer the objectives and questions in part **I.1, 2 and 3** during our investigations diatom samples from the substrate were preserved in situ with 4% formaldehyde. In the laboratory, the silica-based frustules of diatoms were cleaned with hydrogen peroxide and hydrochloric acid. The cleaned frustules were embedded in Naphrax resin with a refractive index of 1.7. Diatom taxa were determined using an Olympus IX70 inverted microscope equipped with differential interference contrast (DIC optics) at 1500× magnification. At least 400 valva individuals per sample were determined at species (or where this was not possible, at least genus) level. Samples with a high number of radially symmetric diatoms or small taxa (e.g. species of the genus *Achnanthidium*) were also examined by scanning electron microscopy (SEM, Zeiss EVO MA 10).

In our studies to answer the first major question (**I.1**), after the microscopic identification of diatom individuals, we assigned each species to the corresponding ecological group (one of the most widespread diatom traits), i.e. the low, high profile, motile (Passy 2007) and planktonic group (Rimet and Bouchez 2012), which includes settled diatoms. Furthermore, we used the IPS biotic diatom index calculated using OMNIDIA 5.3 (Lecointe et al. 2008) software on the basis of our diatom relative abundance data. Subsequently, analyses were performed using statistical procedures (calculation of coefficients of variation, redundancy analysis (RDA)).
To answer our second major question (**I.2**), we followed a similar approach to question 1 (**I.1**), with the addition of evaluating our samples for other traits (nitrogen uptake metabolism, pH,

oxygen demand, trophicity, saprobiting, and cell size (van Dam et al. 1994)) beside the diatom ecological group. In addition, the following statistical procedures were used: calculation of coefficient of variation, ANOVA, PERMANOVA, non-parametric multivariate analysis of variance (NMDS).
For the third question (**I.3**), I used light and scanning electron microscopy to examine the morphological characters of the invasive species in question. Weighted mean regression and NMDS analysis were performed for statistical evaluation of our data.

III. New scientific results - Theses

III.1 Comparison of the commonly used diatom index (IPS) in ecological status assessment for the Danube River and diatom ecological groups (mid-term collection from the drift line, cross-section sampling, surface sampling of mussel shells)

Based on studies in the Danube, I have shown that diatom ecological groups can be mainly uable to confirm hydromorphological effects and do not provide reliable information on nutrient content. In the case of phytobenthos, ecological group-based approach cannot replace the taxon-based approach in high flow rivers.

III.2 Impact of Budapest on the Danube benthic diatom community

We found that the impact of the capital due to wastewater treatment had not shown significant differences in the environmental variables used to determine the physico-chemical status of water, either by north-south orientation or by bank. The differences obtained in the statistical analysis were mainly related to hydrological conditions. However, based on the benthic diatom species composition, which integrates environmental effects in the longer term, and on the diatom traits (cell size, pH, N-adaptation, saprobity, trophity, oxygen demand traits), except for the diatom ecological group, the samples upstream to and downstream to Budapest and the samples on the right and left bank indicated different environments. However, among the traits, the samples differed mainly by ecological groups according to the bank, confirming the hydromorphological stressor dependence of this trait. The hydromorphological effects of urbanisation were reflected by diatom ecological groups, while nutrient loading was primarily reflected by species composition.

III.3 Rapid spread of an invasive diatom species, *Achnanthidium delmontii* Pérès, Le Cohu & Barthès 2012 in the Danube and its tributaries

We were the first to show the presence of the species in the Danube, and thus its occurrence in Hungary.
We have highlighted the invasiveness of the species, using for the first time the SBC index for phytobenthos, estimating the extent of invasion. We highlighted the connection between the distribution of the species and the increase in the average temperature of our surface waters due to global warming. We also drew attention to the morphological stamps needed to accurately identify a species, which is important for a dominant species because the ecological status assessment is species-based, species may have different ecological needs, and the result of the assessment may be greatly affected by misidentification.

IV. References

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V. Publications on the topic of the thesis

V.1. Articles published in refereed scientific journals

I.1 Comparison of the commonly used diatom index (IPS) in ecological status assessment for the Danube River and diatom ecological groups (mid-term collection from the drift line, cross-section sampling, surface sampling of mussel shells):

Trábert, Zs.; Kiss, K.T.; Várbíró, G.; Dobosy, P.; Grigorszky, I.; Ács, É.

Comparison of the utility of a frequently used diatom index (IPS) and the diatom ecological guilds in the ecological status assessment of large rivers

FUNDAMENTAL AND APPLIED LIMNOLOGY 189: 2 pp. 87-103, 17 p. (2017)

DOI: https://doi.org/10.1127/fal/2016/0933

I.2 Impact of Budapest on the Danube benthic diatom community:

Trábert, Zs.; Duleba, M.; Bíró, T.; Dobosy, P.; Földi, A.; Hidas, A.; Kiss, K.T.; Óvári, M.; Takács, A.; Várbíró, G.; Záray Gy. és Ács É.

Effect of land use on the benthic diatom community of the Danube River in the region of Budapest

WATER 12: 2 pp. 1-20. Paper: 479, 20 p. (2020)

DOI: https://doi.org/10.3390/w12020479

I.3 Rapid spread of an invasive diatom species, Achnanthidium delmontii Pérès, Le Cohu & Barthès 2012 in the Danube and its tributaries:

Buczkó, K.; Trábert, Zs.; Stenger-Kovács, C.; Tapolczai, K.; Bíró, T.; Duleba, M.; Földi, A.; Korponai, J.; Vadkerti, E.; Végvári, Zs. és Ács, É.

Rapid expansion of an aquatic invasive species (AIS) in Central-European surface waters; a case study of Achnanthidium delmontii

ECOLOGICAL INDICATORS 135 Paper: 108547, 11 p. (2022)

DOI: https://doi.org/10.1016/j.ecolind.2022.108547

V.2. Conference abstracts

The research results were presented at the following conferences:

I.1 Comparison of the commonly used diatom index (IPS) in ecological status assessment for the Danube River and diatom ecological groups (mid-term collection from the drift line, cross-section sampling, surface sampling of mussel shells):

Szilágyi, Zs., Szekeres, J., Csányi, B., Kiss, K.T., Tóth, B., Ács, É. (2013): Spatially extended sampling methosd of benthic diatoms and macroinvertebrates for ecological status assessment of large rivers. – SIL XXXII. Congress. Budapest, 4-9. August 2013. (poster presentation)

Szilágyi, Zs., Szekeres, J., Csányi, B., Kiss, K.T., Tóth, B., Ács, É. (2013): A few important factors effecting on the ecological status of a Hungarian river (River Danube) based on phytobenthos. – 7th Central European Diatom Meeting (CE-Diatom), Thonon-les-Bains, France, 18-20. September 2013. (oral presentation)

Szilágyi, Zs., Kiss K.T., Ács, É. (2015): Might there be any hydromorphological impacts of ecological guilds of phytobenthos and the IPS diatom index? – a cross-section study in the River Danube, in Hungary. – 9th Central European Diatom Meeting (CE-Diatom), Bremerhaven, Germany, 10-13. March 2015 (oral presentation)

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