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***entitled***

# TRAIT-BASED APPROACHES IN FRESHWATER BENTHIC MICROALGAE COMMUNITIES

**By**

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# 1. INTRODUCTION

Freshwater ecosystems—including rivers and lakes—have been essential to humanity (Porter et al., 2012). These extremely sensitive ecosystems to environmental changes demand close observation because of their several benefits (Morandín-Ahuerma, 2019; Eliasz-Kowalska). These surroundings are seriously threatened by nutrient enrichment, forestry, agriculture, and climate change (Ognjanova-Rumenova, 2019). Good management of these systems entails protecting habitats and enhancing freshwater environments (WFD; European Parliament and Council, 2000), ensuring sustainable use of ecosystem services (Daily et al., 1997), and following national and international conservation laws (Ministerial Order, 2001).

The US Clean Water Act (1972) and the European Water Framework Directive (WFD: European Parliament and Council, 2000) utilize important bioassessment tools, such as diatoms. Diatoms are essential algae in aquatic food chains that produce oxygen. These species are significant indicators of human impacts and climate change due to their quick response to environmental changes (Rühland et al., 2015). Understanding the complex relationship between environmental factors and diatom communities can assist in predicting the diversity of ecosystems and their ability to recover from disturbances (Rühland, 2015).

Recent studies focus on trait-based diatom analyses rather than conventional species-based indicators, with an emphasis on size and growth. These studies include the works of e.g. Rimet and Bouchez (2012) and Kahlert et al. (2021). These approaches provide an improved assessment of the impacts caused by both human activities and environmental factors (Krasznai et al., 2010; Bácsi, 2016). Utilizing a combination of guilds and traits as eco-morphological functional groups provides a more efficient and predictable method for assessing diatom assemblages and comprehending organism adaptations to environmental limitations (Lange et al., 2016; B-Béres et al., 2016; Tapolczai, 2017).

The use of combined eco-morphological groups has been shown to be an effective and reliable approach for detecting both quantitative and qualitative variations in diatom assemblages (Tapolczai et al., 2016). Trait-based approach could use over a wider geographical area, overcoming differences in taxa composition (Tapolczai et al., 2017). Additionally, morphological characteristics of species can offer ecological insights into how these organisms adapt to environmental constraints (Statzner and Bêche, 2010).

## 2. MAIN OBJECTIVES

The aims of this dissertation were to study the impact of environmental variables and morphological factors, raised temperatures, as well as the effect of salinization on benthic diatoms.

*1$^{st}$ chapter: Lake morphology as an important constraint for benthic diatoms in temperate, humic forest ponds*

The aims of this study were to examine diatom communities, as well as the physical and chemical environments and morphological variables (water depth and surface area) of temperate and humic forest ponds in two different parts of Hungary. The primary concern was whether, in addition to environmental influences, morphological characteristics are important and whether they might significantly influence the composition and functioning of the diatom communities in these ponds. It also aimed to determine the individual and shared impacts of environmental and morphological factors on diatom communities. For these purposes, we investigated the main factors that can have a significant effect on the species-, guild-, and trait composition of diatom communities, as well as traditional and functional diversity indices.

*2$^{nd}$ Chapter: Impacts of global warming on freshwater lake phytobenthos and their functions*

Understanding of the full impact of increased temperatures predicted by climate change on the entire phytobenthos in lentic ecosystems remains incomplete. The primary objective was to empirically investigate the potential impacts of elevated temperatures projected by several climate scenarios (SPP2-4.5 and SPP5-8.5) on the lentic phytobenthos using both conventional and functional methodologies.

*3$^{rd}$ Chapter: An examination of the phenotypic reaction of diatoms to salinization*

I provided a review of how diatoms can morphologically adapt to salinization, focusing on the potential impact of salinity on diatoms at the cellular level, aiming to gather and synthesize relevant information.

# 3. METHODS

*3.1 Physical and chemical parameters*

**1st chapter** showed 70 small lakes of two regions TD and NH in Hungary. Samples of water and phytobenthos were gathered in winter and spring. Hach Lange HQD4 handheld multimeter was used to measure water temperature, pH, and conductivity. Shading intensity of the given pond, based on field observation, was estimated on a scale of 0-100.Water samples at every site were taken for both water chemical analyses. Nutrient forms ($NO_3^-$, $NO_2^-$, $NH_4^+$, TP, and SRP), anions ($Cl^-$, $SO4^{-2}$ and $HCO_3^-$), COD and Pt color were measured in line with international guidelines using titration and spectrophotometry (APHA, 1998; Wetzel and Likens, 2000).

**2nd chapter** the experiment was conducted in an outdoor mesocosm system located at the HUN-REN Balaton Limnological Research Institute in Tihany, Hungary (Pálffy et al., 2021). The system comprises 12 identical cylindrical plastic tanks, each with a diameter of 2 meters and a depth of 1.5 meters. Every tank was furnished with a collection of sensors to record significant environmental factors directly at the location (such as temperature, irradiance, pH, redox potential, dissolved oxygen concentration, oxygen saturation, conductivity, TDS, salinity). For the colonization and development of phytobenthos, we utilized clean limestone (travertine) slabs and cubes as a substratum. The slabs measured 5 × 5 × 1 cm, while the cubes measured 1 × 1 × 1 cm. Before filling the tanks, a total of 48 slabs were set in a circular pattern around the center of each tank base. Furthermore, a total of 8 little cubes were positioned on flat, plastic supports and thereafter submerged to the depths of the tanks. The experiment was conducted concurrently with three temperature treatments. Each treatment was applied to four randomly chosen mesocosm tanks:

- Treatment 1: The temperature remains unheated and follows the natural fluctuations of the ambient temperature (A);
- Treatment 2: The temperature is constantly increased by 3 ˚C compared to the unheated control, simulating the SSP2-4.5 emissions scenario of IPPC (A+3);
- Treatment 3: The temperature is constantly increased by 5 ˚C compared to the unheated control, simulating the SSP5-8.5 emissions scenario of IPPC (A+5).

### *3.2 Benthic algal samples analysis and applied traits*

In accordance with the guidelines made by King et al. (2006), diatom samples were obtained using a toothbrush from the distinctive substrates (macrophytes or mud). The water supply of the ponds determined the frequency of the samplings. In order to preserve the diatom samples until their oxidation treatment with the hot hydrogen peroxide, the samples were stored in ethanol (CEN, 2003). Diatom valves were identified to species level under light microscopy (Zeiss, Axio A1) at 1000x magnification using immersion oil (Zeiss, 518 N) and applying international taxonomic guides (Krammer and Lange-Bertalot, 1991; Lange-Bertalot et al., 2017; Stenger-Kovács and Lengyel, 2015; Lange-Bertalot and Metzeltin, 1996a, 1996b; Bey and Ector, 2013; von Berg et al., 2004; Komárek and Anagnostidis, 2007; Komárek, 2013; Moestrup and Calado, 2018; Coesel and Meesters, 2014; Buczkó, K., 2002). A total of 400 valves were observed and classified at the species level using a light microscope (magnification of 400x, Zeiss Axiovert 100).

For the **1st chapter** focused on humic forest, species were classified into a variety of ecological groups, such as guilds and attribute categories, including the following: I. four diatom guilds (L - low profile, H - high profile, M - motile, and P - planktic guilds; Passy, 2007; Rimet and Bouchez, 2012); (ii) six groups of the length-to-width ratio (LW, Tapolczai et al., 2017); (iii) five categories of the biovolume (BV, Berthon et al., 2011); and (iv) the combination of these characteristics as ecomorphological groups (B-Béres et al., 2016; **Table 1**).

**Table 1:** The applied guilds and traits.

| Guilds | Biovolume | Length-width ratio | Combined ecomorphological groups |
|---|---|---|---|
| High profile (H) | BV1<100 µm$^3$ | LW1<2 | HBV1, HBV2, HBV3, HBV4, HBV5 |
| Motile (M) | 100µm$^3$≤BV2<300 µm$^3$ | 2≤LW2<4 | HLW2, HLW3, HLW4, HLW5, HLW6 |
| Low profile (L) | 300µm$^3$≤BV3<600 µm$^3$ | 4≤LW3<6 | LBV1, LBV2, LBV3, LBV4, LBV5 |
| Planktic (P) | 600µm$^3$≤BV4<1500 µm$^3$ | 6≤LW3<12 | LLW1, LLW2, LLW3, LLW4 |
| | BV5≤1500 µm$^3$ | 12≤LW4<20 | MBV1, MBV2, MBV3, MBV4, MBV5 |
| | | LW6≤20 | MLW1, MLW2, MLW3, MLW4, MLW5 |
| | | | PBV3, PBV4, PBV5 |
| | | | PLW1, PLW2, PLW6 |

**2nd chapter** The study involved measuring Chl-a content in samples of benthic algae using spectrophotometry after methanolic extraction (Tett et al., 1975). Subsamples were preserved in

Lugol's solution until microscopic examination. 400 cells were observed and classified using a light microscope. Each species was assigned to specific trait categories based on research. Biofilm thickness was determined using a Leica SP8 confocal microscope (Leica, Wetzlar, Germany) equipped.

The assignment of each species to specific trait categories, such as cell volume, motility, life-forms, and kind of attachment, was determined based on the research conducted by Berthon et al. (2011), Lange et al. (2016), and Lukács et al. (2018). These categories are described in **Table 2**.

**Table 2**: The applied traits and their categories

| Cell size | Motility | Life forms | Attachment |
|---|---|---|---|
| <100 µm3 (S1) | Slow Movement (SM) | Unicellar (U) | Weak attachment (Wa) |
| 100 – 299 µm3 (S2) | Fast Movement (FM) | Colonial (C) | Moderate attachment (Ma) |
| 300 – 599 µm3 (S3) | Non-Motile (NM) | Filamentous (F) | Strong attachment (Sa) |
| 600 – 1499 µm3 (S4) | | | |
| <1500 µm3 (S5) | | | |

### *3.3 Statical analysis*

All statistical analyses were performed using R statistical software (R Core Team, 2021; version 4.1.2 and version 4.2.1.) using the ‘vegan’ (Oksanen et al., 2022, 2019), ‘cluster’ (Maechler et al., 2022), ‘indicspecies’ (De Cáceres et al., 2022), ‘nlme’ (Pinheiro et al., 2022), and ‘FD’ (Laliberté et al., 2022), “metaMDS“ (McCune and Grace, 2002), “varpart” (Legendre and Legendre, 2012) packages.

In the **1$^{st}$ chapter**, the Welch test examined variations in morphological and environmental characteristics across different regions. Non-metric multidimensional scaling (NMDS) was applied to study regional differences based on environmental and morphological variables, species and trait compositions, and traditional and functional diversity indices. Redundancy Analysis (RDA) was used to explore species-and trait composition, traditional and functional diversity indexes, and the link between ponds' morphology and their environment. Variance partitioning analyses were used to determine the effect of environmental and morphological variables on diatom communities.

The study of the **2nd chapter** used Bray-Curtis and Jaccard indices to calculate species composition differences among sample pairs. Linear regression models were developed to evaluate changes in dissimilarity over time. Nonmetric Multidimensional Scaling (NMDS) was applied to analyze benthic algae composition. ADONIS2 analyses were performed to compare treatments. The SIMPER test identified species, algal groups, and combined traits contributing to differences. Functional diversity index (RaoQ) was calculated based on combined traits. Indicator species analysis (IndVal) was performed to determine associated phytobenthos species. Welch's tests were conducted to examine species richness, Shannon- and functional diversity, Chl-a content, biofilm thickness, and maximum quantum yield. Mixed-effects linear models were developed to clarify interrelationships.

## 4. NEW SCIENTIFIC RESULTS – THESIS

**1st Chapter: Lake morphology as an important constraint for benthic diatoms in temperate, humic forest ponds**

1. The response of species-based communities in temperate forest ponds is primarily influenced by environmental parameters, such as nutrients and conductivity, rather than morphological factors.
2. However, morphological features of lakes causes a functional response of diatoms even along a small scale. Variations in water depth and surface area lead to the development of a variety of species that are well-suited in terms of their size, shape, and lifeforms.
3. This study demonstrates that multiple methods are not interchangeable, as they provide different but complementary insights and data from an ecological, conservation, and management standpoint. To ensure the preservation of these distinct aquatic environments, it may be essential to use a combination of these strategies.

**2nd Chapter: Impacts of global warming on freshwater lake phytobenthos and their functions**

1. The rise in water temperature, particularly under the intermediate emissions scenario (SSP2-4.5) might lead to substantial biological problems in freshwater ecosystems, contributing to the loss of biodiversityin phytobenthos..
2. The most pessimistic, very high emissions scenario (SSP5-8.5) will probably imply drastic impacts on the benthic algae endangering even the function of the ecosystem. Namely,

   (i) It will induce very high variability in species composition and compositional changes even in phylum level.

   (ii) It will cause a shift in trait composition (benefits for smaller cell volume, filamentous life-forms, non-motile and weakly attached taxa), consequently it will reduce functional diversity.

   (iii) The increased water temperature will increase biofilm thickness (1.4 μm/◦C) and decrease maximum quantum yield of photosystem.

## 3rd Chapter: An examination of the phenotypic response of diatoms to salinization

Based on the existing knowledge collected from the literature, diatoms have developed a number of ways to deal with salt, allowing them to survive in salty environments.

1. Osmotic stress leads to morphological changes like shape resistance, post-auxospore formation, and several micro- and nano-sized sometimes species-specific variations.
2. Salinity can have an impact e.g. on the surface area and pore size of diatoms, thereby influencing nutrient exchange through the cell wall. The complex effect of high-level concentrations of salt and heavy metals can cause abnormal forms of diatoms, thus affecting their physiological functions and cellular motility.

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## 7. List publication related to the dissertation

**Al-Imari, T.J.K.,** Lengyel, E., Korponai, J., Padisák, J. and Stenger-Kovács, C., 2023. Lake morphology as an important constraint for benthic diatoms in temperate, humic forest ponds. Ecological Indicators, 155, 110939. **IF: 6.9, SJR: Q1.**

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