



Article

Long-Term Changes of Species Composition and Functional Traits of Epiphytic Diatoms in the Szigetköz Region (Hungary) of the Danube River

Éva Ács ^{1,2,*} , Tibor Bíró ^{2,*}, Csaba Berta ³, Mónika Duleba ¹, Angéla Földi ^{1,4}, István Grigorszky ³, András Hidas ^{1,4,5} , Judit Knisz ², János L. Korponai ² , Zsuzsa Trábert ^{1,4}, Edit Vadkerti ² and Krisztina Buczkó ^{1,6} 

¹ MTA Centre for Ecological Research, Danube Research Institute, Karolina út 29, H-1113 Budapest, Hungary; duleba.monika@okologia.mta.hu (M.D.); foldi.angela@okologia.mta.hu (A.F.); hidas.andras@okologia.mta.hu (A.H.); trabert.zsuzsa@okologia.mta.hu (Z.T.); buczo.krisztina@okologia.mta.hu (K.B.)

² National University of Public Service, Faculty of Water Sciences, Bajcsy-Zsilinszky utca 12-14, H-6500 Baja, Hungary; knisz.judit@uni-nke.hu (J.K.); korponai.janos@uni-nke.hu (J.L.K.); vadkerti.edit@uni-nke.hu (E.V.)

³ Department of Hydrobiology, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary; berta.csaba@science.unideb.hu (C.B.); grigorszky.istvan@science.unideb.hu (I.G.)

⁴ Doctoral School of Environmental Sciences, Eötvös Loránd University, Pázmány P. s. 1/A, H-1113 Budapest, Hungary

⁵ MTA Centre for Ecological Research, Sustainable Ecosystems Group, Klebelsberg Kuno u. 3, H-8237 Tihany, Hungary

⁶ Hungarian Natural History Museum, Department of Botany, Könyves Kálmán krt. 40, H-1087 Budapest, Hungary

* Correspondence: acs.eva@okologia.mta.hu (E.A.); biro.tibor@uni-nke.hu (T.B.)

Received: 9 January 2020; Accepted: 4 March 2020; Published: 11 March 2020



Abstract: Here we report the results of our decades-long study on epiphytic communities from two tributary systems of the Szigetköz section of the Danube River. The main goal of the investigation was to detect changes in the epiphytic communities at structural (core species, changes in the relative abundance of common species) and functional (trait changes) levels as a result of the most important anthropogenic effects on Szigetköz, i.e., hydro-morphological modifications. We also examined the impact of rehabilitation on the tributary systems in terms of ecological potential. We discovered that mainly motile diatom species characterized the epiphyton due to reduced water volume were introduced into the tributary system after the diversion of the Danube. The ecosystem stabilized in the rehabilitated section, while the non-rehabilitated section showed a worsening tendency, mainly in the parapotamic branches. Our long-term data sets may provide a good basis for comparisons of different aquatic ecosystems, to define changes in the abundance of core species and in the structure of community in response to different anthropogenic pressures. It is fundamental to determine adaptive traits in assessing the impact of global warming stressors on biodiversity.

Keywords: core diatoms; functional traits; hydro-morphological modifications; ecological potential

1. Introduction

Riverine ecosystems are among the most vulnerable to anthropogenic effects [1]. While eutrophication is still the most common water quality problem on Earth, the reversal of eutrophication is also becoming a global issue [2]. Nutrient overload is only one of the many anthropogenic impacts on riverine ecosystems. The rate and/or intensity of global climate change is increasing on the short-, medium-

and long-term scale [3] and poses a serious threat not only to natural, but also to anthropogenic communities and human society as well [4,5].

The Danube—the second longest river in Europe—has a catchment area of more than 800,000 km² and is shared by 19 nations, making it one of the most international rivers in the world. It has long served social and economic purposes (shipping, hydropower generation, irrigation, recreation, etc.), still, it is a riverine ecosystem with good ecological potential [6]. A wastewater treatment program implemented in the early 2000s resulted in re-oligotrophization of the Hungarian section of the Danube, while the effects of global warming (rise in water temperature, altered water flow) also contributed to detectable changes in the riverine ecosystem [7]. These changes may lead to processes that can fundamentally shape the composition and diversity of actual communities. As environmental conditions change, so does the composition of the community. This may manifest not only in species composition but also in functional traits, which are proxies for adaptation strategies. The spatial distribution of diatom species is subjected to micro-evolutionary constraints, while the distribution of traits is primarily driven environmentally, therefore its use may be more appropriate in detecting global environmental changes [8]. As predicted by different climate change scenarios, any further temperature increase and decrease in precipitation pose a major challenge for water management experts. In the upcoming period, the minimum amount of water needed to maintain good ecological status/potential of waters should be determined considering ecological, engineering, and economic aspects.

The Danube enters the Carpathian basin at Devin (Slovakia). According to traditional sectional division, Devin is located where the upper section ends. There, the velocity of the stream slows down and creates a special inner delta in the Szigetköz-Csallóköz region.

Szigetköz has always been an important tourist destination. In the early 90s, research focused on the construction of a weir at Dunacsún. In 1991 we sampled the Cikola tributary system belonging to the Upper Szigetköz, in 1992 sample collection was extended to the Ásvány tributary system, an area belonging to the Lower Szigetköz. After the diversion of the Danube, the Szigetköz Working Group [9] was organized under the auspices of the Hungarian Academy of Sciences to assess and monitor the environmental status of Szigetköz. A number of geochemical and biological elements were studied, including regular phytobenthos investigations started in 1994. The primary purpose of phytobenthos monitoring was to investigate the effects of water replenishment rather than to compare it with water chemistry data, as water chemistry measurements were less frequent and the data obtained was not sufficient for long-term analyses. Recently, a new water management problem emerged in the area related to both global warming and altered relationship between the river and groundwater as a consequence of Danube diversion [10]. Due to the altered water regime of the Danube (increasing difference between minimum and maximum water levels, increasing variability of water regime [6]), drying out of certain tributaries of the Lower Szigetköz floodplain is becoming more frequent during low water periods and wetland extent is gradually shrinking. As a result, lateral connectivity between branches is eliminated over time and water supply to branches is impaired. This phenomenon is exacerbated by floodplain recharge, as well as by the decrease in the level of small and medium-sized water areas. For instance, a significant decrease in water level is detected in the Ásvány tributary system accelerating branch system aging [11].

Again, these issues point to the urgency to investigate changes taking place in the Szigetköz, which can be interpreted as a huge mesocosm experiment wherein the effects of climate change can be studied. In the current study, we evaluated our epiphytic diatom data collected during the biomonitoring of the Bős/Gabčíkovo power station between 1994 and 2009 (supplemented with data collected sporadically before the start-up of the plant and with records of current status) to provide a basis for further investigations and analyses.

Our 18-year-long (1991–2009) data set collected with unified method provides a rare opportunity for analysis on a decade scale and comparison to the present state. This is one of the greatest contribution of the work. To the best of our knowledge, there is no data set of phytobenthos collected regularly from the Danube spanning for such a long time and investigated by the same researcher.

The aims of our study are:

(1) To understand the long-term changes of benthic diatom assemblages in a riverine branch system exposed to significant anthropogenic influences, we investigated the change of abundance of the most common and abundant diatom species and of the evenness of the communities over time and spatially along environmental gradients. We hypothesized that there would be greater spatial differences in cumulative diatom core species abundance than in time due to spatial differences in environmental conditions. We also hypothesized that relative abundance of common species would increase with increasing water discharge, as faster water flow would help species dispersion.

(2) During our investigation we also sought to determine whether the effects of changing flow conditions during the last few decades can be detected in diatom attachment strategies, one of the most frequently studied traits. We hypothesized that, after the diversion of Danube River, significant changes took place, but subsequent artificial water supply stabilized the ecosystem with smaller changes in time, especially for traits associated with water volume.

(3) We also examined the branch systems of the rehabilitated Upper Szigetköz and the Lower Szigetköz not rehabilitated at that time. We hypothesized that there is a slight difference in the ecological potential of parapotamic and eupotamic branches in the rehabilitated Upper Szigetköz, while the ecological potential of parapotamic branches is lower than in the eupotamic branches in the non-rehabilitated Lower Szigetköz.

(4) The changes in Szigetköz also provided a good opportunity to answer the question of how much water is needed to achieve good ecological potential in this area. Less water flowed into the branch systems between 1995 (year of weir completion) and 1998 than after the beginning of Upper Szigetköz rehabilitation. We hypothesized that rehabilitation improved the ecological potential of the branch system because of an increase in water quantity.

2. Materials and Methods

2.1. Study Site

Szigetköz, with an area of 375 km², is one of the most beautiful and important recreational areas on the Hungarian section of the Danube and a popular tourist destination. In the early 90s, a drastic intervention drew attention to the vulnerability of the river, when in October 1992 the Dunacsún reservoir was constructed by diverting the Danube to a side channel at Dunacsún. The most important consequences of the diversion were that discharge rate and water level of the main arm decreased drastically and water from the tributary system drained towards the main arm, causing the drying out of the tributary system [9,11]. After establishing temporary facilities for water replenishment, a weir was constructed at Dunakiliti (1843 rkm) in 1995 to raise water level in the main arm; as a result, water can flow into the upper branch system and through the entire tributary system (called gravity water supply). In the side branches, smaller baffles and spreaders were built ensuring water level and controlling water flow direction. In this way, 32–160 m³ s⁻¹ of water could be introduced into the tributary system, which had been synchronized with internal sluices and engineering structures (e.g., at Denkpál) by 1998, and a certain amount of water is introduced into the branch system (Figure A1), adjusting to the water regime of the main branch [11]. With this artificial water supply, the water velocity of branches became variable and branches with high water flow and branches with almost standing water conditions developed. Although branch systems were transformed, the water supply resulted in mosaic water bodies, which can be better adapted to the requirements of nature conservation. At the same time, former water dynamics changed and annual water level fluctuations of up to 6–7 m decreased significantly (on average to 1.5 m). Flushing of the tributary system by floods of the Danube has become rarer. Initially, these interventions effected only the upper end of the Ásvány tributary system, but after 2010, interventions were extended to the lower part of it [11].

2.2. Sampling Design

At the time of our investigation, there were no standard protocols for phytobenthos studies (they became available after the implementation of the EU WFD in 2000 [12]). Several parallel studies were conducted on substrate dependence (substrate neutrality), spatial heterogeneity, and for the estimation of hydro-morphological modification [13–16], but the results remained largely unevaluated and only in the form of annual work reports.

2.2.1. Sampling Design for Monitoring the Long-Term Changes

In 1991, we started studying the epiphytic diatoms in the Szigetköz region of the Danube. As hydro-morphological modification is the most important anthropogenic effect in the Szigetköz, we regularly investigated it by deploying “floating reed islands” at two sites in 1994. One was located in the Cikola tributary system of the Upper Szigetköz (c6 on Figure 1) and the other one was placed in the Ásvány tributary system in the Lower Szigetköz (a6 on Figure 1). Hereinafter, these are referred to as deployed reeds (Figure A2). The reed stalks were secured in a compartment and the whole was secured to a buoy. We decided to use floating reed islands in case natural reeds would dry out due to the diversion of the Danube. An arrangement allowing sample collection constantly from the same depths (10 cm below the surface of the water) was used. The density of the reeds on the reed island was similar to that of natural reeds. Reed islands were deployed in spring (late May) and sampled weekly in replicates of 3 at 10–20 cm intervals on the reed stalks below the water surface from June to September. For a control, samples were collected from rooting reeds near the reed island twice a year, in replicates of 5 from the reed stalks at the same 10–20 cm intervals below the water surface. Due to the termination of funding, monitoring was stopped in 2009. In order to record present conditions, reed epiphytic diatoms were collected at sample points of a6 and c6 twice in July 2019 (3 weeks apart) (Figure 1). Altogether, 157 samples from Ásvány and 130 samples from Cikola were investigated.

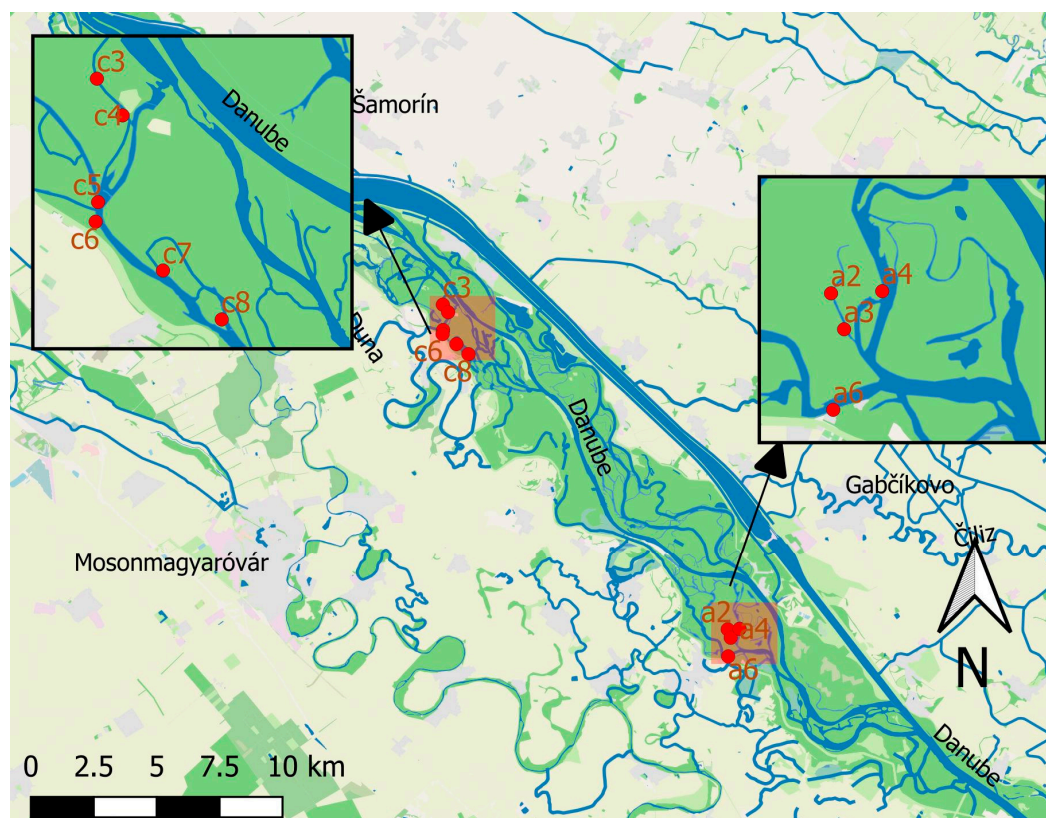


Figure 1. Location of sampling points in the Ásvány (dots marked with a) and Cikola (dots marked with c) tributary systems.

2.2.2. Sampling Design for Ecological Potential Assessment

To determine the ecological potential of the Szigetköz region of Danube River, sampling sites were selected from both eupotamic (Figure 1; a4–7) and parapotamic (Figure 1; a2–8) branches.

While examining the effect of water supply on ecological potential, samples from both eupotamic (a4 and c5; a total of 58 samples) and parapotamic branches (a2, a3, c3, c4, and c8; a total of 66 samples) were included in the analysis, but only samples collected from natural reeds.

2.3. Sample Processing

Samples were fixed with formaldehyde (4%) on site, washed with a toothbrush and then transported to the laboratory. Frustules were cleaned with hydrochloric acid and hydrogen peroxide, then rinsed in distilled water and mounted with Naphrax® mounting medium [17]. A minimum of 400 valves was identified to species or genus level. Identification was based on the literature available at the time [18–22] and later the work of Lange-Bertalot et al. [23] was also used.

2.4. Diatom Index, Trait Data, Core and Common Species

In order to assess the ecological potential of branch systems, the Specific Pollution Sensitivity Index (IPS, [24]) was calculated using OMNIDIA 6.0.2 [25]. Index boundaries for this region are as follows: P/B 4.3; M/P 8.7; G/M 13.0; H/G 15.6 (B: bad, P: poor, M: moderate, G: good, H: high) [26].

We used ecological guilds proposed by Rimet and Bouchez [27] under the term ecological groups following the suggestion of Tapolczai et al. [28]. According to the system of Rimet and Bouchez [27], the low profile group (LPG) attach to the substrate in adnate form, the high profile group (HPG) has erected form, the motile group (MG) can move on the substrate, while the planktonic group (PG) contains diatom species settled from the phytoplankton. For other traits (such as oxygen, nutrient, and moisture requirements), classifications of van Dam et al. [29] were applied. See more details in Földi et al. [30]. Values of other traits used were obtained from the OMNIDIA 6.0.2 database [25].

Top1 (the most abundant species), core species (present in >90% of samples and whose average dominance is >5%), and common species (present in >50% of samples with any relative abundance [31]) were determined according to Marazzi & Gaiser [31].

2.5. Statistical Analyses

A two-tailed F-test was used to check variance of traits showing significant changes and to detect significant differences between deployed and natural reed epiphytic diatoms. As no significant differences were observed ($p > 0.05$), deployed and natural reed epiphytic diatoms were treated simultaneously.

As the calculated ecological index values were not of normal distribution and their density function differed, we compared the values with a non-parametric Brunnel-Munzel test.

Trait changes between 2009 and 2019 were modelled (knowing the water discharges) to better understand trends in the rehabilitated Cikola tributary system and the model was verified by data from 2019. A linear regression (generalized linear model; GLM) model was fitted to water discharge values using the ordinal least squares method. To approximate normal distribution, the relative abundance of traits was transformed into a square root in the model, which corresponds to a Hellinger transformation [32,33]. In the case of IPS, we also fitted a linear regression model to the discharge, where index values were transformed into a square root to approximate normal distribution. For the trait and IPS values after 2009, we made predictions with the help of these models. Values of the 2019 samples are also shown in the prediction figures to validate the model.

Discharge data were provided by the North Transdanubian Water Directorate. As the Directorate only performs regular flow measurements in the Upper Szigetköz, we could have access to only these data. Due to the gravitational nature of water supply, however, the dynamics of water regime in the eupotamic branches of the Ásvány branch system are similar to that of the Cikola branch system, except

that water quantity (and because of this also water discharge) differs, thus, results can be interpreted without precise knowledge of the discharge.

The effect of water replenishment after the diversion of Szigetköz branches was investigated by subdividing the samples into three groups: initial low discharge period 1995–1997, stabilized discharge period 1998–2009 (Figure A1), and samples from 2019 used to validate the model. Group separation was analyzed by standardized principal component analysis (PCA), where objects were samples, variables were taxa, and values were the square root of relative abundance of taxa (Hellinger transformation). In the figures, a convex hull was fitted to the dots to better visualize the separation of groups.

For statistical analyses, R software package [34] was used. We used R ggplot2 [35] package for generating the figures.

The map showing Szigetköz and sampling points was prepared with QGIS [36] software using OpenStreetMap (OSM) layers.

3. Results

3.1. Structure of the Diatom Assemblage

3.1.1. Differences of Diatom Assemblages before and after the Alteration of Water Quantity

A total of 218 diatom taxa were identified from the eupotamic (c5 and c6) branches of the Cikola tributary system and 208 diatom taxa were identified from the eupotamic branches of the Ásvány tributary system (a4 and a6). Based on species and their relative abundance, samples of lower discharge period (1995–1997) were clearly distinct from higher discharge period (1998–2009) of the Cikola tributary system. Samples collected in 2019 fit well with the sample group of the rehabilitation period (Figure 2).

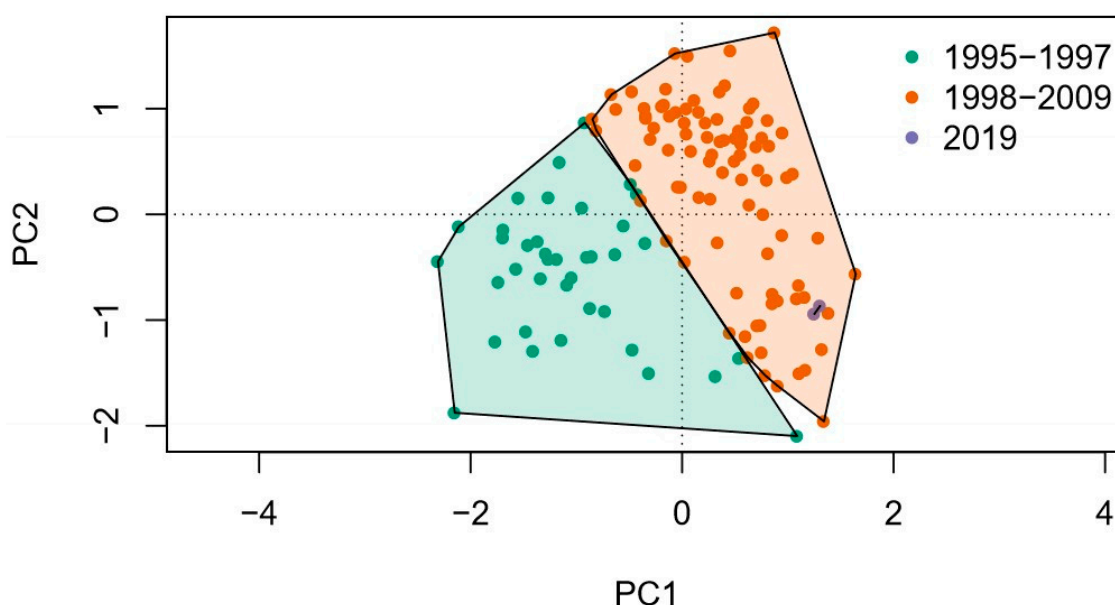


Figure 2. Plot of principal component analysis of deployed reed and natural reed samples from points c5 and c6 in the Cikola branch system. Different colors denote sampling periods.

In the Ásvány tributary system, low discharge samples from 1995 to 1997 and after overlapped, and samples from 2019 were in a marginal position compared to the 1998–2009 sample group (Figure 3).

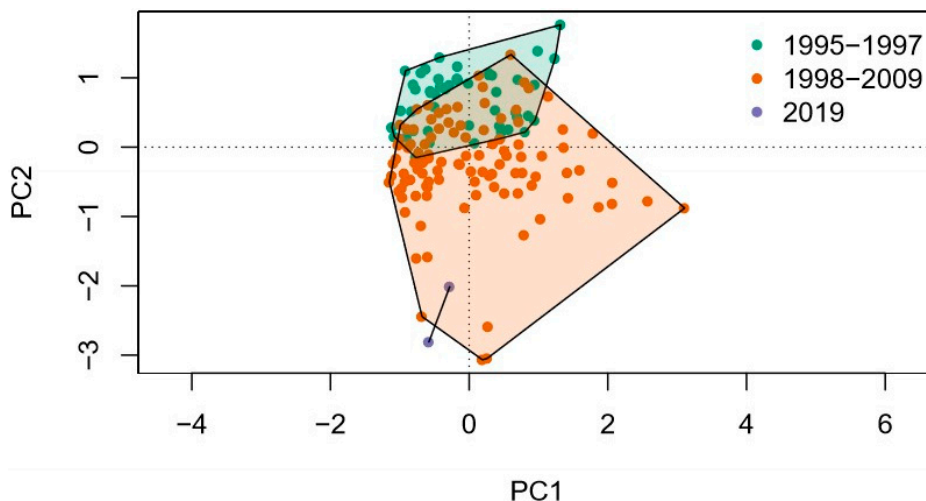


Figure 3. Plot of principal component analyses of deployed reed and natural reed samples from points a4 and a6 in the Ásvány branch system. Different colors denote sampling periods.

3.1.2. Spatial Variance of the Diatom Assemblage Structure

To analyze spatial differences, we compared epiphytic diatom composition of deployed reed and natural reed of eupotamic (c5 and c6) branches of the Cikola tributary system to that of the Ásvány tributary system (a4 and a6) in terms of core species, common species, and evenness.

The Cikola tributary system was characterized by 3 core species (*Achnantheidium minutissimum* (Kütz.) Czarnecki; *Amphora pediculus* (Kütz.) Grunow and *Cocconeis placentula* Ehr.) and 6 common species (*Cymbella affinis* Kütz. s.l. *Gomphonema parvulum* (Kütz.) Kütz., *Navicula cryptocephala* Kütz., *N. veneta* Kütz., *Nitzschia dissipata* (Kütz.) Grunow, *Rhoicosphenia abbreviata* (Agh.) Lange-Bert.) during the investigated period. The Ásvány tributary system was characterized by 2 core species (*Achnantheidium minutissimum*, *Amphora pediculus*) and 5 common species (*Amphora ovalis* (Kütz.) Kütz., *Cocconeis placentula*, *Navicula veneta*, *Nitzschia dissipata*, *Rhoicosphenia abbreviata*) during the study period. In both tributary systems, the Top1 species was *Achnantheidium minutissimum*. The abundance of Top1 species was greater and significantly different in the Ásvány tributary system compared to the Cikola tributary system (Brunner–Munzel Test Statistic = -2.4195 , $df = 21.854$, $p = 0.024$), and the mean and cumulative core species abundance in the Ásvány tributary system was higher and also differed significantly (Brunner–Munzel Test Statistic = -7.1598 , $df = 241.36$, $p < 0.001$). Mean cumulative common species abundance (Brunner–Munzel Test Statistic = 4.5762 , $df = 269.8$, $p < 0.001$) and mean evenness (Brunner–Munzel Test Statistic = 9.1706 , $df = 220.7$, $p < 0.001$) were higher in the Cikola tributary system (Figure 4).

3.1.3. Temporal Variance of Diatom Assemblage Structure

To analyze temporal changes, we examined the temporal variation of the mean relative abundance of Top1 species, cumulative core species, cumulative common species and evenness in the case of epiphytic diatom communities formed on deployed reeds and natural reeds in the eupotamic branches of both the Cikola tributary system (c5 and c6) and the Ásvány tributary system (a4 and a6). Relative abundance of Top1 species increased slightly in both tributary systems (Figure 5a), while relative abundance of common species and evenness showed a slight decrease over time (Figure 5c,d). Cumulative relative abundance of core species showed a decreasing tendency in the Ásvány tributary system and a slight increase in the Cikola tributary system (Figure 5b). Changes were not significant, although there were significant differences between years. Relative abundance of Top1 species ranged from 1.5% to 93.5% and that of cumulative core species ranged from 5.5% to 93.5%. Relative abundance of cumulative common species was smaller, ranging from 0% to only 59.5%, while evenness ranged from 0.2 to 0.9.

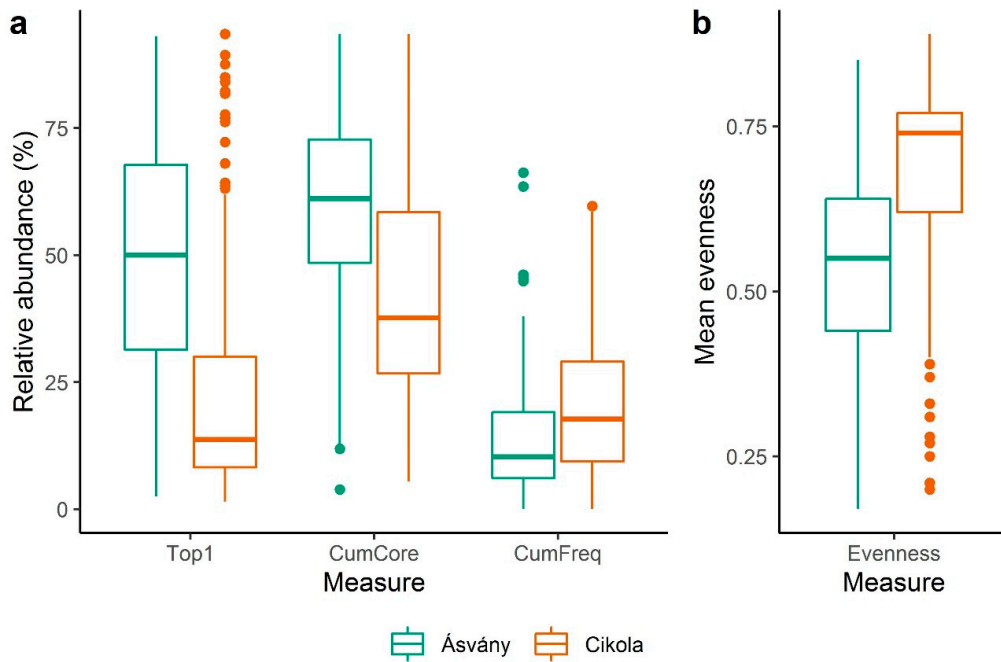


Figure 4. Relative abundance of Top1 species, cumulative core species (CumCor), cumulative common (CumFreq) species (a) and evenness (b) at points c5 and c6 of the Cikola tributary system on deployed reeds and natural reeds (orange) and at points a4 and a6 in the deployed reeds and natural reeds of the Ásvány tributary system (green).

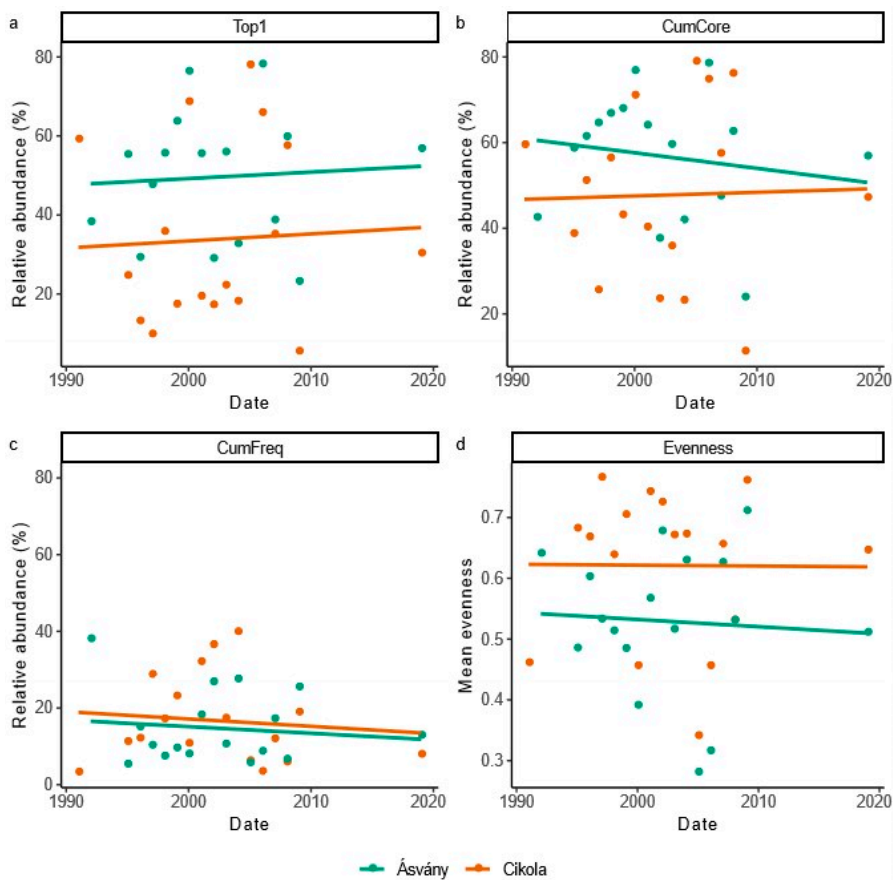


Figure 5. Annual cumulative relative abundance in Top1 (a), core (b), and common (c) diatom species and evenness (d) in the Ásvány (green) and Cikola (orange) tributary systems with fitted linear trend line.

According to the coefficients of variation, relative abundance of Top1 species, cumulative relative abundance of core and common species and evenness varied more across sites than over years (Table A1).

3.1.4. Correlation Between Water Discharge and Diatom Assemblage Structure

Change in cumulative relative abundance of common species was positively correlated with discharge (Figure 6), and the correlation was significant (cor-test: $r = 0.47$; $p < 0.05$). Relative abundance of Top1 species, cumulative relative abundance of core species, and evenness did not show significant correlations with discharge.

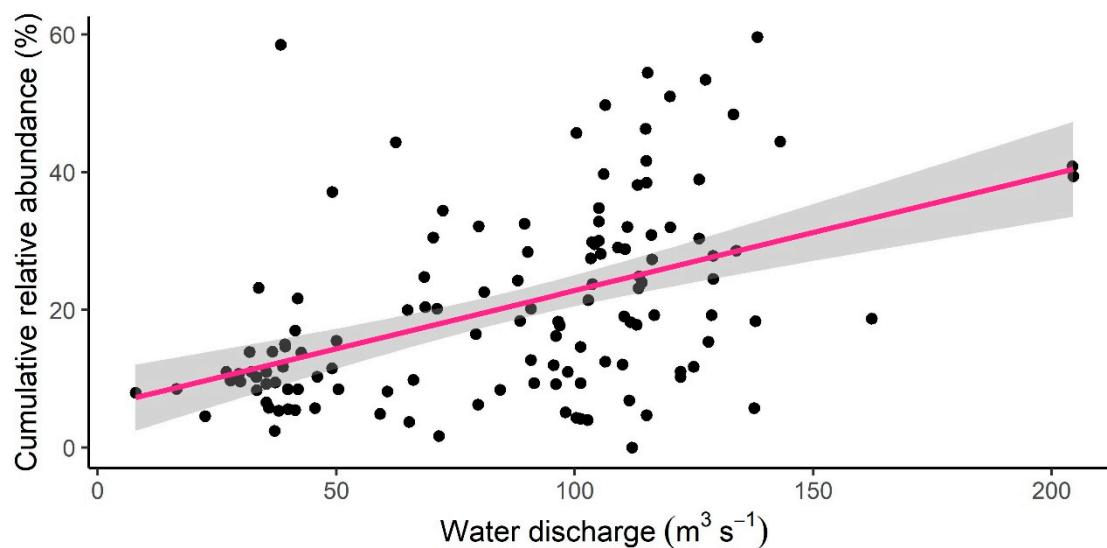


Figure 6. Relationship of cumulative relative abundance of common species with discharge and confidence intervals of fitted linear trend line fitted to points at c5 and c6 sampling sites of the Cikola tributary system for epiphytic diatom assemblages from deployed and natural reeds.

3.2. Traits of the Diatom Assemblage

Changes in diatom traits were also investigated in the eupotamic branches of the two tributary systems (in the case of epiphytic diatoms from natural and deployed reeds at a4, a6, c5, and c6). Among ecological groups, the proportion of high profile group (HPG) (Figure 7a,e), although typically small in running waters, was slightly higher in low-discharge periods than in other periods, especially in the Cikola tributary system (Figure 7e). Practically, low-profile (LPG) diatoms were present in the highest amount in all samples (Figure 7b,f). Proportion of diatoms belonging to the motile group (MG) showed a strong increase in the Ásvány tributary system until 2002 (Figure 7c) and in the Cikola tributary system until 2004 (Figure 7g), then decreased significantly. Proportion of diatoms belonging to the planktonic group (PG), which derives from the phytoplankton, was also considerably higher until 2004 than in the subsequent period (Figure 7d,h).

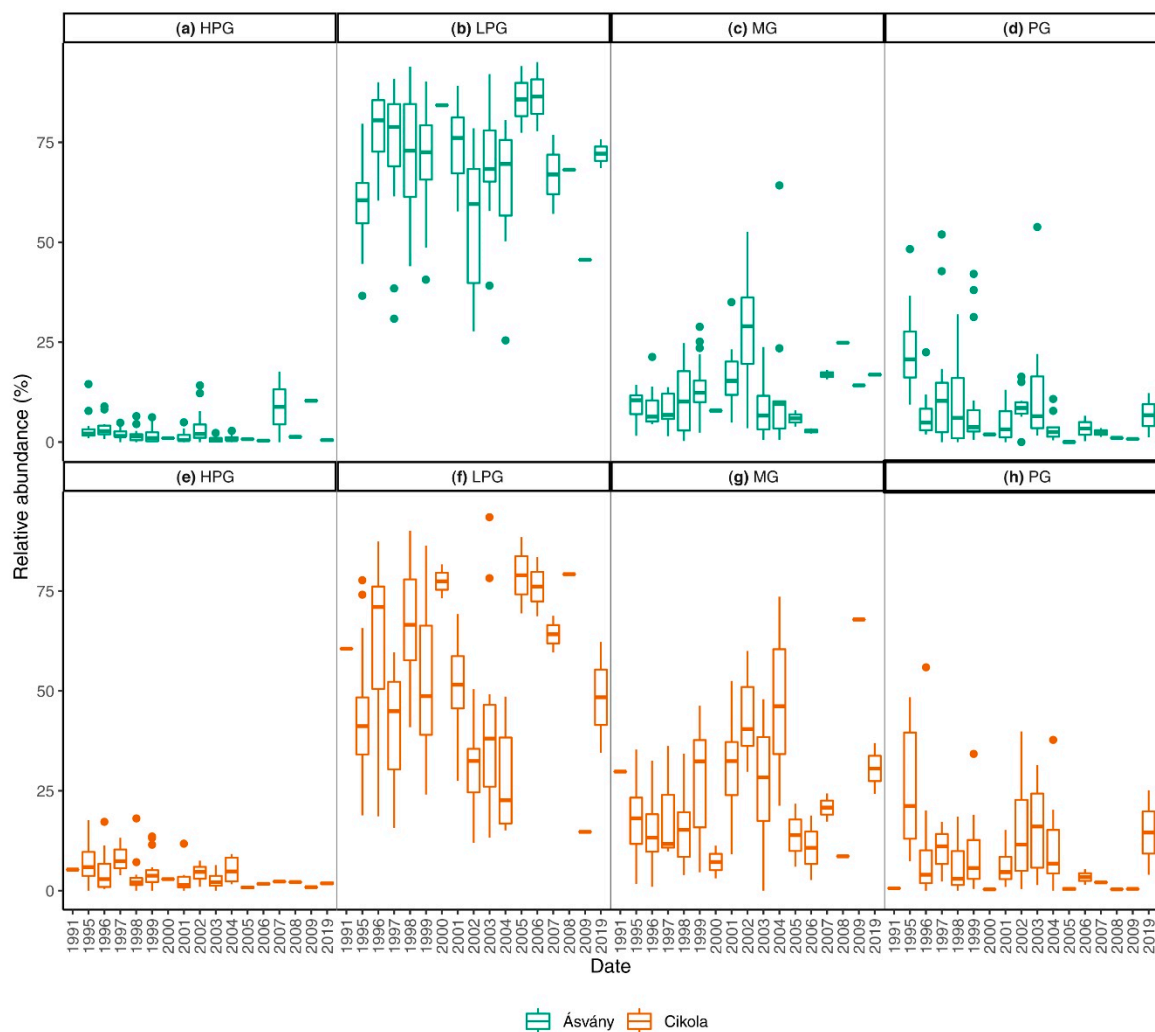


Figure 7. Long-term changes in relative abundance of ecological groups of epiphytic diatom assemblages from deployed and natural reeds at a4 and a6 (a–d) sampling sites in the Ásvány tributary system (green) and at c5 and c6 (e–h) sites in the Cikola tributary system (orange). HPG: high-profile group; LPG: low-profile group; MG: motile group; PG: planktonic group.

The proportion of species with a moderate oxygen requirement in the Ásvány tributary system was high throughout the studied period (Figure 8b) and the proportion of periodically aerophilic taxa increased (Figure 8a), indicating frequent water scarcity in the branch. During periods of low water discharge, the proportion of intermittent aerophilic taxa occasionally increased sharply in the Cikola tributary system, and then gradually decreased as rehabilitation began (Figure 8d). The proportion of species with moderate oxygen requirement showed a decreasing tendency (Figure 8e) and simultaneously they were replaced by species with higher oxygen requirement. An increase in the proportion of meso-eutrophic species (Figure 8c,f), in parallel with a decrease in the proportion of eutrophic species, is in line with decreasing nutrient load of the main arm of the Danube.

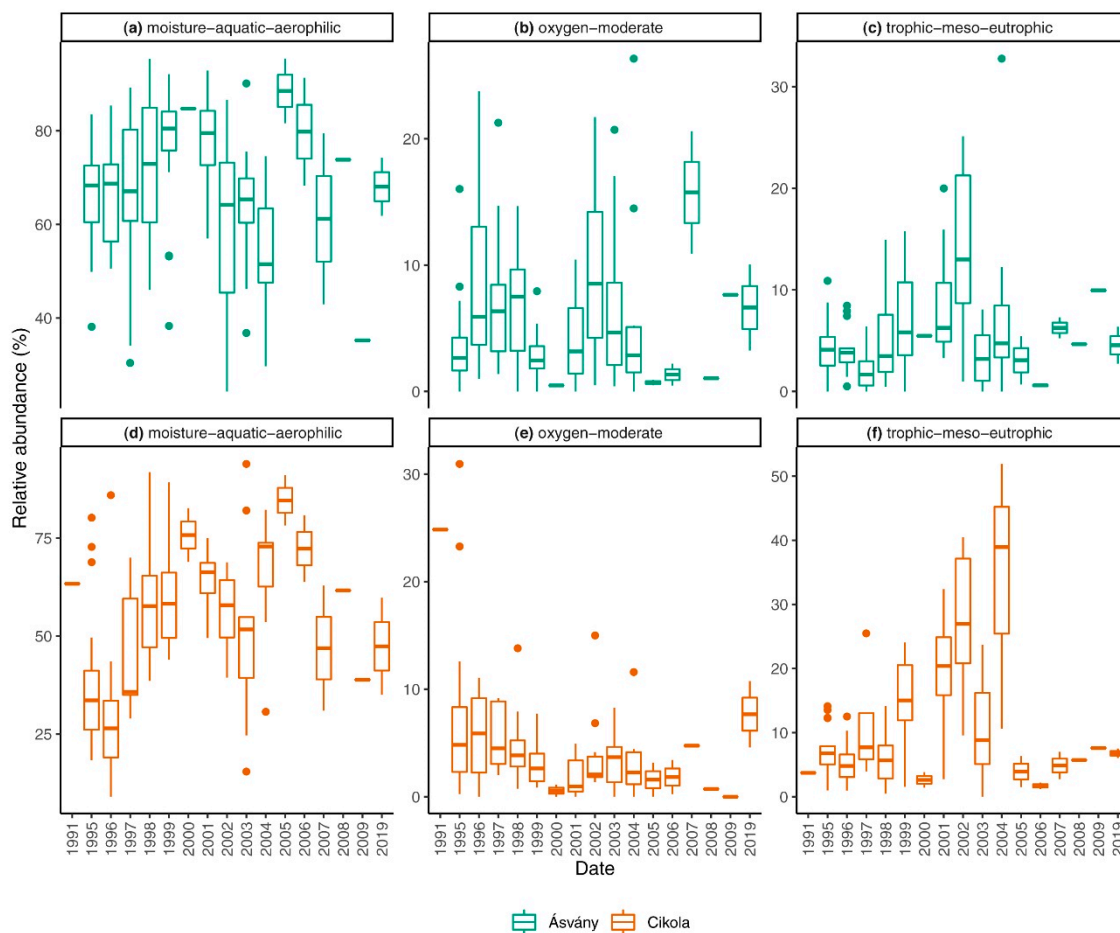


Figure 8. Long-term changes in relative abundance of significant traits of epiphytic diatom assemblages from deployed and natural reeds at a4 and a6 (a–c) sites in the Ásvány tributary system (green) and at c5 and c6 sites (d–f) in the Cikola tributary system (orange).

3.3. Ecological Potential Assessment

3.3.1. Temporal Changes in the Eupotamic Branches of the Tributary Systems

In the Cikola tributary system, the ecological potential of eupotamic branches during low discharge periods (1995–1997) often did not reach good potential; due to rehabilitation, an improving tendency was observed (Figure 9). Eupotamic branches of the Ásvány tributary system mostly have good ecological potential, although they show a slightly worsening tendency (Figure 10).

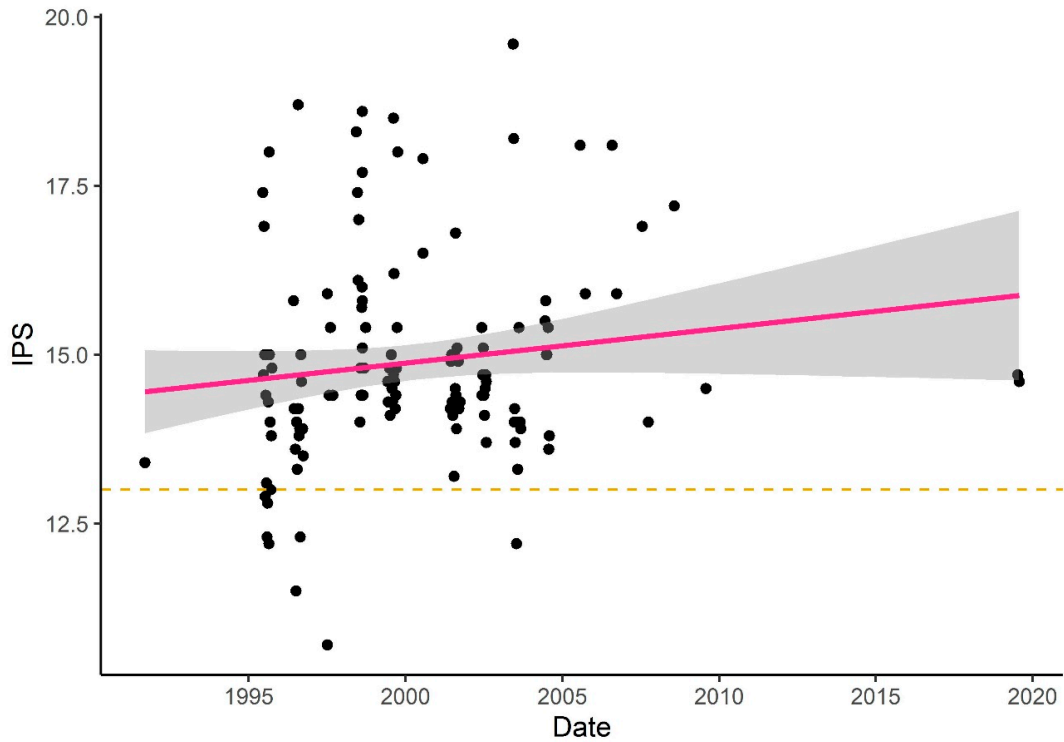


Figure 9. Values of the qualifying index are based on the composition of epiphytic diatom assemblages of deployed and natural reeds at c5 and c6 sites of the Cikola tributary system. Confidence intervals of the linear trend line is fitted to the points ($R^2 = 0.02$, $p = 0.120$). Orange dashed line indicates good/moderate boundary of the index.

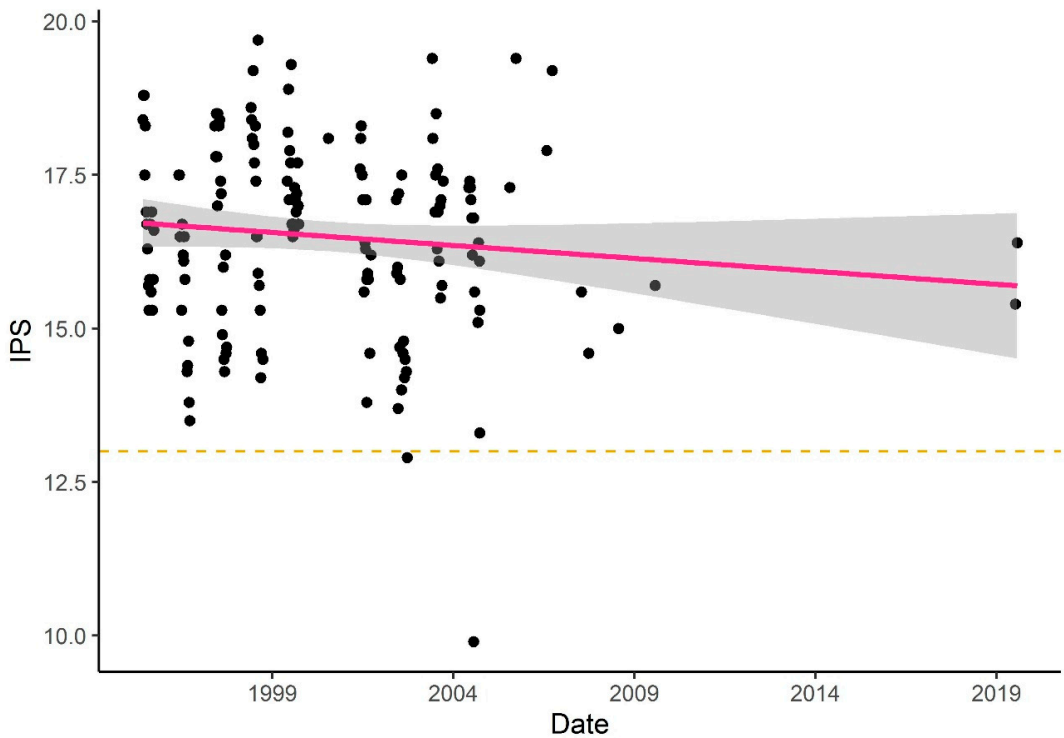


Figure 10. Values of the qualifying index are based on the composition of epiphytic diatom assemblages from deployed and natural reeds at a4 and a6 sites of the Ásvány tributary system. Confidence intervals of the linear trend line is fitted to the points ($R^2 = 0.01$, $p = 0.176$). Orange dashed line indicates good/moderate boundary of the index.

3.3.2. Differences in Eupotamic and Parapotamic Branches of the Tributary Systems

To examine the effects of rehabilitation, we compared the ecological potential of eupotamic (natural reed epiphytic diatoms from a4 and c5) and parapotamic (natural reed epiphytic diatoms from a2, a3, c3, c4, and a8) branches in the period of 1998 to 2009. No significant differences were found between calculated IPS index values of natural reed epiphytic diatoms of eupotamic and parapotamic branches in the Cikola tributary system (Brunner-Munzel Test Statistic = 0, $df = 59.238$, $p > 0.05$, n.s), while values in parapotamic branches of Ásvány tributary system (Brunner-Munzel Test Statistic = 4.942, $df = 33.937$, $p < 0.001$) were significantly lower (Figure 11).

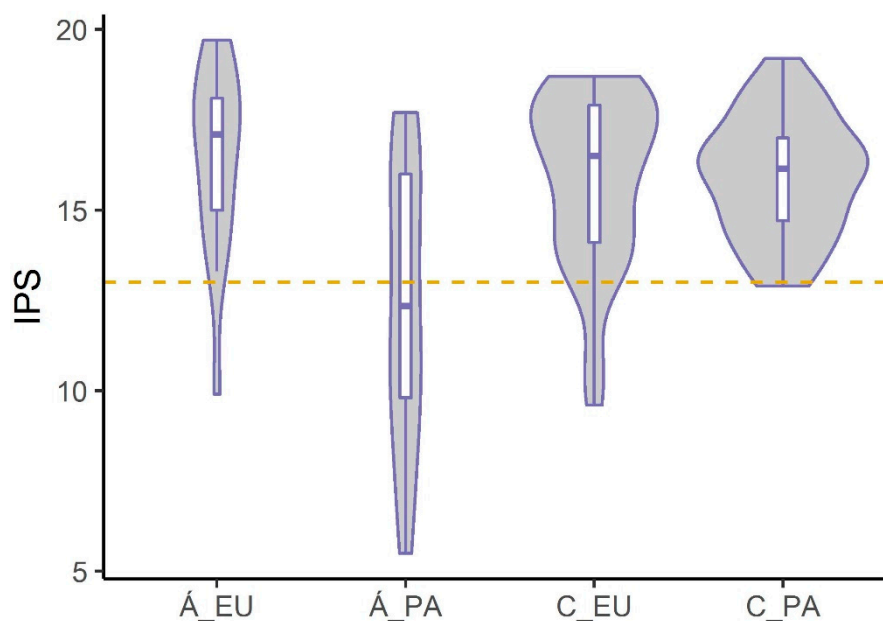


Figure 11. Violin plot of the IPS index in the eupotamic (EU) and parapotamic (PA) branches of the Ásvány (Á) and Cikola (C) tributary systems in the period of 1998–2009. Orange dashed line indicates good/moderate boundary of the index.

3.3.3. Correlation between Water Discharge and Ecological Potential

There was a weak, but significant correlation ($R = 0.2$; $p < 0.05$) between water discharge and qualifying metrics (IPS) applied to this section of the Danube. Thus, we have identified the water discharge threshold above which “not-good” ecological potential is not present. Results indicated that good ecological potential can be achieved at water volumes above $70 \text{ m}^3 \text{ s}^{-1}$, considering the thresholds of metrics currently developed for this section of the Danube (Figure 12). $70 \text{ m}^3 \text{ s}^{-1}$ is necessary for good ecological potential, but it is not per se a sufficient threshold.

The average of indices for water discharge of less than $70 \text{ m}^3 \text{ s}^{-1}$ (IPS average = 14.4) was significantly lower (Brunner-Munzel Test Statistic = -3.0235 , $df = 62.091$, $p < 0.01$) than indices for water discharge of greater than $70 \text{ m}^3 \text{ s}^{-1}$ (IPS average = 15.2) (Figure 13).

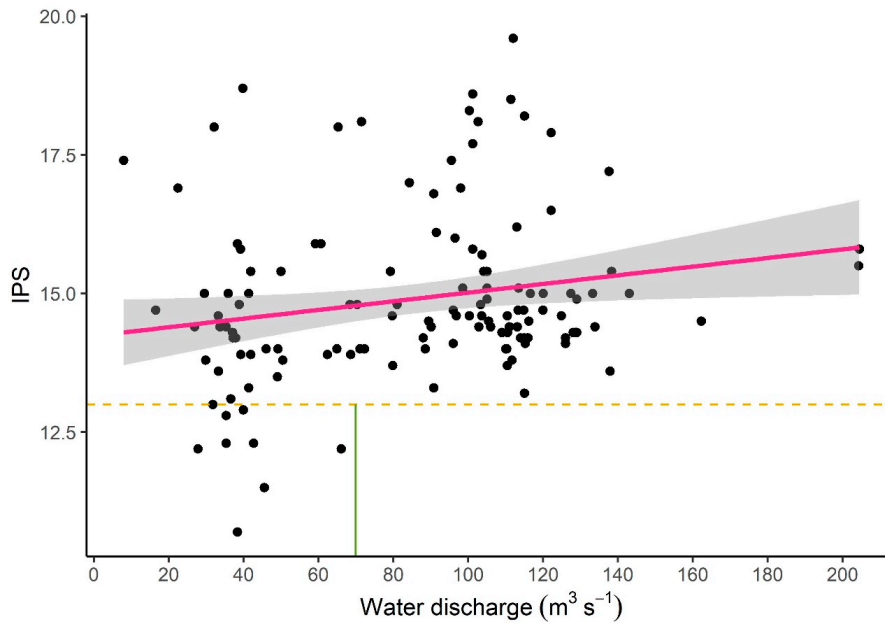


Figure 12. Changes of the IPS index values as a function of water discharge, based on the composition of diatom assemblages from both deployed and natural reed at c5 and c6 sampling points of the Cikola tributary system with a linear trend line fitted to the points. Dashed orange line represents good/moderate boundary of the index, green line indicates water discharge threshold above which the index always reached good value.

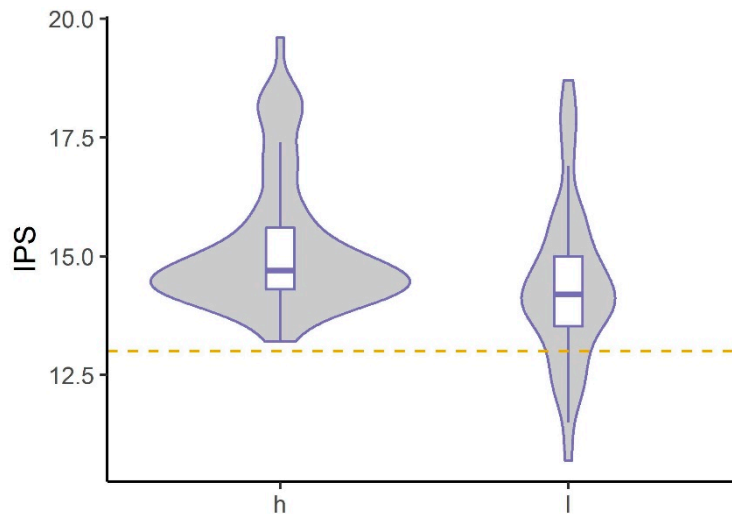


Figure 13. Violin plot of IPS index values based on the composition of deployed and natural reeds' epiphytic diatoms at c5 and c6 sampling points of the Cikola tributary system during periods of low water discharge (l) and water discharge above $70 \text{ m}^3 \text{ s}^{-1}$ (h). Orange dashed line indicates good/moderate boundary of the index.

3.4. Model—Effect of Rehabilitation

For traits that showed significant relationship with water discharge in the Cikola tributary system, we used a linear regression model (GLM) to estimate relative abundance of those periods when no phytobenthos monitoring was performed due to lack of funding. The fit of the model was validated with data from samples collected in 2019. Relative abundance of species that occasionally tolerate lower water coverage (aero-adapt-3) initially showed a slight increase, then slightly decreased during the rehabilitation period and subsequently stabilized (Figure 14a). The oxygen requirement of species is also related to improved water supply due to rehabilitation. Relative abundance of species with moderate

oxygen requirement (oxygen-adapt-3) decreased during rehabilitation (Figure 14d), while that of species with higher oxygen requirement (oxygen-adapt-2) increased (Figure 14c). Relative abundance of the motile ecological group (MG) and the trophic-trait meso-eutrophic species (trophic-meso-eutrophic) increased at the beginning of rehabilitation and then stabilized at a higher relative abundance compared to years with low water discharge (Figure 14b,f). Percentage of phytoplankton derived species (PG) in the epiphytic diatoms decreased due to rehabilitation (Figure 14e).

We also estimated the IPS diatom index for the missing period using GLM. Based on the model for the index, it can be seen that by keeping a good water level, the Cikola tributary system can achieve good ecological potential (Figure 15).

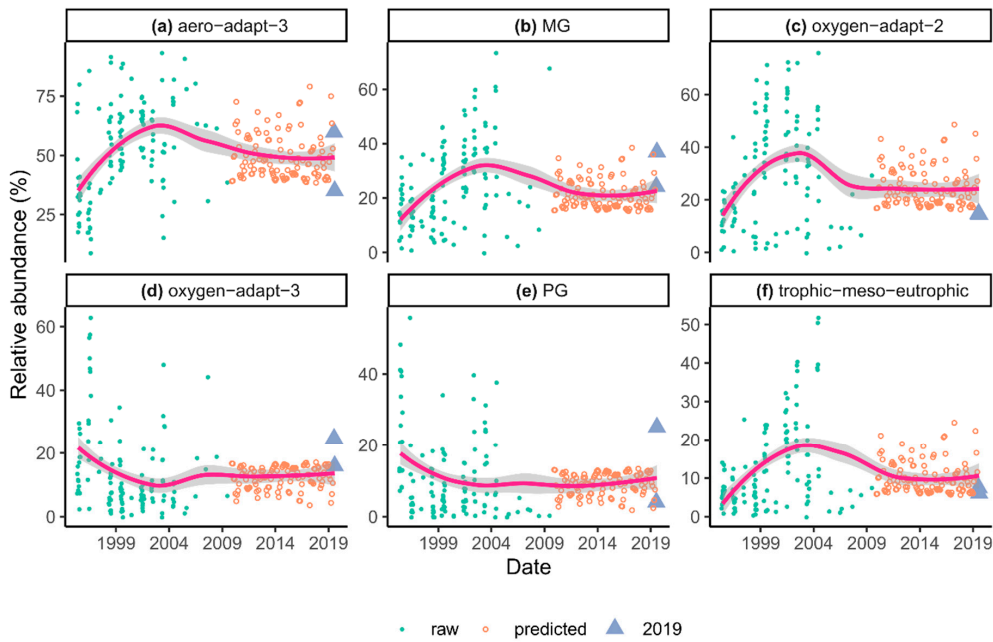


Figure 14. Relative abundance of significant traits of the diatom assemblage on deployed and natural reeds at c5 and c6 sampling points of the Cikola tributary system. Relative abundance is calculated using the model for missing period in the function of water discharge and LOESS (locally estimated scatterplot smoothing) curve is fitted to them. (See Figure 9. and text for abbreviations).

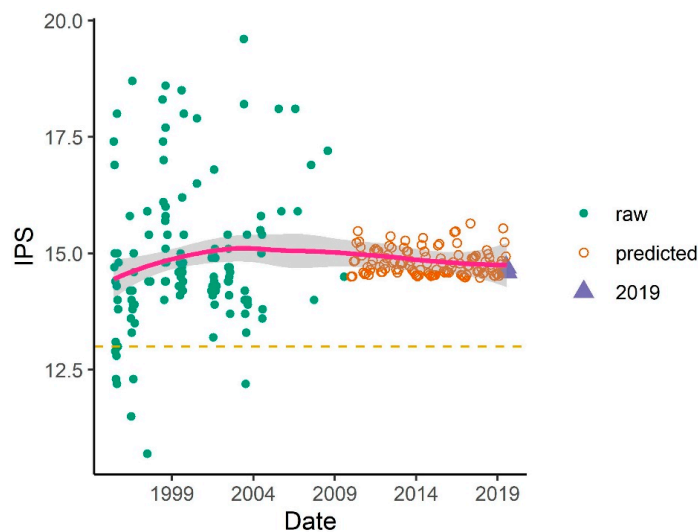


Figure 15. Calculation of IPS values based on the composition of diatom assemblages on deployed and natural reeds at c5 and c6 sampling points of the Cikola tributary system and calculations using the model for the missing periods in the function of water discharge and LOESS curve is fitted to them.

4. Discussion

4.1. Structure of Diatom Assemblage

The river continuum concept (RCC, [37]) describes longitudinal changes in communities of riverine ecosystems as a response to changes in physical and hydrological characteristics of water courses along continuous and longitudinal gradients. The river continuum concept did not consider factors (such as dams and reservoirs) that bypass longitudinal continuity, but it provides a comprehensive description of the role of longitudinal interactions in riverine ecosystems. Riverine systems can be considered as discontinuities with hierarchically nested and interacting elements [38]. In running waters, phytobenthos can be interpreted in four dimensions (horizontal, vertical, longitudinal, and temporal) and are related to hydrological characteristics and nutrient changes [31].

Confluences of tributaries are the focal points of physical and ecological processes [39], therefore, we performed regular and long-term epiphyton investigations at the confluence of two large tributaries of Szigetköz. The results of our surveys carried out before the diversion of the Danube clearly demonstrate that the most important factor in determining the composition of epiphytic diatoms was water velocity [13,14,16]. Due to the reduction of water scarcity following the diversion, the Upper Szigetköz has undergone significant rehabilitation interventions in recent decades. Based on the results of our present study, the effect of rehabilitation is clearly visible in the separation of epiphyton assemblages in the Cikola tributary system, as it was shown by the principal component analysis plot (Figure 4) and in the similarity of samples from the then non-rehabilitated Lower Szigetköz in the Ásvány tributary system (Figure 5). Diatom assemblages respond to stressors by altering their composition and relative species abundance [40,41]. For successful restoration of a riverine ecosystem, it is important to set achievable ecological goals and to use appropriate assessment strategies based on a comprehensive understanding of biotic reactions of manipulated variables, with particular reference to hydrology, as hydrology is an important driver of changes in aquatic ecosystems [42].

Microbial communities are often characterized by the dominance of some key species and these common and dominant species determine the composition of the whole community; the role of rare species is subordinate [43]. In addition, the impact of locally abundant species on dispersal occurs on a regional scale, as these key species reaching high numbers may populate additional habitats (mass effect) [44]. These key or core species likely colonize vacant or newly created spaces (substrates) easily and their populations may survive in an area for long [45]. The dynamics of core algal species have been poorly studied [31]. It is important to understand which species, under which environmental conditions, and when, will become dominant to predict changes in community structures, especially in connection with global warming water discharge, temperature conditions, and even the ice regime of rivers change [6,46].

The distribution of core species is shaped by interspecies interactions and niche division processes [47] and may significantly be modified as environmental conditions change [48]. Although the community structure showed significant variation in both time and space in the tributary systems studied, species composition was primarily determined by site and water discharge changes. Marazzi and Gaiser [31] found that the cumulative abundance of core species increased with decreasing nutrient content [31], because these species were the most adapted to nutrient limitation. In Szigetköz, there was a decrease in nutrient content (the proportion of meso-eutrophic species increased with time), but as we cannot speak of nutrient limitation, nutrient decrease did not cause a trend change in the temporal dynamics of core species. The success of the Everglades restoration, an oligotrophic subtropical wetland in South Florida, USA, has been associated with the abundance of core species and studies have shown that high diatom core species abundance must be maintained for success [42]. Changes in the relative number of common species showed a significant correlation with water discharge. Faster water flow promotes species dispersion allowing common species to appear in greater number in the samples. At the same time, with increasing water flow the selection pressure and species sorting is more pronounced and community structure is increasingly determined by

abiotic environmental gradient [44]. Significant variability in community structure also indicates that flowing water can maintain a dynamic structure in riverine systems. Junk et al. [49] suggest that taxon diversity dynamics are primarily influenced by high fluctuations in water discharge and lateral water exchange between the main river and the floodplain. At the same time, core species are more adapted to prevailing environmental conditions than less abundant species [45].

Our results show that changes in water discharge have a significant impact on benthic diatom species. Although R coefficient values are weak or moderate, correlations were significant. It allows us to predict further negative effects of changing water regime on the composition of the community due to global warming. Decreasing tendency of relative abundance of core species of the Ásvány tributary system can be attributed to the fact that rehabilitation had not yet affected the tributary system at the time of our investigation.

4.2. Traits of Diatom Assemblages

The ability of some algae to actively attach to the substrate plays a key role in the formation of phytobenthos. In lotic waters, water flow is one of the most important drivers in shaping benthic assemblages [50]. Nutrient is also a key factor that, together with hydrological factors, determines species dominance and distribution. During the investigated period, the most significant changes in the Szigetköz were caused by change in water quantity, and as a result, traits associated with it showed the most significant change. Beside abiotic factors, biotic factors (e.g., interspecific competition) also affect the relative abundance of these traits. Regarding traits related to the attachment mechanism and lifeform of diatoms, the proportion of high-profile groups in running waters is typically low because they have a long stalk that attach to the substrate and is easily torn off by flowing water [51,52]. During periods of low water discharge, their proportion was slightly higher in the epiphyton, partly because they are adapted to low current velocities [53]. Also, during periods of higher water discharge, there is more suspended sediment in the water, resulting in lower transparency, and HPG cannot grow under reduced light intensity [54]. Diatoms of the low-profile group are resistant to currents because they attach adnatly to the substrate [51]. Therefore, the amount of this group is typically the largest in flowing aquatic habitats. This group is also the dominant ecological group in the epiphyton of the main arm of the Danube [52]. In addition, LPGs are more common in shaded environments [54] and are able to withstand washing out and physical damage, explaining their domination of unstable habitats [27,53].

Traits such as motility contribute to species' access to light or nutrients; they can move to resource-rich microhabitats, avoiding stress or siltation [53,55]. Thus, increase in the amount of the motile group is often associated with sediment deposition, siltation or the risk of dehydration [56].

The appearance of planktonic ecological group members in the epiphyton is related to sedimentation of individuals [27]. Their proportion decreased with the increase of water discharge, as faster flowing water washes out deposited planktonic species more easily, or prevents them to settle [57].

Oxygen requirement of the species present and the ratio of temporally aerophilic taxa are good indicators of trends related to change in water discharge. Studies of transient small water courses revealed that the proportion of aerophilic species in drying out sites was higher than in sites with constant water coverage [58,59]. During rehabilitation, increased water velocity associated with increased water discharge transported better mixed, oxygen-rich water into the tributary system, also indicated by the increase in the proportion of species with higher oxygen requirement. In contrast, species with moderate oxygen requirement dominated in the Ásvány tributary system that was not rehabilitated at that time.

4.3. Ecological Potential Assessment

Benthic algae produce oxygen and organic matter, they provide habitat and food for other organisms who would not thrive without them, i.e. benthic algae are cornerstones of energy flow and

metabolism in the food web [60,61]. Diatoms are particularly suitable for monitoring environmental changes, as changes in both the composition and quantity of species are related to them. Diatoms are widely used to detect changes in water quality and to evaluate ecosystem status (e.g., Kelly et al. [62], Tan et al. [63]). In accordance with the requirements of the Water Framework Directive (WFD, [12]), artificial and heavily modified water bodies should be assigned ecological potential rather than ecological status. The rationale behind this is that during the development of an artificial water body or in a heavily modified water body, an ecosystem (complexity, functional and species richness, etc.) cannot develop, as would be expected in natural standing or running waters of a similar water type [64]. In Hungary, the specific pollution sensitivity index (IPS, Coste in Cemagref 1982) has been applied to evaluate the ecological status/potential of the Danube River and its branch systems [65], this index was used alongside the Danube for ecological status assessment [66]. Although the IPS index has been primarily developed for the detection of basic inorganic nutrients and organic loads in water courses, its value is also associated with hydro-morphological changes [52]. Diatoms are also good ecological indicators of the effect of altered hydrological conditions, as they react with great sensitivity not only to chemicals but also to physical conditions [67]. They reflect water changes well, although some traits only change slowly. In the long term, water scarcity could place selection pressure on species, i.e., species that can tolerate these conditions become abundant. However, the epiphyton has significant self-regulating properties due to exopolysaccharide (EPS) matrix, which holds the community together, protects it from drying out, and retains nutrients. This can reduce the fluctuation of relative abundance of species over time, so they can better withstand periods with adverse environmental conditions [31].

In our study, both parapotamic and eupotamic branches achieved good ecological potential as a result of rehabilitation in the Cikola tributary system, while in the Ásvány tributary system there was a significant difference in the ecological potential between parapotamic and eupotamic branches. Eupotamic branches showed good ecological potential at the time of our study, although a slight worsening trend can be observed. Parapotamic branches, on the other hand, did not reach good ecological potential. The protected side of the Lower Szigetköz only received limited amount of water from the Upper Szigetköz. As a consequence, stagnant water, water quality deterioration, occasional dehydration, and degradation of local communities could be observed in the parapotamic branches during long periods of low water, which required additional water supply. Thus, the rehabilitation of the Lower Szigetköz became necessary.

Climate change challenges the traditional assumption that past hydrological experience provides a good guidance for future conditions, and has now impacts on the distribution of water resources, including changes in precipitation, evapotranspiration, and in the quantity, quality, and timing of water flows [68]. This creates new challenges for scientists, water managers, both those concerned for human uses and those regarding the environment to adapt to changing hydrology [69]. Based on the classification using benthic diatoms, at least $70 \text{ m}^3 \text{ s}^{-1}$ of water should be introduced into the Cikola tributary system in the present climate conditions. In order to have good ecological potential in the tributary system of the Lower Szigetköz, certainly more water is needed. In a study published in 2018, the International Commission for the Protection of the Danube River (ICPDR) pointed out that the average temperature in the Danube basin [70] had risen markedly in the 21st century. According to the study, a further rise of $1.3\text{--}2.5 \text{ }^\circ\text{C}$ in the Central Danube region can be predicted between 2021 and 2050, which may reach $5.4 \text{ }^\circ\text{C}$ in the long term (2070–2100). Furthermore, as the temperature rises, the amount and distribution of precipitation also changes. While in the Danube-basin rainfall is expected to decrease by 5–10% in the long term, in the Central Danube region it may reach 20% in the long run. The greatest fall is expected in summer (17–58% depending on the model), while in winter it is expected to increase by 13–34%. This will also bring further changes in the Danube's water regime (e.g., increasing length of low water period), which will also affect the water supply of the Szigetköz tributary systems. During periods of low rainfall, it will become increasingly difficult to maintain the quantity of water needed to maintain good ecological potential in the tributary systems. Based on our results, it is already apparent that the minimum water volume of $32 \text{ m}^3/\text{s}$ [11] is insufficient to keep the

Cikola tributary system in good ecological potential. In the light of the forecasts, regular studies are needed to minimize the adverse effects of climate change on the ecosystem of Szigetköz.

4.4. Model–Effect of Rehabilitation

Most traits showing significant changes over time also showed significant association with water discharge. This confirms Munteanu and Maly's [50] finding that water flow in lotic waters is one of the most important drivers and most involved in shaping the epiphyton. In addition to traits associated with the mechanism of attachment and lifeform of algae, as well as to oxygen requirement categories and proportion of aerophilic taxa, cell size also showed significant correlation with water discharge. As water discharge increased, the proportion of large species decreased. In the Danube, significant decrease in nutrient has taken place in recent decades [7], which is also reflected in the increase in species with lower nutrition demand. Based on the model, it is clear that rehabilitation of the Cikola tributary system has stabilized the ecosystem, at least in terms of the epiphyton, but regular examination cannot be avoided because of new challenges described in the introduction (global warming, long-term effects of the diversion). Climate predictions suggest that the Danube will have a water shortage of 22% by the end of the 21st century [71] compared to measured water regimes at the early 21st century. This will bring significant changes not only in the investigated area, but in all affected aquatic ecosystems.

5. Conclusions and Implications for Management

1. Our results revealed greater spatial differences in the relative abundance of core species than in time due to spatial differences in environmental condition (first of all because of differences in water quantities between the Upper and Lower Szigetköz). The relative abundance of common species increased with increasing water discharge, as faster water flow could support species dispersion.
2. After the diversion of Danube River, significant changes took place in diatom traits as well, but after the introduction of artificial water supply smaller changes occurred, especially for traits associated with water volume.
3. Our results confirmed the need for rehabilitation of the Szigetköz tributary systems of the Danube, as rehabilitation improved the ecological potential of the branch system because of the increased water volume. There was a slight difference in the ecological potential of eupotamic and parapotamic branches in the rehabilitated Upper Szigetköz, while the ecological potential of parapotamic branches was lower than that of eupotamic ones in the Lower Szigetköz not rehabilitated at that time.
4. In order to maintain good ecological potential, at least $70 \text{ m}^3 \text{ s}^{-1}$ of water should be introduced into the branch system based on the phytobenthos study. Although, $70 \text{ m}^3 \text{ s}^{-1}$ is necessary for good ecological potential, it is not per se a sufficient threshold. The method used here provides the basis for further targeted studies to clarify a lower threshold of good ecological potential and to fine-tune water management.
5. Regular investigations and a research program are needed to better understand how communities respond to anthropogenic impacts and rehabilitation, as well as to predict how the ecosystem of the Szigetköz will respond to the effects of global warming and adapt to changing environmental conditions (temperature rise, extreme water regimes).
6. Our long-term data sets may provide a good basis for comparing, for example, the abundance of core species and community structures in different aquatic ecosystems as they respond to different anthropogenic pressures (e.g., nutrient loads). Using such and similar data sets, we can understand and predict how algal core species will cope with the effects of accelerating climate change (temperature rise, altered water regime, water level fluctuation, etc.).

7. Our results support the finding that different levels of organisms (both at species and trait levels) need to be explored to gain a deeper understanding of the role of environmental, climatic, regional, and temporal factors in the structure of biological communities [8].
8. We studied a “natural mesocosm” site where different hydrological changes occurred in the same catchment area and these effects could be studied on traits. In light of the impact of global warming stressors on biodiversity, the identification of adaptive traits is of great importance.

Author Contributions: K.B., É.Á. contributed to the fieldwork and sampling design, K.B. carried out the sampling and counting of the diatoms. Z.T. and A.F. applied the trait-based approach. A.H., J.L.K. and T.B. performed statistical analyses. É.Á., T.B., I.G., E.V., M.D., C.B., K.B., J.L.K., J.K. contributed to the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The regular monitoring was financed by the late Ministry of Environmental Protection and Regional Development, later Ministry of Environment and Water; and organized by MTA Szigetköz Working Group. The research was supported by the GINOP-2.3.2-15-2016-00019 and National Research, Development and Innovation Office—NKFIH, 119208 grants and the Higher Education Institutional Excellence Programme (NKFIH-1150-6/2019) of the Ministry of Innovation and Technology in Hungary, within the framework of the 4th thematic programme of the University of Debrecen.

Acknowledgments: Map data copyrighted OpenStreetMap contributors and available from <https://www.openstreetmap.org>.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

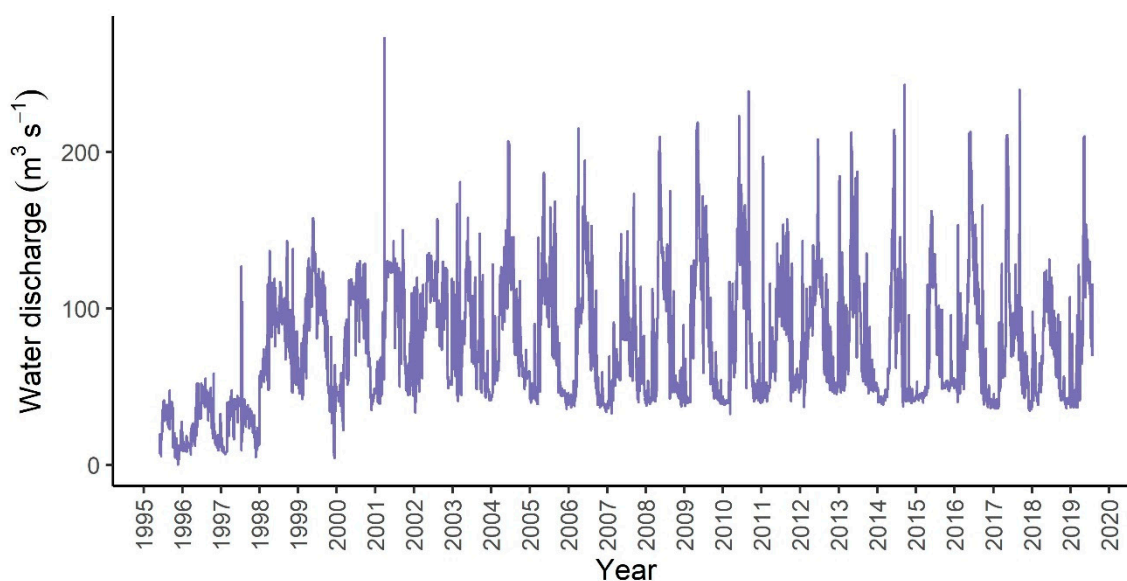


Figure A1. Discharge rates in the Cikola tributary system from 1995 to present.



Figure A2. Picture of the deployed reed island.

Table A1. Mean coefficients of variation (CV %) of cumulative core and common species abundance and evenness, across sites and over years in the Ásvány and Cikola branch systems.

	Years	Sites
Top1 CV% mean	48.1	74.7
Cum. core CV% mean	25.9	40.1
Cum. com. CV% mean	44.3	75.2
Evenness CV% mean	17.9	23.7

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