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Exploring Key Determinants of the Periphytic Diatom Community in a Southern Brazilian Micro-Watershed

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Abstract: Associating anthropogenic effects with variations in biodiversity is key to understanding how anthropogenic impacts are extrapolated in public supply micro-watersheds. The structure and dynamics of metacommunities in aquatic environments depend not only on the river network itself, but on a multitude of factors. Therefore, we associate the density and species richness of diatoms, assessed in a micro-watershed, with the following driver factors: local environmental variables, spatial variables, landscape characteristics, and the historical community, comparing their possible dispersal routes. Variance partitioning was performed using partial RDA models, with prior selection of predictor variables, to estimate the relative role of each predictor in the diatom community. The small scale of the micro-watershed resulted in a small spatial gradient, reflecting in the low variation in community richness across sampling stations. However, temporal heterogeneity associated with fluctuating precipitation throughout the year may cause temporal variation in the relative abundance of species. This pattern is a result of the supply of resources that increases biodiversity over time, as it allows the coexistence of species that alternate between dominance and persistence. Thus, even on a small scale and during one year of sampling, we demonstrated that predictors of different natures act together to explain diatom communities in micro-watersheds.

Keywords: lotic environments; macro and microscale; metacommunity; dispersal routes; diatoms

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1. Introduction

Water resources have been considered the most important of all natural resources [1]. Despite being essential for human survival, whether for consumption or other applications, drinking water is poorly distributed across the planet [2,3]. In addition, territories with high water availability such as Brazil are facing a generalized water crisis, with an imminent reduction in the level of water resources [4]. In the Brazilian scenario, these consequences are maximized by population expansion, affecting the generation of electricity and, above all, the potability of water, especially in rivers used for public supply [5]. By providing environmental and ecosystem services central to human society, natural resources are indispensable in maintaining the communities present in lotic environments and all biodiversity [6]. However, anthropic activities are modifying natural characteristics and threatening ecosystems associated with water bodies [7]. Some important discussions in ecological studies aim to understand which factors explain species variation in aquatic communities [8,9]. Possible dispersal routes, environmental characteristics, and landscape configuration are often considered predictors of metacommunity distribution patterns [10,11].

Although it is agreed that the aforementioned factors act at different spatial scales [12], the relative importance of these factors is likely to change [13]. On a regional scale, the landscape tends to become homogenized as a consequence of the history of this territorial cutout [14]. At finer scales, local environmental factors (abiotic variables and biological interactions) are probably more important in explaining environmental filtering over smaller territorial extents [15]. In addition, the smaller the territorial extent, the greater the cumulative anthropic pressure on aquatic ecosystems due to the significant portion of land use that mixes agricultural and urban purposes, which contribute to reduced biodiversity [16]. High population densities imply the entry of domestic, industrial, and agricultural effluents into streams, loss of riparian vegetation, conversion of forested areas to agricultural use, and increases in impermeable areas in urban settlements [17]. The sum of these factors not only simplify the landscape, but also affect aquatic communities, altering limnological characteristics and impairing water quality [18]. With this in mind, linking anthropogenic effects to biodiversity variation is critical to understanding how anthropogenic impacts are extrapolated in public supply micro-watersheds (see [19]).

Among aquatic communities, periphytic diatoms are commonly used to assess the effects of environmental and spatial predictors [20], since they are algae that easily adhere to some substrate through mucilage production, which can restrict their dispersal and maximize the influence of these factors [21]. Furthermore, these microorganisms respond efficiently to changes in water characteristics, as well as in climate, geology, and land use in micro-watersheds [22,23]. Despite this, few studies use periphytic diatoms as a model of metacommunities in lotic environments [24].

The development of computational tools and increased data availability allows for better comparisons of the relative role of different predictors in structuring metacommunities. Nevertheless, studies comparing landscape descriptors, local descriptors, and spatial configuration for diatoms are rare [25–27]. Spatial configuration is commonly used as an indicator of dispersal routes [28]. However, dispersal of microalgae can occur via multiple vectors: by air [29], by flow-independent water (e.g., [30]), and by flow-dependent water (e.g., [31]). Considering each of these different dispersal hypotheses may more clearly reveal the relative role of predictors of metacommunities [32].

In Brazil, research involving dispersal routes of diatoms related to environmental and spatial filtering are usually developed in reservoirs addressing phytoplankton communities. Bortolini et al. [33] demonstrated that connectivity and dispersal by water flow in a reservoirs–river–floodplain gradient maximize dispersal rates of phytoplankton communities, contributing to increased biomass and homogenization of neighboring communities. Zorzal-Almeida et al. [34] observed that environmental and spatial factors together have a strong influence on variation between planktonic and surface sediment diatom communities in tropical reservoirs, indicating mass effects as a strong structuring predictor. Oliveira et al. [35] also evaluated the relative importance of local and spatial environmental processes on planktonic diatoms in streams in central Brazil, indicating that both act together in affecting metacommunity structuring. Medeiros et al. [36], however, evaluated the response of the periphytic diatom community through environmental predictors along a linear gradient using a distance-by-watercourse matrix. There is greater interest in the study of reservoirs due to their applicability; however, it is also crucial to understand the dynamics of river metacommunities belonging to micro-watersheds. Although these ecosystems are heavily impacted by human actions, they are still essential for public supply, and biomonitoring them provides information on water quality, consequently helping to maintain social health.

Indeed, it is agreed that the structure and dynamics of metacommunities in aquatic environments depend not only on the river network itself, but on a multitude of factors [37]. Understanding how these factors reflect on diatom community structure can help to understand ecological processes in river systems, which are important for the maintenance of the entire ecosystem, but suffer severe threats [38]. Our aim was to compare possible determinants of the periphytic diatom community in a micro-watershed: (i) determinants generated to represent different dispersal routes, (ii) determinants referring to local micro-habitat filtering, and (iii) determinants related to landscape-scale environmental filtering. Thus, diatom community structure was explained both by considering the relative density of species (representing dominance and rarity relationships), and by the simple presence and absence of species (representing local occurrence capacity). Finally, we also considered the community composition from a previous period to explain the diatom community of the following period, thereby considering previous colonization as one of the determinants of the metacommunity (see [39]).

2. Materials and Methods

2.1. Study Area

Cascavel city (24°57′21″ S and 53°27′19″ W) is located in the western region of the State of Paraná, a subtropical area with a mean annual temperature of 21 °C [40]. The Cascavel River (24°32′ and 25°17′ S; 53°05′ and 53°50′ W) has a drainage area of 17.50 km^2 divided in similar percentages between agricultural/urban areas and reserve or forest areas [41] (Figure 1). The main riverbed is 17.85 km long, with the main headwaters located in urban areas [42].

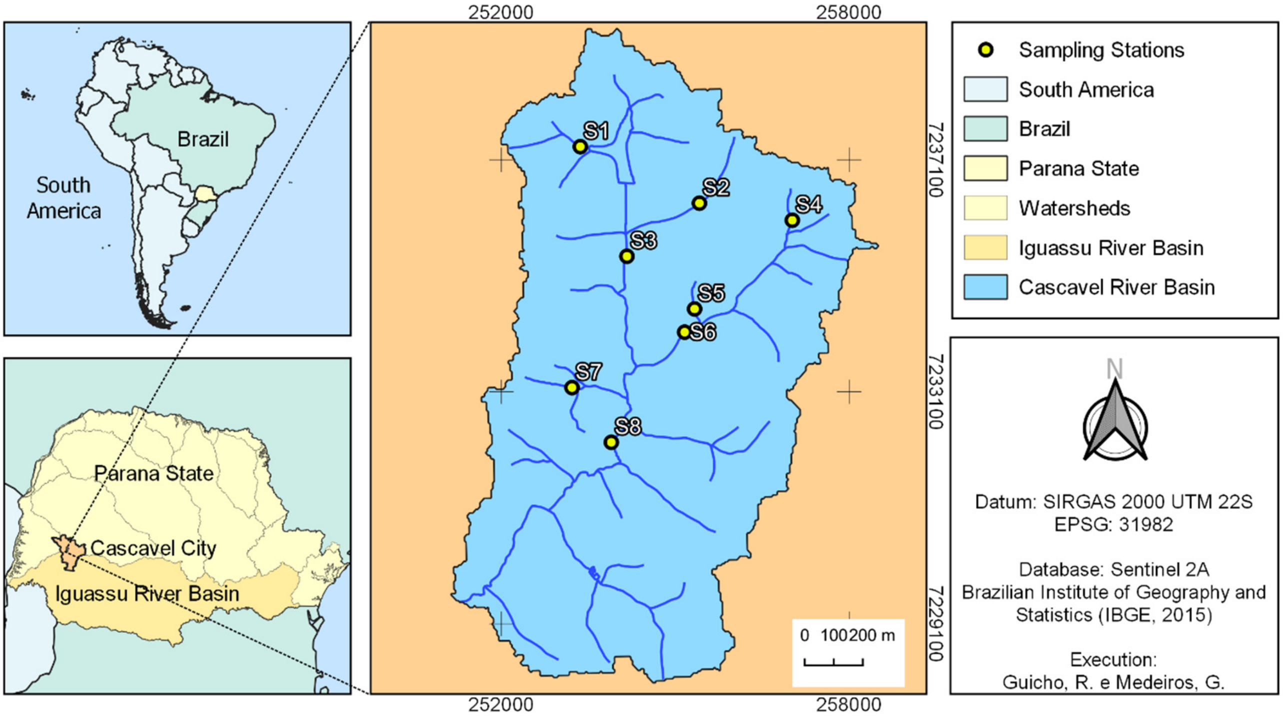

Figure 1. Location of sampling stations (S) in Cascavel River micro-watershed, Cascavel City—PR, Brazil.

2.2. Sampling Design

Eight sampling stations were selected in the micro-watershed of the Cascavel River (Table 1), considering the land use and occupation for agricultural, industrial, extractive activities, and urban settlements, following the head–mouth direction. Samplings were performed quarterly (one per season) during 2018, totaling four samplings for each community, with communities 1, 2, 3, and 4 being associated with March, June, August, and December, respectively.

Table 1. Location with geographic coordinates of the collection points along the Cascavel—PR river, Brazil.

Sampling Sites	**Geographic Coordinates**	
	Latitude	**Longitude**
S1	24°57′32.93″ S	53°26′33.32″ W
S2	24°58′35.81″ S	53°26′6.85″ W
S3	25°0′13.36″ S	53°26′19.12″ W
S4	24°58′17.29″ S	53°24′24.75″ W
S5	24°59′5.99″ S	53°25′23.83″ W
S6	24°59′18.90″ S	53°25′32.20″ W
S7	24°59′48.82″ S	53°26′42.08″ W
S8	25°0′13.36″ S	53°26′19.12″ W

2.3. Landscape and Climate Variables

The land use and occupation measurements were performed by manual classification in QG is software (available in open licensing), using images obtained from the Sentinel 2A satellite with a spatial resolution of 10 m. The study area was classified according to five main classes: Forest, Mining, Water, Rural, and Urban. The divisions were based on the Land Use Technical Manual [43], which provides information about the types of land use included in each class (Figure 2). Climatological variables such as temperature, air humidity, wind speed and direction, solar radiation, and rainfall were provided by the Paraná Meteorological Institute (SIMEPAR).

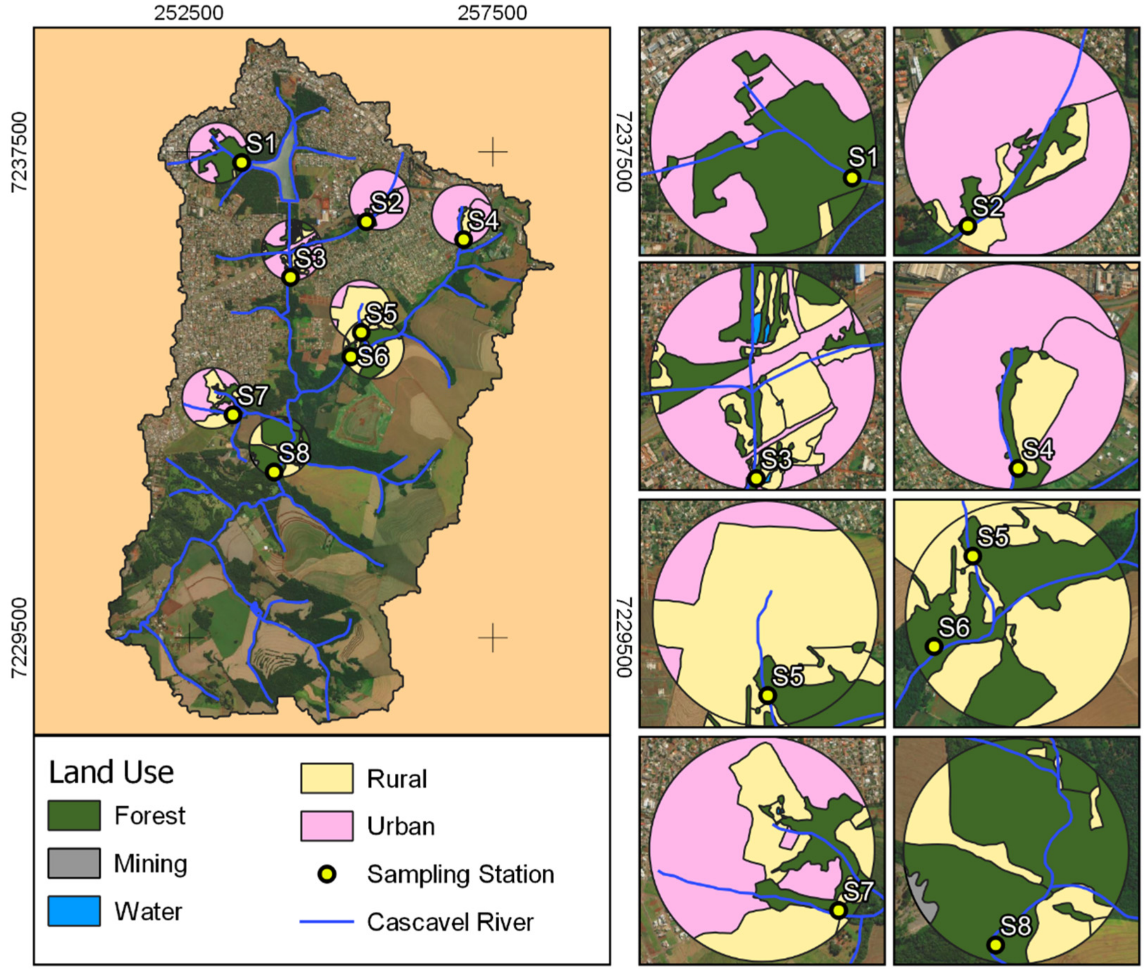

Figure 2. Area of influence containing detailed land use, and sampling stations following the upstream-downstream flow, with a diameter of 1 km.

2.4. Local Environmental Variables

The physical and chemical variables such as temperature, electrical conductivity, dissolved oxygen, pH, and turbidity were measured in situ by a HORIBA multiparameter probe, model U-5000.

We collected water samples for physical–chemical analysis by filling 5 L polyethylene gallons, and also 100 ml of water using dark glass bottles for microbiological analysis, both cooled and kept in the dark until their use in the laboratory. The oxygen consumption due to chemical oxidation (COD) and organic matter (BOD), concentrations of total Kjeidahl nitrogen (NT), total dissolved phosphorus (TDS), orthophosphate (PO_4^-), nitrate ($N\text{-}NO_3$), ammonium ($N\text{-}NH_4$) and chlorophyll *a* were analyzed by the Environmental and Agronomic Analyses Laboratory (ACQUASOLLUS-Campo Mourão), following Standard Methods [44].

The superficial sediment was collected following the protocol of the Environmental Company of the State of São Paulo and the National Water Agency [45]. Samples with approximately 500 g of superficial sediment (0–5 cm depth) were collected utilizing plastic bags. Three sediment samples were collected at each sampling station and mixed to constitute a composite sample. In the laboratory, the samples were dried at room temperature in a closed place, protected from sunlight, wind, and excessive light. Then, the sediments were sieved using a 63 μm mesh to perform metal analyses. The sediment processing and analyses were performed in the laboratory of Unioeste—Cascavel campus. Aluminum (Al), barium (Ba), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), sodium (Na), and zinc (Zn) were read, according to U.S. Environmental Protection Agency method USEPA 3050B [46], analyzing the silt/clay fraction of the soils using atomic absorption spectrometry (AAF) or inductively coupled plasma atomic emission spectrometry (ICP-AES).

2.5. Dispersal Route Indicators

Possible dispersal routes were generated considering the following methodologies:

(1) To test "overland", we considered the Euclidean distances resulting from the Principal Coordinate of Neighbor Matrices (PCNM, [47]), an ordination matrix generated using the coordinates of the sampling stations, creating a spatial connection network with links at all evaluated sites without directionality (i.e., the sampling stations were connected by two directions, upstream–downstream and downstream–upstream).

(2) To test asymmetric eigenvector mapping "AEM", the latitude and longitude of the sites were used to relate the points, considering whether the sites had connectivity or not [48].

(3) To test "watercourse", the distance matrix was generated by drawing a dendritic system over a real river map with Quantum GIS software. Thus, we modeled a spatial network with directional links over the watercourse (considering that the sites were connected only as a function of the river water flow).

2.6. Periphytic Community

Three substrates (stones), three meters apart from each other, were collected at all sampling stations, making a composite sample in order to evaluate the periphytic diatom community. The biofilm containing the diatoms was scraped from all the rocks using a toothbrush and distilled water. The density and relative abundance of taxa were estimated based on standard scraping of the stones (area equal to 9 cm^2), considering the down-stream face of each substrate. Samples were preserved in 1% Lugol's acetic solution (Bicudo and Menezes 2017) and stored at the Herbário da Universidade Estadual do Oeste do Paraná (UNOPA). Permanent slides were prepared with oxidized subsamples according to Simonsen's [49] technique, modified by Moreira-Filho & Valente-Moreira [50], with known volume (1.0 mL) and Naphrax® resin (refractive index = 1.74) as the mounting medium. Quantitative analyses were performed in light microscopy (LM) at 1000× magnification using an Olympus B × 34 microscope equipped with a DP 71 capture and phase contrast camera. Individuals were identified and counted according to Kobayasi & Mayama [51], (1982), ensuring a minimum efficiency of 90% [52]. Diatom valves were counted in an optical microscope (1000×), as oxidation tended to separate frustules (Moro and Bicudo, 2002), including at least 50% of the full-size broken ones. Cells in pleural view were identified when possible. We followed the recommendations of Battarbee [53] and Lobo et al. [54] for counting under light microscopy, calculating densities per cm^2. Abundant (valve density exceeds the average density for the sample) and dominant species (valve density is over 50% of the total density) were determined according to Lobo and Leighton (1986).

2.7. Data Analysis

Environmental variables that were not collinear and previously standardized by standard deviation were subjected to Principal Component Analysis (PCA) (see [55]) in order to characterize the sampling sites. Spatial (between sampling sites) and temporal (between periods) variation in species density and occurrence, as well as in environmental variables, were assessed using non-parametric permutational multivariate analysis (PERMANOVA, [56]). A Bray–Curtis matrix was used for species density after Hellinger transformation, a Sorensen matrix for occurrence, and a Euclidean distance matrix with environmental data previously standardized by standard deviation (see [55]).

The diatom community matrix was related to matrices with the following factors: local environmental variables, spatial variables (connectivity by dispersal routes), landscape characteristics, and the community from the previous period. The effect of previous community colonization was considered in the explanation by using matrices summarized in a principal coordinate analysis applied to the Bray–Curtis or Sorensen matrix (abundance or occurrence, Principal co-ordinates analysis (PCoA)), [57], with the diatom community from the previous period considered as a predictor of diatoms from the following period (except for the first collection period, see also [39]).

In PCoA analysis, data are divided into components to maximize the linear correlation between data points in a dissimilarity matrix. Through a "coordinate transform", the observed values in the sample stations are replaced with newly derived coordinates. Thus, we reduce the dimensionality of a dataset by discarding coordinates that may not capture a threshold of variance in the community data. This technique preserves the global structure of the data while projecting it in low dimension. The predictor variables, i.e., environmental and landscape variables (standardized by standard deviation), were also previously scaled using a PCoA applied to the Euclidean distance matrices. The PCoA was used in order to improve the correlations of the predictor matrices with the community matrices [55,58].

Variance partitions were then performed using partial RDA models (pRDA, [55]), with prior selection of predictor variables (i.e., environmental, landscape, dispersal hypotheses, and previous community) through a stepwise approach [48,59], seeking to estimate the relative role of each predictor in the diatom community. Models were generated separately for each period, considering relative densities or presence and absence.

All analyses were performed using the R Development Core Team language [60] and its environment for computational statistics, along with the vegan [61], adespatial [62] and spdep [63] packages.

3. Results

The characterization of the sampling stations regarding the landscape variables pointed out the differences between these environments and the dominance of urban and agricultural areas in the Cascavel River micro-watershed. Sampling stations 2, 3, 4 and 7 are predominantly urban, stations 5 and 6 are predominantly used for agriculture and livestock, and stations 1 and 8 have a larger area of forest than the others (Figure 2). The urban influence area strongly influenced the sampling stations in the headwaters of the micro-watershed, although they have natural vegetation, which was also observed for points under the rural extension influence area (Figure 2).

Throughout 2018, the Cascavel River micro-watershed suffered great interference from seasonal variation, showing high accumulated precipitation in the first sampling period when compared to the others (Figure 3).

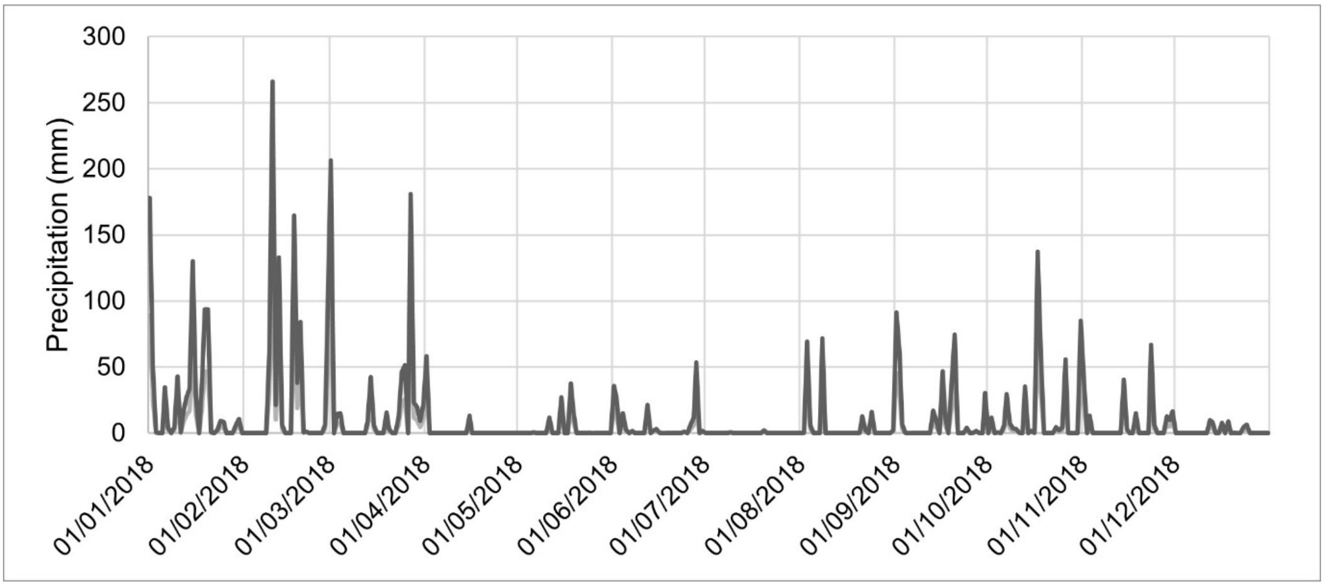

Figure 3. Daily precipitation (mm) in Cascavel city—PR throughout the year 2018. Data provided by SIMEPAR.

The environmental variables' spatial variation (F = 2.07; R^2 = 0.38; p = 0.001) and temporal variation (F = 3.21; R^2 = 0.26; p = 0.001). The Principal Component Analysis (PCA) summarized 31.91% of the total variability of the sampled data in the first two axes (Figure 4). The scores showed the separation of the environments according to the sampling period and the land use at the sampling points. We highlight the interference of the macroscale in the March sampling period (the highest accumulated precipitation among the evaluated periods—557.2 mm in 30 days). This period was associated with higher values of Al, Cu, Nitrate and Chlorophyll *a*. In contrast, June had a lower association with metals in the surface sediment, as well as higher COD and total phosphorus values. Additionally, the analysis grouped stations 1, 4, and 7 (all first-order streams, and heavily impacted by urbanization) with higher values of Na, Zn, Cr, and Pb.

We found 118 infrageneric taxa, distributed in 37 genera. *Eunotia* Ehrenberg, *Pinnularia* Ehrenberg (11 spp. each), *Gomphonema* Ehrenberg and *Navicula* Bory (9 spp. each) showed high representative taxa richness among all sampling stations (see the means of relative abundance for each species according to sampling stations in Appendix A). The variation in species density for the four samplings showed spatial variation (F = 1.82; R^2 = 0.35; p = 0.001), but not temporal variation (F = 1.17; R^2 = 0.11; p = 0.168). In contrast, there was no spatial variation in species occurrence (F = 1.02; R^2 = 0.23; p = 0.4), but occurrences varied in time (F = 1.98; R^2 = 0.18; p = 0.001). However, some species differentiate the sampling stations: *Achnanthidium modestiforme* (Lange-Bertalot) Van de Vijver (S1, S2, S4, S6, and S8, with the greatest relative abundance at S4), *Cymbopleura naviculiformis* (Auerswald) Krammer (S3), *Eunotia yberai* Frenguelli (S4), *Navicula cryptocephala* Kützing (S1, S2, S3, S6, and S8, with the greatest relative abundance at S2 and S8), *Navicula cryptotenella Lange-Bertalot* (S1, S2, S3, S5, and S7, with the greatest relative abundance at S5), *Navicula salinicola* Hustedt (rare at S1, common at S2), and *Spicaticribra kingstonii* Johansen, Kociolek & Lowe (S3 and S8). We also observed the dominance of *Eunotia botulitropica* Wetzel & Costa (June, S7), *Eunotia veneris* (Kützing) De Toni (December, S1, with relative abundance higher than 50%,), and *Gomphonema parvulum* (Kützing) Kützing (December, S3). Species variation between sampling stations is described in the Appendix A. The explanatory power of the selected predictor variables was relatively low, and most of the variability in diatom community structure remained unexplained in pRDA (see fraction containing residues [U] in Figure 5).

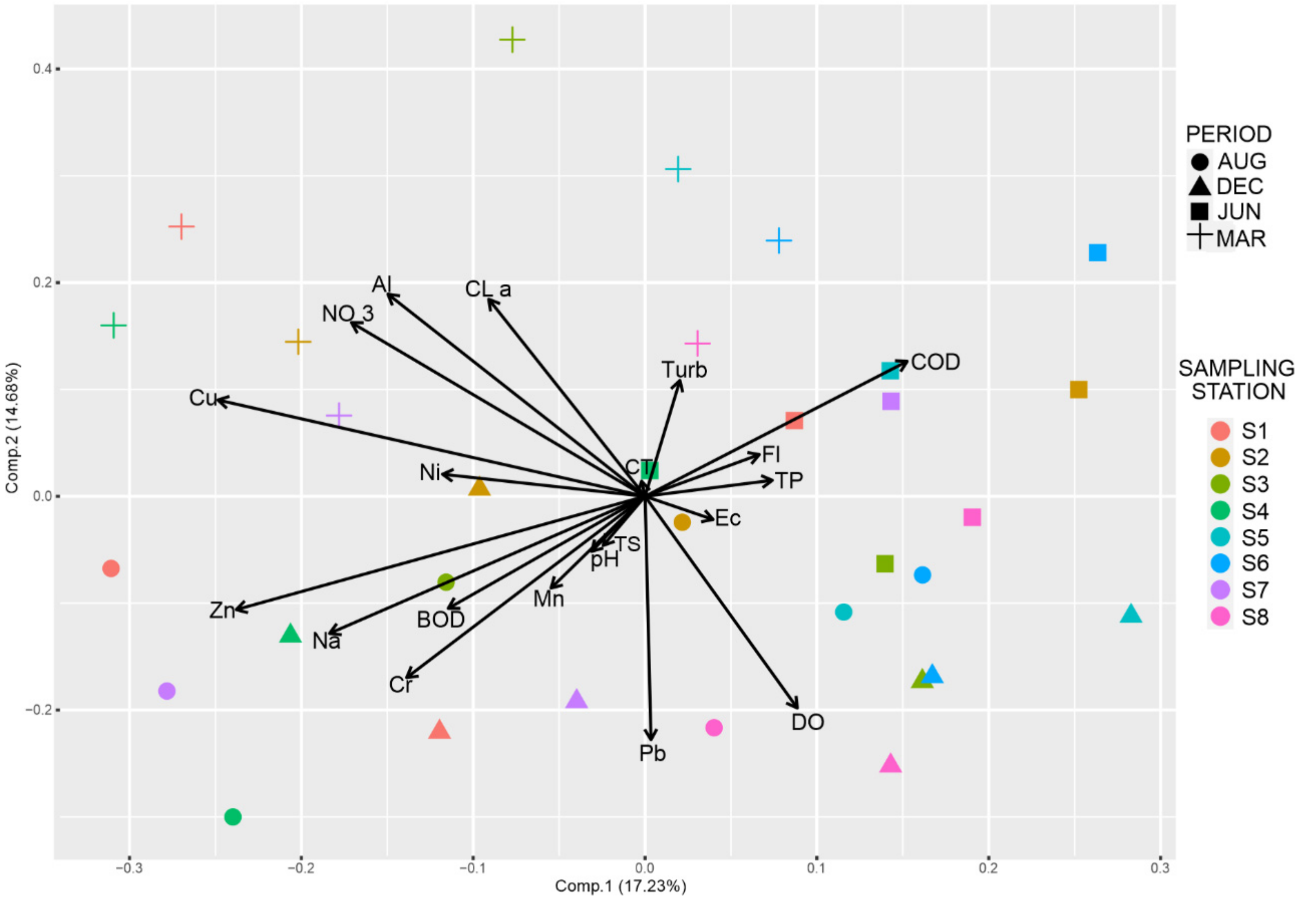

Figure 4. Principal Component Analysis (PCA) for the data evaluated at the sampling stations (S) of the Cascavel River, Cascavel city—PR, Brazil. Limnological data (Ec: electrical conductivity, DO: dissolved oxygen, Turb: turbidity, Fl: flow, COD: chemical oxidation demand, BOD: biochemical oxygen demand, NO_3: nitrate, TP: total dissolved phosphorus, CL_a: chlorophyll a, TS: total solids, CT: total coliforms), metals measured in the surface sediment (Cu: copper; Al: aluminum; Ni: nickel; Zn: zinc; Na: sodium; Mn: manganese; Pb: lead; Cr: chrome).

Figure 5 illustrates the importance of considering different dispersal hypotheses in explaining the structure of the periphytic diatom metacommunity in the Cascavel River. Overall, the predictors generated by AEM had the greatest ability to explain the metacommunity (Figure 5).

In general, the species density matrix was better explained than the abundance matrix. The June community was best explained by the predictors, with 18% of the variance explained by the AEM spatial filters (Figure 5). Overland explained the March community best, with 8% explaining species occurrence and 7% explaining species density. Watercourse was best able to explain the dispersal of the community in December 2018 (2%), also associated with the environmental filters and the historical community (Figure 5). Finally, the historical community becomes influential in explaining species dispersal starting in August, with the June community being influenced by the other factors, but not by the historical community (March community), which was impacted by the high accumulated precipitation (Figure 5).

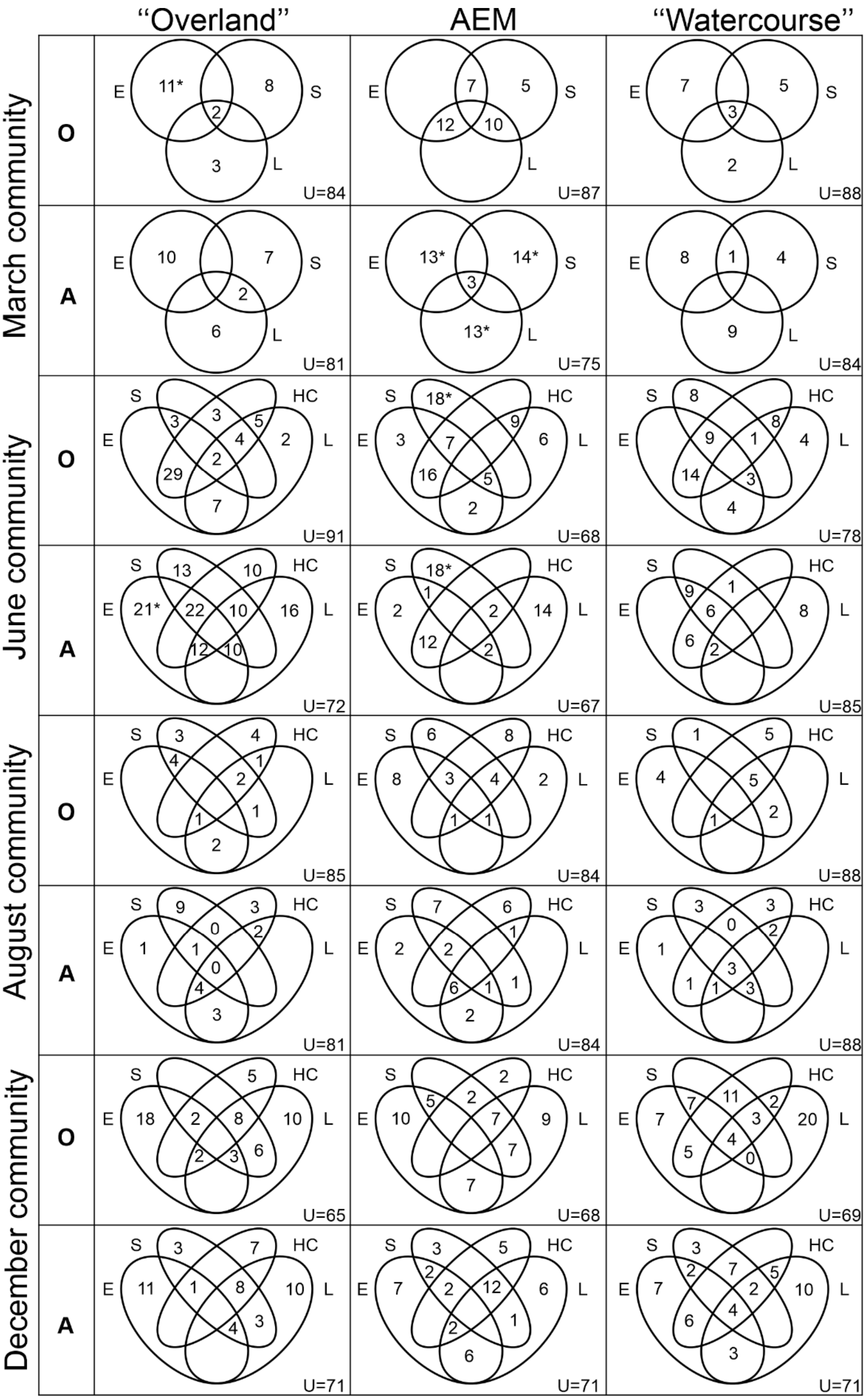

Figure 5. Results of the Partial Redundancy Analysis (pRDA). Relative contributions (% explanation) of environmental (E), spatial (S), historical community (HC) and landscape (L) that explain the variation in relative abundance (A) and Occurrence (O) of periphytic diatom metacommunities. We used possible dispersal routes "overland", "AEM", and "Watercourse" as spatial predictors (see methods). U = unexplained component. Values <0.5% were not shown. Explanation percentages significantly different from a null model ($p < 0.05$) are represented by "*".

4. Discussion

In this study, we showed that the structure of the diatom metacommunity of a micro-watershed is partially dependent on variables related to local environmental variables, landscape variables, dispersal hypothesis, and historical community. More importantly, we show that the relative importance of these factors varies temporally and depending on how the dispersal hypothesis is generated (see also [19,32,39]). The micro-watershed is located in a predominantly agricultural region of Brazil. The much of the city is structured around open, flat fields or fields with some barriers, mainly characterized by areas used for soybean and corn monocultures [64,65]. In addition, our characterization of land use and occupation of the micro-watershed area showed that the headwaters are surrounded by the urban center of the city. This combination of macro influence with direct influence at the sampling stations summarized the impacts along the micro-watershed, especially in the rainy season, when greater leaching occurs [16,66]. This may be associated with the community not having variation in richness between sampling stations, since diffuse pollution causes homogeneity in the spatial structuring of niches.

The landscape transition between urban and agricultural areas is reflected in different levels of anthropic interference on lotic systems [67]. When comparing micro-watersheds in Paraná State, Peres et al. [68] found that the Cascavel River micro-watershed has high environmental fragility, which refers to the sensitivity and resilience of an ecosystem. This high fragility occurs due to the effect of urban and agricultural effluents present in the river fluvial network in urban areas, which alter the limnological characteristics and promote environmental degradation, reducing forest areas and riparian vegetation [24,69,70]. Medeiros et al. [36], when evaluating the driving factors of the metacommunity in a linear design in the same micro-watershed, recorded the real effect of the continuous river theory, highlighting the effects of urbanization and agriculture on environmental variables, which had the greatest effect on the community.

Our results pointed to several interesting pieces of information about the spatial variability of the diatom community along the micro-watershed. Overall, no single spatial factor explained most of the variation in species density or occurrence in this model. Instead, we highlight the complexity to describe the variability of the metacommunity, as the variance partition did not show a significant percentage of association (see also [71]). The spatial autocorrelation structure without flow connection (overland) can be useful for modeling community abundance, which can actively move both upstream and downstream, overcoming dispersal not only by the lotic environment of connected rivers, but also by other means such as ectozoochory, involving adherence to fish and bird feathers, for instance [29,72].

The size of the spatial extents also interferes with the influence of local and landscape variables on community assembly, based on environmental heterogeneity (see more in [19,36,73–75]). The small scale of the micro-watershed probably results in a small spatial gradient that is reflected in the low variation in community richness depending on the sampling station. In fact, spatial and temporal variation in communities have not always been observed (i.e., see results for occurrence matrices), but the relative role of predictors was quite different between periods. This indicates that even with relative temporal stability in the communities, ecological relationships are poorly predicted (see in [76]). Spatial extent is proportional to the importance of niche selection, and the strength of these scale effects depends on the configuration of the environment, dispersal capacity, and niche breadth [74]. However, temporal heterogeneity associated with fluctuating precipitation throughout the year may cause temporal variation in the relative abundance of species. Passy et al. [71] associate this pattern with competition theory, which posits that temporal heterogeneity in resource supply increases biodiversity because it allows species that alternate between dominance and persistence to coexist.

Spatial variables generated using AEM resulted in better estimates, especially when spatial factors were the main drivers of metacommunities. Studies have already shown that it is necessary to consider the connectivity of river networks to model the dispersal routes of metacommunities, as they are important in explaining their effect on community formation [32,48]. However, when considering predictors of hydrologic connectivity across the watercourse, the percentages of total variation explained by spatial and shared effects were reduced, likely reflecting the small spatial gradient [77].

Studies show that environmental gradients may be poorly evident at small scales due to the mass effect [19,20]. This may reflect the high connectivity between sampling stations, which promotes facilitated community dispersal, interfering with the filtering of predictors [77,78]. The influence of metacommunity predictors is more difficult to distinguish at small scales due to the likely effects of niche versus neutral processes on community composition [79]. Possible causes of the complexity to explain these models could be stochastic colonization, ecological drift, and biological interactions or the homogenization of landscape effects itself [19,21,32,73].

Although landscape characteristics, especially the presence of riparian vegetation, are important in providing a favorable environment for community species richness, the results suggest that factors other than land cover may contribute to community restructuring. Previous studies point out that in urban streams, increased surface and subsurface connectivity to drainage networks can lead to water quality impacts, and the presence of forested areas can mitigate these stress pathways [80,81]. Landscape variables end up being suitable driver factors in small-scale models, helping to structure a multidimensional model.

The historical community as a predictor of the metacommunity became significant from the August community onwards. This may be related to the strong influence of precipitation on the March community, which may have needed a period to stabilize again. Foets et al. [82] pointed out that communities took one to two months to re-establish themselves after a significant change in the environment. However, even if the community responds to this temporal variation, in our study, the predominance of both the richness and density of *Eunotia* specimens is a consequence of the spatial homogeneity, which highlights environmental conditions that favor the occurrence of this genus (acidic and oligotrophic waters) [83,84]. Diatom species such as *Eunotia rhomboidea* Hustedt, *E. botulitropica*, *Fragilaria pectinalis* (Müller) Lyngbye, *Gomphonema parvulum*, *Achnanthidium macrocephalum* (Hustedt) Round & Bukhtiyarova, and *Sellaphora nigri* (De Notaris) Wetzel & Ector did not have specific environmental filtering, occurring throughout the entire micro-watershed; these are species commonly reported in polluted urban environments with high concentrations of solids and nutrients [85–88].

The deterioration of forested areas, which support the conservation of micro-watersheds within large agricultural extensions, is reflected in the increased concentration of nutrients and metals associated with fertilizers and pesticides. In addition, the sparse riparian vegetation increases siltation and turbidity in the riverbeds. These changes tend to favor medium to large species with cosmopolitan characteristics and competitive advantage, such as *Gomphonema lagenula* Kützing, *Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt, and *Gomphonema parvulum* [85,87,89–93]. These taxa are commonly registered as tolerant to pollution, occurring in conditions of elevated nutrient and light availability [94–96]. This environmental filtering supports the homogenizing effect of agriculture, which has been highlighted in other studies [93,97].

Another important factor is that the micro-watershed belongs to a region with soil classified as distropheric red latosol, predominated by Fe and Al ions, and acidic conditions [98]. These and other metals are carried to the aquatic environments, especially in the absence of riparian vegetation, situations in which silting facilitates the accumulation of sediments [99]. These conditions may be harmful to diatoms (see references in [100]), leading to changes in the community structure [101,102]. Thus, the streams studied here are characterized by an acidic pH and high conductivity [103], contributing to the occurrence of species belonging to the genus *Eunotia* [104].

Some of the effects of urbanization could be observed in stations with sparse and degraded riparian vegetation, such as S2, S4, and S7, reflecting higher concentrations of metals in the surface sediment (Figure 4), especially in March when high accumulated precipitation was observed. The representativeness of some species also helped differentiate these environments, such as *Navicula salinicola* on S2, *Achnanthidium modestiforme* and *Eunotia yberai* on S4 (also see the previously described *Nupela semifasciata* Amaral, T.Ludwig et Bueno species in [105]), and *Eunotia botulitropica* on S7 (dominant). These conditions also likely contributed significantly to higher leaching of metals into the surface sediment (Figure 4), reflecting intensified human activities [106,107]. These sampling stations were represented by species such as *Navicula salinicola* (S2) and *Navicula cryptocephala* (S2). Therefore, diatom communities are being affected directly or indirectly by a point or diffuse source of pollution. We suggest that other factors need to be considered in future studies, such as the bioavailability of these metals, as well as changes in diatom morphology. On a regional scale, spatial factors can be significant in the distribution of diatoms [108,109], considering land use throughout the micro-watershed [110]. In micro-watersheds, branching rivers contribute significantly to the dispersal dynamics of populations, especially when tributaries are not directly connected to the main river course [31,38]. In these cases, the contribution of the connection can be drastically limited in regions where native vegetation is suppressed, mainly due to anthropic actions [111]. Thus, the temporal maintenance of biodiversity is mainly due to asynchrony, that is, ideal conditions for different species, alternated by the seasonality of climatic events [71,112].

The diatom communities assessed along the micro-watershed presented a variety of growth forms, due to macroinfluence and to the evolutionary process itself, providing dominant profile characteristics of the benthic periphyton biomass [113,114]. The evolutionary aspect of growth may have influence on the comparison results of the methods evaluated, since its dispersal on a small scale (such as the studied micro-watershed), has reached an equilibrium that surpasses the dispersal only by the lotic environment of connected rivers, causing a mass effect [115]. However, it is agreed that time-replicated metacommunity studies are scarce; and that long-term standardized data are essential to clarify the determinants of ecological metacommunities with complex models [116]. Thus, it is necessary that monitoring of ecological communities, such as the one in this study, be continued. Even on a small scale and during one year of sampling, we demonstrated that predictors of different natures act together to explain diatom communities in micro-watersheds.

Due to the limitations of our study, we suggest that future works include a monitoring of the Cascavel River, increasing the number of sampling sites and including data from a larger historical series. It is also suggested to compare these results with other regional micro-watersheds to identify a pattern of anthropic behavior and its influence on lotic environments.

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Appendix A

Epilithic diatom species identified from Cascavel River microbasin.

Table A1. Analysis of variance (Anova) to compare the means of relative abundance of each diatom species according to the sampling stations. Species that had significantly different means across sampling stations, $p < 0.05$, have *p*-value highlighted in bold.

Sampling Station		**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**R^2**	**F**	***p*-Value**
Achnanthidium catenatum (J. Bily & Marvan) Lange-Bertalot	mean	-	-	-	7.5%	0.9%	-	-	-	0.220	0.966	0.478
	standard deviation	-	-	-	14.9%	1.8%	-	-	-			
Achnanthidium exiguum (Grunow) D.B.Czarnecki	mean	-	-	-	-	-	-	-	1.1%	0,.26	1.000	0.455
	standard deviation	-	-	-	-	-	-	-	2.2%			
Achnanthidium macrocephalum (Hustedt) Round & Bukhtiyarova	mean	1.1%	-	3.2%	2.7%	-	-	-	2.8%	0.259	1.198	0.341
	standard deviation	2.2%	-	3.2%	5.3%	-	-	-	3.3%			
Achnanthidium minutissimum (Kützing) Czarnecki	mean	5.8%	3.6%	3.0%	5.5%	-	7.8%	5.0%	9.1%	0.172	0.714	0.661
	standard deviation	8.7%	4.4%	3.7%	7.1%	-	9.1%	6.0%	9.0%			
Achnanthidium modestiforme (Lange-Bertalot) Van de Vijver	mean	1.9%	1.4%	-	11.1%	-	1.4%	-	2.2%	0.477	3.130	**0.017**
	standard deviation	2.6%	2.9%	-	9.9%	-	2.8%	-	4.3%			
Achnanthidium tropicocatenatum G.C.Marquardt, C.E.Wetzel & Ector	mean	0.4%	0.7%	3.6%	-	-	-	-	-	0.274	1.291	0.297
	standard deviation	0.7%	1.5%	6.0%	-	-	-	-	-			
Actinella hermes-moreirae Ruwer, Ludwig & Rodrigues	mean	-	0.2%	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	0.5%	-	-	-	-	-	-			
Aulacoseira ambigua (Grunow) Simonsen	mean	-	0.5%	4.4%	-	0.3%	-	-	2.8%	0.379	2.089	0.085
	standard deviation	-	1.0%	3.2%	-	0.6%	-	-	5.6%			
Aulacoseira granulata (Ehrenberg) Simonsen	mean	-	2.0%	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	3.9%	-	-	-	-	-	-			
Aulacoseira pusilla (Meister) Tuji & Houki	mean	2.1%	-	1.3%	-	-	-	-	-	0.230	1.026	0.439
	standard deviation	4.3%	-	1.6%	-	-	-	-	-			
Aulacoseira tenella (Nygaard) Simonsen	mean	-	-	1.4%	-	0.8%	-	-	0.8%	0.179	0.747	0.635
	standard deviation	-	-	2.8%	-	1.6%	-	-	1.5%			
Brachysira brebissonii Ross	mean	-	0.2%	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	0.5%	-	-	-	-	-	-			
Brachysira microcephala (Grunow) Compère	mean	-	-	1.4%	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	2.9%	-	-	-	-	-			
Caloneis hyalina Hustedt	mean	-	-	-	1.0%	0.9%	-	-	-	0.200	0.858	0.552
	standard deviation	-	-	-	2.1%	1.8%	-	-	-			
Caloneis westii (Smith) Hendey	mean	-	-	-	-	-	-	0.7%	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	-	1.4%	-			
Craticula riparia (Hustedt) Lange-Bertalot	mean	-	0.2%	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	0.5%	-	-	-	-	-	-			
Craticula submolesta (Hustedt) Lange-Bertalot	mean	-	-	-	2.6%	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	5.2%	-	-	-	-			
Cymbopleura naviculiformis (Auerswald) Krammer	mean	-	-	0.7%	-	-	-	-	-	0.465	2.979	**0.021**
	standard deviation	-	-	0.9%	-	-	-	-	-			
Discostella stelligera (Cleve & Grunow) Houk & Klee	mean	3.5%	0.2%	13.7%	1.9%	-	1.0%	1.6%	7.2%	0.356	1.897	0.115
	standard deviation	7.0%	0.5%	17.1%	3.7%	-	1.9%	3.1%	0.8%			
Encyonema minutiforme Krammer	mean	-	-	-	-	-	2.1%	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	4.2%	-	-			

Table A1. *Cont.*

Sampling Station		S1	S2	S3	S4	S5	S6	S7	S8	R^2	F	*p*-Value
Encyonema neomesianum Krammer	mean	-	-	0.5%	-	-	0.7%	-	-	0.202	0.867	0.546
	standard deviation	-	-	1.0%	-	-	1.4%	-	-			
Encyonema perpusillum (Cleve-Euler) Mann	mean	0.4%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	0.7%	-	-	-	-	-	-	-			
Encyonema silesiacum (Bleisch) Mann	mean	-	0.2%	-	0.5%	-	0.6%	-	2.0%	0.259	1.199	0.341
	standard deviation	-	0.5%	-	1.0%	-	1.1%	-	3.2%			
Eunotia bilunaris (Ehrenberg) Schaarschmidt	mean	3.4%	-	7.0%	-	0.3%	5.8%	4.2%	0.3%	0.330	1.686	0.160
	standard deviation	6.8%	-	4.0%	-	0.6%	7.2%	6.4%	0.5%			
Eunotia botulitropica Wetzel & Costa	mean	11.3%	2.0%	4.9%	0.5%	11.9%	6.3%	16.8%	0.5%	0.231	1.029	0.437
	standard deviation	16.6%	2.9%	7.3%	1.1%	9.6%	11.4%	23.6%	1,1%			
Eunotia georgii Metzeltin & Lange-Bertalot	mean	-	-	-	-	1.4%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	2.7%	-	-	-			
Eunotia intricans Lange-Bertalot & Metzeltin	mean	-	-	-	-	-	0.4%	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	0.9%	-	-			
Eunotia kruegeri Lange-Bertalot	mean	0.7%	0.8%	-	1.6%	2,4%	-	-	-	0.168	0.693	0.677
	standard deviation	1.4%	1.5%	-	3.2%	4.8%	-	-	-			
Eunotia meridiana Metzeltin & Lange-Bertalot	mean	3.5%	5.6%	6.8%	2.0%	6.1%	3.5%	0.8%	2.2%	0.135	0.535	0.799
	standard deviation	4.2%	4.9%	9.2%	2.3%	10.4%	4.2%	1.6%	4.3%			
Eunotia rhomboidea Hustedt	mean	7.0%	5.6%	2.8%	1.9%	15.8%	3.1%	5.8%	4.8%	0.333	1.716	0.153
	standard deviation	5.2%	4.4%	4.1%	3.7%	14.7%	3.6%	3.9%	6.0%			
Eunotia subarcuatoides Alles, Nörpel & Lange-Bertalot	mean	-	-	-	-	-	0.7%	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	1.4%	-	-			
Eunotia tropico-arcus Metzeltin & Lange-Bertalot	mean	-	-	-	-	-	0.3%	0.2%	-	0.201	0.860	0.551
	standard deviation	-	-	-	-	-	0.6%	0.5%	-			
Eunotia veneris (Kützing) De Toni	mean	19.6%	-	1.6%	-	1.4%	-	0.2%	0.7%	0.239	1.075	0.409
	standard deviation	36.7%	-	3.1%	-	2.7%	-	0.5%	1.4%			
Eunotia yberai Frenguelli	mean	-	-	-	2.0%	-	-	-	-	0.465	2.983	**0.021**
	standard deviation	-	-	-	2.3%	-	-	-	-			
Fragilaria gracilis Østrup	mean	-	-	2.4%	-	0.3%	0.9%	-	-	0.362	1.943	0.107
	standard deviation	-	-	2.9%	-	0.6%	1.8%	-	-			
Fragilaria pectinalis (Müller) Lyngbye	mean	-	1.2%	1.3%	-	-	2.7%	0.8%	1.0%	0.152	0.614	0.739
	standard deviation	-	2.4%	1.5%	-	-	5.4%	1.6%	2.0%			
Fragilaria tenera var. *tenera* (Smith) Lange-Bertalot	mean	-	-	-	-	-	2.0%	-	1.4%	0.202	0.866	0.547
	standard deviation	-	-	-	-	-	3.9%	-	2.8%			
Fragilaria sp.	mean	-	-	0.5%	-	-	3.1%	-	-	0.218	0.957	0.483
	standard deviation	-	-	1.0%	-	-	6.3%	-	-			
Fragilariforma javanica (Hustedt) Wetzel, Morales & Ector	mean	-	-	-	-	1.0%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	2.0%	-	-	-			
Frustulia acidophilissima Wydrzycka & Lange-Bertalot	mean	1.4%	-	-	0.5%	1.0%	1.5%	1.7%	0.5%	0.107	0.410	0.887
	standard deviation	2.7%	-	-	1.1%	2.0%	2.9%	3.4%	1.1%			
Frustulia crassinervia (Brébisson ex W.Smith) Lange-Bertalot & Krammer	mean	1.2%	-	-	1.5%	5.4%	-	-	0.7%	0.331	1.698	0.157
	standard deviation	0.9%	-	-	3.1%	7.2%	-	-	1.4%			

Table A1. *Cont.*

Sampling Station		S1	S2	S3	S4	S5	S6	S7	S8	R^2	F	p-Value
Frustulia guayanensis Metzeltin & Lange Bertalot	mean	-	0.8%	0.4%	-	0.7%	1.7%	-	-	0.171	0.706	0.667
	standard deviation	-	1.5%	0.8%	-	1.4%	3.4%	-	-			
Frustulia neomundana Lange-Bertalot & Rumrich	mean	-	-	-	-	-	-	0.8%	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	-	1.6%	-			
Frustulia saxonica Rabenhorst	mean	-	-	-	-	-	0.5%	-	0.5%	0.200	0.858	0.552
	standard deviation	-	-	-	-	-	1.0%	-	1.1%			
Frustulia undosa Metzeltin & Lange-Bertalot	mean	0.2%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	0.4%	-	-	-	-	-	-	-			
Frustulia vulgaris (Thwaites) De Toni	mean	-	-	-	-	0.5%	1.0%	-	-	0.206	0.890	0.530
	standard deviation	-	-	-	-	1.0%	2.1%	-	-			
Frustulia weinholdii Hustedt	mean	0.4%	-	-	-	-	-	-	0.9%	0.368	1.993	0.098
	standard deviation	0.7%	-	-	-	-	-	-	1.1%			
Geissleria punctifera (Hustedt) Metzeltin, Lange-Bertalot e García-Rodríguez	mean	-	0.2%	-	-	0.5%	-	-	1.1%	0.195	0.830	0.573
	standard deviation	-	0.5%	-	-	1.0%	-	-	2.3%			
Gogorevia exilis(Kütz.) Kulikovskiy and Kociolek	mean	-	-	-	-	-	-	-	1.4%	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	-	-	2.8%			
Gomphonema angustatum (Kützing) Rabenhorst	mean	-	-	-	-	-	-	-	1.6%	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	-	-	3.3%			
Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichardt	mean	-	-	0.6%	-	-	6.3%	-	1.5%	0.209	0.904	0.520
	standard deviation	-	-	1.1%	-	-	12.5%	-	3.1%			
Gomphonema guaraniarum Metzeltin & Lange-Bertalot	mean	-	-	-	-	-	1.1%	-	0.8%	0.201	0.864	0.548
	standard deviation	-	-	-	-	-	2.1%	-	1.5%			
Gomphonema graciledictum Reichardt	mean	-	-	0.8%	-	-	-	-	0.3%	0.207	0.894	0.527
	standard deviation	-	-	1.6%	-	-	-	-	0.7%			
Gomphonema lagenula Kützing	mean	3.3%	7.0%	3.8%	1.0%	-	2.7%	0.8%	4.5%	0.202	0.869	0.544
	standard deviation	4.1%	7.4%	6.7%	2.1%	-	4.2%	1.6%	7.3%			
Gomphonema naviculoides Smith	mean	0.4%	-	0.5%	-	-	1.3%	-	-	0.195	0.829	0.574
	standard deviation	0.7%	-	1.0%	-	-	2.7%	-	-			
Gomphonema obtusatum (Kützing) Grunow	mean	-	-	0.4%	1.1%	-	-	-	-	0.209	0.908	0.517
	standard deviation	-	-	0.8%	2.1%	-	-	-	-			
Gomphonema parvulum (Kützing) Kützing	mean	5.9%	13.0%	19.7%	2.8%	9.6%	22.7%	3.1%	10.3%	0.236	1.060	0.418
	standard deviation	5.8%	9.4%	33.2%	3.2%	8.0%	11.7%	6.3%	11.4%			
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	mean	-	2.2%	0.7%	-	-	0.9%	2.7%	-	0.208	0.900	0.522
	standard deviation	-	2.9%	0.8%	-	-	1.8%	5.4%	-			
Halamphora montana (Krasske) Levkov	mean	-	0.7%	-	1.0%	-	0.3%	3.9%	0.7%	0.185	0.781	0.610
	standard deviation	-	1.5%	-	2.1%	-	0.6%	7.8%	1.4%			
Hantzschia amphioxys (Ehrenberg) Grunow	mean	-	0.2%	-	-	-	0.3%	-	-	0.200	0.859	0.552
	standard deviation	-	0.5%	-	-	-	0.6%	-	-			
Humidophila contenta (Grunow) Lowe et al.	mean	0.4%	4.8%	-	1.5%	-	-	7.4%	-	0.309	1.533	0.204
	standard deviation	0.9%	8.9%	-	3.1%	-	-	8.5%	-			
Humidophila subtropica (Metzeltin, Lange-Bertalot & García-Rodríguez) Lowe et al.	mean	-	1.5%	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	2.9%	-	-	-	-	-	-			

Table A1. *Cont.*

Sampling Station		**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**R^2**	**F**	***p*-Value**
Iconella tenuissima (Hustedt) D.Kapustin & Kulikovskiy	mean	-	-	-	1.6%	-	0.5%	-	0.3%	0.342	1.779	0.138
	standard deviation	-	-	-	2.0%	-	1.0%	-	0.7%			
Luticola acidoclinata Lange-Bertalot	mean	-	0.2%	-	-	2.8%	1.9%	-	0.9%	0.262	1.214	0.333
	standard deviation	-	0.5%	-	-	3.8%	3.9%	-	1.1%			
Luticola goeppertiana (Bleisch) Mann	mean	-	-	0.4%	-	1.5%	-	-	2.5%	0.194	0.826	0.576
	standard deviation	-	-	0.8%	-	2.9%	-	-	5.0%			
Luticola hustedtii Levkov, Metzeltin & Pavlov	mean	0.2%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	0.4%	-	-	-	-	-	-	-			
Luticola permuticoides Metzeltin e Lange-Bertalot	mean	-	0.7%	-	-	-	1.0%	-	-	0.202	0.866	0.546
	standard deviation	-	1.4%	-	-	-	1.9%	-	-			
Navicula angusta Grunow	mean	-	0.2%	-	-	1.1%	-	-	-	0.215	0.938	0.496
	standard deviation	-	0.5%	-	-	2.1%	-	-	-			
Navicula cryptocephala Kützing	mean	1.5%	7.5%	2.3%	-	-	0.7%	-	4.3%	0.475	3.102	**0.018**
	standard deviation	1.5%	6.8%	2.8%	-	-	1.4%	-	3.7%			
Navicula cryptotenella Lange-Bertalot	mean	1.3%	0.2%	1.5%	-	10.0%	-	1.2%	-	0.472	3.063	**0.019**
	standard deviation	2.0%	0.5%	2.2%	-	10.4%	-	1.5%	-			
Navicula eichhorniaephila Manguin ex Kociolek & Reviers	mean	-	-	-	0.5%	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	1.1%	-	-	-	-			
Navicula leptostriata E.G.Jørgensen	mean	-	-	0.4%	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	0.8%	-	-	-	-	-			
Navicula radiosa Kützing	mean	-	2.0%	0.3%	-	0.5%	0.6%	-	-	0.186	0.783	0.608
	standard deviation	-	4.1%	0.7%	-	1.0%	1.3%	-	-			
Navicula rostellata Kützing	mean	-	-	-	-	-	1.1%	-	0.3%	0.215	0.939	0.496
	standard deviation	-	-	-	-	-	2.3%	-	0.5%			
Navicula salinicola Hustedt	mean	0.2%	3.6%	-	-	-	-	-	-	0.431	2.596	**0.038**
	standard deviation	0.4%	4.4%	-	-	-	-	-	-			
Navicula symmetrica Patrick	mean	-	1.7%	-	-	-	-	-	1.4%	0.201	0.860	0.551
	standard deviation	-	3.4%	-	-	-	-	-	2.8%			
Neidium essequiboanum Metzeltin & Krammer	mean	-	-	-	-	-	0.5%	-	0.8%	0.203	0.876	0.540
	standard deviation	-	-	-	-	-	1.0%	-	1.7%			
Neidium gracile f. *aequale* Hustedt	mean	0.4%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	0.7%	-	-	-	-	-	-	-			
Neidium iridis (Ehrenberg) Cleve	mean	-	-	-	-	0.5%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	1.0%	-	-	-			
Neidium tenuissimum Hustedt	mean	0.7%	-	-	-	-	-	1.4%	-	0.205	0.886	0.533
	standard deviation	1.4%	-	-	-	-	-	2.7%	-			
Nitzschia amphibia Grunow	mean	0.7%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	1.4%	-	-	-	-	-	-	-			
Nitzschia brevissima Grunow	mean	-	-	-	0.5%	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	1.0%	-	-	-	-			
Nitzschia palea (Kützing) Smith	mean	1.6%	4.5%	0.9%	2.1%	1.0%	0.6%	-	1.3%	0.268	1.255	0.313
	standard deviation	2.6%	3.7%	1.0%	4.1%	2.0%	1.1%	-	2.1%			

Table A1. *Cont.*

Sampling Station		S1	S2	S3	S4	S5	S6	S7	S8	R^2	F	*p*-Value
Nitzschia perminuta Grunow in Van Heurck	mean	0.4%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	0.7%	-	-	-	-	-	-	-			
Nupela pardinhoensis Bes, Torgan & Ector in Bes et al.	mean	2.4%	2.6%	-	3.9%	2.4%	2.1%	8.3%	2.1%	0.223	0.982	0.467
	standard deviation	2.8%	3.6%	-	3.5%	4.8%	4.2%	10.1%	3.1%			
Nupela praecipuoides Tremarin & Ludwig	mean	1.1%	-	-	2.0%	4.3%	1.9%	5.4%	7.3%	0.284	1.363	0.266
	standard deviation	2.1%	-	-	2.9%	6.5%	3.9%	4.5%	8.7%			
Nupela semifasciata Amaral, T.Ludwig et Bueno	mean	2.7%	2.7%	-	2.4%	1.5%	2.6%	8.3%	2.4%	0.141	0.564	0.777
	standard deviation	5.4%	5.4%	-	3.5%	2.9%	3.9%	14.4%	4.9%			
Nupela wellneri (Lange-Bertalot) Lange-Bertalot	mean	-	-	-	-	-	-	1.1%	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	-	2.3%	-			
Orthoseira roeseana (Rabenhorst) Pfitzer	mean	-	-	-	-	2.0%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	4.1%	-	-	-			
Pinnularia brauniana (Grunow) Studnicka	mean	-	-	0.4%	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	0.8%	-	-	-	-	-			
Pinnularia divergens W.Smith	mean	0.4%	-	1.4%	0.5%	-	-	-	-	0.195	0.832	0.572
	standard deviation	0.7%	-	2.9%	1.1%	-	-	-	-			
Pinnularia graciloides var. *latecapitata* Metzeltin & Krammer	mean	-	-	-	-	-	0.6%	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	1.1%	-	-			
Pinnularia laucensis Lange-Bertalot	mean	0.4%	-	-	-	0.7%	1.5%	-	0.5%	0.172	0.712	0.662
	standard deviation	0.7%	-	-	-	1.4%	3.0%	-	1.1%			
Pinnularia microstauron var. *rostrata* Krammer	mean	0.4%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	0.7%	-	-	-	-	-	-	-			
Pinnularia obscura Krasske	mean	-	-	-	-	2.0%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	4.1%	-	-	-			
Pinnularia subanglica Krammer	mean	-	0.8%	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	1.5%	-	-	-	-	-	-			
Pinnularia subgibba Krammer	mean	-	-	-	0.5%	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	1.0%	-	-	-	-			
Pinnularia subinterrupta Krammer & Schroeter	mean	-	-	-	-	0.3%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	0.6%	-	-	-			
Pinnularia sp.	mean	1.4%	0.2%	-	-	-	-	-	-	0.217	0.951	0.488
	standard deviation	2.7%	0.5%	-	-	-	-	-	-			
Pinnularia sp.2	mean	0.2%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	0.4%	-	-	-	-	-	-	-			
Pinnularia tabellaria Ehrenberg	mean	1.1%	-	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	2.1%	-	-	-	-	-	-	-			
Placogeia kriegeri (K.Krasske) Bukhtiyarova	mean	-	-	-	0.5%	-	0.3%	-	-	0.204	0.880	0.537
	standard deviation	-	-	-	1.0%	-	0.6%	-	-			
Placoneis elginensis (W.Gregory) E.J.Cox	mean	-	-	-	-	0.7%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	1.4%	-	-	-			
Placoneis hambergii (Hustedt) K.Bruder	mean	-	-	-	-	-	-	-	1.1%	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	-	-	2.2%			
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	mean	-	-	-	-	0.7%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	1.4%	-	-	-			

Table A1. *Cont.*

Sampling Station		**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**R^2**	**F**	***p*-Value**
Psammothidium hustedtii (Krasske) Mayama	mean	-	-	-	-	-	-	0.2%	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	-	0.5%	-			
Rhopalodia gibberula var. *vanheurckii* O. Müller	mean	-	-	0.4%	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	0.8%	-	-	-	-	-			
Sellaphora laevissima (Kützing) D.G.Mann	mean	-	-	-	-	0.5%	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	1.0%	-	-	-			
Sellaphora nigri (De Notaris) Wetzel & Ector	mean	9.1%	9.3%	-	18.8%	6.0%	1.0%	14.5%	4.4%	0.324	1.641	0.172
	standard deviation	8.0%	10.7%	-	20.9%	5.6%	1.9%	11.5%	5.4%			
Sellaphora sassiana (Metzeltin & Lange-Bertalot)	mean	-	-	2.4%	1.5%	-	-	-	-	0.202	0.870	0.544
	standard deviation	-	-	4.8%	3.1%	-	-	-	-			
Sellaphora saugerresii (Desmazières) Wetzel & Mann	mean	-	7.9%	1.2%	10.1%	0.3%	-	3.0%	3.7%	0.159	0.649	0.712
	standard deviation	-	15.9%	1.8%	20.2%	0.6%	-	6.0%	5.1%			
Sellaphora sp.	mean	-	-	-	1.8%	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	3.6%	-	-	-	-			
Sellaphora sp.2	mean	-	0.1%	-	-	-	-	-	-	0.226	1.000	0.455
	standard deviation	-	0.2%	-	-	-	-	-	-			
Spicaticribra kingstonii Johansen, Kociolek & Lowe	mean	-	-	1.0%	-	-	-	-	0.3%	0.616	5.509	**0.001**
	standard deviation	-	-	0.7%	-	-	-	-	0.5%			
Stauroneis gracilis Ehrenberg	mean	-	-	-	-	-	0.6%	-	-	0.226	1.000	0.455
	standard deviation	-	-	-	-	-	1.1%	-	-			
Surirella angusta Kützing	mean	0.2%	-	-	1.5%	-	-	-	-	0.219	0.961	0.481
	standard deviation	0.4%	-	-	3.1%	-	-	-	-			
Surirella roba Leclercq	mean	-	0.4%	-	-	0.3%	-	-	-	0.200	0.859	0.551
	standard deviation	-	0.7%	-	-	0.6%	-	-	-			
Ulnaria ulna (Nitzsch) Compère	mean	0.2%	0.2%	0.1%	1.5%	-	-	-	2.1%	0.247	1.126	0.380
	standard deviation	0.4%	0.5%	0.3%	3.1%	-	-	-	3.1%			

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