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„Longitudinal distribution patterns of Ephemeroptera, Plecoptera and Trichoptera in a lake outlet in Lunz (Lower Austria)"

verfasst von / submitted by

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Abstract

Lake outlet streams have long been known to facilitate high densities of aquatic invertebrates in their first hundred metres which decrease rapidly downstream. This is especially prevalent for filter feeding invertebrate larvae which are assumed to exploit the resources induced by the lake. Although this effect has been observed for a long time, the underlying mechanisms behind it are still debated. One of the common explanations is that high quality lacustrine food sources create a nutritional gradient in the lake outlet stream due to consumption or degradation. To test this, more than 13800 specimens belonging to the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) were collected over four seasons from nine sites along the Unterer Lunzer Seebach, which is the outlet of the Lunzer Untersee (Lower Austria), using a modified variant of the MHS method as well as CPUE sampling. Additionally, a set of environmental parameters was assessed, which included plankton densities, C/N ratios, stream velocity, water temperature, choriotope composition, oxygen concentration and conductivity. The first hundred metres of the Unterer Lunzer Seebach were found to harbour high abundances of trichopteran filter feeders which declined significantly downstream. C/N ratios and plankton densities did not change significantly over the sampled stretch and fail to explain the observed filter feeder gradient. The study's findings instead suggest that habitat composition and stream velocities strongly influenced EPT community composition and correlated well with abundances of filter feeding taxa.

Zusammenfassung

Seeausrinne beherbergen hohe Dichten aquatischer Invertebraten in ihren ersten hunderten Metern, welche stromabwärts drastisch abnehmen. Besonders ausgeprägt ist dieses Phänomen bei filtrierenden Insektenlarven, bei denen angenommen wird, dass sie die Auswirkungen des Sees auf den Ausrinn besonders gut ausnutzen können. Obwohl dies seit langem bekannt ist, sind die unterliegenden Mechanismen nicht vollständig verstanden. Ein prominenter Erklärungsansatz ist, dass der See einen Nährstoffgradienten im Ausrinn hervorruft, bedingt durch hochqualitative Nährstoffpartikel, welche durch Aufnahme oder Degradation ausgedünnt werden. Um diese Annahme zu testen, wurden mehr als 13800 Larven von Ephemeroptera, Plecoptera und Trichoptera (EPT) über vier Jahreszeiten an neun Untersuchungsstellen im Unteren Lunzer Seebach gesammelt, der den Ausrinn des Lunzer Untersees (Niederösterreich) repräsentiert. Angewandt wurden dabei modifizierte MHS- sowie CPUE-Sammelmethoden. Zusätzlich wurden verschiedene Umweltparameter erhoben, darunter Planktondichte, C/N-Ratios, Strömungsgeschwindigkeit, Wassertemperatur, Choriotopzusammensetzung, Sauerstoff-konzentration und Leitfähigkeit. In den ersten hundert Metern des Seebachs wurden große Mengen von filtrierenden Trichopterenlarven gefunden, die stromabwärts signifikant abnahmen. C/N-Ratios sowie Planktondichten veränderten sich über den untersuchten Abschnitt nicht signifikant und konnten den beobachteten Filtrierergradienten nicht erklären. Stattdessen weisen die Ergebnisse dieser Studie darauf hin, dass Habitatzusammensetzung und Strömungsverhältnisse wesentlich die EPT-Gesellschaften beeinflussen und stark mit den Filtriererdichten korrelieren.

1. Introduction

Lake outlet streams have long been known to harbour invertebrate communities distinct from those of either lake or stream (Müller, 1954; Brönmark and Malmquist, 1984; Robinson and Minshall, 1990). Such communities are characterised by high densities of filter feeders whose abundance decreases rapidly along the stream's longitudinal gradient (Richardson and Mackay, 1991). The ecological basis for this compositional turnover has been a continuous matter of research interests since first concepts for lake outlets were proposed (e.g., Briggs, 1948; Illies, 1956). Most of the explanatory models available so far identify gradients of environmental parameters such as food quality, water temperature, stream velocity and discharge as driving forces for the longitudinal patterns observed (Richardson and Mackay, 1991).

1.1. Lake outlets

Two distinct theories linking food quality to observed species patterns exist, and both are based on the increased production of lakes and their output of seston and other food particles with high nutritional values into the outlet stream (Richardson and Mackay, 1991). The food depletion theory assumes that lake outlet filter feeders are selective in their ingestion and thereby remove the food particles offering the highest nutritional values along the stream's longitudinal gradient. Thus, filter feeders relying on food particles of a particular size and yield may only occur close to the lake in dense assemblages, where these conditions are met (Sheldon and Oswood, 1977; Morin et al., 1988). A rivalling concept is the food dilution theory, stating, that even though concentrations of high-quality food particles may not change, the additional input of particles unsuited for filter feeder growth resulting from allochthonous sources or from the streambed itself, reduces the proportion of high-quality food items filter feeding organisms ingest, thereby putting them at a disadvantage (Richardson, 1984; Morin and Peters, 1988).

Lakes in temperate regions affect the temperature regime in their outlets in unique ways due to their often stratified nature. During the spring and summer, the warmest water is located at the surface of the lake while colder, denser water sinks to the ground (Hynes, 1970). In the case of surface release outlets this causes summer temperatures to be warm and constant, thereby lowering the day-night temperature extremes which, in combination with the overall higher temperatures, might lead to increased growth rates (Richardson and Mackay, 1991; Wotton,

1995). Additionally, growth rates of aquatic insect larvae are positively correlated with higher temperatures, potentially allowing for more generations of filter feeders and therefore, higher densities (Wotton, 1987; Wotton, 1995).

Stable discharge rates associated with lake outlets have been shown to favour certain filtering blackfly larvae (Morin and Peters, 1988), with stable conditions at the outlet favouring a set of key species dominating this habitat (Ward and Stanford, 1983). While increased stream velocities do not influence filter feeder density by them self, they do increase the frequency of transported seston and might therefore allow filter feeders to occur in denser assemblages. Such conditions are well documented in some lake outlets with high continuous discharge (Matczak and Mackay, 1990; Richardson and Mackay, 1991; Eriksson, 2001).

1.2. Study aims and overview

Although a large body of literature exists on lake outlets elsewhere, there is a lack of recent data for Austria. In order to improve this situation, the lake outlet of the Lunzer Untersee was studied. This outlet provides an ideal model reach to test a set of main hypotheses associated with this special type of lotic communities. A special emphasis was laid on macroinvertebrates belonging to the EPT fraction (Ephemeroptera, Trichoptera and Plecoptera), because they are known to react very sensible to changes of their habitat templates (AQEM, 2002). The set of biota is based on two cross-supporting collecting methods, and a number of environmental factors commonly not included in the analysis of lake outlet communities were assessed as well, such as choriotope composition, planktonic densities and C/N ratios.

1.3. Hypotheses

Following the definition of lake outlet communities as outlined by Richardson and Mackay (1991), this study hypothesizes (1) a **shift in functional feeding guild composition** away from filter feeders, downstream of the lake outlet. A consequence of this is (2) an anticipated **increase in diversity of invertebrate taxa** due to the decreasing dominance of filter feeders and an increase of other competing taxa. In addition, (3) **the abundance of macrozoobenthos taxa** is expected to decrease as well, with the high-density populations at the outlet gradually being replaced with communities more typically riverine in nature. This shift away from filter feeding

taxa is expected to be mirrored by a decrease of food particles of high nutritional value or otherwise an increase of particles with low nutritional value explaining the pattern.

2. Material and Methods

2.1. Site selection and sampling design

The study was conducted at the Unterer Lunzer Seebach (henceforth Seebach) which is the outlet of the Lunzer Untersee. The lake (elevation: 608 m a.s.l.) is situated close to the town of Lunz am See in south-western Lower Austria (Figure 1). It is a deep (34 m), oligotrophic (Malicky-Schlatte, 1991) lake and the only natural one in Lower Austria. This makes its outlet, the Seebach, an ideal research site.

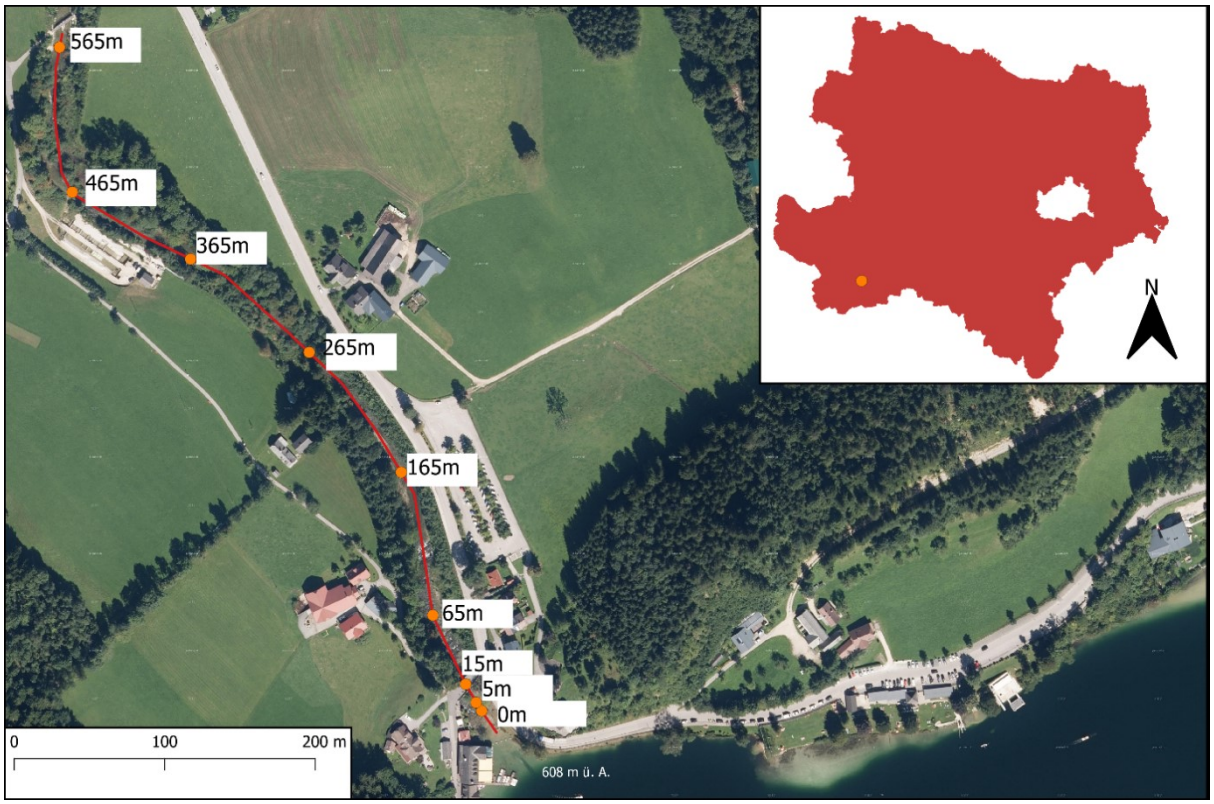

Figure 1: Overview of the Seebach (red) and the study reach. The Lunzer Untersee and the Seebach are situated in south-western Lower Austria. Along the Seebach, nine sites along a stretch of 565 m were sampled for this study (Figures: distance from lake outlet in m). Upper right insert: location of the study area within the borders of Lower Austria. Data source: basemap.at (https://basemap.at/).

Nine sites along the uppermost stretch of the Seebach were selected for this study, covering a distance of 565 m from the lake outlet. A site was defined as a stretch of stream three meters long. The first site was additionally subdivided into three sampling areas situated above, in, and below the lake outlet cascade (Figure 2).

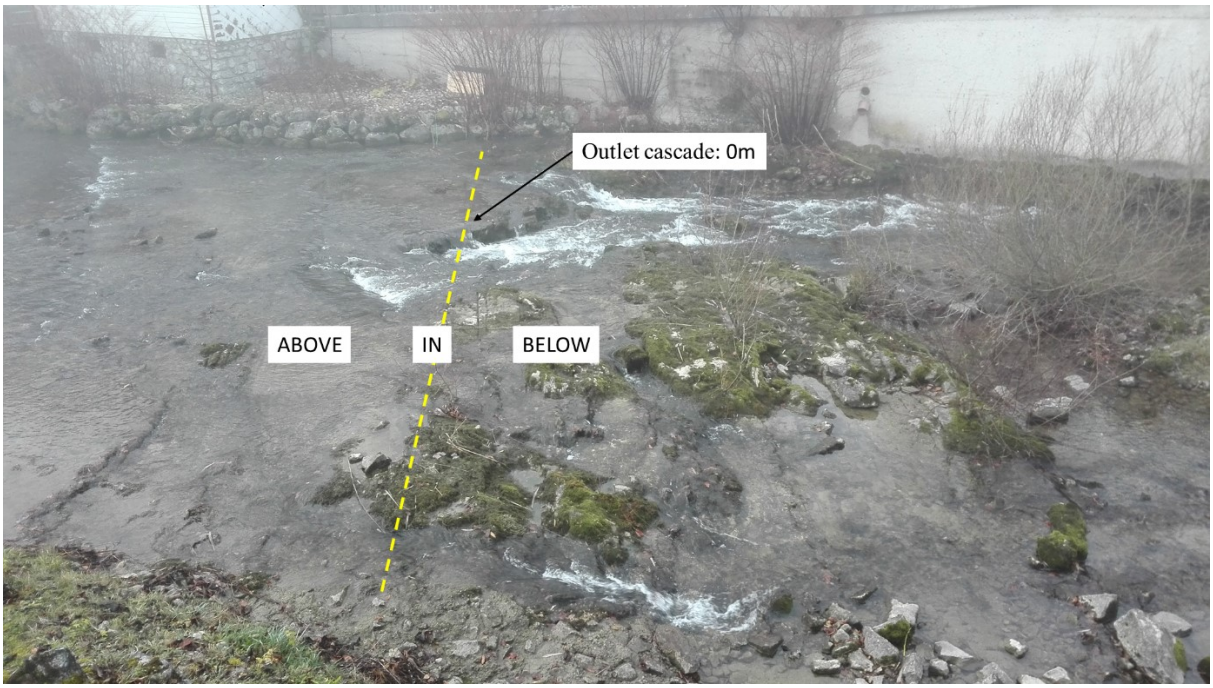

Figure 2: The first sampling site was placed at the outlet cascade and subdivided into three parts (above, in and below the cascade). The Lunzer Untersee is situated to the left.

This was done in order to obtain a more detailed knowledge of the taxa set occurring at this site as it was located right at the streams' head and assumed to be lacustrine in character. The second site was situated five metres downstream from the first, and the third ten metres below the second. The fourth site was located 50 metres downstream of the third site, and the subsequent five sites were each set 100 metres apart (Table 1). In total, a stretch of 565 metres was analysed along the Seebach. The impoundment of the Seebachbad is situated downstream from the ninth site. The sites were mapped in detail during the first sampling week.

Table 1: Distance of the selected sites from the lake outlet (m). Nine sites were sampled for this study, with the first one being subdivided into three subsections (above, in, and below the outlet cascade).

Site	Outlet cascade			2	3	4	5	6	7	8	9
Distance from lake (m)	above	in	below								
	0	0	0	5	15	65	165	265	365	465	565

In order to fully explore the phenology of the macrozoobenthos in the lake outlet over the course of a year, sampling was conducted every three months starting in autumn 2019 (Table 2).

Table 2: Seasonal sampling procedure in the Seebach; each sampling covered a period of one week.

Season	Autumn	Winter	Spring	Summer
Date	24 Nov - 1 Dec 2019	25 Feb - 1 Mar 2020	8 – 14 Jun 2020	17 - 23 Aug 2020

2.2. Abiotic parameters

At each site and sampling date, electric conductivity (µS cm^{-1}), oxygen concentration (mg l^{-1}, %) and water temperature (°C) were measured with handheld WTW electrodes. For discharge and velocity measurements a propeller-meter (Ott $C_{2;}$ propeller diameter = 30 mm) was used. Discharge measurements followed the principles of the velocity-area method, with individual measurements spaced one meter apart along a measuring tape spread at right angles to flow. All stream velocity measurements were conducted at 40% depth (Dingman 1984). Discharge for each site (Q; m^3 s^{-1}) was calculated by multiplying the current velocity at 40% depth (v; mean over profile) with the transect area (A), derived from transect width (m) times mean depth over the profile:

$$Q = A * v$$

During the first sampling, HOBO dataloggers were exposed at each site, programmed to measure water temperatures twice a day. The times of measurement were set at 2 am and 2 pm for each day in order to ascertain the daily minimum and maximum temperatures.

2.3. Macrozoobenthos sampling

A steel Hess sampler (barrel diameter = 0.25 m; mesh size = 250 µm) was used for sampling macrozoobenthos. For obtaining representative samples, a modified multi-habitat sampling procedure was used (modified after AQEM 2002). For this, first the composition of ground cover types (choriotopes) at each site was assessed. According to this standard, five mineral (Akal = 2–20 mm grain size; Microlithal = > 20–63 mm; Mesolithal = > 63–200 mm; Macrolithal = > 200–630 mm; Megalithal and Bedrock = > 630 mm) and two biotic choriotopes (Phytal = water mosses; submerged dead wood such as logs, roots, twigs = Xylal) were mapped at each section, and area percentages calculated. The number of samples per choriotope reflected the corresponding area percentages. While assessing the habitats, locations where

samples were to be taken, were not disturbed. The three choriotope types with the highest area percentage were chosen at each site, and three replicates with the Hess sampler were taken.

For sampling, the sampler was firmly attached to the bottom of the stream, and the substrate thoroughly disturbed with hands and/or a screwdriver. In loose choriotopes (mesolithal, akal), the uppermost five centimetres of sediment inside the barrel were disturbed. This was done for five minutes until most invertebrates inside the diameter had been swept inside the catching net. If the stones at the riverbed were larger than the diameter of the Hess sampler (megalithal), they were scrubbed clean of algae, moss, and macrozoobenthic organisms using a screwdriver. The samples were preserved in ethanol (diluted to approximately 50%) and transported to the laboratory.

In order to supplement the quantitative samples taken by the Hess sampler, a second sampling run was conducted throughout each site using the CPUE (catch per unit effort) method. For this the whole surface area of a given sampling site was collected during a pre-defined time period of 20 minutes. While collecting using the CPUE method, stones and debris were picked up and searched for invertebrates which were subsequently transferred into containers with the help of tweezers. This was done by starting on one bank and proceeding in a zigzag pattern to the other in order to obtain a representative sample. The three metre boundaries of the sampling stretch were not crossed and special attention was given to microhabitats ignored by the MHS method. These microhabitats included woody debris as well as near-bank structures, such as tree roots. The collected material was then preserved in ethanol in the same manner as that collected with the Hess sampler.

2.4. Plankton sampling

At each site, a plankton net of 28.5 cm diameter and a mesh size of 40 µm was deployed upstream of the sampling location. The net was fastened to the stream bed so that it was stretched by the current. Each time the net was deployed, the submerged amount of the net's diameter was recorded so that the sampled water volume could be calculated. On each site the net was exposed for 10 minutes. Plankton samples were transferred to a storage bottle using a funnel and additional water from a spray bottle, preserved in diluted 96% ethanol, and transported to the laboratory for further analyses of the taxa composition and plankton density. A second set of samples was taken at each site, using the same procedure. These samples,

however, were subsequently transferred to a drying oven at 80°C for several days as a preparation for C/N analyses which were later performed at the limnology unit of the University of Vienna using an elemental analyser utilizing the combined methods of Pregl and Dumas whereby dried samples are placed in tin capsules and mineralize at high temperatures. The produced gasses are then analysed in a gas chromatographic system (Kudzin and Waśkowski, 2004).

2.5. Identification of Ephemeroptera, Plecoptera and Trichoptera (EPT taxa)

The EPT fraction of macrozoobenthos obtained by Hess and CPUE samples were analysed using a Nikon SMZU stereo microscope. After carefully emptying the sample bottles into a white plastic tray all EPT specimens visible by naked eye were picked out and placed into Petri dishes filled with 98% ethanol. Having done that, most of the sampled EPT specimens still remained inside the tray and had to be quantitatively picked out under the stereo microscope. Each individual EPT specimen was removed from the tray and put into a Petri dish filled with ethanol. In cases where a lot of debris covered the tray's bottom the debris was stirred using tweezers to access the specimens. Otherwise, the contents of the trays were not disturbed. In cases where the amount of soil and debris was excessive, the contents were filtered through a fine mesh (mesh size=100 µm) so that soil particles were washed off while specimens and larger debris particles remained. Checking of each tray under the stereo microscope was conducted three times for each sample to ascertain that all EPT specimen were removed. Identification of EPT taxa was performed under the stereo microscope using keys by Bauernfeind and Humpesch (2001) for Ephemeroptera, Graf and Schmidt-Kloiber (2008) for Plecoptera, and Waringer & Graf (2011) for Trichoptera. Identification was performed to the lowest taxonomical level possible.

2.6. Analyses of feeding guild composition

In order to perform analyses of feeding guilds along the sampling reach of the Seebach, the EPT taxa lists, and abundances obtained by the Hess and CPUE samples were transferred into a format readable by the ECOPROF program. ECOPROF (Version 5.0) is a tool developed by the Institute of Hydrobiology and Water Management of the University of Natural Resources and sponsored by the Austrian Federal Ministry of Agriculture, Regions and Tourism. The tool

was designed to allow for analysis of datasets based on aquatic biota. It is based on ecological data from the reference work Fauna Aquatica Austriaca (Moog & Hartmann (Eds.), 2017).

2.7. Plankton densities

Counting of planktonic organisms was performed under a Nikon SMZU stereo microscope. A petri dish was filled with a preserved sample and the specimens per 1 cm^2 counted. For this analysis, the samples were grouped into phyto- and zooplankton. In cases where the amounts of planktonic organisms were manageable the total number of individuals was counted. Sometimes however, this approach was unfeasible and subsampling was conducted. This was done by shaking the total sample for three minutes and then pipetting off 20 ml of the sample. This approach allowed for a representative subsampling, since the planktonic organisms were assumed to be distributed equally in the liquid after shaking. The remaining sample volume was measured afterwards to allow extrapolating from the subsample.

2.8. Statistical analysis

All statistical analyses were conducted using “RStudio” (1.4.1103) and the “vegan” package (version 2.5.7., Oksanen et al., 2020). In addition, the packages “dplyr” (version 1.0.8., Wickham et al., 2022) and “ggplot2” (Wickham, 2016) were used extensively for data management and graphs respectively. For overview maps as well as for calculating the distribution of choriotopes QGIS was used (version 3.10.10., QGIS, 2020). Data management and formatting was conducted in Excel as well (version 2110).

2.8.1. Temperature

The time information provided by the HOBOwarePro program (version 3.2.2.) was converted into date classes using basic “R” functions. A daily mean temperature was calculated using the mean of the two daily readings. From that, monthly mean temperature values were derived. In order to investigate longitudinal temperature gradients, regression analyses were conducted for all sites and a Wilcoxon rank-sum test for differences between the first and last site.

2.8.2. Oxygen and conductivity

Oxygen and conductivity data were examined for seasonal differences using Kruskal-Wallis rank sum tests followed by Bonferroni corrected Dunn's rank sum tests. All data were checked for normality using the Shapiro-Wilk test of normality. Longitudinal gradients were obtained using regression analyses.

2.8.3. Velocity and discharge

Measured propeller-meter values were multiplied with their appropriate constants in order to obtain actual velocity data, and arithmetic means calculated over each profile. Mean discharge of sites was then calculated by multiplying mean velocity with the width and mean depth of each site for each season. Differences between seasons were tested for in "R" by first testing for normality using the Shapiro-Wilk test and then conducting Kruskal-Wallis rank sum tests followed by Dunn' rank sum tests. Longitudinal gradients were subject to regression analyses.

2.8.4. EPT abundances, diversity indices, evenness, and species richness

Data derived from Hess and CPUE samplings were used for generating a community data matrix at genus level using "Excel" and subsequently imported into "R" and analysed with tools from the "vegan" package. First, a species richness for each site was established using the "specnumber" command. Afterwards, Shannon's index of diversity (H') was calculated using the "diversity" command allowing finding out Pielou's evenness (J) by dividing H' with the natural logarithm of the species richness. Abundance of individuals was arrived by using the aggregate function of base "R" and calculating the sums of all EPT specimens determined to genus level for each season and site.

Density of EPT specimens was calculated by taking taxa-specific numbers for each site and dividing the raw count data with the area of the sampler multiplied by the number of times sampling was conducted on the site. Afterwards, longitudinal trends were investigated for these parameters using regression analyses.

Abundance and density of EPT specimens were also investigated for seasonal differences using Kruskal-Wallis rank sum tests followed by Bonferroni corrected Dunn's rank sum tests.

2.8.5. Beta diversity

Analyses of community dissimilarity measures were conducted by calculating Bray-Curtis dissimilarity indices between all sites with the "vegan" command "vegdist()". This distances were then scaled and normalised using the base "R" "scale()" command. Afterwards, Euclidian distances were calculated from the scaled matrix, and a hierarchical cluster was performed using the base "R" "hclust()" command set to Ward's clustering.

2.8.6. Feeding guild analyses

ECOPROF provided a matrix containing feeding guild values ranging from 0 to 10 for each site and feeding guild present. These values were transformed into percentages by multiplying by 10. A Shapiro-Wilk test was conducted on the data which were then normalized using natural logarithms. Regression analyses were then used to investigate longitudinal gradients.

2.8.7. Electivity

A measure of electivity was calculated for each site and choriotope, based on Ivlev's electivity (Ivlev, 1961; Lechowicz, 1982; Martini and Waringer, 2021). For this, the following equation was used:

$$E_i = \frac{r_i - p_i}{r_i + p_i}$$

where E_i is the electivity index ranging from -1 to 1, derived from r_i which denotes the relative proportion of EPT specimens of a given site per choriotope, and p_i which denotes the relative proportion of a choriotope at that site. For r_i MHS data abundances of all EPT specimens regardless of taxonomic level were used. For p_i, the ratio was calculated by dividing the area percentages of the choriotopes present at a given site by total sampled area.

2.8.8. C/N analysis

After data processing including -1outlier elimination in the base "R" boxplot function data were tested for normality using a Shapiro-Wilk test of normality, and afterwards differences in C/N content as well as C/N ratios between seasons investigated using a Kruskal-Wallis rank sum test followed by a Dunn's rank sum test.

After testing for normality, a ln-ln (natural logarithm) transformation was applied to the data, and regression analyses performed for obtaining longitudinal gradient information.

2.8.9. Plankton

The calculation of plankton density was based on discharge per net and exposition time, and on total counts. A Shapiro-Wilk test for normality was applied to the data which were then log-transformed. For seasonal differences Kruskal-Wallis rank sum tests were performed, followed by Dunn's rank sum tests. The longitudinal gradient was investigated by regression analyses.

2.8.10. Constrained correspondence analysis

In order to prepare the data obtained from MHS sampling for ordination analysis, the rarest species (those with ten individuals or less) were dropped from the dataset. Constraining variables were selected from the above-mentioned environmental parameters. Variables where datapoints were missing (mean temperature and C/N ratio) had to be disregarded for the analysis. The final set of constraining variables consisted of yearly mean oxygen concentration (mg l^{-1}), yearly mean conductivity (µS cm^{-1}), yearly mean velocity (m s^{-1}), mean zooplankton density (n l^{-1}), mean phytoplankton density (n l^{-1}) as well as the area percentages of choriotopes (except akal which was dropped due to collinearity).

CCA was conducted in "R" using the "cca()" function of the "vegan" package. The resulting cca object was then plotted using "ggplot2". Tests for significance were conducted using the "anova()" command in "R"

3. Results

3.1. Abiotic parameters along the sampling reach

3.1.1 Water temperature

Of the nine dataloggers exposed at the beginning of the study, six yielded data suitable for further analysis. One was manipulated by strangers in autumn 2019 compromising the dataset during that time. Another datalogger failed to read, and a third device was removed from its anchoring point and lost. Fortunately, the rest of the dataloggers collected good data enabling an analysis of the water temperature in the Seebach albeit with a shortened timespan (Figure 3).

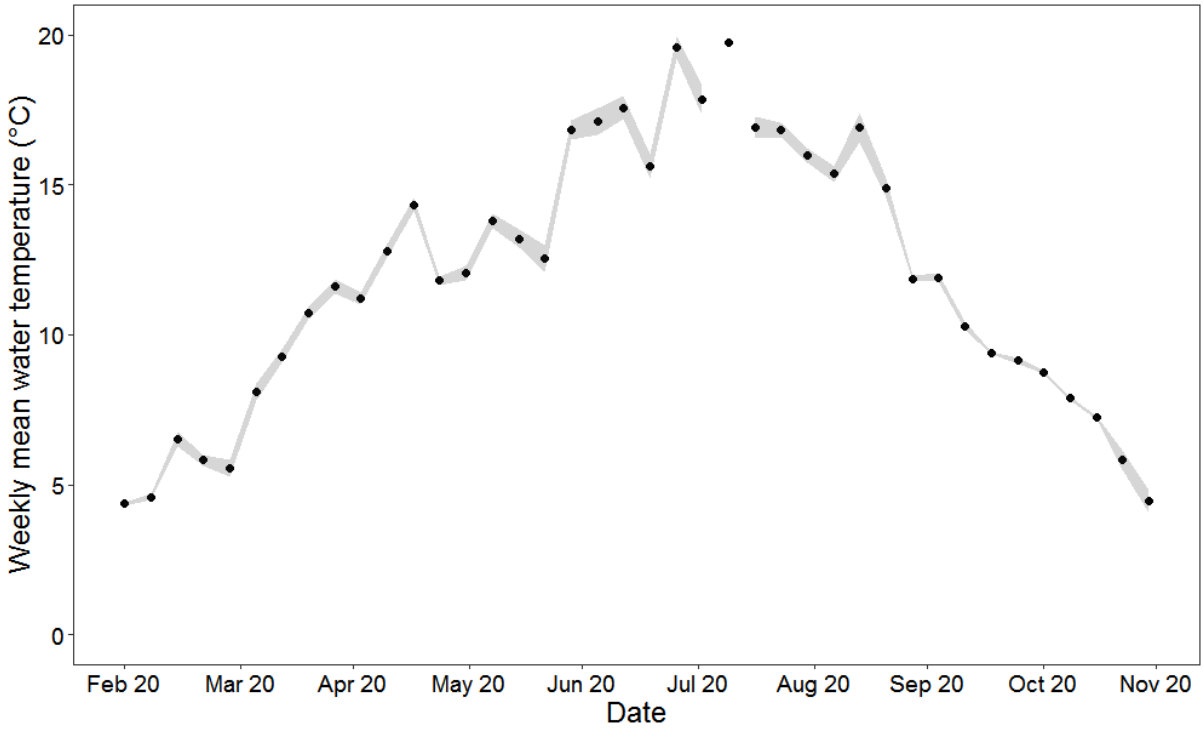

Figure 3: Weekly mean water temperatures (with 95% CL; pooled data from all sites), based on HOBO data logger data. The duration of the investigated timespan was shortened due to the lack of temperature measurements from autumn to winter at the first site.

Average mean temperature of the sites followed a yearly pattern with high temperatures during July and low temperatures during late autumn and early spring (Figure 3). With a yearly mean temperature of 11.99 °C the last site (situated 565m downstream) was slightly warmer than the first one (mean=11.95 °C). A Wilcoxon rank sum test revealed no significant differences

(p>0.05) in daily mean temperature between the first and the last site. A regression analysis involving yearly mean temperature of all sites indicated that there was no significant decrease of temperature along the longitudinal gradient (p>0.05) (Figure 4).

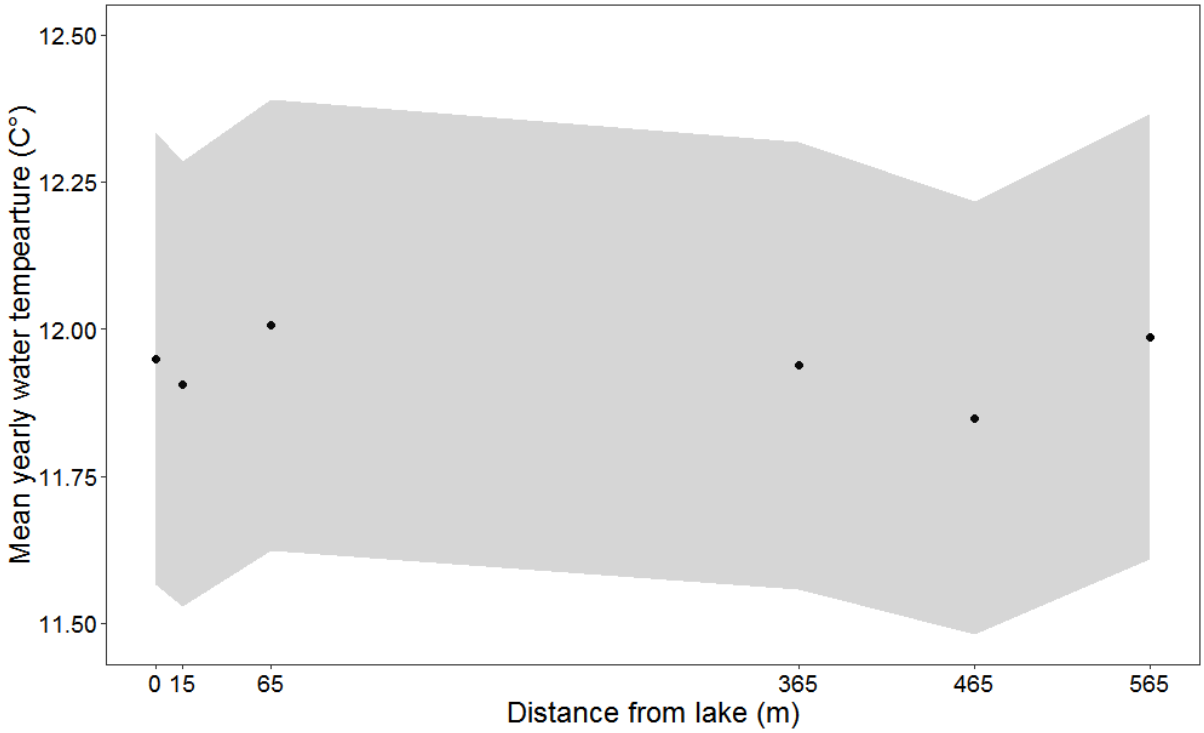

Figure 4: Mean yearly water temperatures (with 95% CL) of the six sites where HOBO dataloggers were exposed. Only small differences in water temperature were observed between the sites, with no correlation between distance from lake outlet and mean temperature.

3.1.2. Oxygen

No significant changes in oxygen concentration (mg l^{-1}) and saturation (%) with distance from the lake outlet could be observed when seasons were pooled. When splitting the seasons, however, significant changes were observed over the sampling stretch (Figure 5, Table 3, and Table 4).

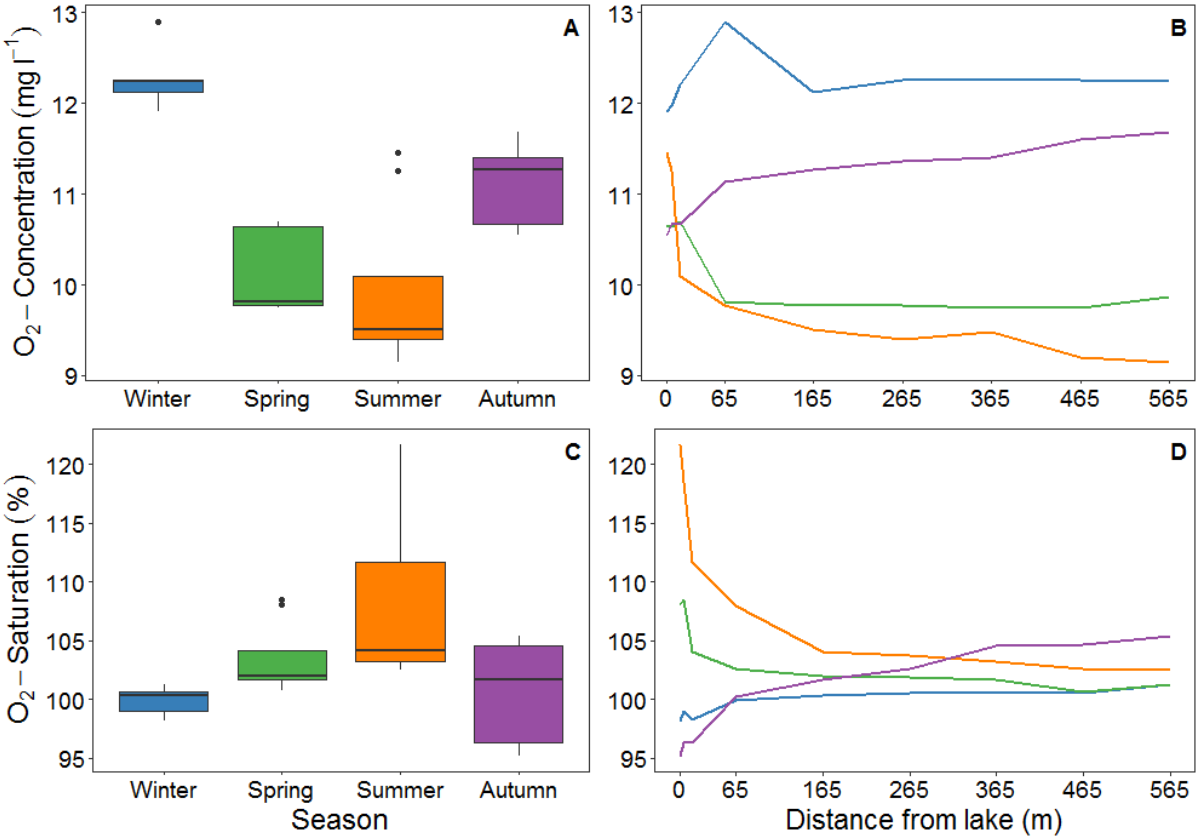

Figure 5: Oxygen concentration and saturation varied significantly between seasons and with distance from the lake outlet: A: oxygen concentration (mg l^{-1}) per season, B: oxygen concentration (mg l^{-1}) over distance (m) from lake outlet, C: oxygen saturation (%) per season, and D: oxygen saturation (%) over distance (m) from the lake outlet. Oxygen data measured during winter is coloured blue, during spring green, during summer orange and during autumn purple.

During the colder months (autumn and winter) oxygen concentration was generally higher and increased with distance from the lake outlet. During spring and summer, oxygen concentration dropped significantly with distance, as did oxygen saturation. A Kruskal-Wallis rank sum test showed significant differences of oxygen saturation between seasons (p=<0.001, chi square=16.87) with a Bonferroni corrected Dunn's post hoc test revealing that oxygen saturation during summer was significantly higher than during winter (p=<0.001). Median oxygen saturation was lowest during winter (Mdn=100.3%) and highest during summer (Mdn=104.1 (Figure 5 C).

Table 3: Linear regression table for oxygen saturation (%) as dependent variable and distance from the lake outlet as independent variable. Variables tested are oxygen saturation during winter, spring, summer, and autumn. B=beta, SE B=standard error of beta.

Dependent variable	R^2	$F_{1,7}$	p	B	SE B
Winter	0.76	22.85	0.002	0.004	0.002
Spring	0.57	9.36	0.018	-0.010	0.003
Summer	0.63	11.88	0.011	-0.027	0.008
Autumn	0.86	45.08	<0.001	0.017	0.003

There were significant differences in oxygen concentration across seasons (Kruskal-Wallis, p=<0.001, chi-square=26.3) and a subsequent Dunn's test showed that significant seasonal differences were present between winter and spring (p=<0.001) as well as winter and summer (p=<0.0001). Oxygen concentration was highest during winter (Mdn=12.25 mg l^{-1}) and lowest during summer (Mdn=9.5 mg l^{-1}) (Figure 5 A).

Table 4: Linear regression table for oxygen concentration (mg l^{-1}) as dependent variable and distance from the lake outlet as independent variable. Variables tested are oxygen concentration during winter, spring, summer, and autumn. B=beta, SE B=standard error of beta.

Dependent variable	R^2	$F_{1,7}$	p	B	SE B
Winter	0.01	0.08	n.s.	<0.001	<0.001
Spring	0.5	6.975	0.033	-0.001	<0.001
Summer	0.6	10.7	0.014	-0.003	<0.001
Autumn	0.86	42.61	<0.001	0.002	<0.001

3.1.3. Conductivity

A Kruskal-Wallis rank sum test revealed significant differences in conductivity across the seasons (Kruskal-Wallis, chi-square=25.43, p=<0.0001). A Bonferroni corrected Dunn's post hoc test showed that significant differences exist between spring and summer (p=<0.001) as well as spring and winter (p=0.0002). The highest overall conductivity was recorded during winter and summer (Mdn=244 µS cm^{-1}) while it was lowest during spring (Mdn=228 µS cm^{-1}) (Figure 6). Even though overall conductivity increased slightly downstream of the lake outlet, this effect was not significant (p>0.05).

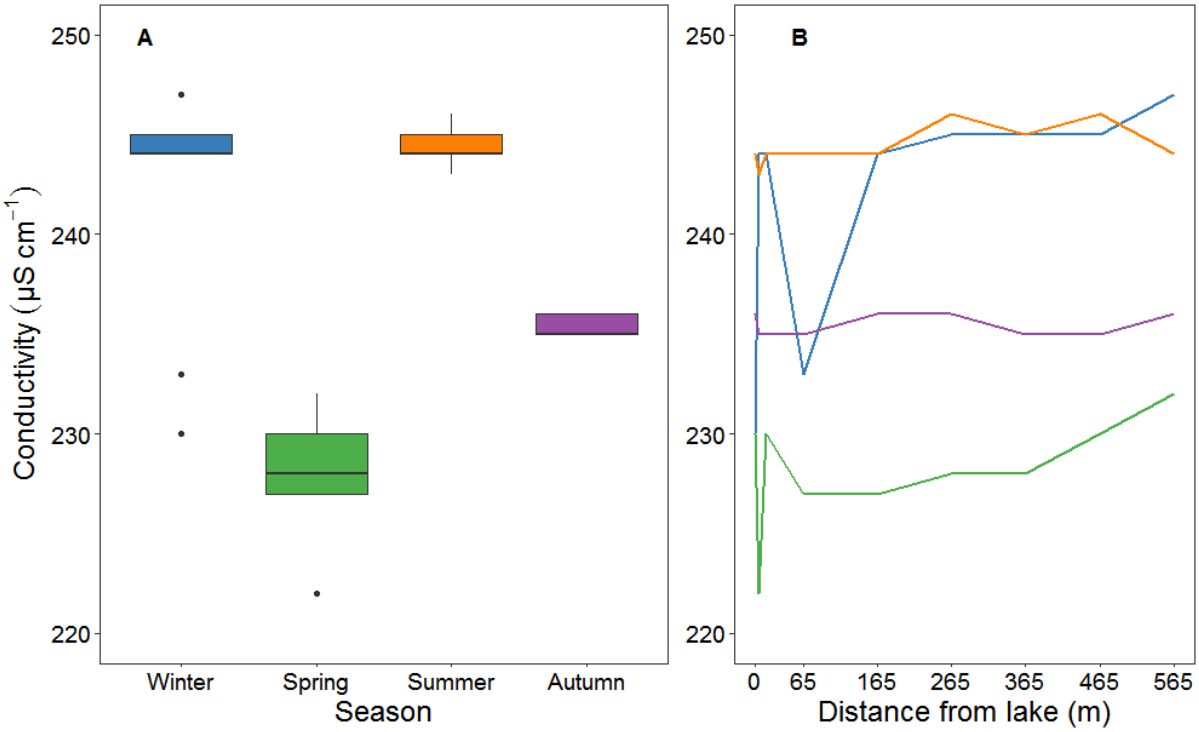

Figure 6: Electric conductivity (µS cm^{-1}) differed between seasons (A) and with distance (m) from the lake outlet (B). There were significant differences ($p<0.05$) in the conductivity between seasons. However, the differences between sampling stations were not significant ($p>0.05$). Conductivity data measured during winter is coloured blue, during spring green, during summer orange and during autumn purple.

3.1.4. Stream velocity and discharge

Mean stream velocities did not significantly change between seasons (Mdn=0.48-0.57 m s^{-1}) except for autumn when they were significantly lower (Mdn=0.17 m s^{-1}). A subsequent pairwise Kruskal-Wallis ranked sum test followed by a Bonferroni corrected Dunn's test revealed chi-square values of 16.37 ($p=<0.0001$; Dunn's test, $p_{spring-autumn}=0.0023$, $p_{summer-autumn}=0.0083$, $p_{winter-autumn}=0.0041$) (Figure 7 A). Although there was a slight decrease in stream velocity with distance, this effect was not significant when considering measurements from all sampling dates ($p>0.05$). However, site seven (365 m downstream of the lake) had very low stream velocities throughout the year (except winter which could not be recorded due to technical problems) (Figure 7 A). Current speeds too high to maintain a standing position and occasional technical problems of the propeller-meter prevented obtaining velocity data at some sites. During the winter sampling, therefore, only three sites could be measured.

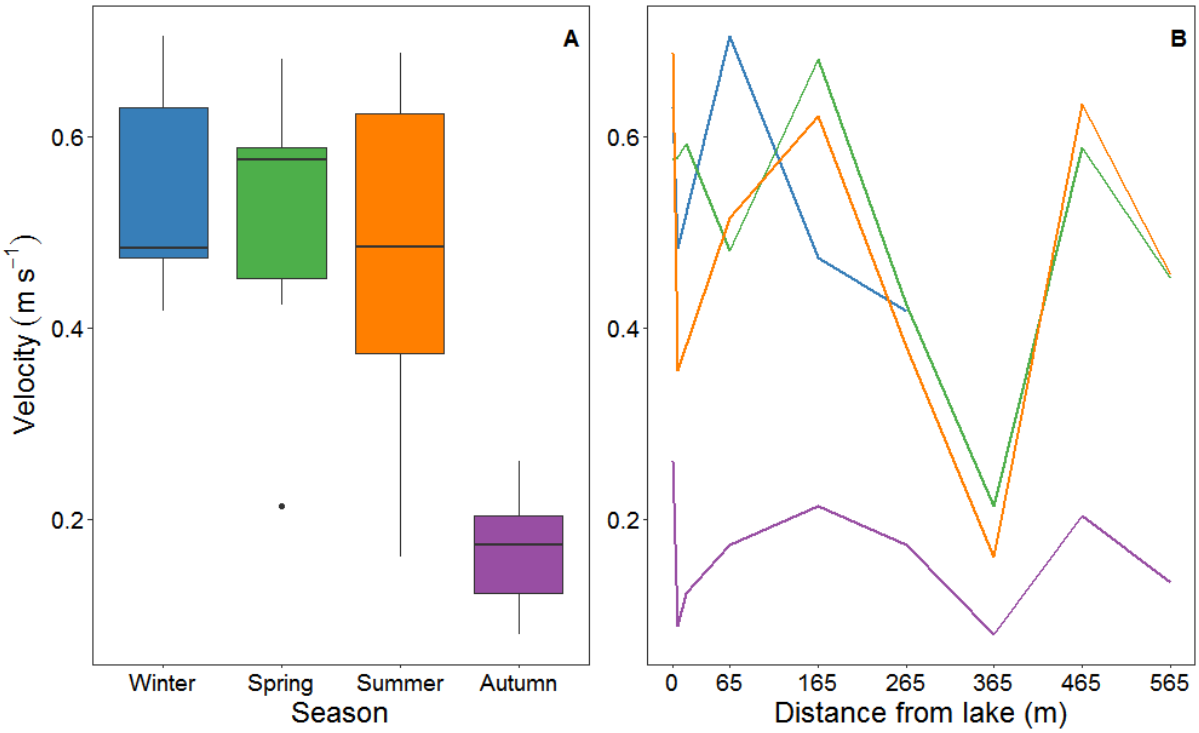

Figure 7: Mean stream velocity (m s^{-1}) differed significantly ($p<0.05$) between seasons (A) and between sites (B), with autumn being the season with the lowest measured velocities and site seven (365 m downstream of the lake outlet) having consistently the lowest mean current velocities. Velocity data measured during winter is coloured blue, during spring green, during summer orange and during autumn purple.

Discharge was not significantly affected by distance ($p>0.05$) and was highest at the lake outlet (Mdn=1.91 m^3 s^{-1}) and lowest 365 metres downstream (Mdn=1.03 m^3 s^{-1}) (Figure 8). Discharge rates varied significantly according to seasons (Kruskal-Wallis test; chi-squared=19.13, $p<0.001$). A subsequent Dunn's post-hoc test revealed that discharge was significantly lower during autumn (all $p<0.005$).

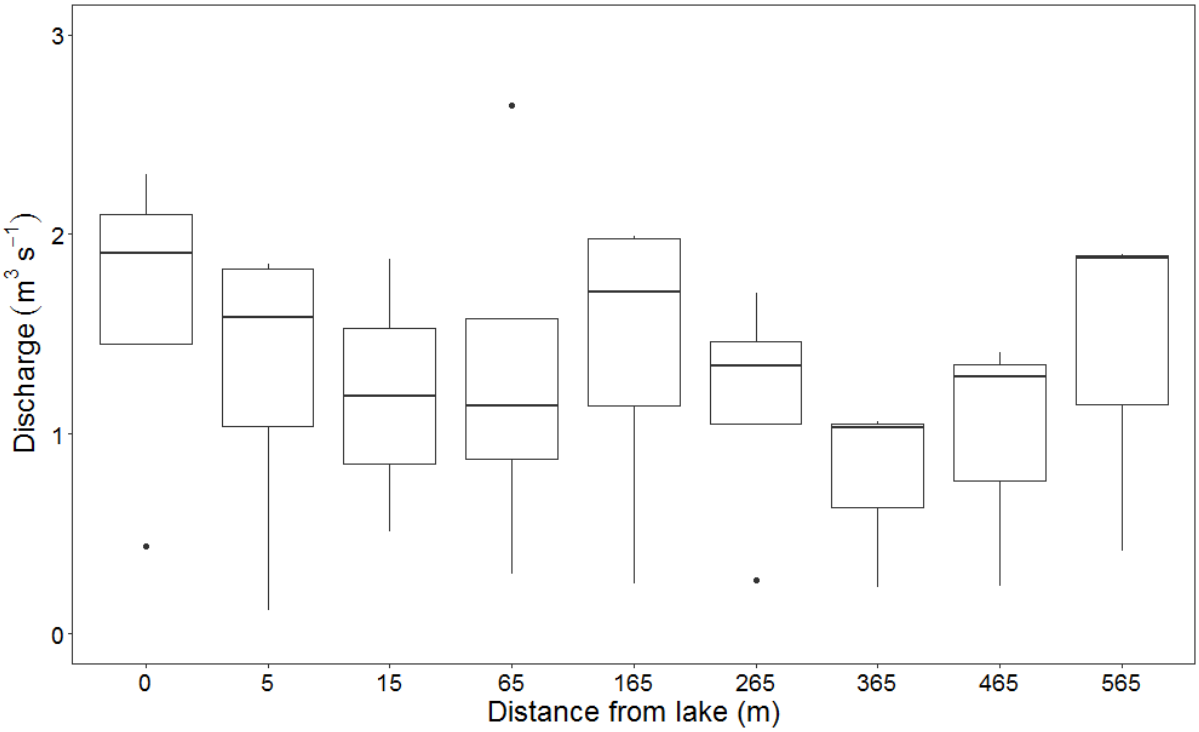

Figure 8: Box-whisker plot showing discharge over the longitudinal gradient (m^3 s^{-1}; seasons pooled). The sites did not differ significantly, and regression analysis revealed that there was no significant change over the longitudinal gradient.

3.2. Longitudinal gradients of Ephemeroptera, Plecoptera and Trichoptera

3.2.1. EPT taxa inventory

A total of 11920 specimens was collected with the MHS method. Of these, 11390 specimens were identified to genus level (total=32 genera), and subsequently 8207 to species level. Specimens not identifiable to genus level were omitted from the subsequent analyses.

Trichoptera were most abundant (63.82% of total), followed by Ephemeroptera (27.41%) and Plecoptera (8.77%). In terms of taxa numbers, 51.43% of genera belonged to Trichoptera, 37.14% to Ephemeroptera and 11.43% to Plecoptera.

The four most abundant taxa were *Micrasema setiferum* (26.74% of the total abundance), *Hydropsyche* spp. (mostly *H. incognita*; 25.67%), *Baetis* spp. (10.93%), and *Ephemerella* sp. (9.15%). *Micrasema setiferum* was common throughout the sampled reach, and particularly common at sites four, five and six. It was the most prominent taxon (3187 individuals). Members of the caseless caddisfly genus *Hydropsyche* dominated within the first 65 metres of

the lake outlet as well as at the last site (565m downstream of the lake outlet). A total of 3084 *Hydropsyche* individuals were sampled. Less common were mayflies of the genus *Baetis* which were prominent within the most downstream 300 metres of the study reach (1303 specimens in total). This taxon might have been underestimated due to the fact that they are very fragile and often largely damaged. Another common mayfly was *Ephemerella* which was common at many sites. Most of the 1091 *Ephemerella* individuals sampled belonged to *E. ignita* (Table 5).

Table 5: Overview of EPT taxa and their abundance obtained using the MHS (n m^{-2}) and the CPUE method (n 20 min^{-1}). The five most prominent taxa are in bold letters.

Species	Family	n m^{-2}	n 20 min^{-1}
Ephemeroptera			
Baetis rhodani	Baetidae	22.90	0.07
Baetis spp.	Baetidae	**266.21**	**1.07**
Centroptilum sp.	Baetidae	8.81	0.09
Baetidae juveniles	Baetidae	71.71	-
Caenis spp.	Caenidae	7.05	-
Ephemerella ignita	Ephemerellidae	**189.39**	**4.48**
Ephemerella major	Ephemerellidae	1.76	-
Ephemerella mucronata	Ephemerellidae	0.18	-
Ephemerella spp.	Ephemerellidae	0.88	-
Ephemera danica	Ephemeridae	1.06	-
Ephemera spp.	Ephemeridae	0.18	-
Ecdyonurus picteti	Heptageniidae	0.18	-
Ecdyonurus spp.	Heptageniidae	1.06	0.14
Epeorus assimilis	Heptageniidae	3.52	1.25
Rhithrogena spp.	Heptageniidae	3.70	0.02
Heptageniidae juveniles	Heptageniidae	1.23	-
Habrophlebia lauta	Leptophlebiidae	9.69	0.02
Paraleptophlebia submarginata	Leptophlebiidae	32.77	0.30
Leptophlebiidae indet.	Leptophlebiidae	1.06	-
Ephemeroptera juveniles	-	10.22	-
Plecoptera			
Leuctra geniculata	Leuctridae	13.39	0.25
Leuctra spp.	Leuctridae	50.39	0.16
Nemoura spp.	Nemouridae	17.79	0.16
Nemurella pictetii	Nemouridae	4.58	0.11
Protonemura spp.	Nemouridae	-	0.02
***Amphinemura* spp.**	Nemouridae	**90.03**	0.09
Nemouridae juveniles	Nemouridae	1.59	-
Plecoptera indet.	-	0.53	-
Trichoptera			
Brachycentrus montanus	Brachycentridae	1.76	0.36
Micrasema setiferum	Brachycentridae	**561.49**	**5.32**

Micrasema spp.	Brachycentridae	-	0.02
Agapetus sp.	Glossosomatidae	0.53	-
Silo nigricornis	Goeridae	0.70	0.23
Hydropsyche incognita	Hydropsychidae	**426.36**	**9.69**
Hydropsyche instabilis	Hydropsychidae	1.41	0.11
Hydropsyche siltalai	Hydropsychidae	26.60	0.48
Hydropsyche spp.	Hydropsychidae	88.97	0.39
Hydroptila spp.	Hydroptilidae	2.29	0.02
Lepidostoma hirtum	Lepidostomatidae	30.48	0.80
Oecetis testacea	Leptoceridae	13,4	0.82
Athripsodes albifrons	Leptoceridae	7.40	0.25
Athripsodes aterrimus	Leptoceridae	0.70	-
Athripsodes bilineatus	Leptoceridae	10.57	0.02
Athripsodes spp.	Leptoceridae	0.18	0.02
Ceraclea dissimilis	Leptoceridae	2.11	0.61
Mystacides azurea	Leptoceridae	0.35	-
Leptoceridae indet.	Leptoceridae	0.53	-
Chaetopteryx fusca	Limnephilidae	-	0.11
Drusus biguttatus	Limnephilidae	-	0.09
Drusus spp.	Limnephilidae	0.18	0.23
Halesus radiatus	Limnephilidae	-	0.05
Halesus spp.	Limnephilidae	-	0.02
Limnephilidae indet.	Limnephilidae	0.18	-
Plectrocnemia conspersa	Polycentropodidae	1.06	-
Plectrocnemia geniculata	Polycentropodidae	0.35	-
Polycentropus excisus	Polycentropodidae	0.35	0.02
Polycentropus flavomaculatus	Polycentropodidae	57.26	**2.00**
Polycentropus irroratus	Polycentropodidae	1.41	0.16
Polycentropus spp.	Polycentropodidae	6.51	0.07
Psychomyia pusilla	Psychomyiidae	1.06	-
Tinodes waeneri	Psychomyiidae	0.70	-
Rhyacophila tristis	Rhyacophilidae	5.29	-
Rhyacophila s. str. sp.	Rhyacophilidae	25.19	0.84
Rhyacophila spp. juveniles	Rhyacophilidae	-	0.11
Sericostoma flavicorne	Sericostomatidae	7.58	0.43
Trichoptrea indet.	-	5.46	-

Additionally, 1926 EPT specimens, belonging to 29 taxa, were collected using the CPUE method. There were some significant deviations from the taxa list based on Hess samples. Some genera such as the limnephilids *Halesus* sp. and *Chaetopteryx* sp. as well as the nemourid stonefly *Protonemura* sp. did not occur in the data obtained with the Hess sampler (Table 5).

There were also differences in relative abundance of taxa: while *Micrasema* sp. was the most common taxon present in the Hess samples, *Hydropsyche* sp. was much more prominent in the CPUE samples.

3.2.2. Densities of EPT taxa

A regression analysis showed that EPT taxa densities obtained using the MHS method (n m^{-2}) and distance from the lake outlet were not significantly related and that EPT taxa densities instead followed an irregular pattern reflecting the life cycles of dominant taxa. (Figure 9 A).

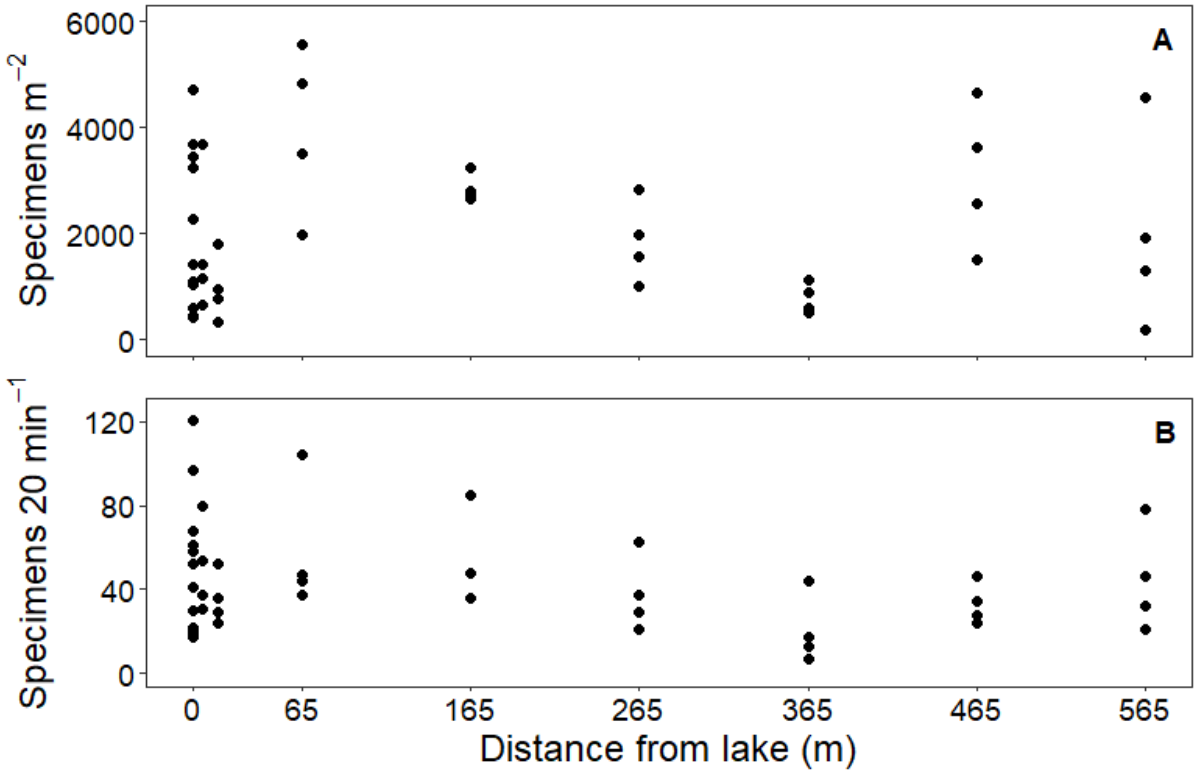

Figure 9: (A) Densities of EPT taxa obtained by the MHS method (n m^{-2}; pooled data over all sampling sites and seasons) along a longitudinal gradient downstream of the lake outlet. (B) Abundances of EPT taxa sampled using the CPUE method (20 minutes collection time) along the same longitudinal gradient including data from all seasons.

During all seasons except spring, densities were high within the first 15 meters from the outlet, dropped to a minimum at 15 meters, and increased again over the next 50-meter stretch. For the following 200 meters densities dropped continuously until they increased again for the last 200 meters of the sampling reach (Figure 10).

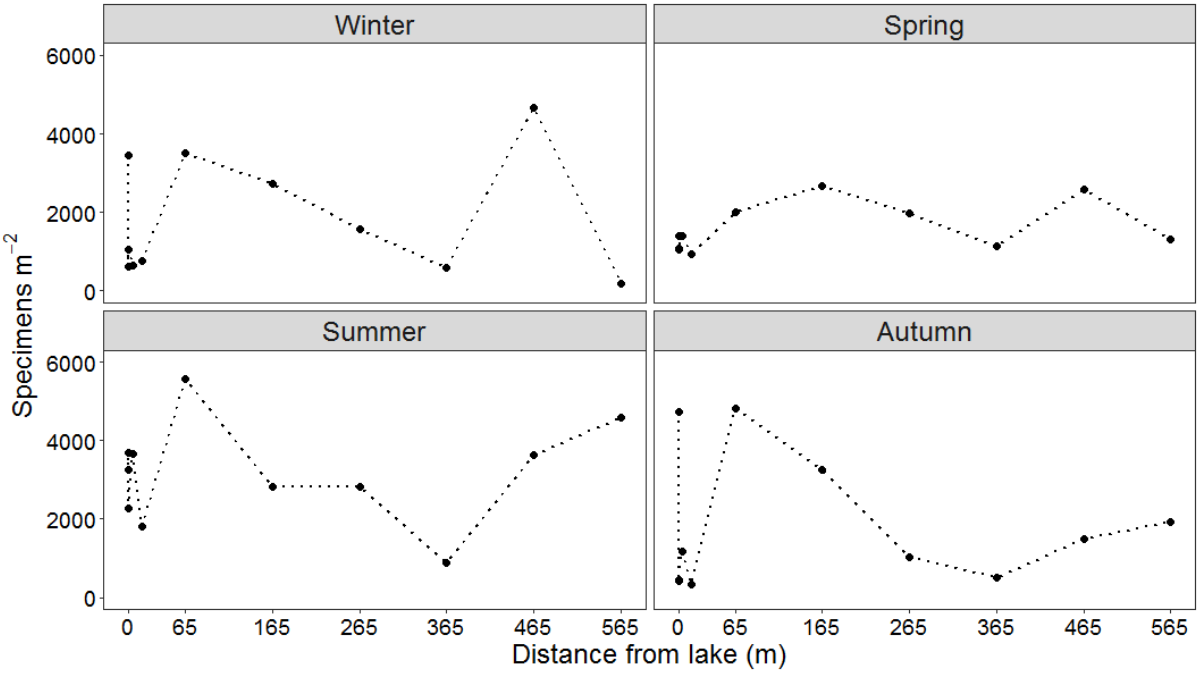

Figure 10: Seasonal densities of EPT taxa obtained by the MHS method (n m^{-2}; pooled data over all sampling sites) along the longitudinal gradient downstream of the lake outlet.

The number of specimens collected at each site with the CPUE method did not decrease significantly over the longitudinal gradient when considering all seasons. Instead, the abundances followed a pattern similar to that of the samples taken with the MHS method, where they increased over the first three sites and then decreased over the following 300 metres. At the last two sites the number of specimens collected increased again (Figure 9 B).

A Kruskal-Wallis test showed no significant seasonal differences in EPT abundances obtained with the CPUE method. The largest number of specimens was observed during winter (Mdn=46) and the lowest during summer (Mdn=32).

A Kruskal-Wallis rank sum test performed on the MHS data showed significant differences in densities between seasons (chi-squared=8.48, p=0.037). A post hoc Dunn's test, however, revealed that the differences between each season were not significant when a Bonferroni correction was applied. The highest median density was observed during summer (Mdn=3248.1 specimens/m²) and the lowest during winter (Mdn=1038.7 specimens/m²) (Figure 11).

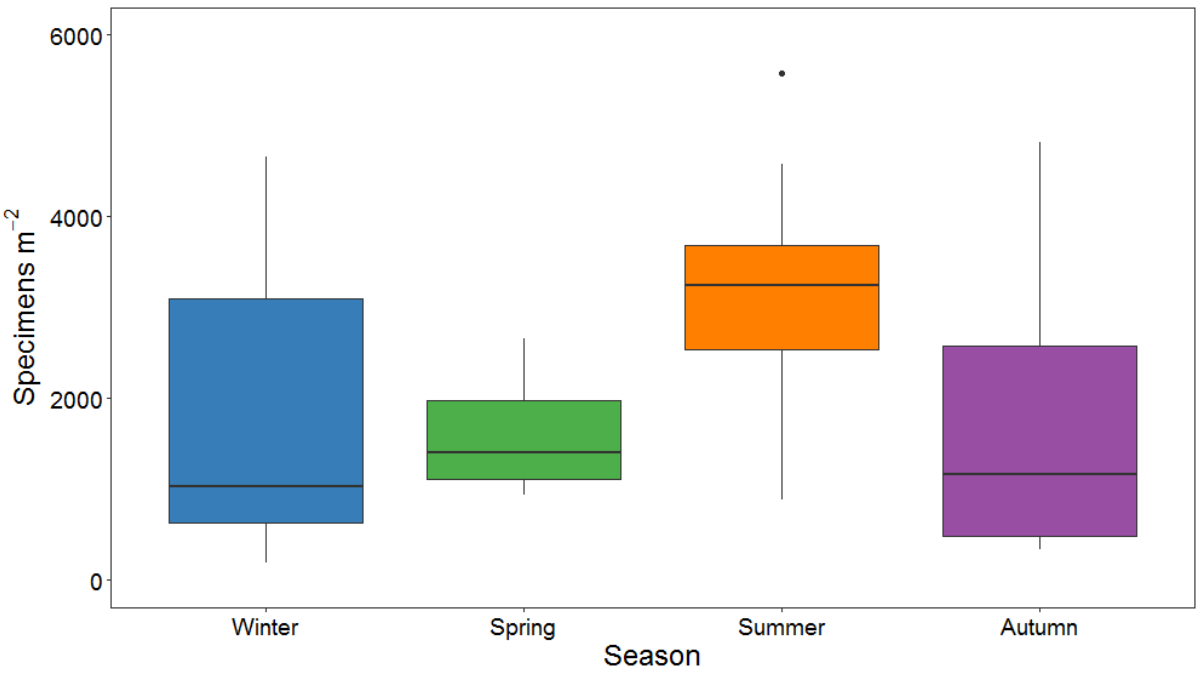

Figure 11: Box-whisker plot of EPT densities (n m^{-2}) sampled with the MHS method over all seasons. Densities were highest during summer and lowest during winter. No significant differences between seasons were observed. Density data obtained during winter is coloured blue, during spring green, during summer orange and during autumn purple.

The number of individuals per site increased with mean stream velocity (linear regression; y=117.7 (±102.7) + 658.4 (±233.0) x; R^2=0.26; $F_{1,23}$=7.98; p=0.0096). This effect was particularly strong during summer (linear regression; y=-195.3 (±242.4) + 1535.7 (±475.3) x; R^2=0.67; $F_{1,5}$=10.44; p=0.023) (Figure 12).

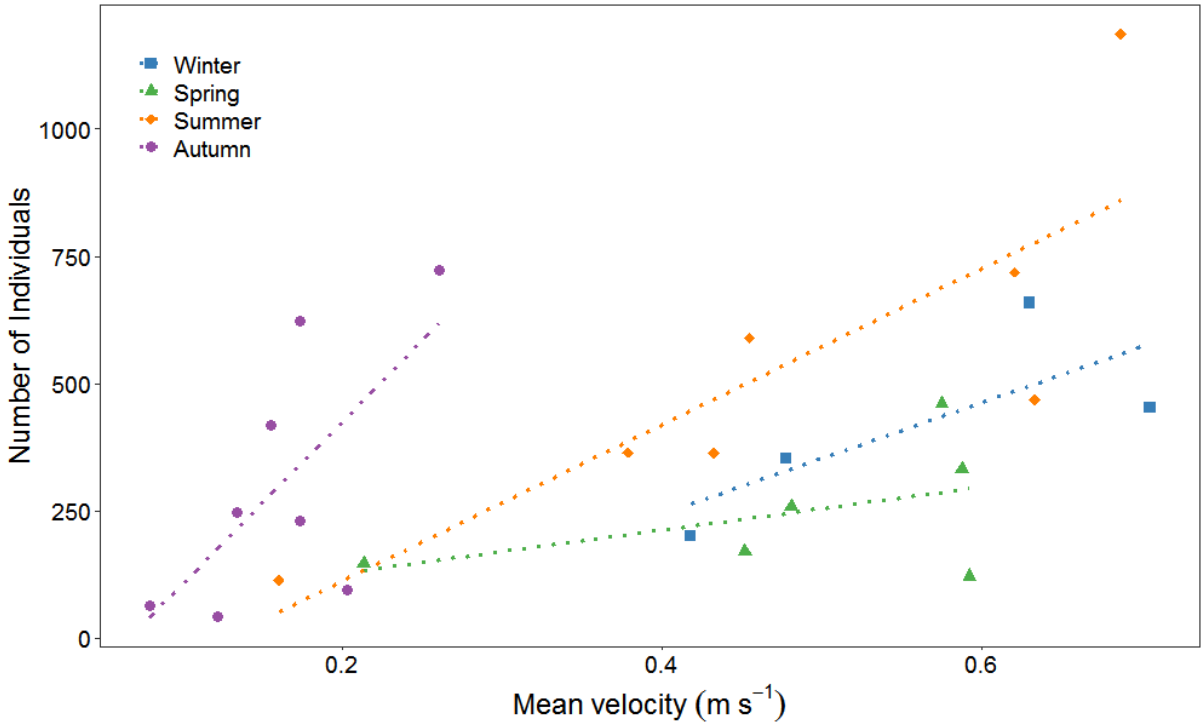

Figure 12: The total number of individuals collected with the MHS method with mean site velocity; the gradient was steepest in autumn and summer, but less so in the remaining seasons. Linear regressions were highly significant ($p<0.001$). Data obtained during winter is coloured blue, during spring green, during summer orange, and during autumn purple.

3.2.3. Taxa richness, diversity, and evenness of EPT taxa

The number of EPT taxa collected with the MHS method at each sampling site increased downstream from the lake outlet. The linear regression between taxa number and distance from the lake was significant ($y=18.88(\pm1.22) +0.015(\pm0.004)\ x$; $R^2=0.535$; $F_{1,9}=10.33$; $p=0.01058$) (Figure 13).

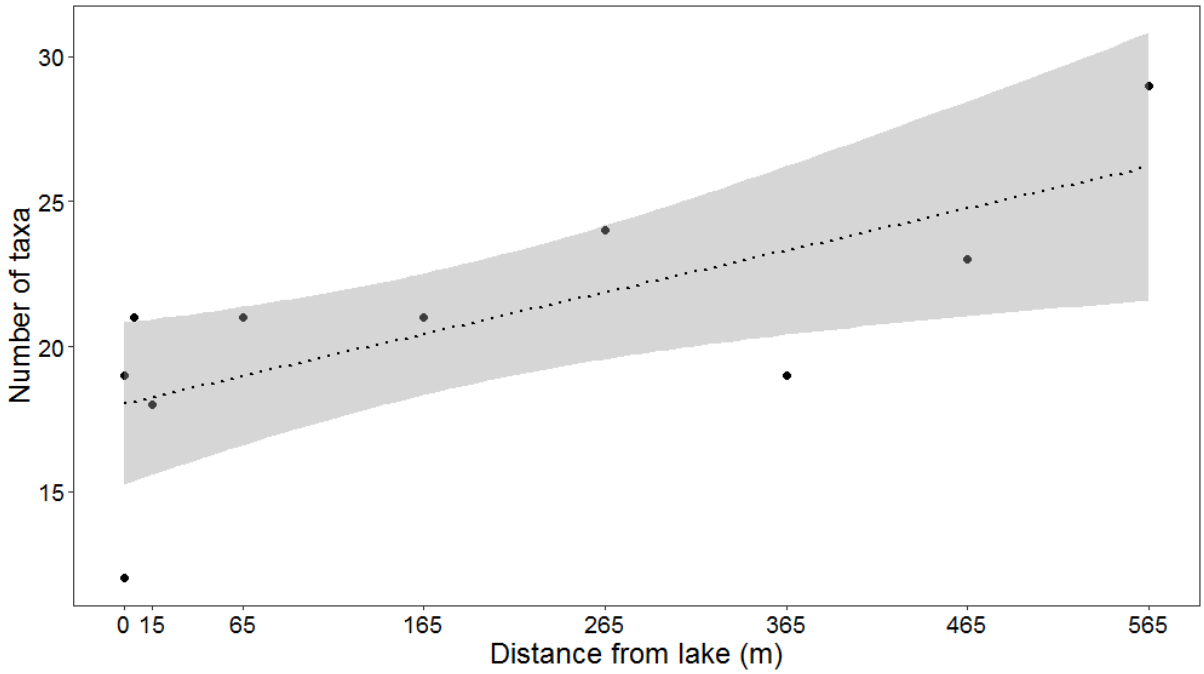

Figure 13: Correlation between numbers of EPT taxa collected using the MHS method and distance (m) from the lake outlet. The increase downstream was significant ($p<0.05$); linear regression line dotted black, 95% CI shaded grey.

The number of taxa obtained with the CPUE method also increased significantly with distance from the lake ($y=11.8(\pm1.05) + 0.01(\pm0.00)\ x$, $F_{1,9}=14.81$, $R^2=0.58$, $p=0.004$).

Shannon's site-specific diversity index H' for taxa collected using the MHS method increased with distance from the lake outlet (Figure 14). The linear regression between H' and distance from the lake outlet was significant and given by $y=1.76(\pm0.10) + 0.001\ (\pm<0.001)\ x$; $R^2=0.45$; $F_{1,9}=7.25$; $p=0.0247$.

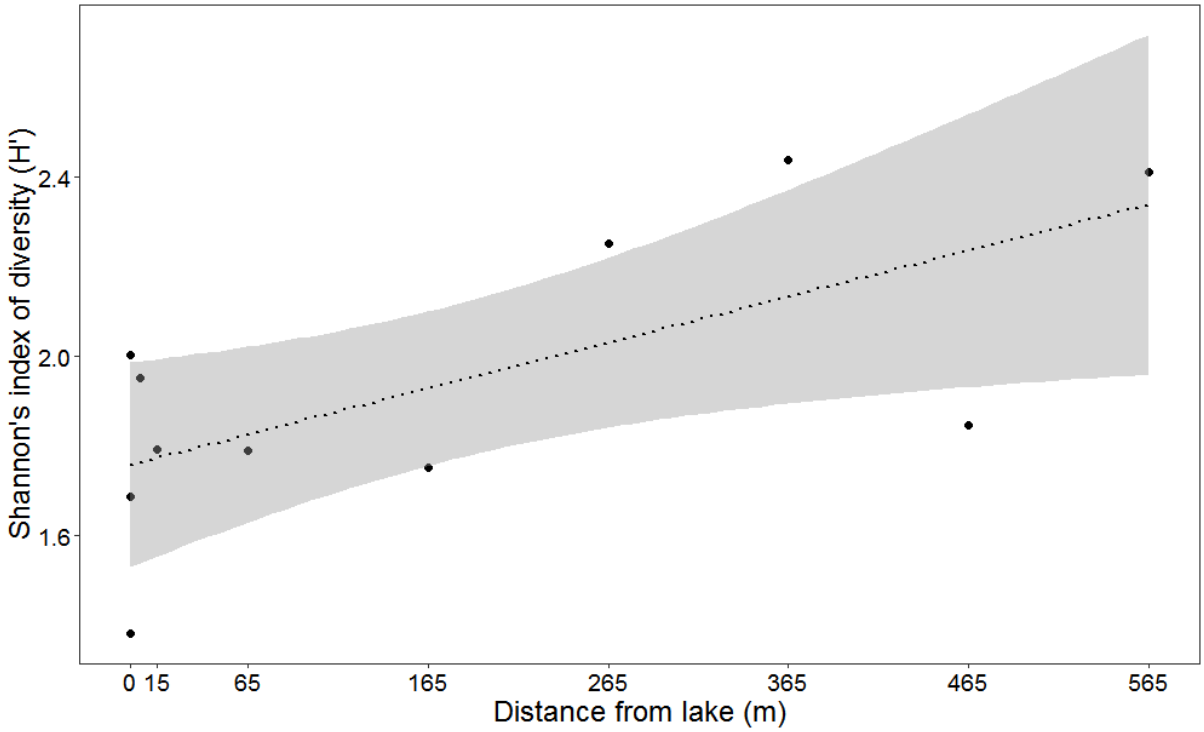

Figure 14: Shannon's diversity index (H'), calculated using MHS data increased with distance (m) from the lake outlet. The linear regression line is dotted black, 95% CL range is in grey.

Linear regression failed to show significant relationships linking diversity (H') and evenness (P') to either zoo- or phytoplankton concentrations ($p>0.05$). Instead, site specific diversity (H') of the MHS samples decreased significantly with velocity (Linear regression: $y=2.77(\pm0.256) - 2.09(\pm0.586)\ x$; $R^2=0.58$; $F_{1,9}=12.71$; $p=0.006$) and megalithal area (Linear regression: $y=2.07(\pm0.109) - 0.01(\pm0.003)\ x$; $R^2=0.4$; $F_{1,9}=6.03$; $p=0.036$). There was a highly significant increase of diversity with depth (Linear Regression: $y=1.14(\pm0.195) + 0.036(\pm0.009)\ x$; $R^2=0.64$; $F_{1,9}=15.99$; $p=0.003$). A similar pattern was observed with Pielou's evenness (P') of sites. It decreased with higher velocities (Linear regression: $y=0.88(\pm0.059) - 0.60(\pm0.135)\ x$; $R^2=0.68$; $F_{1,9}=19.61$; $p=0.001$) and there was a highly significant increase of evenness at deeper sites (Linear regression: $y=0.42(\pm0.045) + 0.01(\pm0.002)\ x$; $R^2=0.73$; $F_{1,9}=24.02$; $p=<0.001$).

Regression analysis revealed a significant decrease of diversity (H') with increasing filter feeder proportions ($y=2.17(\pm0.126) - 0.15(\pm0.052)\ x$; $R^2=0.47$; $F_{1,9}=8.11$; $p=0.019$). Taxa number and filter feeder proportions were negatively linked as well (Linear regression: $y=23.87(\pm1.461) - 1.83\ (\pm0.599)\ x$; $R^2=0.51$; $F_{1,9}=9.37$; $p=0.013$).

Pielou's evenness derived from MHS data, calculated for all sites, showed no significant relationship with distance from the lake outlet (Figure 15 A). When excluding the two most downstream sites, however, a significant increase in evenness was observed ($y=0.58(\pm0.02) + 0.0005(\pm<0.001)\ x$; $R^2=0.57$; $F_{1,7}=9.19$; $p=0.0191$) (Figure 15 B). Generally, evenness increased slightly over the longitudinal gradient and dropped sharply over the last 200 metres.

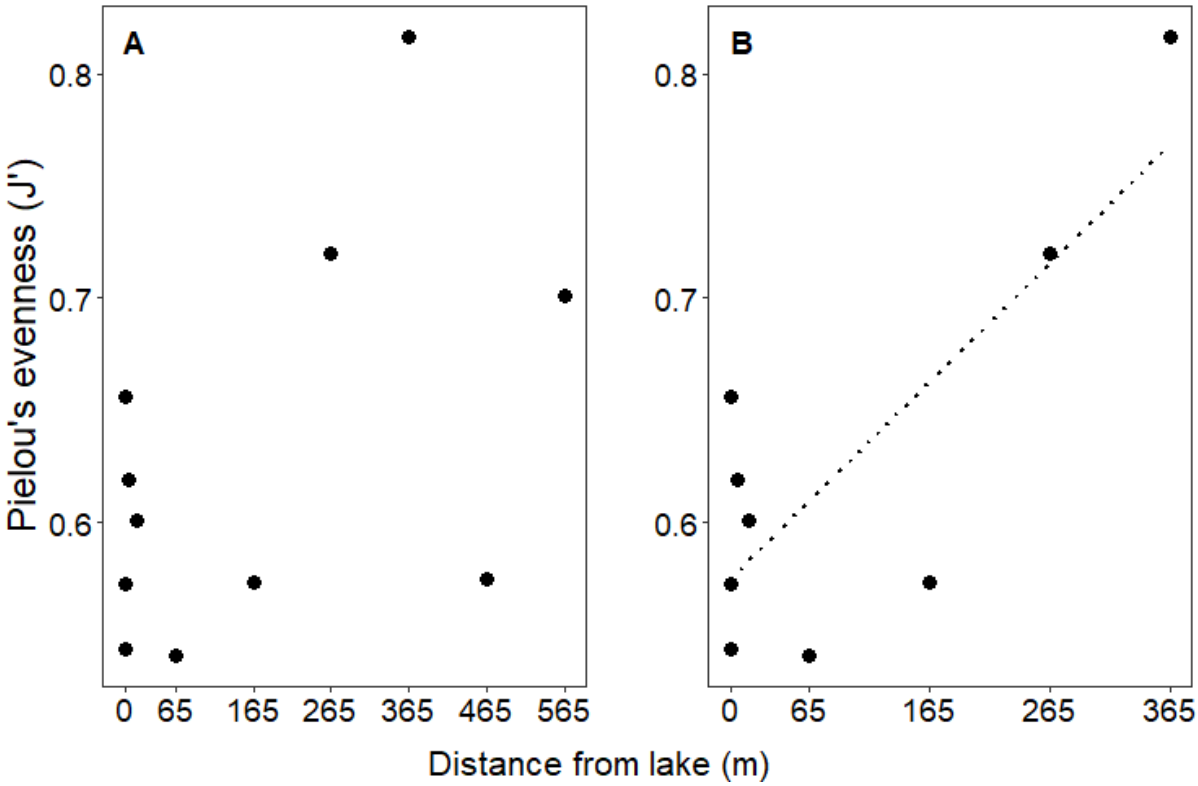

Figure 15: Pileou's evenness (J') calculated from MHS data increased along the longitudinal gradient downstream of the lake outlet, however only insignificantly (p>0.05) when considering all sites (A). In turn, by excluding the two most downstream sampling stations, the linear regression between evenness and distance was significant (B). Regression line dotted black.

There was a highly significant increase of diversity (H') for specimens collected using the CPUE method along the longitudinal gradient when variables were linear-log transformed ($y=1(\pm0.12) + 0.19(\pm0.03)\ \ln(x+1)$, $F_{1,9}=45.5$, $R^2=0.81$, $p=<0.0001$) (Figure 16). Evenness of the CPUE EPT community increased significantly in the downstream sites ($y=0.52(\pm0.04) + 0(\pm0)\ x$, $F_{1,9}= 15.81$, $R^2=0.59$, $p=0.003$) (Table 6).

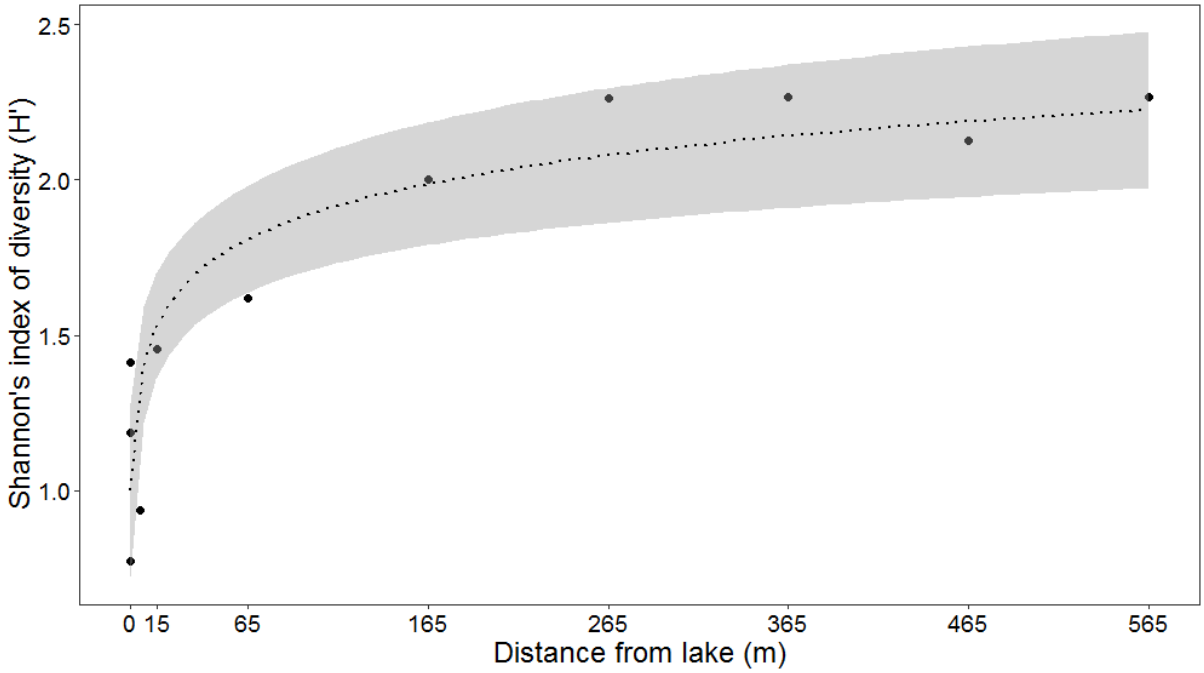

Figure 16: Linear-log model showing the increase of Shannon's diversity index (H') of the CPUE samples along the longitudinal gradient. Regression line is dotted black. 95% confidence intervals are coloured grey.

Table 6: CPUE method. Site-specific Pielou's evenness (J'), Shannon's index of diversity (H') as well as the number of genera present along the longitudinal gradient of the study reach.

Site	Distance (m)	J'	H'	Genera
Above	0	0.45	1.19	14
In	0	0.37	0.77	8
Below	0	0.61	1.41	10
2	5	0.43	0.99	10
3	15	0.59	1.46	12
4	65	0.57	1.58	16
5	165	0.72	1.99	16
6	265	0.76	2.26	20
7	365	0.84	2.27	15
8	465	0.74	2.13	18
9	565	0.76	2.27	20

3.2.4. Dissimilarity

A similar effect as in evenness was also observed for MHS samples when calculating Bray-Curtis dissimilarity indices as a measure of the compositional dissimilarity of EPT taxa between sampling sites. Bray-Curtis dissimilarity was calculated and revealed that the most downstream site 9 (565 m from the lake outlet) was more similar to the first three upstream sites than to those in between. Site eight (465m downstream) was grouped closer to other downstream sites. With the exception of site one (in) there was a clear separation between upstream and downstream sites at the distance of 15 m from the lake outlet (Figure 17 A). Dissimilarity (Bray-Curtis) was calculated for CPUE data as well. A subsequent cluster analysis using the Ward method and Euclidian distances for the matrix revealed a pattern distinctly different from the pattern obtained with Hess sampler data: the study reach was grouped in to an upstream (sites 1-2), central (sites 3-5) and a downstream section (sites 6-9), the latter also including site 1 immediately below the outflow cascade (Figure 17 B).

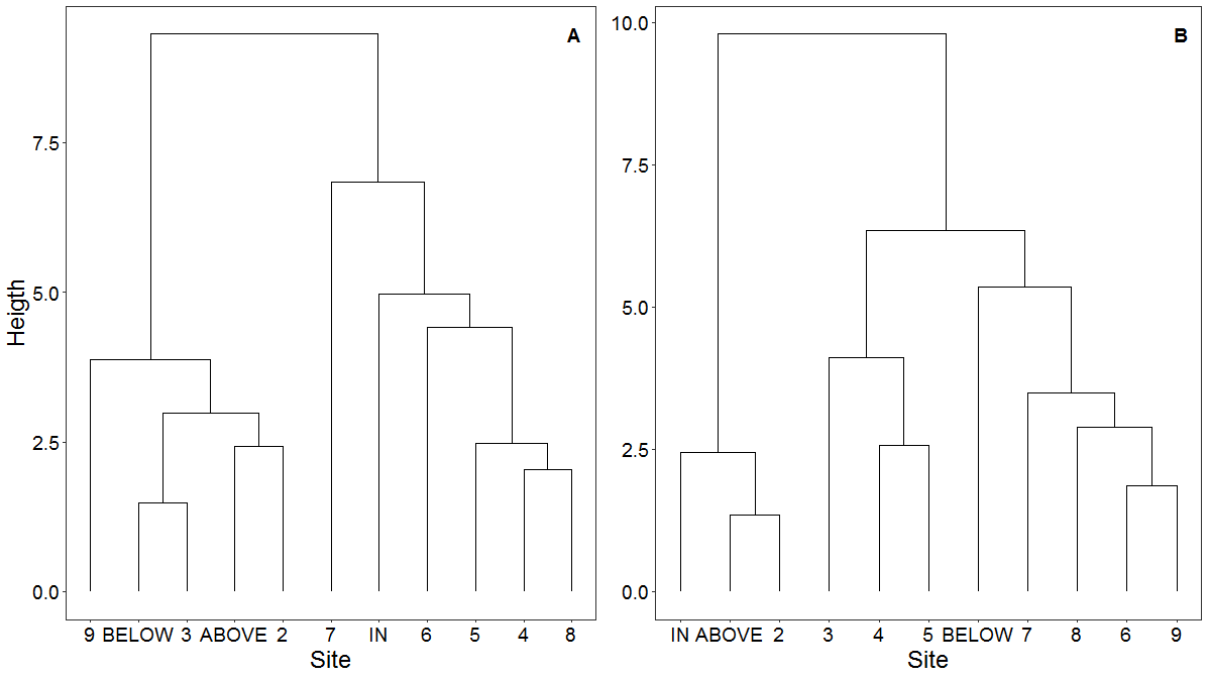

Figure 17: Cluster dendrogram of Bray-Curtis dissimilarities of all sites using Hess sampler data (A) and CPUE data (B) (Ward method, Euclidian distances). The most downstream site (nine) in the Hess data was more similar to upstream sites than to other downstream sites. Site one (in) appears more similar to those further downstream. The CPUE data reveals a tri-partition of the sites, with the first two sites making up a cluster (except site one (in)) and another cluster composed of sites 15 to 165 metres downstream.

3.2.5 Feeding guild patterns

The most prominent feeding guilds when considering all seasons and sites were the grazers (Mdn=32.2%), followed by the detritivores (Mdn=25.9%) and predators (Mdn=18.3%). Passive filter feeders made up a smaller fraction of the feeding guild composition (Mdn=14.1%). Active filter feeders occurred only rarely and constituted only a small fraction of the feeding guild composition. Another rare feeding guild, the xylophages, was present at more sites but in small numbers (Figure 18).

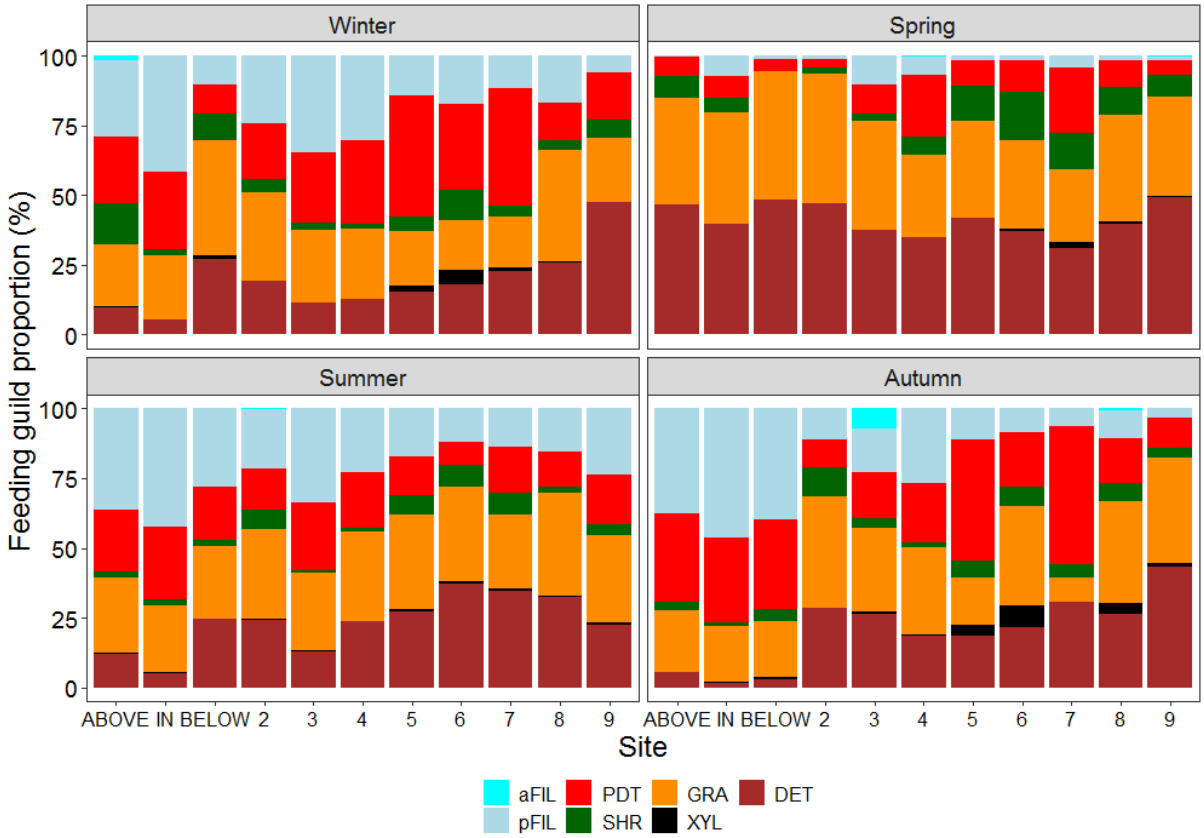

Figure 18: The proportion of feeding guilds during all four seasons and for all nine sampling sites; aFIL (cyan) = active filter feeders, pFIL (blue) = passive filter feeders, PDT (red) = predators, SHR (green) = shredders, GRA (orange) = grazers, XYL (black) = xylophages, DET (brown) = detritivores. During all seasons except spring, where they remained relatively unchanged, the proportions of passive filter feeders decreased significantly, while the detritivores increased. The most important guilds throughout the study reach and across seasons were grazers (Mdn=32.2%) detritivores (Mdn=25.9%) and predators (Mdn=18.3%). Proportions calculated using MHS-data.

The proportion of filter feeders decreased significantly with distance from the lake during summer and autumn but not during winter and spring (Table 7). Where significant, the decrease followed a natural logarithmic pattern (Figure 19). A Kruskal-Wallis rank sum test revealed that there were significant seasonal differences in the proportion of passive filter feeders (chi squared=22.25, $p<0.001$) and a subsequent Bonferroni corrected Dunn's test showed that spring proportions were significantly lower from other seasons (all $p<0.01$). Detritivores made up larger proportions during spring (Kruskal-Wallis rank sum, chi squared=19.54, $p<0.001$; Dunn, all $p<0.01$).

Table 7: Linear regression table showing the proportion of passive filter feeders (%) as dependent variable and distance from the lake outlet as independent variable. All dependent variables were transformed using natural logarithms. Variables tested are the proportion during winter, spring, summer, and autumn. B=beta, SE B=standard error of beta.

Dependent variable	R^2	$F_{1,9}$	p	B	SE B
Winter	0.29	0.29	n.s.	-0.2	0.12
Spring	0.01	0.01	n.s.	-0.01	0.04
Summer	0.65	16.6	0.003	-0.3	0.07
Autumn	0.86	45.08	<0.001	-0.5	0.09

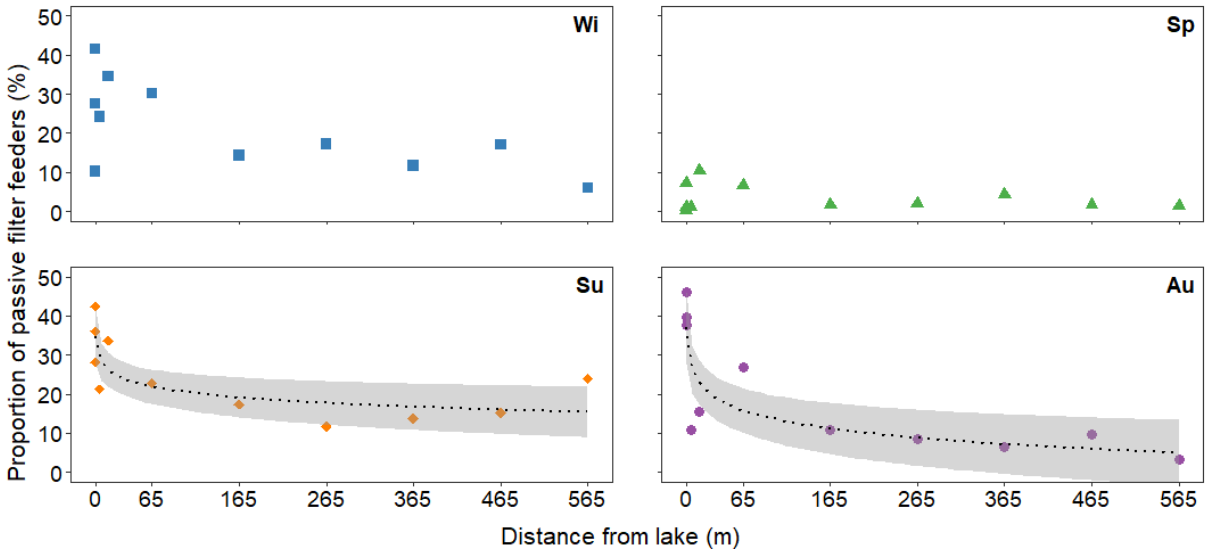

Figure 19: Relationship between the proportion of passive filter feeders and distance (m) from the lake outlet. Winter = Wi, spring =Sp, summer = Su, autumn = Au. Passive filter feeders decreased significantly with distance during summer and autumn following a logarithmic relationship. No significant relation was observed during winter and spring. 95% confidence intervals shaded grey.

The proportions of filter feeders increased significantly with average yearly stream velocity of sites, the proportion of megalithal coverage of sites, yearly mean oxygen concentrations of sites and decreased with increasing depth (Table 8).

Table 8: Linear regression table showing the proportion of passive filter feeders (%) as dependent variable and environmental factors as independent variables. Independent variables are yearly mean stream velocity (m s^{-1}), megalithal area of sites (%), yearly mean oxygen concentration (mg l^{-1}) and mean depth (cm). B=beta, SE B=standard error of beta.

Dependent variable	R^2	$F_{1,9}$	p	B	SE B
Velocity (m s^{-1})	0.51	9.37	0.014	9.11	2.98
Megalithal area (%)	0.72	23.24	0.001	0.01	0.06
Oxygen (mg l^{-1})	0.63	15.10	0.004	5.82	1.50
Depth (cm)	0.38	5.59	0.042	-0.13	0.06

Besides passive filter feeders, no other feeding guild's proportions changed significantly with distance, except for detritivores which increased downstream, inversely mirroring the decrease of passive filter feeders: ($y=4.17(\pm0.147) - 0.91(\pm0.07)\ x$; $F_{1,42}=178.4$; $R^2=0.81$; $p<0.0001$).

Hydropsychids were the most abundant taxon of passive filter feeders obtained with both collection methods. They dominated the upper reaches of the Seebach during all seasons except spring, where the grazing and detritivorous Ephemerellidae prevailed on all sites (Figure 20).

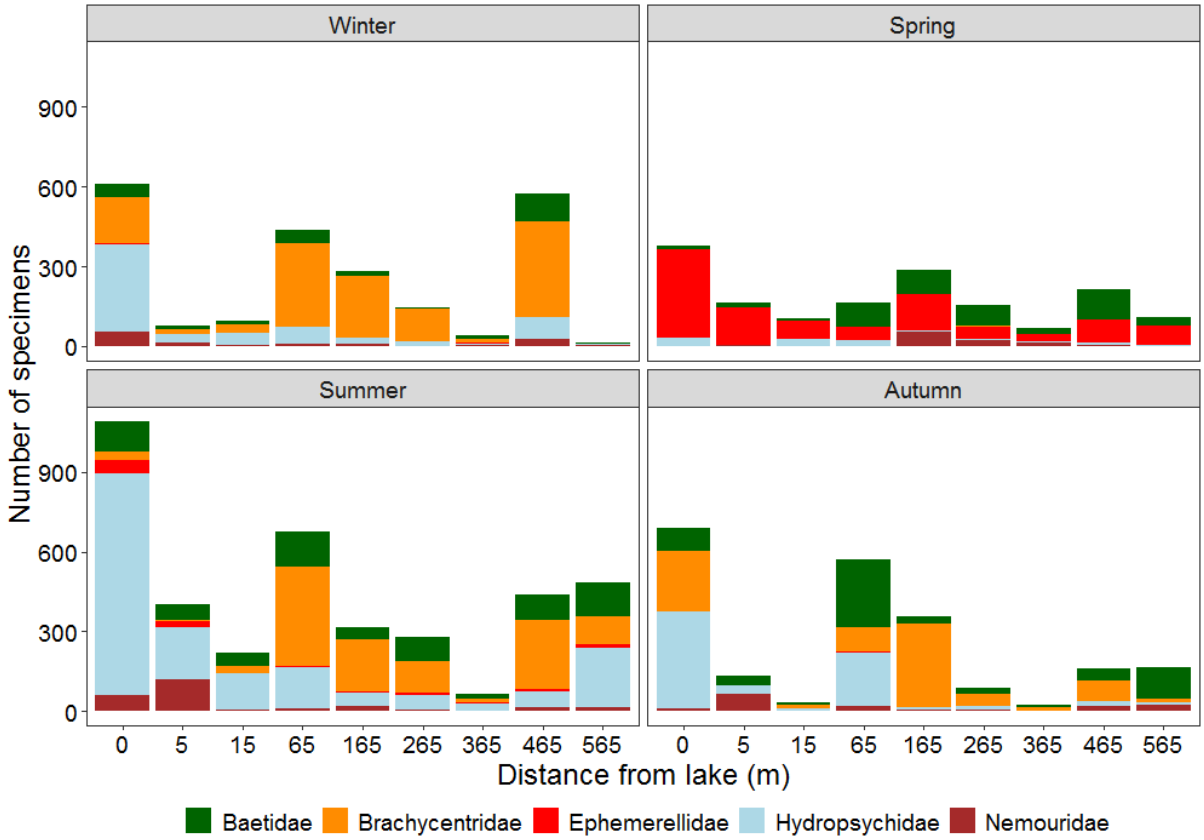

Figure 20: Number of specimens belonging to the five most numerous taxa obtained with the MHS method along the longitudinal gradient. Baetidae are coloured green, Brachycentridae orange, Ephemerellidae red, Hydropsychidae blue and Nemouridae brown. Hydropsychidae were among the most prominent taxa within the first 15m stretch from the lake outlet in all seasons except spring, where all sites were dominated by Ephemerellidae.

Hydropsychid density was unaffected by oxygen concentration during all seasons. When viewed at a season-by-season basis, however, linear regression analyses showed that summer densities increased significantly with oxygen concentration ($y=-4985.8(\pm2021.1) + 608.2(\pm197.2)\ x$; $R^2=0.51$; $F_{1,9}=9.51$; $p=0.013$). This correlation was not significant during other seasons (Figure 21).

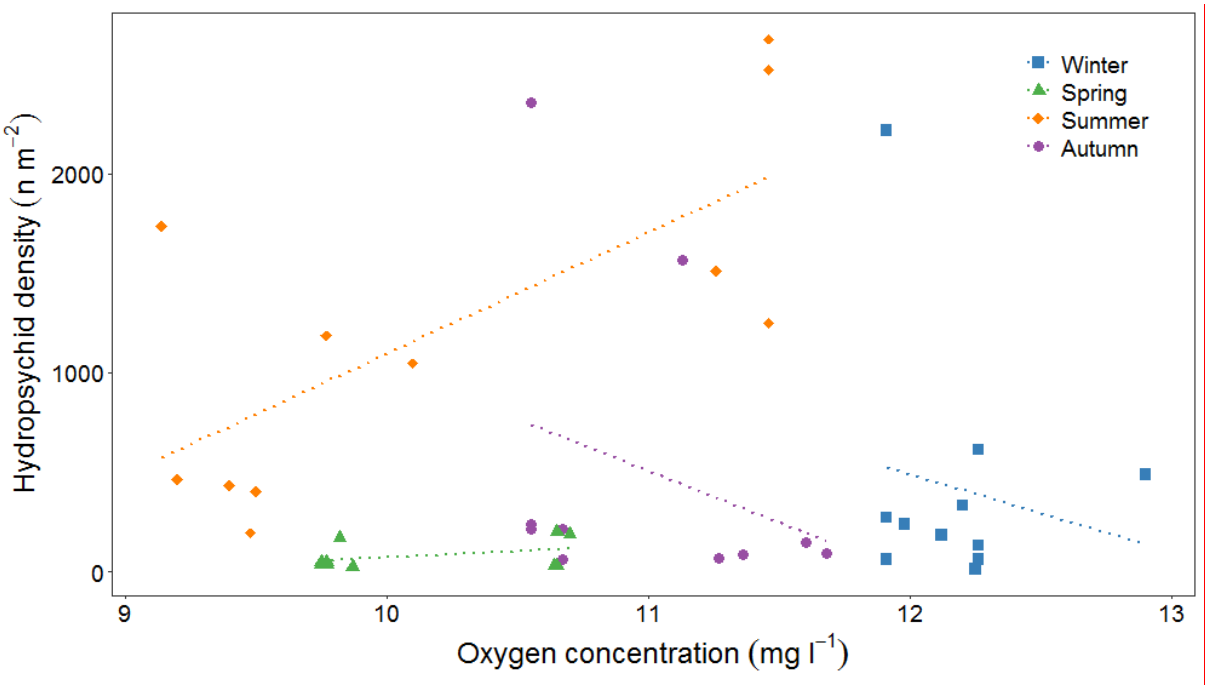

Figure 21: Relationship between hydropsychid density (n m^{-2}) and oxygen concentration (mg l^{-1}). Data points constitute densities per site and are colour-coded for season. During summer hydropsychid density increased significantly ($p<0.05$) with oxygen concentration. Winter = blue, spring = green, summer = orange, autumn = purple.

3.2.6. C/N ratios

A log-log transformation applied to both axes of the relationship between distance from the lake outlet (m) and C/N ratios yielded a significant decrease of the ratios with distance ($y=3.01088$ (± 0.14977) - 0.06769 (± 0.03149) x; $R^2=0.13$; $F_{1,30}=4.62$; $p=0.03976$) (Figure 22).

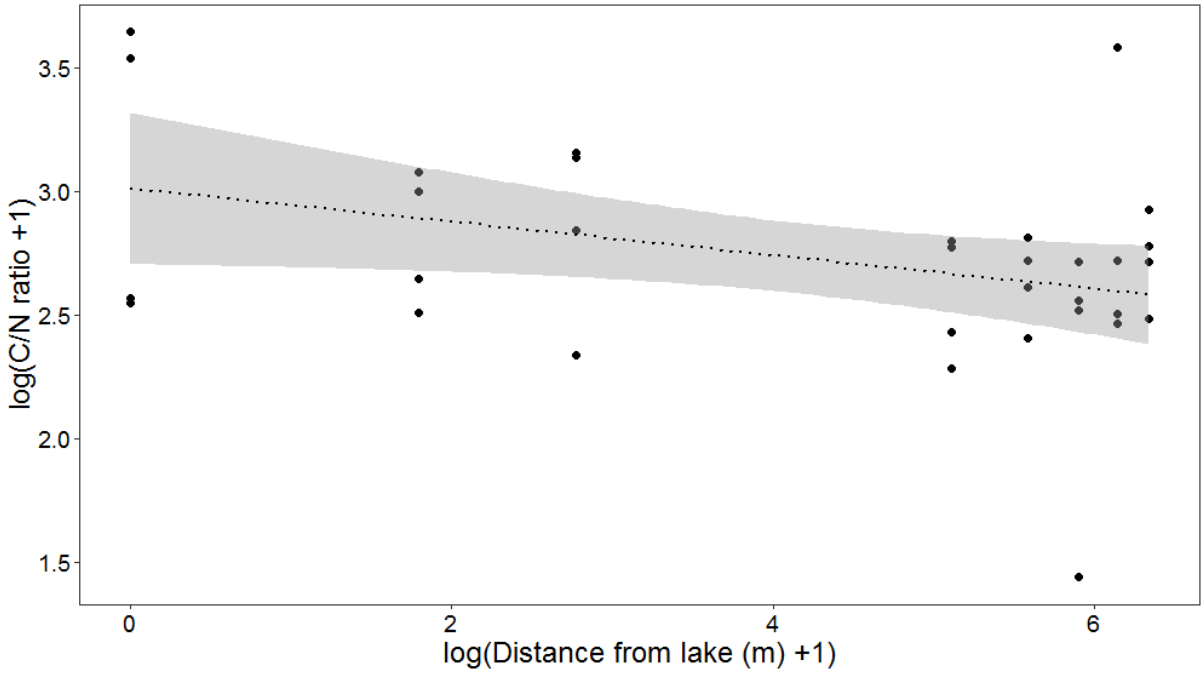

Figure 22: Relationship between log- transformed distance from the lake outlet (m) and log-transformed C/N ratios (y=3.01088 (±0.14977) - 0.06769 (±0.03149) x; R^2=0.13; $F_{1,30}$=4.62; p= 0.03976). Regression line dotted black, 95% CL confidence space in grey.

A Kruskal-Wallis rank sum test indicated significant differences in the C/N ratios between the seasons (chi-squared=11.52, p=0.009). In addition, a Bonferroni corrected Dunn's post hoc test revealed significant differences between summer and spring (p=0.0110) as well as summer and winter (p=0.0120), with summer having the lowest median ratio (Mdn=10.6) and spring the highest (Mdn=17.3) (Figure 23 A). The N ratio differed significantly between the seasons as well (Kruskal-Wallis, chi-squared=19.07, p=0.00026). Here, summer differed significantly from autumn (Dunn, p=0.0056) and spring (p=0.0001). During all seasons, N values were very low, with the highest median ratios (Mdn=0.81) being recorded during summer (Figure 23 B). Differences in the C ratio differed according to seasons as well (Kruskal-Wallis, chi-squared=16.95, p=0.0007). A post hoc Bonferroni corrected Dunn's test revealed significant differences between summer and spring (p=0.0005), summer and autumn (p=0.0154) as well as spring and winter (p=0.0250). The highest median ratios of C were recorded during summer (Mdn=7.32) and the lowest during spring (Mdn=0.63) (Figure 23 C).

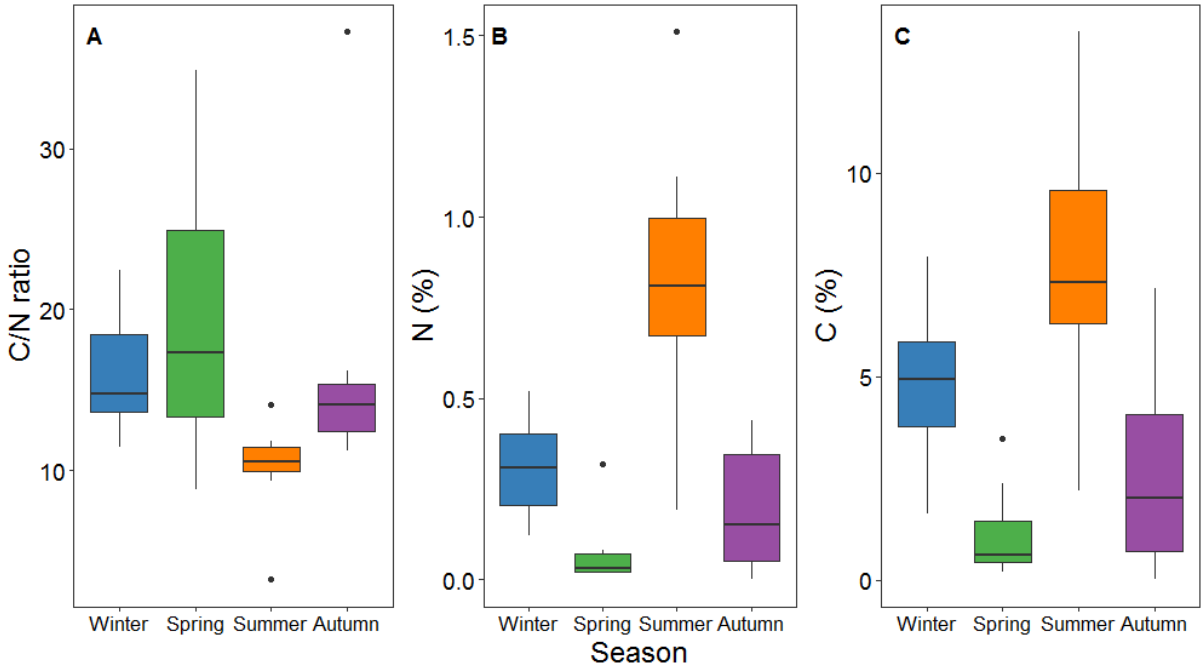

Figure 23: Seasonal differences of C/N ratios (A) and proportions of N (B), and C (C) with values pooled from all sites. Blue = winter, green = spring, orange = summer, violet = autumn.

3.2.7. Plankton

The planktonic organisms caught with the plankton net were counted and standardized as numbers per litre. This was done by taking the measured stream velocity at the net site, the net's coverage by water as well as the net's diameter for each season and site into account. A calculated total of 1131692 planktonic organisms were sampled with the nets. Of this total, 1016227 were phytoplankton and 115464 zooplankton. The standardized values per litre revealed that site four harboured the lowest (Mdn=0.036 specimens l^{-1}) and site eight the highest median densities (Mdn=1.16 specimens l^{-1}). No significant correlation between plankton densities and distance was found. This was also true for the fractions of phytoplankton and zooplankton densities as well.

Significant differences existed, however, between sampling seasons (Table 9). The highest densities of plankton were observed during summer (Mdn=6.1 specimens l^{-1}) and the lowest during autumn (Mdn=0.01 specimens l^{-1}). Generally, densities were highest during summer, followed by spring, and winter. Autumn consistently had very low plankton densities.

Table 9: Results of Kruskal-Wallis rank sum tests and Dunn's multiple comparison tests, showing p and chi-squared values. The grouping variable for the tests are the seasons: Sp = spring, Sm = summer, Au = autumn, Wi = winter. The Dunn's test results were Bonferroni corrected. The variables tested are the plankton densities as well as the pure plankton counts.

Variable	Kruskal-Wallis test		Dunn's test of multiple comparisons					
			Sm-Sp	Sm-Au	Sm-Wi	Sp-Au	Sp-Wi	Au-Wi
	chi-squared	p	p	p	p	p	p	p
Plankton * L^{-1}	28.88	<0.0001	ns.	0.0000	0.0052	0.0070	ns.	ns
Phytoplankton * L^{-1}	28.33	<0.0001	ns.	0.0000	0.0009	0.0070	ns.	ns.
Zooplankton * L^{-1}	23.12	<0.0001	0.0109	0.0000	ns.	ns.	ns.	ns.
Plankton count	30.51	<0.0001	ns.	0.0000	0.0028	0.0024	ns.	ns.

3.2.8. Choriotope analysis

The most prominent choriotope present in the study reach was mesolithal (mean area percentage=52.22%), followed by makrolithal (mean=26.67%) and megalithal (mean=13.89%). Akal was rare (mean=6.67%), and xylal was only present at site seven (0.56%) (Figure 24).

Makrolithal and Akal was lacking at site 1, and Xylal was only recorded at site 7. Megalithal gradually decreased downstream (Figure 24).

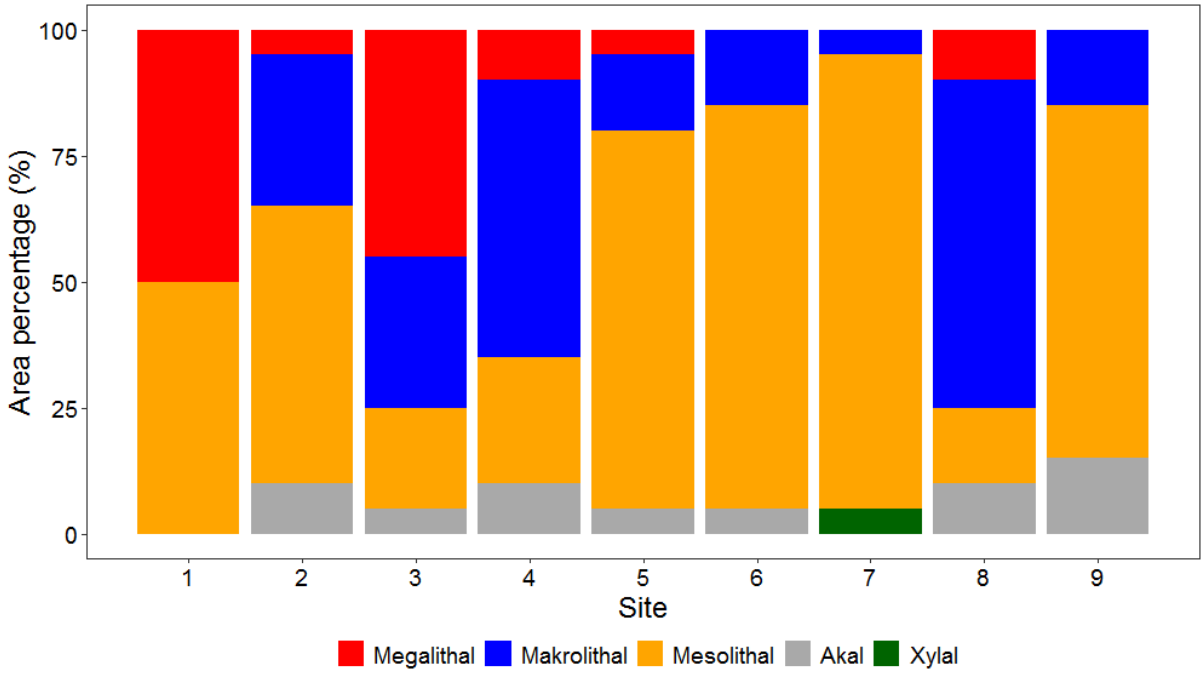

Figure 24: Site-specific choriotope composition. Megalithal = red, makrolithal = blue, mesolithal = orange, akal = grey, xylal = green.

Most EPT specimens were collected in mesolithal (6396 specimens), and 34 out of 35 taxa present in the outlet were also recorded in this choriotope. In mesolithal, the second-highest EPT taxa diversity was observed (H'=2.23). The highest EPT diversity was present in akal (H'=2.42), but only 367 individuals and 22 taxa were sampled in this choriotope (Table 10).

Table 10: Choriotope-specific data on Shannon's index of diversity (H'), abundance of individuals and taxa richness.

Choriotope	H'	Abundance	Taxa richness
Megalithal	1.74	2575	21
Makrolithal	1.77	1944	27
Mesolithal	2.23	6396	34
Akal	2.42	367	22
Hygropetric	1.64	115	10

The calculated electivity showed that mesolithal was not only the most prominent choriotope present (in terms of area percentage), but also preferred by EPT taxa at most sites. In turn, megalithal was strongly rejected. Although diversity of EPT specimens at akal sites was rather high, this substrate type was also only unfrequently chosen (Figure 25).

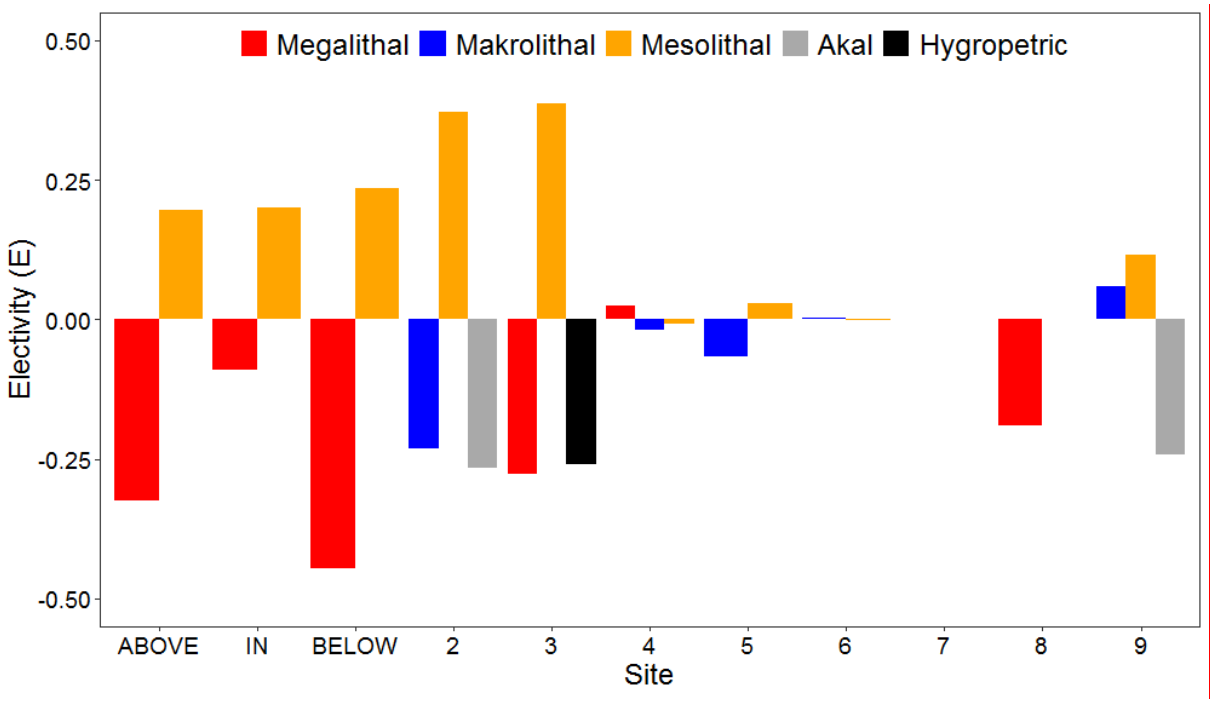

Figure 25: Barplot showing choriotope-specific electivity index values (E) at each site. Red=megalithal, blue=makrolithal, orange=mesolithal, grey=akal, black=hygropetric.

3.2.9. Constrained correspondence analysis

A constrained correspondence analysis was conducted using a species matrix of common EPT taxa obtained with the MHS method. Excluded from this matrix were taxa represented with fewer than 10 specimens due to the sensitivity of CCA to rare species. It was constrained by a

matrix of mean environmental factors measured over the course of the year (oxygen concentration, conductivity, stream velocity, zooplankton density, phytoplankton density, choriotope area). Although temperature data were measured, it was not implemented in the analysis due to missing measurements on multiple sites. C/N ratios were excluded for the same reason. Area percentages of the akal choriotope was dropped from the constraints due to collinearity. The model was highly significant (ANOVA, chi square=54.21, p=0.009), and the two components extracted explained 60.36% of the variation. Of the constraining variables oxygen concentration, conductivity, mean flow velocity, zooplankton density, and megalithal area percentage were significant (ANOVA, $p<0.05$) (Figure 26 A).

A second CCA was conducted using yearly feeding guild data constrained by oxygen concentration, phytoplankton density, choriotope area, velocity and depth. Zooplankton concentrations were collinear and excluded. An ANOVA showed that the model was highly significant (chi square=0.37, p=0.001), and the three components were significant (ANOVA, $p<0.05$). The first three components cumulatively explained 88.3% of the variation. All constraining variables were also significant (ANOVA, $p<0.01$) (Figure 26 B).

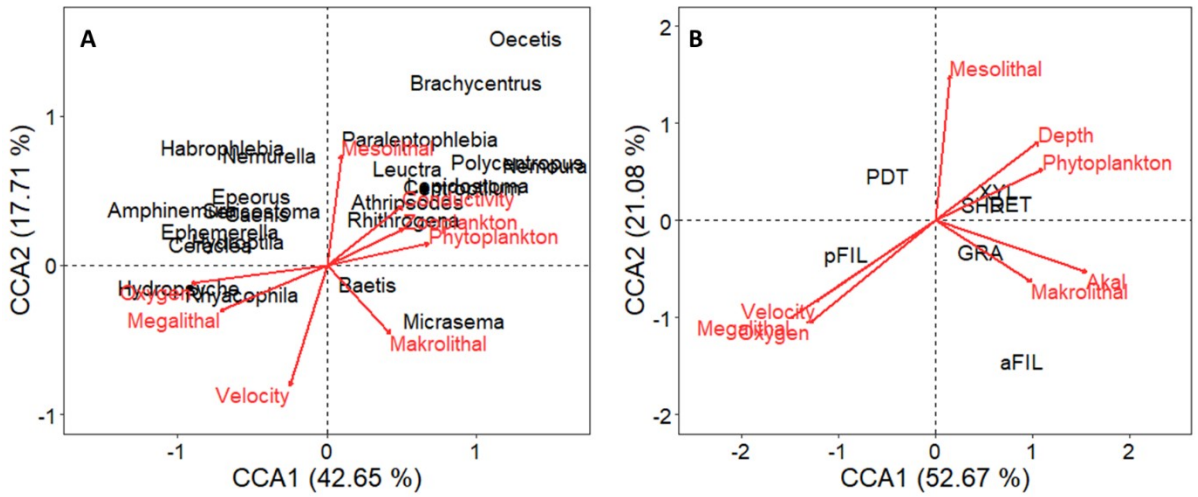

Figure 26: Canonical correspondence analysis (CCA) biplots showing the first two axes for EPT community data based on MHS (A) and for feeding guild proportions (B). The variance explained by each component is indicated in parentheses following the axis label. For the MHS Community data (A), constrains used are the environmental variables (oxygen concentration, conductivity, stream velocity, zooplankton density, phytoplankton density, choriotope area) which are indicated as red vectors. Community data points are indicated as black labels. For the feeding guild data (B), constrains used are the environmental variables (oxygen concentration, conductivity, stream velocity, phytoplankton density, choriotope area and depth) which are indicated as red vectors. All feeding guild data points are indicated as black labels: aFIL = active filter feeders, pFIL= passive filter feeders, PDT = predators, SHR = shredders, GRA = grazers, XYL = xylophages, DET= detritivores.

4. Discussion

4.1. Longitudinal feeding guild composition and food quality

As well documented also in previous surveys (Krawany, 1928; Schranz, 2012; Malicky 2014), high abundances of passive filter feeders were found in the study area. Most prominent of these were the hydropsychids, mainly *Hydropsyche incognita* (Schuhmacher, 1970), but less common Trichopteran taxa such as *Polycentropus flavomaculatus* (Edington and Hildrew, 1981) were present as well. The logarithmic decrease of filter feeder density during most seasons is congruent with the lake outlet model as outlined in Richardson and Mackay (1991) and first proposed by Sheldon and Oswood (1977) (Figure 19). An initial increase of filter feeder populations from low densities in a horse-shoe shaped manner as described by some authors for oligotrophic lake outlets (Wotton, 1979; Eriksson, 2001) was not observed. If this downstream increase was present in the Seebach it happened already over the first 10 metres of the sampled stretch (Figures 18 & 19). Interestingly, while filter feeders were numerous, they constituted only a small fraction of total feeding guild proportions, although *Hydropsyche spp.* specimens were the second most abundant taxon and contributed a quarter of all sampled specimens. This relatively low share of feeding guilds may be caused by the lake's trophic status and relatively low surface temperature (Malicky-Schlatte, 1991) which limits the lake's production and therefore the amount of lake-derived seston available to filter feeding larvae.

Macroinvertebrates including EPT taxa are an important part in the decomposition of organic materials (Cuffney et al., 1990, Malmqvist and Oberle, 1995), and several groups are specialised detritivores (Short et al., 1980). The most common detritivores in the Seebach were part of the mayfly families Baetidae and Ephemerellidae as well as the stonefly genera *Leuctra*, *Nemoura*, *Nemurella* and *Amphinemura* (Table 5) (Bauernfeind and Humpesch, 2001; Graf and Schmidt-Kloiber, 2008). The significant downstream increase of detritivores may be explained with a corresponding accumulation of allochthonous material over the longitudinal gradient, a factor described in previous studies (Maciolek and Tunzi, 1968; Sheldon and Oswood, 1977). While the first 20 metres of the Seebach are wide and comparatively unshaded, and only small quantities of organic matter are retained, the channel subsequently narrows and is partially nearly completely enclosed by riparian vegetation allowing for a progressively larger accumulation of leaves and other allochthonous debris. Remarkably, the proportion of

detritivores was highest during spring, potentially due to the absence of filter feeders and predators as well as the continuing presence of already processed debris from the shed leaves of past autumn (Richardson, 1991).

The known decrease of filter feeders with distance in lake outlets has often been explained by a decrease of the availability of lake-derived high-quality food resources (Ulfstrand, 1968; Carlsson et al., 1977; Valett and Stanford, 1987; Benke and Wallace, 1980; Merritt et al., 1982; Richardson and Mackay, 1991). This could not be confirmed by the findings of this study, as the concentrations of zooplanktonic organisms, assumed to be of high nutritional value, did not decrease over distance and the C/N ratio decreased contrary to this model (Figure 22). While the design of this study does not allow to analyse the ingestion rates of zooplanktonic organisms, the findings suggest that differences in the concentrations of planktonic organisms remained statistically insignificant over the sampled stretch and that the nutritional value of seston even increased slightly. This increase might be facilitated by terrestrial input (Morin and Peters, 1988) and might not be utilised by filter feeders for a variety of reasons such as particle size, since some filter feeders are known to be selective feeders (Wotton, 1979; Fuller and Mackay, 1981; Richardson and Mackay, 1991). Since plankton size was not assessed, this study is unable to determine, whether a reduction in particle size happened as reported by some authors for other lake outlets (Richardson, 1984; Voshell and Parker 1985). Dilution of high-quality food items by particles of lesser nutritional value (Richardson 1984; Morin and Peters, 1988) through allochthonous sources can also not be ruled out and might offer an alternative explanation for the downstream decrease of filter feeders.

While measured seston quality and quantity remained mostly unchanged or even increased over the longitudinal gradient, filter feeder densities declined nonetheless, thus making pure concentrations of high-quality food particles unlikely to be the sole cause of filter feeder distribution.

Water temperature is one the main abiotic drivers in aquatic habitats (Wotton, 1987; Ward and Stanford, 1979). This also fully applies to lake outlet communities (Wotton, 1995) where most available information deals with hypolimnetic water release (Ward and Stanford, 1979; Wotton, 1995; Richardson and Mackay, 1991, Waringer 2003). In contrast, the Lunzer Untersee provides a natural, surface release outlet due to its natural dam which creates an impressive, steep outflow cascade. The stabilising and cooling effects of hypolimnetic water release are not

present at the study site; instead, the lake outlet community investigated in this study is exposed to significantly higher ambient water temperatures in summer when compared to other stream biota. Although no significant temperature gradient was detected in the 565 m long study reach in the Unterer Lunzer Seebach, differences become apparent when comparing its annual temperature range of more than 17°C (Figure 3) with the lake inflow of the Upper Lunzer Seebach where annual fluctuations of water temperatures are very narrow (4-10°C; Waringer, 1986). A cooling of the warmer lake water in the Untere Seebach must happen either very gradually or further downstream of the study reach. This means that a potential growth-promoting effect of the warm lake water is impacting the whole sampled stretch and cannot be said to facilitate the observed lake outlet community.

Instead, the proportion of filter feeders in the Seebach appears to be strongly influenced by several abiotic factors, namely stream velocity, oxygen concentration, depth, and the availability of solid substrates (Table 8, Figure 26 B).

Of these, stream velocity is of special ecological importance, since it influences the relative number of food particles per time unit. Even though no linear gradient of current velocities was found in the Seebach, sites with faster current velocities had more filter feeders. While differences in seston concentrations between sites in the Seebach did not differ significantly over the sampled stretch, changes in current velocities impacted the amount of nutrients available to filter feeding taxa. Thus, differing delivery rates of food particles can facilitate higher densities of filter feeders on certain sites. This view has been proposed by Eriksson (2001) who argued that delivery rate rather than food quality are the main drivers of filter feeder density in lake outlets.

One difficulty associated with the C/N ratio and plankton density assessment conducted in this study lies in the fact that the seston samples were obtained relatively close to the surface of the water body. Current velocities between different strata of the water body are not always proportional (Hart et al., 1996), and the availability of seston between top and bottom layers of the water column can differ significantly (Smith-Cuffney and Wallace, 1987). It is hard to estimate, therefore, how the composition of the seston actually sampled differs from that available to filter feeding invertebrates situated at the stream bed.

The diminishing proportion of filter feeders can be linked well to the amount of megalithal ground cover (Table 8). Large substrates offer several advantages to filtering organisms, such as providing stable colonization substrates and platforms for biofilm growth (Mackay and Waters, 1986; Martini and Waringer 2021). This stability is especially important in combination with increased stream velocities which are often present at lake outlets which wash away less solid substrates (Carlsson et al., 1977) and force macrozoobenthic organisms to seek out stable anchoring points (Richardson and Mackay, 1991). Solid substrates thus allow filter feeders to utilize the elevated delivery rate of food particles associated with increased velocities (Carlsson et al., 1977, Thorp, 1983; Thorp et al., 1986; Statzner et al., 1988). The efficiency of filtering is increased at shallow depths where filter feeders can reach a greater proportion of the water body (Morin et al., 1988). At shallow depths laminar flows can further increase delivery rates of food particles (Wetmore et al., 1990; Statzner, 1981; Smith-Cuffney and Wallace, 1987). Smith-Cuffney and Wallace (1987) also found, that these laminar flows tend to be disturbed by larger rocks breaking the even ground surface which is another advantage provided by megalithal choriotopes where this is not the case.

While chemical parameters have rarely been considered in the context of lake outlet communities (Richardson and Mackay, 1991), some of them were measured for this study, nonetheless. One, sometimes perhaps overlooked aspect, is the presence of sufficient amounts of oxygen. While the concentration of oxygen was significantly higher during the colder seasons (Figure 5 A and B) it is interesting to note, that even during spring and summer, the first 65m of the sampled stretch were remarkably well oxygenated (Figure 5 B). This becomes even clearer, when looking at the oxygen saturation during summer, where the water in the first 165m was saturated well in excess of 100% (Figure 5 D). This pattern is probably induced by the autotrophic production taking place in the oligotrophic lake (Malicky-Schlatte, 1991) combined with the high discharge rates during the first 15m (Figure 8) which bring fresh oxygen into the stream during summer. The relatively high concentration of oxygen is then diminished downstream by respiration and diffusion into the atmosphere. Oxygen concentration correlated to a high degree with the summer densities of hydropsychids (Figure 21) who are prominent filter feeding Trichoptera in the Seebach and constitute 25.67% of specimens found. This relationship was only present during summer when overall oxygen concentration was at its lowest (Figure 5 A). A study conducted by Pîrvu et al. (2015) analysing the ranges and tolerances of *Hydropsyche incognita* in the Carpathian Mountains found that *H. incognita*,

which is the most prominent filter feeding species obtained from the Seebach, prefers a concentration of dissolved oxygen between 10 and 12 mg l^{-1}. While oxygen concentrations below that value did not preclude the presence of this species, the authors' findings suggest that *H. incognita* considers this an optimum (Pîrvu et al., 2015). In the Seebach, oxygen concentrations dropped below 10 mg l^{-1} only during summer and only 65m downstream of the lake. At sites further downstream, therefore, oxygen might become a limiting environmental factor for members of this important filter feeding species. Since high densities of *H. incognita* were present at sites further downstream as well it is unlikely, however, that low oxygen concentrations alone regulate the presence of lake outlet communities.

4.2. Diversity gradients and filter feeder dominance

Site specific diversity (H') and number of taxa increased significantly downstream of the lake outlet regardless of sampling method (Figures 12 & 13). Sites with higher filter feeder proportions contained fewer species and were less diverse, lending credibility to the initial hypothesis that filter feeder dominance decreases diversity. The low taxa numbers, often observed in lake outlets, have usually been explained with the presence of a few (mostly filtering) species dominating the headwaters. Downstream, environmental conditions in the stream shift from lentic to lotic and the dominance of filtering taxa diminishes, allowing for the increased presence of more generalist and typically riverine species (Ward and Stanford, 1983; Lomond and Colbo, 2000; Donath and Robinson, 2001; Eriksson, 2001). This study's findings suggest, that site-specific diversity in the Seebach is not necessarily influenced by the amount and quality of food particles but by current velocity, depth, and the availability of solid substrates. Sites with higher mean stream velocities and megalithal choriotope proportions had lower diversity indices and evenness. This indicates that sites in the Seebach with stable substrate conditions and high flow velocities were dominated by fewer, but more abundant taxa. Higher velocities at these sites allow for the establishment of dense aggregates of filter feeders through the mechanism of increased delivery rates. Depth is relevant insofar as current velocities tend to be higher at shallower depths. Wallace and Merritt (1980) found, that filter feeding caddisflies appeared in high abundances where water is fast-flowing and depths are shallow. It is known that substrate type influences insect diversity (Rabeni and Minshall, 1977; Morin, 1991) and that stable environmental conditions, as caused by solid streambeds and

constant velocities, reduce the incidences of disturbance (Richardson and Mackay, 1991; Myers et al., 2007) which allows few, well adapted taxa to dominate (Rabeni and Minshall, 1977; Resh et al., 1988; Jones, 2010). The special conditions in these environments allow species well adapted to them to outcompete and potentially displace taxa less adapted to lake outlet environments (Matczak and Mackay, 1990; Richardson and Mackay, 1991). Competitive behaviour has been well observed in filter-feeding caddisflies (Hemphill, 1988; Matczak and Mackay, 1990), and the displacement of unsuccessful competitors and their subsequent downstream drift helps to explain the diversity gradient observed in lake outlets (Matczak and Mackay, 1990). In the Seebach, these environmental traits seem to influence site diversity and evenness to a significant degree, as shown by the present study. Richardson and Mackay (1991) rightfully argue, that substrate conditions alone cannot explain the formation of lake outlet communities, since similar compositions of choriotopes are found in non-lake outlet streams as well where these communities are not present. The findings of this study do not contradict this statement since the availability of large amounts of lake-derived food particles are present over the whole sampled stretch. The high density of filter feeders in the Seebach, however, cannot be explained by a diminishing availability of food particles which was not observed, but requires suitable habitat conditions in addition to the availability of sufficient nutrients.

The increase of site-specific diversity over the longitudinal gradient differed between the MHS data where it showed a linear relationship, and CPUE data where the increase followed a natural logarithmic increase (Figures 12 & 14). This suggests that the composition of sampled taxa differed between sampling methods. Similarly, the Bray-Curtis dissimilarity clusters varied according to sampling method (Figure 17). MHS data suggest a paring of the most downstream site 9 with the lake outlet sites and places site 7 separately, while CPUE data creates a dendrogram with a more regular branching between upstream and downstream sites.

A total of 29 genera were collected employing the CPUE method, three fewer than with the MHS method (Table 5). Some rarer genera such as *Agapetus, Mystacides* and even *Oecetis* were absent from the CPUE taxa list while limnephilid genera such as *Drusus*, *Chaetopteryx* and *Halesus* were absent or vanishingly uncommon in the MHS specimens. When taking into account the unequal sample sizes it becomes apparent, that the adapted MHS method utilised in this study was less effective in collecting certain rarer genera. CPUE sampling allowed for selecting microhabitats which the unwieldy Hess sampler could not reach. It appears reasonable

to assume that, while CPUE sampling is a procedure well suited for finding rare specimens as well as those found in microhabitats unexplored by the MHS method, it carries an inherent bias due to the limitations of the user. In contrast, multi-habitat-sampling (even a modified procedure as employed in this study) is less susceptible to human bias, and collected specimens appear to be more proportional to the sampled population. While differences between sampling methods became apparent, the overall findings of both methods remained mostly consistent. With both possessing unique advantages and disadvantages it becomes difficult to argue for the merits of either, and their differences warrant further exploration in future projects.

4.3. Longitudinal abundance gradients

The assumption that high abundances are caused by filter feeder presence was not confirmed by this study. The oligotrophic conditions of the Lunzer Untersee might not suffice for the production of seston in quantities large enough to allow filter feeder dominance as observed in other studies (Malicky-Schlatte, 1991).

Downstream changes in specimen densities did not closely resemble the filter feeder gradient and cannot therefore be said to be caused by their numbers alone (Figs. 9 & 19). Even though hydropsychids, the main filter-feeding taxon in the Seebach, made up 25.67% of total specimens, their numbers alone do not explain the distribution of specimen densities. Abundances of EPT organisms were influenced by stream velocity (Figure 12). Sites with particularly high velocities harboured proportional specimen densities. This suggests, that not only filter feeders profit from increased current velocities, but other feeding groups as well, perhaps through the increased delivery rate of food as well as debris. The question then remains, why these sites are not exploited by-filter feeders similar to those upstream. The author argues, that habitat parameters such as depth and choriotope composition downstream of the lake outlet do not provide a competitive advantage for these taxa such as they do upstream.

Conclusion

While a longitudinal pattern of filter feeder distribution, in accordance to the model described by Sheldon and Oswood (1977), was found to be present in the Seebach, a degradation of food quality alone does not appear to be the cause of this decline. Instead, hydrological factors such as current velocity, depth and substrate composition appear to shape the feeding guild

composition in the studied sites. The findings of this study suggest that, in the absence of nutritional gradients, food delivery rate, caused by varying current velocities, may be a leading cause for the establishment of dense aggregates of filter feeding EPT larvae and that solid and stable substrates appear to facilitate their presence. Furthermore, this study revealed that filter feeding specimens such as hydropsychids were present in large abundances and had a significant impact on taxa numbers and diversity, although filter-feeding EPT larvae did not constitute the largest share of feeding groups and are not the sole drivers of EPT densities in the Seebach.

This study's findings show that the distribution of larvae of EPT taxa in the Seebach is not governed by a food quality gradient but instead by a complex combination of hydrological parameters. Further research is required, however, to discern the individual importance of these parameters and their interaction. The methods employed in this study will have to be refined and complemented with new approaches. In addition, the generality of these findings will have to be tested on different lake outlet systems.

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