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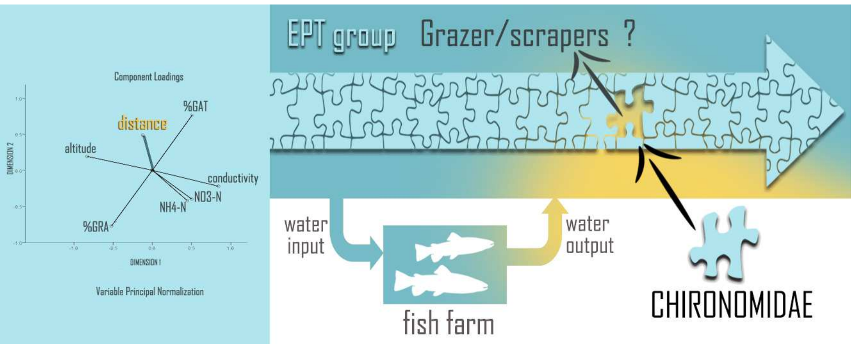
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DIMENSION 2
DIMENSION 1
%GAT
distance
altitude
conductivity
NO3-N
NH4-N
%GRA
Variable Principal Normalization
EPT group
Grazer/scrapers ?
water
input
water
output
fish farm
CHIRONOMIDAE

The response of chironomid alpha taxonomic and functional diversity to fish farm effluent pollution in lotic systems

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Abstract

The lotic habitats affected by trout farm waste are colonized with a particular invertebrate community of which chironomids are the most abundant group. However, there is little information available regarding how chironomid community structures respond to this type of pollution at the highest taxonomic resolution. Eight fish farms, together with their lotic systems as recipients, were used to test the variability of the chironomid community and its surrogates (taxonomic and functional metrics) across spatially arranged sampling sites to form a gradual decrease in the trout farm influence. The self organizing map (SOM) classified six different types of chironomid communities which were characteristic for both the control and affected habitats. The species indicator analyses listed 32 taxa as positive indicators of water pollution. The SOM

and Kruskal-Wallis test revealed that the pattern of chironomid community structure obtained was mainly driven by six environmental parameters (Altitude, conductivity, distance from the outlet, hardness, HN_4-N, NO_3-N). Categorical principal components analysis (CATPCA) derived three models for each type of biotic metric, in which for diversity-, taxonomy- and functional feeding group-based metrics, the first two dimensions explained 55.2%, 58.3% and 55.4%, of the total variance respectively for 296 sampling sites. According to this analysis, the total number of taxa (S), abundance and the Shannon-Wiener index (H') (as a diversity metric), as well as the proportion of Tanypodinae (as taxonomic group) and grazers/scraper (GRA) and gatherer collector (GAT)(as FFG metrics), were related to the outlet distance gradient, thus showing great potential to be used in the multimetric approach in bioassessment.

Capsule The taxonomy- and functional chironomid community structure acts as a fine-tuned bioindicator of the impact of fish-farm effluent.

Key words: trout farm, Chironomidae, community, metric, bioassessment, Self organizing map

Introduction

The substantial increase in fish farming, predominantly in mountainous and hilly regions of developing countries, presents a potential stress for mainly undisturbed aquatic ecosystems, usually situated in protected areas. Low ordered headwater streams, which are a crucial habitat for the main processes in the whole catchment, as nitrogen uptake and processing; (Bernot and Dodds, 2005), are the only recipient for the effluents originating from freshwater land-based salmonid farms (Tello et al., 2010). This constant and steady influence is characterized as a disturbance since via an understood mechanism it affects the structural and functional ecological endpoints in aquatic ecosystems (USEPA, 1997). Coming from the flow-through system, the effluents, which are discharged into streams, are composed of suspended solids and dissolved phosphorus and nitrogen based nutrients (Guilpart et al., 2012). All these components are important for the key energy pathways in lotic systems, and uncontrolled changes in their proportions could have ecological consequences and alter the properties of hydrobiocenosis.

Many previous papers have presented how trout farm waste output influences the aquatic biota, and all these studies have been in the light of searching for reliable biological indicators. Various analytical approaches have been used to test the biota responses for different taxa groups (different hydrobiocenosis) at different ecological levels (Camargo and Gonzalo, 2007; Doughty and McPhail, 1995; Fabrizi et al., 2010; Guilpart et al., 2012; Kırkağaç et al., 2009; Rueda et al., 2002; Selong and Helfrich, 1998; Webb, 2012). The most investigated aquatic group was macroinvertebrates, whereby communities were tested with the taxonomical resolution mainly at the level of family or genera. Since the goal was to find a cost-effective approach that was appropriate for the rapid bioassessment of aquatic systems exposed to fish farm pollution, many of these studies presented communities by means of well-known metrics used in routine monitoring programs (e.g., traditional diversity indices, percentage of particular group, feeding groups). The results were consistent, and in all cases macroinvertebrates regularly changed their structure along the nutrient enrichment gradient. Also, the most prominent change was a significant increase in the abundance of chironomids. This result is expected since in general some tolerant taxa of chironomid larvae occur in large numbers in poor quality habitats (McCord and Kuhl, 2013). However, this widely distributed and diverse group, as one of the most dominant and abundant within macroinvertebrates, offers a huge spectrum of ecological profiles. These engineers of aquatic ecosystems, which is what they are popularly known as, occupy a wide variety of niches and play a very important role in organic matter recycling processes. Also, their larvae are an important part of the food chain (Armitage et al., 1995). Being a member of different feeding groups, their species take part in all of the important ecological processes (energy pathways), which are interrupted by the constant input of uneaten feed, fish feces and excretion coming from land-based salmonid fish farms. Thus, one could consider chironomids as the most ecologically relevant group for indicating fish farming impairments in aquatic ecosystems. Bearing all these advantages in mind, an examination of the influence of fish farming on the chironomid community composition and structure could lead to the development of biological metrics which can respond in a fine-tuned manner. Despite these facts, no previous studies have analyzed the chironomid community structure in fish farm-affected lotic systems at a high taxonomic resolution, and instead they have considered them as a single entity (the family of Chironomidae or sub-family level).

In addition, macroinvertebrates are the most commonly applied group in routine monitoring programs all over the globe (Hering et al., 2006). Besides their many advantages in term of bioassessment, some shortcomings do exist, of which the most prominent is their sensitivity to spatial and temporal scales (Rosenberg and Resh, 1993). More precisely, the variability caused by human degradation is confounded by natural degradation generated on temporal and spatial gradients. This is especially true for the Chironomidae family whose larvae, due to their strong natural variability, have been excluded from the majority of bioassessment programs (Milošević et al., 2013). For example, their natural variability can be so strong that differences between two temporal units within one community structure can exceed site-to-site differences (Kerans and Karr, 1994). To trace such variability and to separate it from that caused by, in our case, fish farm effluent pollution, it is necessary to ordinate the community structure, presented with the high taxonomic resolution, within the spatial and temporal dimension.

Having this in mind, we examined how the chironomid community reacts to fish farming and tested its potential as a bioindicator to respond in a fine-tuned manner. The main goal of the study was to describe the pattern of the chironomid community along temporal and spatial scales represented by 8 lotic systems over seven months. Sampling sites were spatially arranged over a decreasing gradient of trout farm influence. In addition, we wanted to elucidate the relationship between the structural properties of the community and relevant environmental factors, disentangling natural and farming -caused variability. Finally, the potential biological metrics were tested along the fish farm effluent-based degradation gradient.

Material and methods

Study area

Trout farming in Serbia is essentially concentrated in the hilly and mountainous part of the country, located south of the Danube and Sava Rivers. In this study, data were collected from 8 selected rivers and streams (see Fig 1) which the upper and middle course trout farms are located on, in order to investigate the ecological impacts of trout farms. All the rivers and streams selected for this study belong to ecoregions 5 (Dinaric West Balkan) and 7 (Eastern Balkan), according to llies (1978) and Paunović et al., (2012). The farms were chosen based on their production capacity as well as the size of the recipient. Their annual fish production ranged from 5.5 to 110 t yr^{-1}, depending on the trout farm (Table 1). Within each trout farm, macroinvertebrate samples were taken at several sampling sites, a priori defined. Sampling points were selected, based on certain criteria. For instance, two sampling sites were selected as control sites, located upstream of the fish farms. More precisely, the first sampling site, labeled 1 in the sample code (fig2) was positioned near the water source, up to a distance of 770m, while the second site was located 20-60m from the water intake of the fish farm (labeled 2). In contrast, when a water source directly supplies water to a trout farm, it can be the only choice for a control site. Downstream of the fish farm water discharge, the positions of sampling sites were defined based on their distance from the fish farm. Thus, the remaining sampling sites downstream of the fish farm were located at a distance of 10-50m (labeled 3), 110-150m (labeled 4), 590-2600m (labeled 5) and 1700-6400m (labeled 6) from the discharge point. Such a study design enables us to estimate the potential of the lotic systems in the self-purification process.

Sampling

We investigated 46 sampling sites (up to two upstream and three or four downstream sites per trout farm). The sampling of all sites for each trout farm occurred on seven occasions: April 2011, June 2011, September 2011, October 2011, December 2011, March 2012 and May 2012. Apart from Studenica River, where sampling was conducted on six occasions due to high water level in October 2011. Also, considering the same river, a sample could not be taken from sample site ST2 in December 2011 because of a dense (thick) snow cover. Taking into account all 46 localities and seven (or six) sampling periods, a total of 315 sites were included in this study. At each sampling site, benthic invertebrates were sampled using a 300cm^2 Surber sampler of 250 µm mesh. All three benthic samples taken at each sampling site on each occasion were composited into a single sample. In the laboratory, Chironomids were separated from the remainder and identified up to the genus or species level using the following identification keys: (Andersen, 2013; Moller Pillot, 1984a, b; Schmid, 1993; Vallenduuk and Moller Pillot, 2007).

On the same occasion as the benthic invertebrate samples were taken, several physical and chemical variables were measured at each sampling site. The dissolved oxygen concentration, water temperature, pH and electro conductivity were measured using a PCE-PHD field device (Germany). The concentration of anions (NO_3^-, Cl^-, SO_4^{2-}) in the water was determined with a DIONEX ICS3000 Ion Chromatography (Thermo Fisher Scientific Inc., USA), in the Water Laboratory at the Institute of General and Physical Chemistry in Belgrade, based on standard EPA methods (Pfaff et al., 1993). The total phosphorus, orthophosphates (PO_4^{3-}) and ionized ammonia (NH_4^+) were measured according to APHA protocols (APHA, 1998) in the Laboratory at the Institute for Chemistry, Technology and Metallurgy, Belgrade, Serbia.

The information about altitude, mean river width and depth, distance from the source, main substrate type, velocity and mean annual discharge was available for each sampling site. Finally, the distance from the outlet was defined as an ordinal variable with 5 as the control sites and 1 to 4 as the site closest and furthest to the outlet, respectively.

Data analysis

The chironomid community structure was examined using the Self organizing map (SOM) method, which is an unsupervised type of artificial neural network (Kohonen, 1982). This visualization technique was applied to construct the model based on biotic (chironomid community) and abiotic (environmental factors) information. In the first phase, during the training process, the SOM imported 315 input vectors (sampling sites), each one comprising 95 variables (chironomid taxa). The output of the training process is a two dimensional neural network composed of hexagonal neurons. In this output layer, each neuron carries sampling sites with similar models of data (community structure), which in total form a particular pattern of community structure. Similarity between the models of neurons are presented with their mutual distance on a two dimensional map. During this process, for each neuron of the trained network (group of sampling sites) the mean value of the parameter tested was calculated. The output of the SOM algorithm was made up of component planes visualizing the distribution of each passive variable across the trained neural network, enabling visual correlation with previously obtained biotic patterns.

The resolution (number of neurons) of the output neural network was defined a priori, relying on the two most commonly applied methods (Park et al., 2003; Vesanto et al., 2000) and trying not to have many empty neurons (Penczak et al., 2012). A network that is too small could miss some important patterns in the data while a network that is too big diminishes the differences obtained, thereby disabling a plausible interpretation. For this study, a 10 X 9 grid was most appropriate for the processed data matrix. The SOM analysis was applied using the Matlab ver. 6.1.0.450 algorithm interface (http://www.cis.hut.fi/projects/som-toolbox).

Since SOM is a visualization technique without any statistical indication, significant differences in the active (chironomid taxa) and passive (environmental factors) variables between groups obtained by the SOM were tested by indicator species analysis (IndVal) and the Kruskal-Wallis test, respectively. More precisely, the IndVal method was applied in order to define taxa which significantly changed their abundance and occurrence between the SOM groups, since such taxa are the main generator of the derived community pattern and, consequently, they are potential bioindicators for fish farm effluent pollution. The Monte Carlo significance test with 1000 permutations was applied in order to identify significant taxa. All indicator species with an

IndVal score over 25 were interpreted as representative taxa of a particular group, with a relative frequency and abundance of at least 50%. On the other hand, the variability of environmental factors which formed a clear gradient in the component planes were further tested by the Kruskal-Wallis test. Analysis of IndVal was conducted using the PC-ORD 4.0 for Windows software (McCune and Mefford, 1999), while non-parametric ANOVA was applied in SPSS version 15.0.

To search for potential indicators of fish farm influence, the following biological metrics were calculated:

- Composition abundance metrics. Six metrics, representing the relative proportion of particular taxa groups (subfamily or tribe) were determined.
- Richness/diversity metrics. Five diversity indices were calculated: taxa richness (S), abundance (N), Shannon index (H'), Simpson index (J) and taxonomic distinctness index (Δ+) (Magurran, 2004)
- Functional metrics. All chironomid taxa were assigned to the five functional feeding group (FFG) categories: gatherers/ collector- (GAT), active filter feeder (AFF), predators (PRE), grazers/scraper (GRA) and shredders (SHR) (Brabec et al., 2017).

All of the metrics were tested along environmental gradients using categorical principal components analysis (CATPCA). The input matrix for all three models were composed of 8 environmental parameters (7 continuous and one ordinal), significant for chironomid community (fig 3a) and composition abundance, Richness/diversity and functional matrices. Since in this study the distance from the outlet was presented as an ordinal variable, the CATPCA method was the most appropriate one, enabling different types of variables (continual and ordinal) to be processed in the same model. Different models were derived for different types of metrics (Composition/abundance, Richness/diversity and Functional model) and the potential metrics were selected if they explained 25% of the variance across the principal components. The analysis of CATPA was conducted using the SPSS version 15.0.

Results

Out of 64944 sampled chironomid specimens, 101 taxa were identified from the following subfamilies: Chironominae (28 taxa), Orthocladinae (53), Diamesinae (8), Prodiamesinae (2) and Tanypodinae (9) (Table S1).

The SOM ordinated and classified 295 sampling sites based on chironomid community data into six groups of neurons (Fig 2). Introducing a passive variable in the model, SOM revealed that eight environmental parameters changed their intensity in accordance with the chironomid community pattern (SOM groups) (Fig 3a). The Kruskal-Wallis test confirmed that these environmental parameters significantly changed ($p<0.05$) among the groups defined by the SOM. Finally, IndVal analysis derived a list of 32 indicators, where 10, 1, 18 and 3 taxa significantly occurred in I, III, V and VI SOM groups, respectively. These are the representative taxa of SOM groups (Fig 3b).

Groups I, III and V clustered the sampling sites located near to the outlet of the fish farms with high production (Table 2, Fig 3a). The three groups were on the lower altitudes with high conductivity and low discharge while the highest hardness was recorded in group III (Fig 3a). The poorest water quality was recorded for the chironomid community from group V, with a high concentration of NO_3-N and NH_4-N. The IndVal method revealed the representative taxa for all three groups of sampling sites (I, III and V; Fig 3b). On the other hand, groups II and IV had a particular pattern for the chironomid community, which occurred mainly at the control sites and those furthest from the outlet (Table 2). In addition, group IV included the lotic systems at high altitudes where a high discharge was also measured (Fig 3a). Group VI had sampling sites evenly distributed along the distance gradient from the outlet, represented by three taxa: *Parametriocnemus stylatus, Conchapelopia* agg. and *Synorthocladius semivirens* (Table 2, Fig 3b). This group of neurons was also characterized by a high discharge and concentration of nutrients (NO_3-N and conductivity; Fig 3a). The trout farm production was the lowest in group IV and VI. Finally, the chironomid community did not change along the temporal scale since for many sampling stations all season occasions were clustered in the same group and even in the same neuron (Fig 2).

CATPCA derived three models for each type of biotic metric, in which for the diversity indices, taxonomy-based metrics and FFG metrics, the first two dimensions explained 55.2%, 58.3% and 55.4% respectively of the total variance between 315 sampling sites. In all three models the first dimension was interpreted as a nutrient gradient (conductivity and NO_3-N), while the second had high loadings of factors regarding the distance outlet gradient, along which NH_4-N was related in the opposite direction (Fig 4)

The total number of taxa (S), abundance and the Shannon-Wiener index (H') were positively related with NH-4N values, which decreased as the distance from the outlet increased (Fig 4a). Out of 5 taxonomy based metrics, the proportions of Tanypodinae and Chironominae were selected for constructing the model, with a VAF of more than 0.25%. Contrary to the diversity metrics, the proportion of Tanypodinae was positively related to the distance from the outlet gradient while Chironominae was associated with the first dimension, since it had high loadings of factors relating to nutrient enrichment (Fig 4b). Finally, GAT and GRA, as FFG metrics, were also related to the second dimension but in opposite directions (Fig 4c). More precisely, as the distance from the outlet increased, the proportion of GRA decreased, being replaced with GAT chironomids.

Discussion

Lotic systems are severely affected by freshwater fish farming, which could also be clearly seen at the community level of aquatic biota. This is especially true for macroinvertebrates, whose predictable changes and specific structure patterns along the stressor gradient have been investigated and presented in many previous studies in the light of fish farming pollution bioassessment (Camargo, 1992; Fabrizi et al., 2010; Guilpart et al., 2012; Kırkağaç et al., 2009). In our study, the chironomid community behaved similarly to macroinvertebrates in previous investigations of fish farm influences but offered missing pieces of information. More precisely, diversity indices (based on species richness) and feeding trait metrics showed inconsistent variation as parameters in previous studies (Guilpart et al., 2012; Minoo et al., 2016; Tello et al., 2010) whereas, when based on chironomid data with high taxonomic resolution, they changed accordingly to the fish farm effluent gradient.

Community structure

According to the community structure, the SOM derived 6 groups of sites which were mainly concordant with the level of fish farm effluent pollution (Fig 2, 3a). However, suites of sampling sites in SOM groups IV and VI were monotonous in terms of the distance from the outlets since none of the distance classes particularly emerged as a dominant one (Table 2). This output could be a consequence of natural variability as well as the variability in annual production between the fish farms tested. More precisely, in group IV and VI, the percentage of impacted sites increased due to the fish farms being situated at higher altitudes (VR (1142-929 m.a.s.l.) and RA (701-637 m.a.s.l.)). The high altitude and consequently the high discharge, which have been recorded in present study (Fig 3a), multiply the potential of the recipient and meliorate the harmful consequences for the chironomid community. Therefore, the particular chironomid communities at these sites were not decoupled in their composition and structure from those sampled at the control sites. In addition, sampling sites from the fish farms with the lowest annual production (RA, RD and VR; Fig 2, 3a) were clustered in the same SOM group, albeit they were differing in the distance from the outlet (RA, RD and VR; Fig 2, 3a). It means that the extent of disturbance, coming from these farms, was probably insufficient to generate significant changes in the chironomid community structure, making it again similar to the control sites.

The Indicator species analysis revealed the dominant and frequent taxa which were responsible for the structural patterns obtained for the communities (Fig 3b). All IndVal taxa indicated poor conditions in the sampling sites close to the outlet. Moreover, some of them have already been reported in previous studies as positive indicators of polluted rivers (Milošević et al., 2013). On the other hand, there were no indicator taxa for the control conditions. This was expected, since in degraded habitats, sensitive taxa first disappear while the tolerant ones become dominant in the community, colonizing empty ecological niches (Allan, 1995). In addition, not all of our control sites can be considered as reference sites, since some of them are exposed to multiple stressors, probably causing an intermediate level of degradation and therefore none of the chironomid taxa were apparently dominant as a significant indicator (Lévêque and Mounolou, 2004).

Environmental parameters

Fish-farm effluents which contain uneaten feed and fish excreta significantly increased the concentrations of nitrates, phosphates and ammonia in the recipients, which, according to many studies, can represent a reliable chemical proxy for fish farm pollution (Tello et al., 2010). The same factors (NO_3-N and NH_4-N), the concentrations of which rapidly increased downstream of the fish farm outlets, appeared to be significant for the community structuring, decoupling particular macroinvertebrate assemblages for different levels of pollution. It was similar in our study since the SOM revealed that the chironomid community reacted to the growth of nutrient concentrations in the water (Fig 3a). Besides fish farm influence, the variability of the community structure was also caused by natural gradients. More precisely, SOM group IV gathered all sampling sites located at higher altitudes, whereas polluted group III differed from the other impacted ones in its higher values of hardness (Fig 3a). This was expected since out of all macroinvetrebrates, chironomids at the community level can show extreme natural variability (Milošević et al., 2013). However, despite this trait, in this study the fish farm influence produced stronger changes in the community structure and provided a clear signal, interpretable for bioassessment purposes.

Biological metrics

The clearly visible response of the chironomid community to the fish-farm effluent gradient was confirmed by testing the biological metrics whose calculation is facilitated by poor taxonomic resolution. This was possible as long as sufficient information was kept for the bioassessment. In previous studies, a significant increase in benthic invertebrate abundance was recorded downstream of the input of outlets (Camargo and Gonzalo, 2007; Guilpart et al., 2012). The groups responsible for these changes were Chironomidae and Oligochaeta. On the other hand, the total number of taxa and other indices based on diversity information were inconsistent, changing their trends in different fish farms. This was not the case in our study, in which the values of the total number of taxa (S), abundance and the Shannon-Wiener index (H') increased as the distance from the outlet decreased, being lowest at the control sites. This could be a consequence of the high taxonomic resolution used in our study, since in previous studies (Camargo and Gonzalo, 2007; Doughty and McPhail, 1995; Fabrizi et al., 2010; Guilpart et al., 2012; Kırkağaç et al., 2009; Rueda et al., 2002; Selong and Helfrich, 1998; Webb, 2012), the Chironomidae family, as one of the most diverse and dominant groups of macroinvertebrates, was considered as a single entity. Such coarse input in terms of taxonomy for analysis significantly underestimated the taxonomic richness of this community. The effect could have been additionally promoted since the majority of chironomids prefer polluted habitats and exhibit highly diverse communities.

Macroinvertebrates are mostly applied in bioassessment programs which are based on the multimetric approach. However, despite there being more than 300 tested biological metrics which describe ecosystem health within (AQEM, 2002) and STAR (Standardisation of River Assessment Methods, www.eu-star.at) research projects none of these include chironomid community data at more refined taxonomic level than family. Due to this practice, no information is available regarding how particular chironomid groups (subfamilies) react to environmental degradation. Nevertheless, some general trends in abundance changes along deterioration gradients using low taxonomic resolution are well known. For example, representants of the Chironominae subfamily, with a high percentage of hemoglobin, are tolerant to organic enrichment and consequently, a lack of oxygen in water. On the other hand, Orthocladiinae and Tanypodinae are dominant in the communities which prefer habitats at higher altitudes with good water quality (Milošević et al., 2013). The results of the present study are consistent with the general pattern of community structure at the subfamily level, since %Chironominae were correlated more with descriptors of nutrient enrichment (nitrates and conductivity) while %Tanypodinae were dominant in communities which were established at habitats distant from the fish farm outlets (Fig 4b).

Poor taxonomic resolution, and consequently, averaging the affinities of the functional traits of species at the family level led this type of biological metric to some unexpected patterns along the fish-farm effluent gradient. Guilpart et al., (2012) presented functional feeding groups as a more efficient biological metric over taxonomy-based indicators to indicate the impact of fish farming. More precisely, the FFG metrics caught the signal of ecosystem impairment at much more distant habitats from the outlets than taxonomy-based indicators, the values of which significantly varied only 100m from the fish-farm effluent inputs (Guilpart et al., 2012). Also, functional trait-based indicators relying on the functional affinity of taxa enable the investigation and monitoring of lotic systems across broader regional scales, which include bigger species pools with wider taxonomic breadth. However, the result of our study was inconsistent with those of Guilpart et al., (2012), detecting a significant decrease in grazers/scraper at most polluted sampling sites. More precisely, the proportion of grazers/scraper within the chironomid community was negatively correlated with the distance from the outlets (Fig 4c), and this functional group was dominant in the most affected habitats. Such differences in FFG trends could be explained by the different taxonomic resolution used in these studies. High taxonomic diversity within the Chironomidae family generates a wide variety of ecological profiles with great variability in feeding behavior within this group. Averaging the FFGs within chironomids obviously leads to a substantial loss of information and biases the real composition of feeding traits in the community. In previous studies, the Ephemeroptera, Plecoptera and Trichoptera taxa, the main representants of grazers, probably disappeared on the impacted sites due to their high sensitivity to the degradation and poor water quality. Since more tolerant grazers, chironomids, can easily colonize empty ecological niches and maintain the algal-grazer pathway, this was probably the case in the present study.

Conclusions

The chironomid community has confirmed that benthic macroinvertebrates act as a fine-tuned bioindicator of the impact of fish-farm effluent. However, the contribution of this Diptera family is essential within macroinvertebrates since chironomids are the most dominant group in habitats affected by fish farming. In addition, like in other bioassessment studies, taxonomic resolution is the main factor governing the extent of precision in the results obtained, and their levels have to be traded off with the aim of the study. However, chironomids, due to their great diversity in both a taxonomical and ecological sense, leave little space for compromising, requiring the highest possible taxonomic level for their implementation.

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Table caption

Table 1 Production area and annual production of trout farms located in the study rivers

Table 2. Relative sampling frequencies of sites per classes of the distance from the outlet for each SOM group. The distance from the outlet was presented with 5 categories (5 as control sites, 1 as sites at the distance of 10-50m, 2 as sites at the distance of 110-150m, 3 as sites at the distance of 590-2600, and 4 as sites at the distance of 1700-6400m from the discharge point).

Table S1. Chironomid taxa recorded during the sampling campaign.

Figure caption

Figure 1. Map of sampling sites distributed along the investigated lotic systems

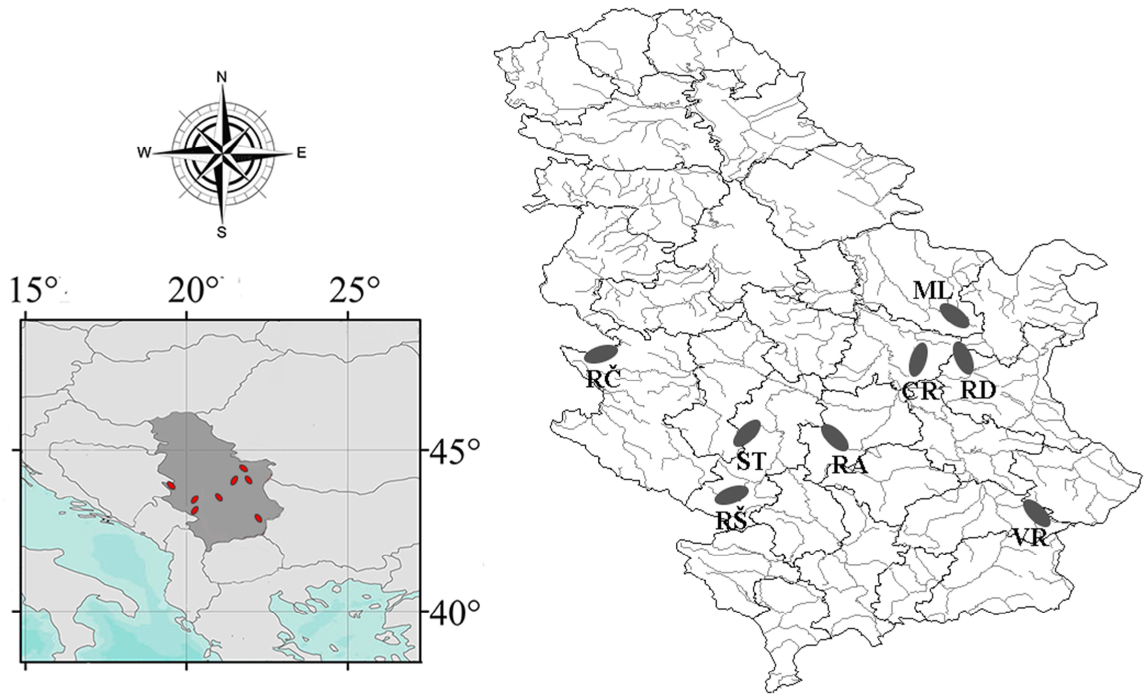
Figure 2. Ordinational and classificational patterns of chironomid community structure on the Self organizing map. Triform site labels attached to the neurons stand for the site and date of sampling in the following way: *code of the site* - *month* - *year*. Capital letters (A, B and C) indicate the groups of neurons on the map.

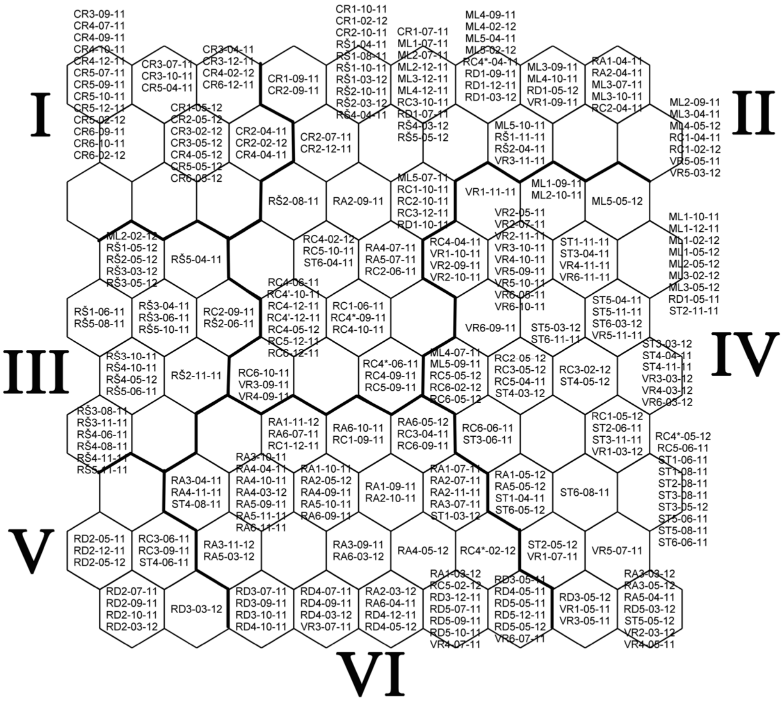
Figure 3. The distributional pattern of (a) environmental parameter intensity and (2) IndVal taxa abundance along the component planes.

Figure 4. CATPCA biplots present how (a) diversity-, (b) taxonomy- and (c)FFG-based biological metrics vary along the distance from the outlet.

Rivers	Production area of fish farms (m^2)	Annual production of trout farms (t)
Crnica (CR)	4200	70.0
Mlava (ML)	13000	110.0
Rača (RČ)	147	5.5
Radovanska reka (RD)	1643	68.5
Rasina (RA)	1400	8.0
Raška (RŠ)	520	28.0
Studenica (ST)	600	30.0
Vrla (VR)	1700	3.6

Distance/SOM group	I	II	III	IV	V	VI
Fish farm outlet (1)	25,92	9,47	34,78	16,67	81,82	11,47
Downstream the outlet (2)	22,22	24,21	21,74	14,10	18,18	22,95
Downstream the outlet (3)	25,92	15,79	21,74	16,67	0	19,67
Downstream the outlet (4)	18,52	9,47	0	17,95	0	24,59
Control site (5)	7,41	41,05	21,74	34,61	0	21,31


N
W
E
S
15°
20°
25°
45°
40°
ML
RČ
CR
RD
ŠT
RA
RŠ
VR


I
II
III
IV
V
VI
CR3-09-11 CR4-07-11 CR4-09-11 CR4-10-11 CR4-12-11 CR5-07-11 CR5-09-11 CR5-10-11 CR5-12-11 CR5-02-12 CR6-09-11 CR6-10-11 CR6-02-12
CR3-07-11 CR3-10-11 CR5-04-11
CR3-04-11 CR3-12-11 CR4-02-12 CR6-12-11
CR1-09-11 CR2-09-11
CR1-10-11 CR1-02-12 CR2-10-11 RŠ1-04-11 RŠ1-08-11 RŠ1-10-11 RŠ1-03-12 RŠ2-10-11 RŠ2-03-12 RŠ4-04-11
CR1-07-11 ML1-07-11 ML2-07-11 ML2-12-11 ML3-12-11 ML4-12-11 RC3-10-11 RD1-07-11 RŠ4-03-12 RŠ5-05-12
ML4-09-11 ML4-02-12 ML5-04-11 ML5-02-12 RC4*-04-11 RD1-09-11 RD1-12-11 RD1-03-12
ML3-09-11 ML4-10-11 RD1-05-12 VR1-09-11
RA1-04-11 RA2-04-11 ML3-07-11 ML3-10-11 RC2-04-11
ML2-09-11 ML3-04-11 ML4-05-12 RC1-04-11 RC1-02-12 VR5-05-11 VR5-03-12
CR1-05-12 CR2-05-12 CR3-02-12 CR3-05-12 CR4-05-12 CR5-05-12 CR6-05-12
CR2-04-11 CR2-02-12 CR4-04-11
CR2-07-11 CR2-12-11
ML5-10-11 RŠ1-11-11 RŠ2-04-11 VR3-11-11
RŠ2-08-11
RA2-09-11
ML5-07-11 RC1-10-11 RC2-10-11 RC3-12-11 RD1-10-11
VR1-11-11
ML1-09-11 ML2-10-11
ML5-05-12
ML2-02-12 RŠ1-05-12 RŠ2-05-12 RŠ3-03-12 RŠ3-05-12
RŠ5-04-11
RC4-02-12 RC5-10-11 ST6-04-11
RA4-07-11 RA5-07-11 RC2-06-11
RC4-04-11 VR1-10-11 VR2-09-11 VR2-10-11
VR2-05-11 VR2-07-11 VR2-11-11 VR3-10-11 VR4-10-11 VR5-09-11 VR5-10-11 VR6-08-11 VR6-10-11
ST1-11-11 ST3-04-11 VR4-11-11 VR6-11-11
ML1-10-11 ML1-12-11 ML1-02-12 ML1-05-12 ML2-05-12 ML3-02-12 ML3-05-12 RD1-05-11 ST2-11-11
RŠ1-06-11 RŠ5-08-11
RŠ3-04-11 RŠ3-06-11 RŠ5-10-11
RC2-09-11 RŠ2-06-11
RC4-06-11 RC4'-10-11 RC4-12-11 RC4'-12-11 RC4-05-12 RC5-12-11 RC6-12-11
RC1-06-11 RC4*-09-11 RC4-10-11
VR6-09-11
ST5-03-12 ST6-11-11
ST5-04-11 ST5-11-11 ST6-03-12 VR5-11-11
RŠ3-10-11 RŠ4-10-11 RŠ4-05-12 RŠ5-06-11
RŠ2-11-11
RC6-10-11 VR3-09-11 VR4-09-11
RC4*-06-11 RC4-09-11 RC5-09-11
ML4-07-11 ML5-09-11 RC5-05-12 RC6-02-12 RC6-05-12
RC2-05-12 RC3-05-12 RC5-04-11 ST4-03-12
RC3-02-12 ST4-05-12
ST3-03-12 ST4-04-11 ST4-11-11 VR3-03-12 VR4-03-12 VR6-03-12
RŠ3-08-11 RŠ3-11-11 RŠ4-06-11 RŠ4-08-11 RŠ4-11-11 RŠ5-11-11
RA1-11-12 RA6-07-11 RC1-12-11
RA6-10-11 RC1-09-11
RA6-05-12 RC3-04-11 RC6-09-11
RC6-06-11 ST3-06-11
RC1-05-12 ST2-06-11 ST3-11-11 VR1-03-12
RC4*-05-12 RC5-06-11 ST1-06-11 ST1-08-11 ST2-08-11 ST3-08-11 ST3-05-12 ST5-06-11 ST5-08-11 ST6-06-11
RA3-04-11 RA4-11-11 ST4-08-11
RA3-10-11 RA4-04-11 RA4-10-11 RA4-03-12 RA5-09-11 RA5-11-11 RA6-11-11
RA1-10-11 RA2-05-12 RA4-09-11 RA5-10-11 RA6-09-11
RA1-09-11 RA2-10-11
RA1-07-11 RA2-07-11 RA2-11-11 RA3-07-11 ST1-03-12
RA1-05-12 RA5-05-12 ST1-04-11 ST6-05-12
ST6-08-11
RD2-05-11 RD2-12-11 RD2-05-12
RC3-06-11 RC3-09-11 ST4-06-11
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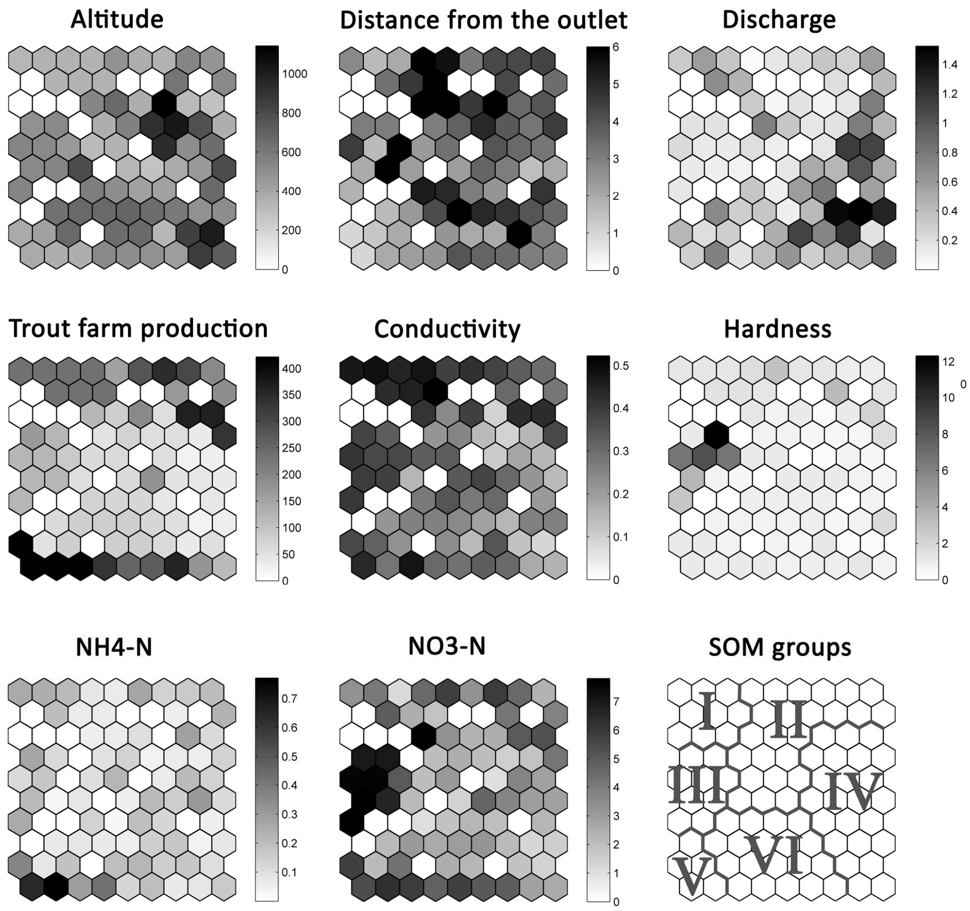
RA3-09-11 RA6-03-12
RA4-05-12
RC4*-02-12
ST2-05-12 VR1-07-11
VR5-07-11
RD2-07-11 RD2-09-11 RD2-10-11 RD2-03-12
RD3-03-12
RD3-07-11 RD3-09-11 RD3-10-11 RD4-10-11
RD4-07-11 RD4-09-11 RD4-03-12 VR3-07-11
RA2-03-12 RA6-04-11 RD4-12-11 RD4-05-12
RA1-03-12 RC5-02-12 RD3-12-11 RD5-07-11 RD5-09-11 RD5-10-11 VR4-07-11
RD3-05-11 RD4-05-11 RD5-05-11 RD5-12-11 RD5-05-12 VR6-07-11
RD3-05-12 VR1-05-11 VR3-05-11
RA3-03-12 RA3-05-12 RA5-04-11 RD5-03-12 ST5-05-12 VR2-03-12 VR4-05-11

a)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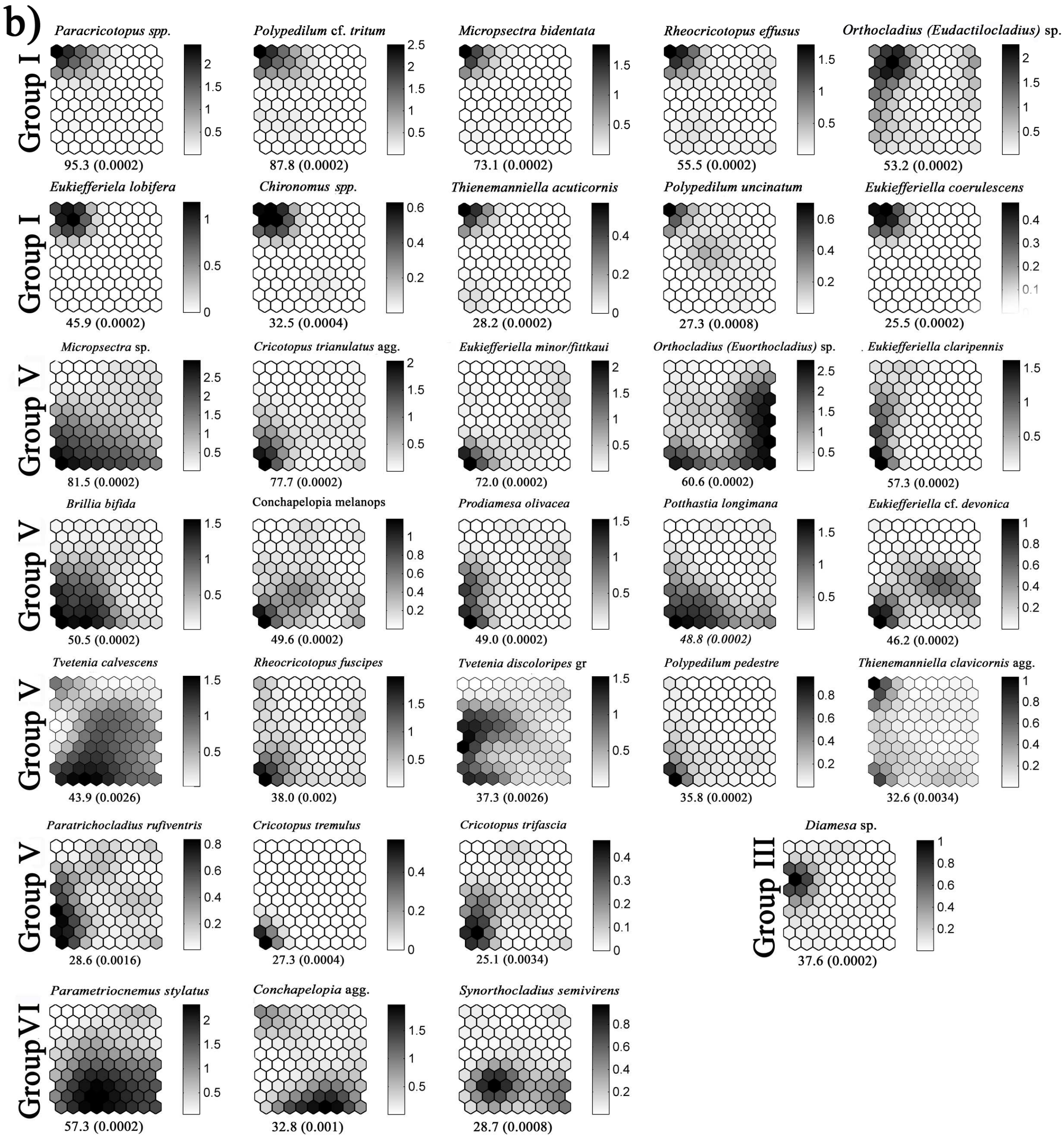
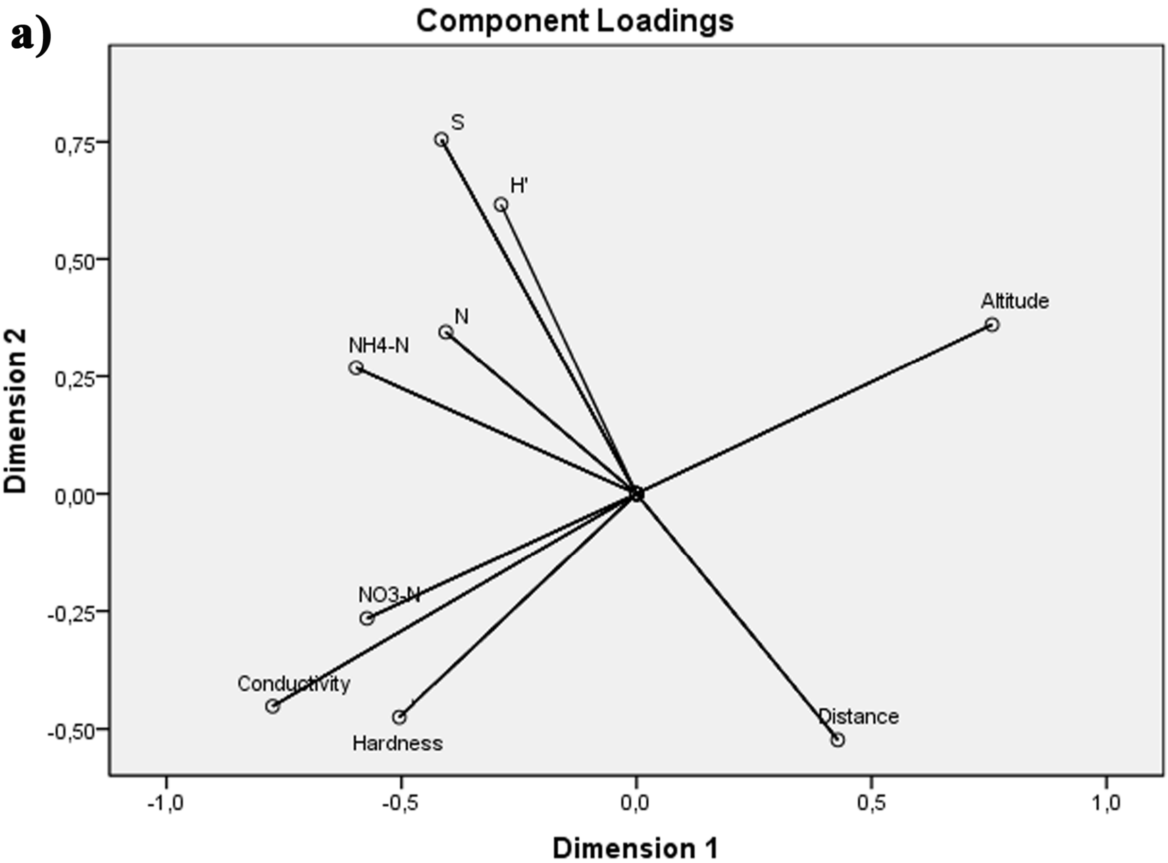
800
600
400
200
0
Distance from the outlet
6
5
4
3
2
1
0
Discharge
1.4
1.2
1
0.8
0.6
0.4
0.2
Trout farm production
400
350
300
250
200
150
100
50
0
Conductivity
0.5
0.4
0.3
0.2
0.1
0
Hardness
12
10
8
6
4
2
0
NH4-N
0.7
0.6
0.5
0.4
0.3
0.2
0.1
NO3-N
7
6
5
4
3
2
1
0
SOM groups
I
II
III
IV
V
VI


b)
Group I
Paracricotopus spp.
95.3 (0.0002)
Polypedilum cf. tritum
87.8 (0.0002)
Micropsectra bidentata
73.1 (0.0002)
Rheocricotopus effusus
55.5 (0.0002)
Orthocladius (Eudactilocladius) sp.
53.2 (0.0002)
Group I
Eukiefferiela lobifera
45.9 (0.0002)
Chironomus spp.
32.5 (0.0004)
Thienemanniella acuticornis
28.2 (0.0002)
Polypedilum uncinatum
27.3 (0.0008)
Eukiefferiella coerulescens
25.5 (0.0002)
Group V
Micropsectra sp.
81.5 (0.0002)
Cricotopus trianulatus agg.
77.7 (0.0002)
Eukiefferiella minor/fittkaui
72.0 (0.0002)
Orthocladius (Euorthocladius) sp.
60.6 (0.0002)
Eukiefferiella claripennis
57.3 (0.0002)
Group V
Brillia bifida
50.5 (0.0002)
Conchapelopia melanops
49.6 (0.0002)
Prodiamesa olivacea
49.0 (0.0002)
Potthastia longimana
48.8 (0.0002)
Eukiefferiella cf. devonica
46.2 (0.0002)
Group V
Tvetenia calvescens
43.9 (0.0026)
Rheocricotopus fuscipes
38.0 (0.002)
Tvetenia discoloripes gr
37.3 (0.0026)
Polypedilum pedestre
35.8 (0.0002)
Thienemanniella clavicornis agg.
32.6 (0.0034)
Group V
Paratrichocladius rufiventris
28.6 (0.0016)
Cricotopus tremulus
27.3 (0.0004)
Cricotopus trifascia
25.1 (0.0034)
Group III
Diamesa sp.
37.6 (0.0002)
Group VI
Parametriocnemus stylatus
57.3 (0.0002)
Conchapelopia agg.
32.8 (0.001)
Synorthocladius semivirens
28.7 (0.0008)

a)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H'
Altitude
N
NH4-N
NO3-N
Conductivity
Hardness
Distance
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0,50
0,25
0,00
-0,25
-0,50
Dimension 2
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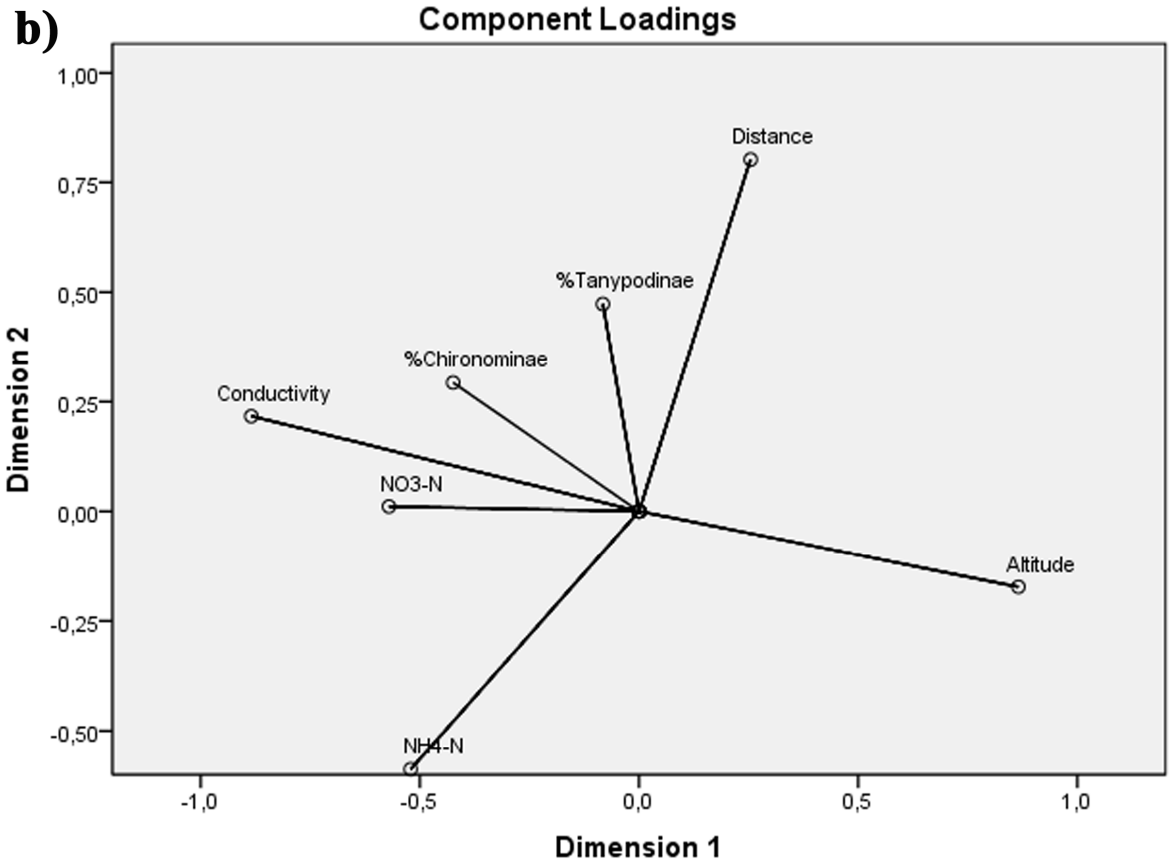
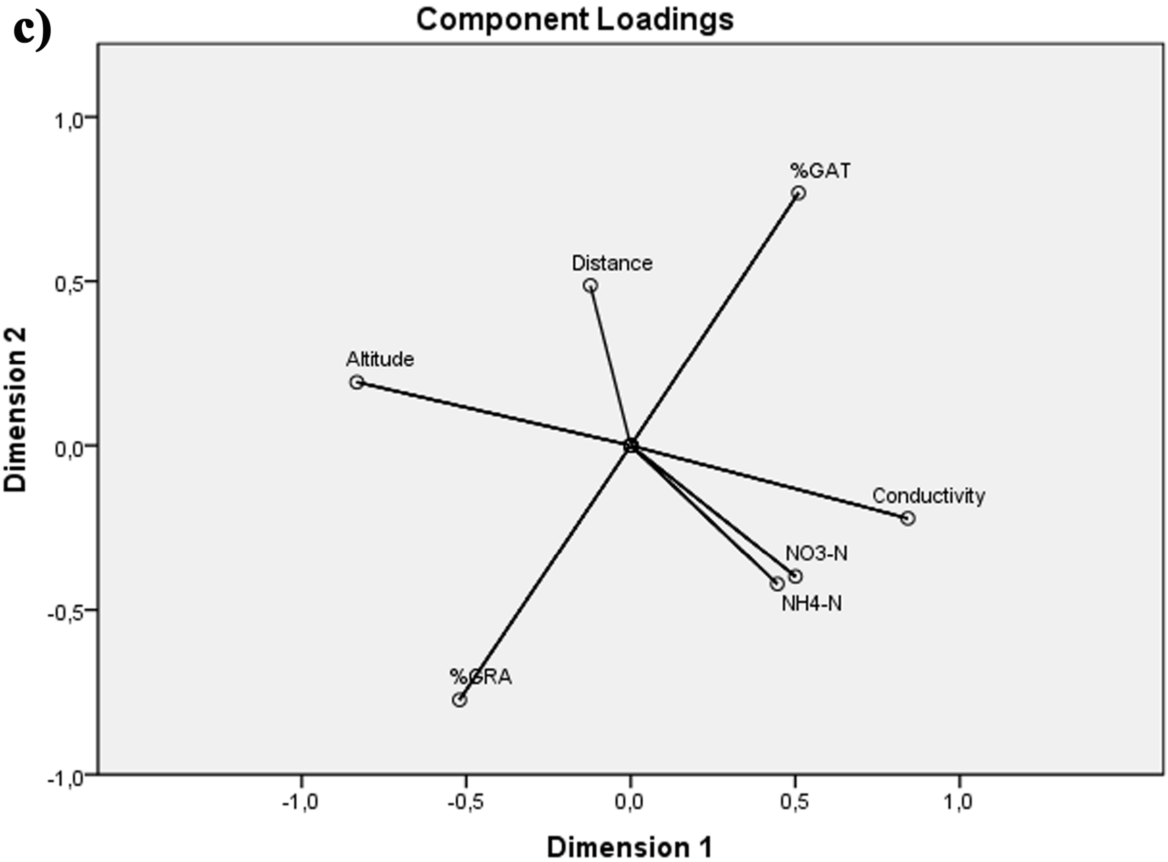
-0,5
0,0
0,5
1,0
Dimension 1

b)



Component Loadings
Distance
%Tanypodinae
%Chironominae
Conductivity
NO3-N
Altitude
NH4-N
1,00
0,75
0,50
0,25
0,00
-0,25
-0,50
-1,0
-0,5
0,0
0,5
1,0
Dimension 1
Dimension 2

c)


Component Loadings
%GAT
Distance
Altitude
Conductivity
NO3-N
NH4-N
%GRA
1,0
0,5
0,0
-0,5
-1,0
-1,0
-0,5
0,0
0,5
1,0
Dimension 2
Dimension 1

- We tested how chironomid community reacts to trout fish farming
- The SOM has revealed that community changes along the fish farm effluent gradient
- Chironomid grazers were negatively correlated with the distance from the outlet
- Diversity indices, taxa groups and FFG can be useful metrics for bioassessment

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