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Research paper

# Community concordance in lotic ecosystems: How to establish unbiased congruence between macroinvertebrate and fish communities

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# ABSTRACT

Community concordance within aquatic biota could provide useful information for improving the methods used in bioassessment and biodiversity conservation management. The main goal of the study was to investigate the mechanism of community concordance between macroinvertebrates and fish in a single river basin (South Morava river Basin, Serbia). In order to achieve this, a Self organizing map (SOM) ordinated and classified sampling sites based on the community structures of five different taxa groups (macroinveretbrates (MIB), fish (FSH), Chironomidae (CHI), Macroinvertebrates without Chironomidae (MWC) and the Ephemeroptera Plecoptera Trichioptera group (EPT)). SOM also revealed 6 environmental gradients along the groups tested that significantly changed their community structures. Using the results of the SOM analysis as the input, the Mantel test quantified the highest community concordance between FSH and MIB (r = 0.42) followed by FSH and CHI (r = 0.29). The lowest concordance was recorded between FSH and EPT (r = 0.14). The indicator species analysis (IndVal) revealed 39 species to be responsible for the community patterns obtained. The Geo-SOM visualized the spatial distribution of the IndVal taxa, revealing the generators of community concordance. The strength of community concordance depends on the variability of the data on the aquatic biota. Thus, having an appropriate sampling and statistical design as well as high taxonomic resolution, as some of the factors which increase the variability in the data set, could present community concordance between fish and macroinvertebrates in an unbiased way.

#### 1. Introduction

Information on the variability of the freshwater community structure is commonly used in bioassessment and biodiversity conservation management (Backus-Freer and Pyron, 2015). Predictable and regular changes in community structures along environmental and degradation gradients enable reliable assessment of an ecosystem health. To construct cost-effective bioindication methods for broad-scale assessments of biodiversity or ecosystem impairment, it is necessary to apply a surrogacy approach and to select representative taxonomic groups (Guareschi et al., 2015). To be usable as a representative, a surrogate group needs to be well-known in terms of its taxonomy and ecology. It should be easily sampled and monitored, and it should occur in a wide range of habitat types (Heino et al., 2009). Finally, and most importantly, the qualitative and quantitative changes in the community structure of the surrogate group, along degradation gradients, should be well-understood and highly correlated with those of the whole hydrobiocenosis. However, a prerequisite for this process is to establish a regional-specific model for a particular type of aquatic system which describes the community structure and dynamic, as well as the relationship between all of the relevant taxonomic groups of aquatic biota. Such a relationship is defined as a community concordance (also known as a community congruence or in the univariate context, crosstaxon congruence), and represents the degree of similarity in the pattern changes of communities along the main environmental gradients, between different taxonomic groups (Heino, 2010). This topic is broadly discussed in the scientific audience, and it includes testing the community concordance through the univariate (diversity indices; Pearson and Carroll, 1999) and multivariate context (community structure; Jackson and Harvey, 1993), in both lotic and lentic systems.

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The relationships between taxonomically close groups (within macroinvertebrates; Bilton et al., 2006), and distant groups (marcroinvertebrates vs. fish; Backus-Freer and Pyron, 2015; Infante et al., 2009; Paavola et al., 2006, macroinvertebrate vs. waterbirds; Guareschi et al., 2015 and macroinvertebrate vs. macrophytes; Traversetti et al., 2014) were compared and quantified using different statistical approaches. However, almost all of these studies excluded less well-known groups whose community patterns are mainly unexplored, or these groups were analyzed, but with poor taxonomic resolution. This is especially true for the Chironomidae family, which is usually included in studies at the family (Heino et al., 2005; Infante et al., 2009; Jackson and Harvey, 1993) or occasionally genus level (Backus-Freer and Pyron, 2015). This broadly distributed and abundant invertebrate group, with one of the most important functional roles in aquatic ecosystems, is characterized with the highest diversity among freshwater macroinvertebrates (Milošević et al., 2013). Ignoring the changes in the community structure, abundance and frequency of this dominant benthic group could lead to the substantial loss of data and consequently, biased results in the study of community concordance.

A statistical approach to concordance analyses between different taxonomic groups depends on the data matrix construction. Cross-taxon congruence, for the communities described using diversity indices, could be simply tested with correlation tests (the Pearson or the Spearman rank test; Gioria et al., 2011). On the other hand, where more variables are simultaneously introduced into the analysis, community patterns have to be tested using a multivariate approach. In previous studies, two main statistical tests have been used: the Mantel test (Manly, 2006) and the Procrustes analysis (Gower, 1971). The first method is a correlation analysis, which uses the resemblance matrix based on any distance measure as its input, and its output shows whether two data matrices are significantly similar and to which extent, using the Mantel r statistic. The second method is a superimposition approach which through the centering, scaling, reflection, rotation and dilation processes compares ordination resolutions and measures the degree of association among data matrices. The main advantage of Procrustes analysis over the Mantel test is that it uses the results of multivariate ordination analysis (e.g. NMDS) as its input. This enables a reduction in the dimensionality of the raw data (Peres-Neto and Jackson, 2001). Community concordance reveals to which extent the variability of different taxonomic groups changes in a similar way along a particular environmental gradient. Accordingly, an appropriate statistical approach should offer the following analytical advantages: to model non-linear relationships in huge data sets, to pattern community structure together with its environment, and to visualize the distributional patterns of each entity (taxa) along the spatial scale and passively introduced environmental gradients. It should also enable a visual evaluation of the degree to which the distribution of indicator taxa or environmental parameters are correlated (Gioria et al., 2011).

In the light of previous studies, we can assume that taxonomic groups within aquatic biota are weakly concordant at the local scale (single river basin; Heino, 2010). Having this in mind, we wanted to investigate the mechanism of community concordance among taxonomically close (within the macroinvertebrate group) and distant (macroinvertebrate vs. fish) groups that are routinely used as surrogates in bioassessment, and also to reveal the main generators of their structural inconsistency. In order to achieve this, the following tasks were set: (1) to visualize community patterns of macroinvertebrates and fish as well as taxonomic groups within macroinvertebrates: the Ephemeroptera, Plecoptera and Trichoptera group, the Chironomidae group and macroinvertebrates without chironomids, (2) to identify significant environmental gradients for the community structure of each taxa group investigated, (3) to quantify the concordance between different taxonomic groups, (4) to define the indicator taxa responsible for community congruence and (5) to visually correlate their spatial distribution.



Fig. 1. Map of the sampling sites distributed along the Southern Morava River basin.

### 2. Material and methods

# 2.1. Study area and sampling

The Southern Morava River, as a part of the Danube basin, is one of the biggest rivers in Serbia, with a catchment area of 15,469 km<sup>2</sup>. Situated in the south of the country, at 344 river km it joins up with the Western Morava River and creates the Great Morava River, a direct tributary of the Danube. Many tributaries flow into the Southern Morava River, the largest of which are included in this study: the Veternica, Toplica and Pusta reka rivers, which are left-hand and the Vlasina, Nisava (the longest) and Sokobanjska Moravica rivers which are right-hand tributaries (Gavrilović and Dukić, 2002).

Field campaigns were conducted at 26 sites along the Southern Morava River basin, covering an altitudinal gradient of 975 m. (Fig. 1). The macroinvertebrates were sampled twice, in September and October 2010, while the fish were sampled once, in September 2010. The sampling sites were selected to include different stream orders and to increase the variability of habitat conditions with a wide range of altitude (Fig. 1).

At each sampling site, three quantitative macroinvertebrate samples were taken from the most common substrate types with a Surber sampler of  $0.0625 \text{ m}^2$  (25 cm  $\times$  25 cm) and a 250 µm mesh. All three benthic samples were composited into a single one. Then, the specimens were manually sorted out from the sediment and preserved in 70% ethanol. Identification of the macroinvertebrates was performed up to the species or genus level, based on the relevant identification keys (Andersen, 2013; Bauernfeind and Humpesch, 2001; Eiseler, 2005; Elliot et al., 1988; Elliot and Humpesch, 2010; Gerken and Sternberg, 1999; Glöer, 2002; Moller Pillot, 1984a,b, 2009; Nilsson, 1997; Pfleger, 2000; Rossaro and Lencioni, 2015; Schmid, 1993; Waringer and Graf, 1997; Zwick, 2004, 2005). At the same sampling sites ichthyofauna was collected using an electrofishing pass, based on two types of unit effort

(CPUE) (along 50 m of the bank in wadeable streams, and along 200 m of the bank when drifting in a boat). Each site was sampled by at least two people operating an anode dipnet. A DC Aquatech IG 1300 electrofisher (2.6 kW, 80-470 V) was used to conduct the sampling procedure.

During each sampling several physicochemical parameters were measured for each site. The water temperature (T), conductivity (EC), pH and oxygen content [dissolved oxygen (DO) and oxygen saturation (DO%)] were measured by a WTW multi 340i probe. The biochemical oxygen demand (BOD5) was estimated using the standard methodology recommended by (APHA, 1999), and the water transparency was measured with a Lovibond PC Checkit. Also, the concentrations of ammonia nitrogen (NH4-H), nitrate nitrogen (NO3-N) and orthophosphates (PO4-P) were estimated using a Shimadzu UV–vis Spectrophotometer.

## 2.2. Data analysis

To test community congruence among taxonomically close (within the macroinvertebrate group) and distant (macroinvertebrate vs. fish) groups, the following data matrices with information on the different taxonomic groups were constructed: Macroinvertebrates (MIB), Fish (FSH), the Ephemeroptera Plecoptera Trichoptera group (EPT), the Chironomidae family (CHI) and Macroinvertebrates without chironomids (MWC). The Kohonen artificial neural network (Self-organizing map, SOM; Kohonen, 1982) was used to model the community structure of different taxonomic groups. This method processes and visualizes ecological data patterns, successfully modeling not only linear but also non linear data matrices (Milošević et al., 2013). Five community models were derived, one for each of the previously defined taxonomic groups. The vectors (sampling sites) from the five data matrices, as the input, were successively introduced into the SOM during the learning process. Once the learning process was complete, the community patterns were visualized in the form of a two-dimensional grid, composed of hexagonal neurons. Each neuron carried the sampling sites with a specific model of community structure. As the distance in the grid increased, the dissimilarity of the models carried by the neurons increased. The clusters of neurons on the trained SOM map, were obtained using the k-means method (Jain and Dubes, 1988). The resolution of the two-dimensional grid (number of output neurons) was determined a priori according to the relevant methods (Park et al., 2003; Vesanto et al., 2000) and the rule of avoiding a huge number of empty neurons (Penczak et al., 2012). In this study,  $5 \times 4$  grid resolution was most appropriate for the data sets.

The main environmental gradients that showed susceptibility along the taxonomic groups in the study were tested using the component planes (visualization technique) of the SOM (Milošević et al., 2014). This technique uses a two-dimensional grid, previously derived by the SOM, to present the distribution of the abiotic factors measured. The environmental parameters were passively introduced into the SOM, which had no influence on the ordination and classification processes based on the community structure of the taxonomic groups in the study. After the normality assumption for the environmental parameters was not met, the non-parametric ANOVA test (the Kruskal-Wallis test) was used to test whether they varied significantly between the groups of neurons obtained by the SOM.

The indicator values analysis (IndVal: Dufrêne and Legendre, 1997) was applied to reveal the taxa most responsible for the classification patterns. The Monte Carlo significance test with 1000 permutations was used to define the significant taxa for the SOM groups. All indicator taxa with IndVal values over 25 appeared in the group they represented with a relative frequency and abundance of at least 50%. A GeoSOM analysis (Bação et al., 2005, 2008) was used to compare the spatial distributions of the IndVal representant taxa. This method is an extension of the SOM, which ordinates and classifies sampling sites with geographic constraints, adjustable by the geographic tolerance parameter k. For k = 0 only geographically closer sampling sites can be attached to the same neuron in the map. As k increases, the radius of a potential neuron also increases, whereby sampling sites carrying a similar community model but which are geographically distant can be attached to the same neuron. In our study we trained the GeoSOM with k = 0 and a map resolution of  $10 \times 10$ , and derived component planes for each taxa with an IndVal over 25. This visualization technique made it possible to visually correlate the spatial distribution of the representant taxa. A more detailed description of the GeoSOM analysis is available in Milošević et al. (2016).

The community concordance between the taxonomic groups was quantified using the Mantel test. This method calculates the correlation between the two resemblance matrices, and using a randomization procedure it evaluates whether the observed correlation differs from random correlation (Manly, 2006). Any type of data and different distance measures can be used as the input for the Mantel test. To eliminate the main disadvantage of this method, which is its analysis of all dimensions in the data matrix (Gioria et al., 2011), the results of the multivariate ordination method (SOM) were used as input for the Mantel test. More precisely, the SOM method, as one of the outputs, creates the resemblance matrix based on the Euclidian distances, and the matrix defines the similarity between the neurons to which particular models of community structure are attached on the map. Finally, for each SOM model (each taxonomic group) the resemblance matrix was derived and used as input for the Mantel test.

# 3. Results

# 3.1. Variability patterns in communities along the environmental gradients

From 29,185 sampled specimens, 254 taxa were identified, and they were distributed within the defined taxa groups in the following pattern: 23 fish species (FSH) and 231 macroinvertebrate taxa (MIB), where 93, 78 and 138 taxa of Chironomidae (CHI), EPT and macroinvertebrates without chironomids (MWC) were present, respectively. The SOM analysis derived five models, which ordinated sampling sites

> Fig. 2. Sampling sites classified into three groups (A, B and C) on the Self Organizing Map, based on different community structures. The first number on the connective line indicates the Mantel r statistic while the last number stands for the percentage of matching sampling sites per group. The labels (numbers) assigned to each neuron on the SOM present different sampling sites.





Fig. 3. Component planes of environmental factors for all taxa groups showing regularity in change along the Self organizing map. According to the Kruskal-Wallis test all of the environmental factors significantly varied (P < 0.05) between the previously defined SOM groups.

and classified them into three clusters, using the structural pattern of community for each taxa group (Fig. 2). Furthermore, the environmental variables were passively introduced in each SOM community model, and component planes visualized their distribution along the previously defined clusters (Fig. 3). From 15 environmental parameters, the SOM and the Kruskal-Wallis test showed that the suite of 6 gradients (altitude, t, EC, NO3-N, PO4-P, NH4-N) significantly influenced the structure of all of the communities investigated (Fig. 3). The oxygen concentration was significantly different between groups of neurons, based on the community structure information for MIB, FSH and CHI (Kruskal-Wallis test p < 0.001), while BOD5 emerged as a significant gradient for MIB and all their taxa groups. EPT was the only taxa group which, besides reacting to the suite of 6 parameters, also significantly reacted to velocity. Finally, MIB, FSH and MWC significantly varied in their community structures along the depth gradient.

According to the environmental characterization of the groups of sites obtained in the SOM maps, different taxonomic groups showed similar classification patterns of the sampling sites. Group A, in all taxa models, was composed of unpolluted sampling sites situated at high altitudes, with low temperatures and poor mineral content. In sampling sites belonging to group B, a moderate increase in water pollution and the mineral content indicating parameters was observed. Finally, group C included sampling sites belonging to the lower reaches of the basin that were exposed to different levels of degradation (Figs. 2 and 3).

# 3.2. Community concordance

The Mantel test quantified the concordance of the community structure between the taxa groups in the study, finding them significant in all cases, but with different correlation strengths (Fig. 2). The highest concordance was recorded between FSH and MIB (r = 0.42, P < 0.05), with 77% of sampling sites classified into the same groups. The community concordance between fish and macroinvertebrates was

conspicuously lower when chironomids were excluded from the analysis (r = 0.21, 57.6% concordant sites.). The lowest cross-taxa congruence was detected between fish and EPT whereby only 46% sites (r = 0.14, P < 0.05) were concordantly clustered.

#### 3.3. Indicator taxa

The IndVal analysis revealed that the taxa of macroinvertebrates and the fish community appeared to be significant for one of the three groups, with relative frequencies higher than 50% (Fig. 4). The spatial distribution of all these IndVal representant taxa was visualized by the GeoSOM (Fig. 4). The only fish indicator taxa of group A, *Salmo trutta* showed concordant distribution along the GeoSOM with 7 MIB taxa (Fig. 4a). Group B was characterized by two FSH indicator species, changing their abundance along the sampling sites in the same manner as 6 MIB taxa (Fig. 4b). Finally, the highest number of indicator fish species (5) emerged for group C, and they shared a concordant pattern of distribution with four macroinvertebrate taxa (Fig. 4c).

## 4. Discussion

Community concordance within aquatic biota can, if it exists, significantly facilitate bioassessment and biodiversity conservation management, thus simplifying the sampling and identification processes and increasing the cost-effectiveness. The main question is to what extent the signals derived from different taxa groups overlap and whether their complementarity is important for the indication of biodiversity and ecosystem quality. Many authors have tried to answer this question by investigating community congruence between different taxa groups and along different environmental gradients (Heino, 2010). The results were inconsistent since all these studies used different study area scales, sampling designs, taxonomic resolution and finally, different statistical designs (Gioria et al., 2011).

According to our findings and SOM analysis, macroinvertebrates and fish community structure classified sampling sites with high concordance (77%) into three groups with similar environmental profiles (Figs. 2 and 3). This result is in accordance with previous studies suggesting that community concordance is mainly driven by the extent of data variability. Any factor (poor taxonomic resolution, small species pool, short environmental gradients or a small study area) which would diminish the variability of the data set can make taxa groups less concordant in their community structures. Paavola et al. (2006) showed that in near pristine lotic systems, fish, macroinvertebrates and bryophytes changed their concordance along the spatial scale, and the highest ones were obtained when the complete data set, with six river basins, was included in the analysis. Similar studies, but at the local scale (Backus-Freer and Pyron, 2015; Infante et al., 2009), revealed no significant community congruence between macroinvertebrates and fish. With poor taxonomic resolution of chironomids, that is, when they were presented only as a single entity in the data set, the authors significantly diminished the variability of the data, which probably affected the extent of community concordance. In our study, the Chironomidae family, one of the taxonomic groups within macroinvertebrates, showed the highest correlation with fish, with a classification pattern coincidence of 50% (Fig. 2), thus supporting the previous assumption. This is not surprising since in many studies regarding the community structure of macroinvertebrates, almost half of the recorded taxa belong to the Chironomidae family (Armitage et al., 1995). Such a huge dominance of chironomids probably increases the variability of the data, as well as the potential for cross taxa congruence through the biotic relationship (Heino, 2010). It seems that Chironomidae larvae, with their role as ecosystem engineers (Milošević et al., 2012), are an important element in the concordance between different communities. However, one of the features of a good surrogate

group is its ease of identification and well-understood taxonomy (Heino et al., 2009), which could present an obstacle for the utilization of chironomids. Due to the difficulties in larvae identification and taxonomy, chironomids have been excluded by default from the majority of community ecology and bioassessment studies. However, over the last decade many new identification keys for chironomid larvae based on a new approach to identification methods have been published (Andersen, 2013; Orendt and Spies, 2012; Rossaro and Lencioni, 2015; Vallenduuk and Moller Pillot, 2007), thereby significantly facilitating the identification process and increasing the cost-effectiveness of their application. Having all this in mind, the Chironomidae family has to be included in defining the surrogate since it meets all the requirements which are set as a prerequisite for a good indicator group.

Macroinvertebrates and fish, as key candidates for freshwater bioassessment and biodiversity conservation studies (Angermeier and Winston, 1999), are expected to be highly sensitive to different environmental gradients. It is also the case in our study, whereby the community structure of all of the groups tested significantly reacts to two main environmental gradients: natural (altitude) and man caused (organic pollution). According to previous studies, it appears that the length of the environmental gradient (existence of the underlying gradient) and the consistency of the set of environmental filters which taxa groups are sensitive to could be crucial for the strength of community concordance (Paavola et al., 2006). In addition, the concordance of macroinvertebrates and fish increases if sampling sites are distributed along a strong deterioration gradient (Kilgour and Barton, 1999) as well as an underlying natural gradient (Ormerod et al., 1994). In our study this was also the case for all of the taxa groups except for EPT, which significantly varied along some other gradients (velocity). On the other hand, EPT showed the lowest correlation with other taxa groups, confirming that a similar complex of environmental factors, significant for



**Fig. 4.** Distributional patterns of IndVal taxa (with IndVal values > 25 and p < 0.05) for the a) A, b) B and c) C SOM groups, visualized on a spatial scale by GeoSOM. The red circles on the component planes and red spots on the geographic map stand for the groups of sampling sites with the concordant occurrence of IndVal representant taxa. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)





The differences in the level of concordance between fish and different taxa groups of macroinvertebrates can also be explained from the aspect of biodiversity distribution following theirs spatial patterns. For instance, the EPT group shows the highest species richness in the upper reaches of the river basin (Allan, 1995). In contrast, the diversity of the fish community increases from upstream to downstream areas due to the greater variety of habitat diversity (Stojković Piperac et al., 2016) according to the well-known mechanism of species addition (Sheldon, 1968). Inconsistency in these patterns reduces both the variability of the data and the potential of these two groups to be concordant in the community structure. Representant taxa for each group of sampling sites, derived by the SOM, could be considered as taxa responsible for the ordination and classification patterns obtained. Consequently these taxa have a main role in the community concordance between the groups tested. Visualizing their spatial distribution on component planes (Fig. 4), we showed the level of overlapping of appearance along the sampling sites. Co-occurrence of the indicator taxa was detected at sampling sites situated at the end of the environmental gradients. More precisely, the indicator taxa for group C overlapped in the lower reaches with highly polluted habitats (Fig. 4c). The indicators for group A and B co-occurred at pristine or moderately polluted sites, situated at higher altitudes (Fig. 4a,b). Such findings confirm the assumption that the presence of an underlying gradient could increase the concordance strength



Fig. 4. (continued)

between the community structures of aquatic biota (Paavola et al., 2006). On the other hand, some macroinvertebrate taxa (Fig. 4), probably as a result of natural variability, did not show any co-occurrence with the spatial distribution of fish species and thus could be considered as the main source of inconsistency between these two groups.

One of the reasons for the diverse results in previous studies regarding cross-taxon congruence patterns could be the choice of statistical design (Gioria et al., 2011). In the relevant literature, quantification of community concordance is mainly done in two ways: using the correlation method of the resemblance matrix without previous reduction of dimensions (e.g. Backus-Freer and Pyron, 2015; Heino et al., 2009; Infante et al., 2009), or presenting the mean residuals of ordination patterns (e.g. Jackson and Harvey, 1993; Paavola et al., 2006). However, all of these studies omitted to interpret the results of concordance quantification, in the way usually used in bioassessment and community ecology studies (presenting the outputs through the classification scheme of sampling sites). In the present study, besides the results of the Mantel r statistic, the congruence of classification patterns was also presented as additional information in order to reveal the meaning of the correlation values and make it possible to set the thresholds of r coefficient for defining a surrogate group. Furthermore, it is very important to model community structure in a proper way before quantification of the concordance strength. Therefore, we decided to use the Self-organizing map as a method which overcomes many limitations in traditional multivariate analysis (e.g. non linear relationship, great variability and huge data sets). The SOM can also visually present the distribution of each dimension (species) along the map, enabling the understanding of patterns of cross-taxon congruence (Gioria et al., 2011).

In conclusion, community concordance is affected by the data variability and specific sampling, and data analysis designs can significantly influence its strength in bioassessment and biodiversity conservation studies. The Chironomidae family represents a key group within macroinvertebrate fauna, which reinforces its congruence with the fish community. For successful implementation of the surrogate approach, while designing the study it is necessary to include all variable aspects which can influence the variability of the data set (high taxonomic resolution, appropriate statistical design and sufficient sampling sites network which include wide environmental gradients).

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