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## Ecological analysis of macroinvertebrate communities based on functional feeding groups: a case study in southeastern Serbia

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#### Abstract:

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To examine if the macroinvertebrate community corresponds to the RCC (River Continuum Concept), which describes longitudinal patterns in allochthonous and autochthonous energy input and the associated feeding categories of macroinvertebrates along the lotic continuum, it was necessary to determine the functional feeding groups - FFGs. The goals of the research were: to examine whether a macroinvertebrate community matches the RCC; to use the functional feeding groups to determine the attributes of the ecosystem; to test if the ITC index is appropriate for use in this part of the world and to determine water quality along the river course of the Nišava River. Macroinvertebrate samples and physicochemical data were analyzed for 10 localities along the 151 km long stretch of the Nišava River in southeastern Serbia, over a one-year period. Out of all the collected specimens (9837 individuals) 49.6% belong to shredders, 31.77% to scrapers, 12.25% to collectors and 6.4% to predators. On the annual level only locality nine belongs to autotrophic type; relation P/R=1.44. Locality four has the most heterotrophic character, the lowest channel stability and the most disturbed predator-prey relationships. The results of the research reveal that the trends found in relative functional group abundance do not correspond with the tendencies predicted by the RCC. The FFG ratios surrogate are consistent with the observations of the properties of the ecosystem at the sampling localities. According to the Index of Trophic Completeness, most of the localities included in the research belong to good water quality (seven out of ten), while other localities belong to moderate water quality (three out of ten).

Key words: macroinvertebrates, functional feeding groups, Index of Trophic Completeness

#### Apstrakt:

# Savić, A., Đorđević, M., Jušković, M., Pešić, V.: *Ekološka analiza zajednice makroinvertebrata na osnovu funkcionalnih grupa zasnovanih na tipu ishrane: primer iz jugoistočne Srbije. Biologica Nyssana, 8 (2), Decembar, 2017: 159-166.*

RCC (River Continuum Concept) opisuje longitudinalne šablone u inputu autohtone i alohtone energije, i njihovu povezanost sa kategorijama ishrane makroinvertebrata duž rečnog kontinuuma. Da bi se utvrdilo da li

zajednica makroinvertebrata prati RCC neophodno je odrediti funkcionalne grupe na osnovu tipa ishrane (FFG). Tokom jedne godine makroinvertebratska zajednica i fizičko hemijski podaci su analizirani na 12 lokaliteta duž 151 km rečnog toka Nišave, koja se nalazi na jugoistoku Srbije. Od ukupnog broja individua (10519) 48.99% pripada sekačima, 31.49% grebačima, 13.02% sakupljačima i 6.5% predatorima. Na osnovu FFG odredjeni su sledeći ekosistemski atributi: autotrofnost/heterotrofnost lokaliteta, stabilnost rečnog kanala, odnos predator-plen i odnos CPOM/FPOM (krupne čestice organske materije/sitne čestice organske materije). Na godišnjem nivou, samo lokalitet 9 ima autotrofnu prirodu (P/R=1.44). Lokalitet 4 je sa najizraženijim heterotrofnim karakterom, sa najmanjom stabilnošću rečnog kanala i sa najnarušenijim odnosom predator-plen. Odnos CPOM/FPOM pokazuje da reka Nišava ima regularnu asambleju sekača (na svim lokalitetima odnos je >0.25) što je povezano sa uslovima u riparijalnoj zoni.

Ključne reči: makroinvertebrate, funkcionalne grupe na osnovu tipa ishrane, Indeks of Trophic Completeness

#### Introduction

Of all the freshwater organisms that have been considered for use in biological monitoring, benthic macroinvertebrates are recommended most often (Carter et al., 2007). Macroinvertebrates have been used to evaluate the effects of anthropogenic stressors at all levels of biological organization, from the molecular to the ecosystem (Rosenberg & Resh, 1993). Functional feeding group (FFG) approach, described more than 40 years ago (Cummins, 1973), has been modified in some details since then (e.g., Cummins & Klug, 1979, Wallace & Merritt, 1980, Cummins & Wilzbach, 1985, Merritt & Cummins 1996, Merritt et al., 1999, 2002), but the basis of FFG relationships remains quite simple. FFGs are based on a direct correspondence between the categories of nutritional resources present in the environment and the populations of freshwater invertebrates that are

adapted to efficiently harvest a given food resource (Merritt & Cummins, 2007). The analysis of the trophic structure of benthic macroinvertebrate communities can be used in biological assessments of the condition of river ecosystems. Using the trophic or functional approach, the Index of Trophic Completeness (ITC) was developed (Pavluk et al., 2000). Also, FFG ratios can be used as surrogates for these aquatic ecosystem attributes and serve as a useful assessment of the ecological condition freshwater ecosystems (Merritt & Cummins, 2007).

In this study, we wanted to examine whether the macroinvertebrate community matched the RCC (River Continuum Concept) (V a n ot e et al., 1980) in the river, or its trophic structure (distribution pattern of FFG) was more influenced by pollution. The goal was to use functional feeding groups to determine the ecosystem attributes: indication of autotrophic/heterotrophic type; ratio between FPOM



Fig. 1. The map of the studied area with localities

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in transport and FPOM in sediment; stability of channels (river channel resistance capacity to the detachment of bed and bank materials); and predator control. Another goal of the research was to test is ITC index appropriate for use in this part of the world, because there was no evidence about its use in the region of the Balkan peninsula. In addition, the study set out to determine water quality along the river course of the Nišava River based on these two types of analysis.

#### Material and methods

The Nišava River (**Fig. 1**) belongs to the Black Sea drainage basin. It originates in western Bulgaria, on the Stara Planina Mt., and flows in a southeastnorthwest direction. It is 218 km long, of which 67 km flows through Bulgaria, and 151 km through Serbia. Ten localities were chosen along the entire course of the Nišava River in Serbia. The odd numbered localities are positioned upstream of the settlements, and even numbered downstream of the settlements. Sampling was performed each month, from May 2006 to April 2007. All localities were sampled on a single day during each field trip.

Biochemical oxygen demand (BOD5) was estimated using the standard methodology recommended by APHA (1999). Dissolved oxygen, pH and conductivity were measured using a WTW®Multi 340i probe. The concentration of total nitrogen (TN) and phosphorus (TP) were determined in the field, using a Photometer – System PC Multi Direct Lovibond ® meter.

The percentage of substrate was observed visually; the classification of mineral substrates by particle sizes according to W e n t w o r t h (1922) and V e r d o n s c h o t (1999) was used. Since larger substrates require greater stream power for movement, they are physically more stable (G u r t z & W all a c e 1984); thus, pebbles, cobbles and boulders consolidated a stable substrate. Water turbidity was measured with a Lovibond® Checkit device. Stream order was determined according to S t r a hl e r (1952).

Macrozoobenthos were sampled at each locality over a 50 m river stretch with a square frame kick net ( $35 \times 35$  cm, mesh size 300 µm). Three 3-minute samples were taken during each visit to include different substrates (boulder, cobble, pebble, sand, silt, and detritus) and flow regime zones at each location. The net was held perpendicular to the flow and the substrate was vigorously disturbed in front of the net. As the substrate was disturbed, sampling moved progressively upstream. The three samples were then pooled, representing a single monthly sample for each site. This sampling procedure was

previously evaluated by preliminary test sampling, and three replicates proved to be sufficient to capture the maximum number of taxa. All samples were elutriated in the field and the organisms were fixed in 4% formaldehyde solution and returned to the laboratory for sorting.

The material identified was using identification keys: for Oligochaeta Brinkhurst & Jamieson (1971) and Hrabe (1981); for Hirudinea Mann & Watson (1964); for freshwater snails Macan & Cooper (1994) and Pfleger (1990, 2000); for Odonata Nilsoon (1997) and Bešovski (1994); for Ephemeroptera Belfiore (1983) and Elliot et al. (1988), for Trichoptera Walace et al. (1990), Edington & Hildrew (1995) and Pescador et al. (1995), for Plecoptera Hynes (1967) and Zwick (2004); for Diptera Nilsson (1997), Vallenduuk & Pillot (2007) and Pillot (2009).

For each taxon the functional feeding group was determined based on:  $M \circ o g$  (1995), Graf et al. (2006) and Pavluk et al. (2000). The ecosystem attributes where calculated according to  $M \circ rritt$  & Cummins (2007) and  $M \circ rritt$  et al. (2002). ITC index and water quality class are determined by de Vaate & Pavluk (2004). Quality class score is calculated by the formula:

$$C_{tot} = \sum_{i=1}^n C_i ,$$

where  $C_{tot}$  is the total score, *n* is the number of trophic guilds present in the data-set, and  $C_i$  is the *ln* transformed indication value of trophic guild *i*. The relation between  $C_{tot}$  and the quality classes is given in de V a a t e & P a v l u k (2004).

#### Results

The community of macroinvertebrates was collected at ten localities along the entire course of the Nišava River. The river is a  $4^{th}$  to  $7^{th}$  stream order (according to Strahler, 1952) (**Tab. 1**).

**Table 1** shows that the concentrations of total nitrogen and total phosphorus are the highest at localities 4 and 10. These localities are under the highest anthropogenic pressure. The  $4^{th}$  locality is under the impact of industrial wastewater from tire factory 'Tigar'. The  $10^{th}$  locality is downstream from the biggest city along the entire length of the river – Niš.

During one-year research 9837 individuals of macroinvertebrates were collected. Out of the total number, shredders were the most present - 4877 individuals. They were followed by scrapers (3125 individuals), collectors (1205 individuals) and finally predators (630 individuals) (**Fig. 2**).

**Table 1.** Average annual values of environmental parameters at each locality (loc) studied along the Nišava River. SO-Stream order; TP-total phosphorus in mg/l; TN-total nitrogen in mg/l; O-oxygen in mg/l; BOD<sub>5</sub>-biochemical oxygen demand in mg/l; TU-turbidity in NTU; CON-conductivity in S/cm; SS-stable substrate in %.

loc 1	loc 2	loc3	loc 4	loc 5	loc 6	loc 7	loc 8	loc 9	loc 10
4	5	6	6	7	7	7	7	7	7
0.02	0.08	0.04	0.10	0.07	0.07	0.07	0.06	0.07	0.11
0.27	0.28	0.08	0.34	0.10	0.12	0.12	0.08	0.09	0.21
6.72	7.28	8.04	7.09	7.85	8.34	8.37	7.98	7.56	6.50
1.68	3.04	2.74	4.16	3.08	3.37	3.61	3.08	3.17	2.99
7.56	6.54	6.17	6.59	6.98	6.56	6.30	7.34	6.15	6.54
26.60	35.92	12.33	18.20	21.98	20.72	5.28	12.90	6.94	3.75
496.00	536.42	460.08	459.42	395.75	403.83	414.17	411.08	413.58	575.67
67.50	79.67	78.33	46.50	76.67	52.58	83.75	89.17	66.67	70.42
	4   0.02   0.27   6.72   1.68   7.56   26.60   496.00	4 5   0.02 0.08   0.27 0.28   6.72 7.28   1.68 3.04   7.56 6.54   26.60 35.92   496.00 536.42	$\begin{array}{c ccccc} 4 & 5 & 6 \\ \hline 0.02 & 0.08 & 0.04 \\ \hline 0.27 & 0.28 & 0.08 \\ \hline 6.72 & 7.28 & 8.04 \\ \hline 1.68 & 3.04 & 2.74 \\ \hline 7.56 & 6.54 & 6.17 \\ \hline 26.60 & 35.92 & 12.33 \\ \hline 496.00 & 536.42 & 460.08 \\ \hline \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$					

**Table 2** shows that the highest number of predators were detected at localities 4 and 10, which have already been mentioned as localities with the highest concentrations of nutrients. The greatest percentage of predators are present at locality 4 (**Fig. 3**).



□ collectors □ predators □ scrapers ■ shredders

Fig. 2. Percentage of FFG on annual level

According to the ratio P/R (**Tab. 3**) at annual level, nine localities on the Nišava River were characterized as heterotrophic (the exception is locality 9). According to the ratio TFPOM/BFPOM, FPOM in transport is greater than normal particulate loading in suspension on locality 7. At other localities, there is more FPOM in sediments (benthos) than in suspension. The stability of the river channel is the lowest at locality 4. Also, it is low at locality 10. The ratio CPOM/FPOM indicates that the River Nišava has normal shedder association linked to functioning riparian system along the whole river stretch. The predator-prey relationship is the most disturbed at locality 4.

According to de Vaate & Pavluk (2004), localities 2, 3, 5 and 7 belong to class II (good quality), subclass I; locality 8 belongs to class II (good quality), subclass II; localities 6 and 9 belong to class II (good quality), subclass III. On the other hand, localities 1, 4 and 10 belong to class III (moderate), subclass II (**Tab. 4**).

#### Discussion

The trends found in relative functional group abundance in the research do not correspond with the tendencies predicted by the RCC. Similar results were obtained in the research conducted by S k a f f (2010). The RCC assumes that the highest proportion of shredders will be present in low- streams order (Vannote et al. 1980). The data obtained in this study illustrate that this is not the case - more shredders (in %) were found at locality 10 (the 7<sup>th</sup> stream order) than at locality 1 (the 4<sup>th</sup> stream order). One possible explanation for the disparity between the observed and the expected feeding group proportions may be relatively small ranges of physical stream characteristics found between stream orders. The 6<sup>th</sup> order (locality 3) has average depth 89.5 cm, while the 7<sup>th</sup> order (the locality 10) has average depth 96.5 cm (Savić, 2012). Similar results were obtained by Skaff (2010). The use of stream order to characterize a stream is sometimes deemed to be a misrepresentation of the true size. Some researchers consider drainage area and discharge to be better indicators (Allen & Hoeksta, 1992). Another explanation could be simply a general lack of riparian cover to supply litter inputs (Cummins et al., 2005) in low stream order parts of the river stretch.

According to the P/R ratio, nine sites (out of ten) were characterized as heterotrophic (**Tab. 3**). It means that the dominant base food chain for the invertebrate communities, at most localities, was judged to be allochthonous detritus, largely from the riparian zone. Locality 4 was the most heterotrophic.



Table 2. Number of specimens of FFG at each locality

Fig. 3. Percentage of FFG at ten localities

This is in accordance with the fact that the highest value of BOD5 and the lowest value of oxygen concentration were found there (**Tab. 1**).

The TFPOM/BFPOM ratio revealed significant amounts of FPOM in transport at locality 7 (**Tab. 3**) which was an indicator of high quality of water (Cummins et al., 2005). This is in accordance with the fact that the highest concentration of oxygen was detected at locality 7 (**Tab. 1**).

The stability of the stream channel is the key attribute of a stream/river ecosystem (Merritt & Cummins, 2007) as reflected in the relative permanence (stability) of various bottom materials. If the bottom is stable, invertebrates that cling to surfaces of stones or large wood, while feeding on attached algae will be more abundant (C u m m i n s et al., 2005). The FFG surrogate ratio for channel stability revealed that localities 1, 2, 4 and 10 were below the threshold of 0.50. Localities 3 and 5 were on the borderline. The lowest values were at locality 4. This is also in accordance with the fact that the lowest percentage of stable substrates were found at locality 4 (**Tab. 1**).

According to Merritt et al. (2002) for the FFG surrogate ratio for predator-prey balance the locality 4 is (**Tab. 3**) the locality with the most deviation from typical values of this ratio (<0.15). In other words, only localities 4 and 10 are not with 'normal' predator-prey balance, or top-down control.

localities	P/R	TFPOM/BFPOM	Stability of the river channel	Top-Down control
1	0.29	0.12	0.32	0.14
2	0.31	0.37	0.43	0.07
3	0.34	0.18	0.47	0.09
4	0.02	0	0.03	1.56
5	0.45	0.07	0.47	0.02
6	0.49	0.38	0.55	0.02
7	0.54	0.62	0.71	0.07
8	0.74	0.1	0.79	0.03
9	1.44	0.23	1.51	0.01
10	0.07	0.02	0.07	0.2

Table 3. Ecosystem attributes calculated using FFG according to Merritt and Cummins (2007).

<b>Table 4.</b> Values for ITC on investigated localities.	N number of species in each guild; A – relative number
of species in each trophic guild; C=100/A.	

		_	-											
Guild	Loc	1	2	3	4	5	6	7	8	9	10	11	12	$C_{tot}$
1	Ν	6	3	3	5	0	9	11	3	1	0	0	0	
	А	14.6	7.32	7.32	12.2	0	21.9	26.8	7.32	2.44	0	0	0	
	С	6.83	13.7	13.7	8.19	0	4.57	3.73	13.7	41	0	0	0	
	Ln C	1.92	2.61	2.61	2.1	0	1.52	1.32	2.61	3.71	0	0	0	18.4
2	Ν	5	4	7	3	0	16	17	5	1	2	0	2	
	А	8.05	6.45	11.3	4.84	0	25.7	27.4	8.05	1.61	3.22	0	3.22	
	С	12.4	15.5	8.86	20.7	0	3.88	3.65	12.4	62.1	31.1	0	31.1	
	Ln C	2.52	2.74	2.17	3.03	0	1.35	1.28	2.52	4.13	3.43	0	3.43	26.6
3	Ν	7	6	8	5	0	15	22	3	1	2	0	1	
	А	10	8.56	11.4	7.13	0	21.4	31.4	4.28	1.43	2.86	0	1.43	
	С	10	11.7	8.75	14	0	4.66	3.17	23.4	69.9	35	0	69.9	
	Ln C	2.3	2.46	2.17	2.63	0	1.54	1.14	3.15	4.25	3.55	0	4.25	27.4
4	Ν	2	1	0	2	0	4	5	1	3	3	0	0	
	А	9.52	4.75	0	9.52	0	19.1	23.8	4.75	14.3	14.3	0	0	
	С	10.5	21.1	0	10.5	0	5.25	4.19	21.1	7	7	0	0	
	Ln C	2.34	3.04	0	2.34	0	1.65	1.42	3.04	1.94	1.94	0	0	17.7
5	Ν	4	2	3	3	1	12	12	2	5	1	0	0	
	А	8.88	4.44	6.66	6.66	2.22	26.7	26.7	4.44	11.1	2.22	0	0	
	С	11.3	22.5	15	15	45	3.75	3.75	22.5	9	45	0	0	
	Ln C	2.42	3.11	2.71	2.71	3.81	1.32	1.32	3.11	2.19	3.81	0	0	26.5
6	Ν	6	3	4	3	0	12	9	0	4	1	0	1	
	А	13.9	6.97	9.3	6.97	0	27.9	20.9	0	9.3	2.32	0	2.32	
	С	7.17	14.3	10.8	14.3	0	3.57	4.77	0	10.8	43.1	0	43.1	
	Ln C	1.96	2.66	2.36	2.66	0	1.26	1.55	0	2.36	3.75	0	3.75	22.3
7	Ν	7	4	7	6	0	23	14	2	4	2	0	1	
	А	10	5.71	10	8.56	0	32.9	20	2.86	5.71	2.86	0	1.43	
	С	10	17.5	10	11.7	0	3.04	5	35	17.5	35	0	69.9	
	Ln C	2.3	2.86	2.3	2.46	0	1.11	1.61	3.55	2.86	3.55	0	4.25	26.9
8	N	5	3	3	3	0	20	15	1	3	1	0	0	
	A	9.25	5.55	5.55	5.55	0	37	27.8	1.85	5.55	1.85	0	0	
	C	10.8	18	18	18	0	2.7	3.6	54.1	18	54.1	0	0	
	Ln C	2.37	2.88	2.88	2.88	0	0.98	1.27	3.98	2.88	3.98	0	0	24.1
9	N	4	4	4	4	0	12	14	2	3	2	0	0	
	A	8.15	8.15	8.15	8.15	0	24.5	28.6		6.12	4.07	0	0	
	C	12.3	12.3	12.3	12.3	0	4.07	3.5	24.6	16.3	24.6	0	0	<b>01</b> 0
10	LnC	2.51	2.51	2.51	2.51	0	1.4	1.25	3.2	2.79	3.2	0	0	21.9
10	N	1	2	0	3	0	12	10	0	1	3	0	0	
	A	3.12	6.25	0	9.37	0	37.5	31.3	0	3.12	9.37	0	0	
	C	32.1	16	0	10.7	0	2.66	3.2	0	32.1	10.7	0	0	4
	Ln C	3.46	2.77	0	2.36	0	0.98	1.15	0	3.46	2.36	0	0	16.5

This is in accordance with the fact that proportion of some FFG could be changed under some pollutants (Pavluk et al., 2000).

The Index of Trophic Completeness (ITC) at locality 3 was with high value (ITC=27.4), at locality 4 (downstream of water discharge point from tire factory 'Tigar') it dropped to ITC=17.7, then after a distance of 34.9 km, at locality 5, it increased again to ITC=26.5; at locality 6 it dropped to ITC=22.3 and 8.1 km further downstream, at locality 7, it increased

to ITC=26.9 (S a v i ć, 2012). On the other hand, the situation was different between localities 8 and 9, where instead of the expected increase of ITC, its value decreased from ITC=24.1 to ITC=21.9. The distance between these two localities is only 4.8 km, which suggest that the minimal distance for the river self-purification processes to become effective is at least 8 km (S a v i ć et al., 2013). The odd-numbered localities are positioned upstream, and even-numbered downstream of the settlements. A clear

pattern can be seen: values of  $C_{tot}$  are higher at oddnumbered localities, except when it comes to localities 1 and 2. This could be explained by facts that all other pairs of localities (3 and 4; 5 and 6; 7 and 8; 9 and 10) belong to the same stream order (**Tab. 1**). Locality 1 belongs to the 4<sup>th</sup> stream order, while locality 2 belongs to the 5<sup>th</sup> stream order. According to that, the Index of Trophic Completeness could be sensitive on river section which is opposite to conclusion to some investigation in different parts of the world.

### Conclusion

The trends found in relative functional group abundance in this study do not correspond with the tendencies predicted by the RCC. A possible explanation for the disparity between the observed and expected feeding group proportions may be the relatively small ranges of physical stream characteristics found between stream orders. Another explanation could be disturbance of riparian vegetation.

Surrogate measures for ecosystem attributes were used for the very first time in this region. The FFG ratios surrogate are consistent with the observations of the properties of the ecosystem at the sampling localities. The results show that the use of surrogates of ecosystem attributes is quite adequate for this purpose since the results obtained in this way match the results obtained by direct measurements of the attributes. However, direct measurements are often more difficult and require additional time and money. Therefore, this principle should be used more often.

The Index of Trophic Completeness is considered to be a promising tool for determination of water quality in our region. According to this index, most of the localities included in the research belong to good water quality (seven out of ten) while other localities belong to moderate water quality (three out of ten).

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