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Effects of nutrients and turbidity on grazer–periphyton interactions: a case study from the Nišava River, Balkan Peninsula

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Abstract. The impact of nutrients and turbidity on the grazer–periphyton interactions was investigated monthly at ten sites along the Nišava River (Balkan Peninsula). Based on the grazers densities, the studied sites were divided into three groups. There was significant difference in average grazer density between these three groups of sites. Our study revealed that the negative effect exerted by grazers on periphyton was statistically significant only at the localities of the site group II, characterized by the highest density of scraper assemblages. The results of SIMPER analysis revealed that the grazer assemblage of the site group II was mostly determined by *Stagnicola palustris*. We found that more diverse assemblages decrease the negative impact of grazers on periphyton biomass. The responses of grazing pressure were not influenced by river order, nor sensitive to season. Our findings show that the high levels of total nitrogen, and to a lesser extent of total phosphorus, led to the decrease of the negative impact that grazers have on periphyton. On the other hand, turbidity increased the impact that scrapers have on the periphyton biomass to the more significant level (from p=0.028 to p=0.015).

Key words: macroinvertebrates, grazers–periphyton interaction, river, nutrients, turbidity.

Introduction

Herbivore effects on producers have been shown at fine, intermediate, and large spatial scales (Holomuzki et al. 2010). In this paper, we restrict our analyses to stream grazers that consume periphyton, one of two dominant food sources (autochthonous and allochthonous) available to consumers in rivers (Feminella and Hawkins, 1995). Although the studies on top-down control of food resources by consumers has been a central tenet in ecology in the last decades (Marshall et al. 2012), there were no intensive studies on grazers–periphyton interactions in freshwaters of Balkan Peninsula.

While numerous studies have revealed that grazers, through consumption, have a direct negative effect on periphyton biomass, some studies have demonstrated that this relationship is more complex than the classic view of the plus–minus relationship (Liess and Hillebrand 2004). For example, some studies address the presence of several types of indirect effects, which is often hard to identify, influencing the relationship between grazers and periphyton, such as habitat facilitation and trophic cascading (Liess and Hillebrand 2004). Moreover, grazers can indirectly improve the habitat of the periphyton by increasing the availability of space (Holomuzki et al. 2010).

The rate of grazing and its relationship to periphyton production depend on many other factors such as nutrient availability and turbidity. Many studies have shown that growth of periphyton is often constrained by the availability of nutrients (Hillebrand and Sommer 1997) and light (Hill 1996; Liess and Hillebrand 2004). Experiments in freshwater and in coastal habitats have shown that the addition of nutrients often results in the increase of periphyton biomass (Hillebrand and Kahlert 2001). On the other hand, some papers highlighted the role of turbidity on grazers–periphyton interactions (Lamberti and Resh 1983, Caraco et al. 1997, Liboriussen et al. 2005, Holomuzki et al. 2010) indicating the latter as one of the most important factor affecting periphyton (Qin et al. 2007, Uehlinger et al. 2010).

The present study aimed to determine the impact which grazers have on periphyton biomass in a stream ecosystem, and to evaluate the impact of nutrients (total phosphorus and total nitrogen) and turbidity on the grazers–periphyton interactions. We postulated that interactions between grazers and periphyton biomass might be affected by the species composition, abundance and diversity of the grazer's assemblage. Moreover, we predicted that periphyton biomass rates depend on the impact that nutrients and turbidity have on the relationship between grazers and periphyton.

Material and Methods

Study area

The field experiments were conducted along the Nišava River, which is the longest tributary of the Južna Morava River in the Danube River basin (Fig. 1). From the total 218 km of river course, 67 km flows through Bulgaria, and 151 km through Serbia, with mean slope of 19.8%. The river in the Serbian part is 4th to 7th order (according to Strahler, 1952). In most of its length, this is an upland type of river. Mean discharge during period of investigations was 1.2 m^3s^{-1} in the station in Dimitrovgrad (st.1.) and 16.8 m^3s^{-1} in Niš (st.10) (data from Republic Hydrometeorological Service of Serbia (RHMZ, yearbook 2006, 2007) and were lower than long term data (Gavrilović and Dukić, 2002). Earlier studies indicated that the Nišava River had a good ecological status, and only in the lower part of the investigated river section were noted moderate hydrochemical changes of anthropogenic origin (Savić et al. 2011, 2013, 2016).

Collecting material

Water sampling, macroinvertebrate and periphyton collection were performed monthly, from May 2006 to April 2007, from ten sites in the same day. The concentration of total nitrogen (N) and total phosphorus (P) in water were determined in the field, using a Photometer-SystemPC MultiDirect Lovibond® meter. Water turbidity (T) was measured with a LovibondR® Checkit device.

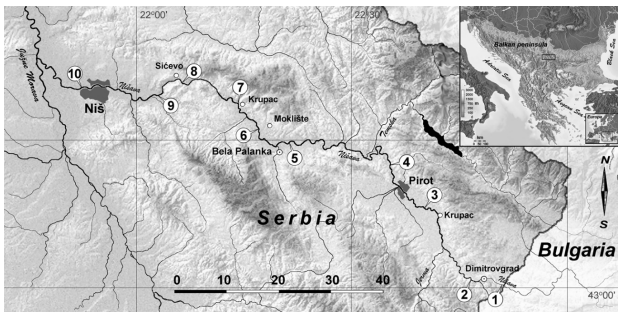

Figure 1. The map of the study area with sites.

At each site macroinvertebrates were sampled with a square frame kick net (35×35 cm, mesh size 300 μm) over a 50 m river stretch. Three 3-minute samples were taken during each sampling to include different substrates and flow regime zones at each locality. 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 cleaned in the field, and the organisms were fixed in 4% formaldehyde and returned to the laboratory for sorting.

Periphyton was sampled from rocks by gentle scraping with a brush (up to 10 cm^2 of surface), three times at the left bank, three times in the middle of the river, and three times at the right bank along the transect, in order to include various water depths. In general, the stone was removed from the stream before scraping in order to prevent scraped algae to be washed away (Lowe and Laliberte, 2007). The periphyton collected in this way was combined into a single sample, which was filtered, using glass fibre filters Whatman GF/F for determination of ash free dry weight values (APHA, 1995).

The material was identified using the following identification keys: Gastropoda after Glöer (2002; 2015); Ephemeroptera after Belfiore (1983), Elliot et al. (1988) and Elliot and Humpesch (2010); Trichoptera after Wallace et al. (1990), Edington and Hildrew (1995), Waringer and Graf (1997, 2011) and Lechthaler and Stockinger (2005); Diptera after Nilsson (1997a), Vallenduuk and Pillot (2007) and Pillot (2009); Coleoptera after Nilsson (1997b). For each taxon the functional feeding group was determined based on: Moog (1995); the Bayerisches Landesamt für Wasserwirtschaft (1996) and Merritt and Cummins (2007).

Statistical analysis

Normality of data was tested with the Kolmogorov-Smirnov test. Sites similar by abundance of scrapers were grouped using cluster analysis with Euclidean distance and the unweighted pair group average method. Difference of average number of scrapers between groups was tested with One-way ANOVA. In order to compare individual and mutual impact of factors on mass of periphyton, we used Pearson's and partial correlation coefficients in correlation analyses and hierarchical linear regression. SIMPER analysis was performed to test differences within faunal composition of the groups of localities. The Shannon diversity index (H') was also calculated (Krebs, 2001). Statistical analyses were performed using PRIMER 7.0 (Clarke and Gorley 2015) and SPSS 19 (SPSS version 19.0 SPSS Inc., Chicago, IL, USA). For all statistical analysis the level of significance p=0.05 was used.

Results

The average concentrations of total nitrogen (N) and total phosphorus (P) (Table 1) are under the maximum allowable concentration for Serbia in this type of rivers (for N < 15 mg dm^{-3}; for P < 0.5 mg dm^{-3} according to Anonymous, 2011; 2012). Hydrochemical data for sites 4 and 10 indicated a moderate anthropogenic impact on water composition. Turbidity varied considerably, from 11.2 NTU to 16.3 NTU. The river section with order 4th to 6th (sites 1 to 4) has a N:P ratio value higher (ranged from 2 to 28) than that of the 7th order (ranged from 0.75 to 1.5).

A total of 33 scrapers species were found (Table 2). Gastropoda accounted for 33.3% (11 species) of the total species number, followed by Ephemeroptera (9 taxa, 27.3%) and Diptera (8 taxa or 24.2%). The highest number of scrapers was detected at the sites 5 and 9. The Shannon's diversity index ranged between 0.05 (site 4) to 1.31 (site 8) (Table 1).
The highest average periphyton biomass was recorded at site 10 (Table 1).

No significant correlation between the density of grazers and periphyton biomass was detected neither regarding data by seasons (spring: p=0.08; summer: p=0.296; autumn p=0.924; winter: p=0.676), neither regarding data by stream order (fourth: p=0.912; fifth: p=0.948; sixth: p=0.244; seventh: p=0.106), neither regarding overall data (whole sample) (p=0.093).
According to scraper density, the studied sites can be divided into three groups (Fig. 2). The first group consisted of five sites (1, 2, 3, 4 and 10) with average density of 21.7 $ind.m^{-2}$ (standard deviation 28.28). The second group includes sites 5 and 9, characterized by the highest average density, 179 $ind.m^{-2}$ (158.32) of scraper assemblage. The third cluster includes three sites (6, 7 and 8) with average density of 86.3 $ind.m^{-2}$ (78.32). ANOVA analysis showed significant differences (F=30.3; p<0.001) for average abundance between

Table 1. Geographical position, characteristic and annual average values of environmental factors: AL – altitude (in m); °N – latitude; °E – longitude; SO – stream order; T – annual average turbidity (in NTU); P – annual average concentration of total phosphorus (in mg/dm^3); N – annual average concentration of total nitrogen (in mg/dm^3); MP – annual average weight of periphyton (mg/m^2); AS – annual average of abundance of scrapers (ind/m^2); H' - Shannon's diversity index.

Sites	Al	°N	°E	SO	T	P	N	MP	AS	H'
1	466	43.27	23.04	4	14.35 (SD: 9.79)	0.02 (SD: 0.01)	0.27 (SD: 0.38)	63.9 (SD: 25.99)	8.62 (SD: 9.12)	0.27
2	445	43.28	22.81	5	16.3 (SD: 10.02)	0.08 (SD: 0.04)	0.28 (SD: 0.12)	93.55 (SD: 76.05)	46.26 (SD: 29.38)	0.36
3	381	43.35	22.81	6	11.97 (SD: 9.73)	0.04 (SD: 0.03)	0.08 (SD: 0.03)	62.81 (SD: 32.2)	43.54 (SD: 35.1)	0.54
4	359	43.30	22.83	6	15.8 (SD: 12.71)	0.1 (SD: 0.04)	0.34 (SD: 0.21)	69.25 (SD: 35.32)	2.27 (SD: 2.80)	0.05
5	289	43.47	22.45	7	13.59 (SD: 15.64)	0.07 (SD: 0.04)	0.1 (SD: 0.06)	65.32 (SD: 33.85)	189.57 (SD: 147.99)	0.52
6	276	43.41	22.36	7	14.9 (SD: 16.55)	0.07 (SD: 0.03)	0.12 (SD: 0.05)	48.15 (SD: 31.68)	75.06 (SD: 111.16)	0.47
7	266	43.46	22.38	7	13.39 (SD: 16.42)	0.07 (SD: 0.04)	0.12 (SD: 0.05)	60.76 (SD: 65.82)	95.24 (SD: 68.94)	1.23
8	233	43.56	22.19	7	11.23 (SD: 8.88)	0.06 (SD: 0.03)	0.08 (SD: 0.04)	58.83 (SD: 23.84)	88.66 (SD: 46.69)	1.31
9	224	43.42	22.11	7	13.00 (SD: 12.43)	0.07 (SD: 0.01)	0.09 (SD: 0.05)	66.97 (SD: 34.97)	168.48 (SD: 173.96)	0.79
10	205	43.41	21.97	7	15.01 (SD: 9.23)	0.11 (SD: 0.03)	0.21 (SD: 0.13)	117.45 (SD: 216.61)	7.94 (SD: 11.04)	0.38

Table 2. List of scraper organisms. Abb – abbrevations.

		Sites									
Species	Abb	1	2	3	4	5	6	7	8	9	10
Gastropoda											
Ancylus fluviatilis O. F. Muller, 1774	Anf	+		+	+	+	+	+	+	+	
Anisus vortex (Linnaeus, 1758)	Anv		+				+				
Fagotia daudebartii (Prevost, 1821)	Fad					+			+	+	+
Planorbarius corneus (Linnaeus, 1758)	Plc		+								
Planorbis planorbis (Linnaeus, 1758)	Plp		+			+	+	+			+
Radix auricularia (Linnaeus, 1758)	Raa		+					+			+
Radix baltica (Linnaeus, 1758)	Rab	+	+	+	+	+	+	+	+	+	+
Stagnicola palustris (O.F. Muller, 1774)	Stp					+	+	+	+	+	+
Theodoxus danubialis (C. Pfeiffer, 1828)	Thd		+			+	+	+	+	+	
Theodoxus fluviatilis (Linnaeus, 1758)	Thf					+	+	+	+	+	
Theodoxus transversalis (C. Pfeiffer, 1828)	Tht					+	+	+	+	+	
Isopoda											
Asellus aquaticus (Linaeus, 1758)	Asa				+						+
Ephemeroptera											
Baetis buceratus Eaton, 1870	Bab		+						+		+
Ecdyonurus dispar (Curtis, 1834)	Ecd			+		+		+			
Ecdyonurus insignis (Eaton, 1870)	Eci	+		+				+	+		+
Ecdyonurus torrentis Kimmins, 1942	Ect							+			
Ecdyonurus venosus (Fabricius, 1775)	Ecv	+		+			+	+	+		
Heptagenia longicauda (Stephens, 1835)	Hel	+									
Heptagenia sulphurea (Muller, 1776)	Hes	+						+			
Rhithrogena germanica Eaton, 1885	Rhg			+							
Rhitrogena semicolorata (Curtis, 1834)	Rhs	+						+			
Trichoptera											
Silo nigricornis (Pictet, 1834)	Sin		+	+				+	+		
Notidobia ciliaris (Linnaeus, 1761)	Noc			+						+	
Sericostoma personatum (Kirby & Spence, 1826)	Sep							+			
Diptera											
Brillia flavifrons (Johansen, 1905)	Brf					+					
Cricotopus annulator Goetghebuer, 1927	Cra							+			
Cricotopus bicinctus (Meigen, 1818)	Crb					+					
Cricotopus trifascia Edwards, 1929	Crt						+				
Diamesa sp. Meigen, 1835	Dis		+				+	+	+	+	
Tvetenia calvescens (Edwards, 1929)	Tvc			+							
Tvetenia discoloripes (Goetghebuer & Thienemann, 1836)	Tvd								+		
Eloeophila maculata (Meigen, 1804)	Elm		+	+				+	+	+	
Coleoptera											
Elmis maugetii Latreille, 1798	Els							+			

scrapers assemblages of site groups I, II, and III. The highest average value of periphyton biomass (81.4 mg m^{-2}; standard deviation 104.1) was recorded in the site group I; average value of periphyton biomass for sites of group II was 66.1 mg m^{-2} (33.7) and for the site group III 55.9 mg m^{-2} (43.4). According to the SIMPER analysis, *Radix baltica* is the characteristic representative for site group I, while *Stagnicola palustris* is characteristic for the assemblages of groups II and III (Table 3).

In order to recognize a pattern between nutrition, turbidity, densities of scrapers, diversity and periphyton in the different groups, correlation analysis was performed. The concentration of total phosphorus was statistically significantly correlated with the periphyton biomass for the localities of the site group I (p=0.023; Table 4; Model 3). On the other hand, concentration of total nitrogen was not significantly correlated with periphyton in any of the all three site groups (Table 4; Model 4). Significant correlation between turbidity and periphyton biomass was detected for the site group II (p=0.031; Table 4; Model 5).

The correlation analysis of the periphyton biomass and densities of scrapers confirmed a lack of correlation between these two parameters for the site group I (R=-0.042; p=0.747) and site group III (R=-0.24; p=0.161). On the other hand, we found statistically significant negative correlation (R=-0.45; p=0.028) (Table 4; Model 1) for the localities of group II.

Since a statistically significant negative correlation be-

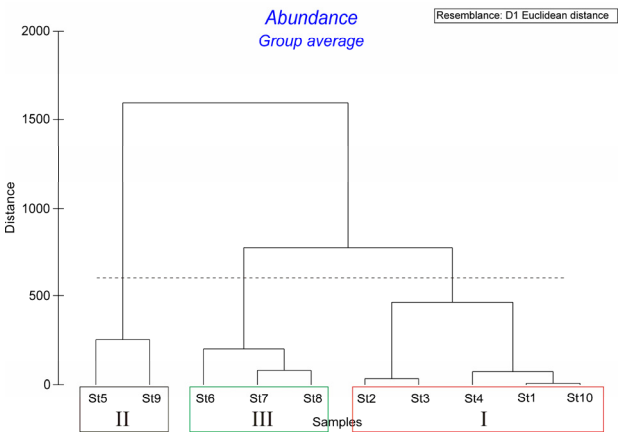

Figure 2. Similarity distance between the sites (St) in the groups I, II, and III based on the scrapers abundance of the investigated localities.

Table 3. Results of SIMPER analysis for scrapers assemblage of site groups I, II and III.

Group I - average similarity=21.08						Groups I and II - average dissimilarity=89.69						
Group II - average similarity=84.23						Groups I and III - average dissimilarity=77.56						
Group III - average similarity=64.50						Groups II and III - average dissimilarity=50.57						
Species	Av. Abund	Av. Sim	Sim /SD	Contrib %	Cum %	Species	Av. Abund	Av. Abund	Av. Sim	Sim/ SD	Contrib %	Cum %
group I						groups I and II						
Rab	72.80	17.09	0.80	81.10	81.10	Stp	0.60	621.00	70.42	8.20	78.52	78.52
Asa	2.20	1.78	0.32	8.44	89.53	Rab	72.80	96.50	8.90	3.06	9.92	88.44
Anf	0.60	0.60	0.46	2.86	92.39	Thd	0.20	26.50	3.01	7.55	3.36	91.80
group II						Groups I and III						
Stp	621.00	68.90	/	81.80	81.80	Stp	0.60	166.33	36.96	2.27	47.66	47.66
Rab	96.50	11.40	/	13.53	95.34	Rab	72.80	138.33	22.24	1.54	28.68	76.34
						Thd	0.20	21.67	4.50	1.03	5.80	82.13
						Anf	0.60	9.67	1.95	2.65	2.51	84.64
						Tht	0.00	9.00	1.87	1.06	2.41	87.05
						Dis	0.40	6.33	1.28	1.39	1.65	88.70
						Thf	0.00	6.00	1.27	1.31	1.64	90.34
group III						groups II and III						
Stp	166.33	34.38	9.09	53.29	53.29	Stp	621.00	166.33	38.56	5.98	76.25	76.25
Rab	138.33	23.41	3.77	36.30	89.59	Rab	96.50	138.33	4.83	1.18	9.54	85.79
Anf	9.67	1.85	3.80	2.86	92.45	Tht	20.00	9.00	1.70	1.29	3.35	89.14
						Thd	26.50	21.67	1.60	2.11	3.17	92.32

Table 4. Values of Pearson simple (R) and partial (R_{part}) correlation coefficients between mass of periphyton and predictors and their significances: Sc-scrapers; H-Shannon's diversity index.

		Group I		Group II		Group III	
		R	p	R	p	R	p
Model 1 with Sc as predictor	Sc	-0.042	0.747	-0.447	**0.028**	-0.238	0.161
Model 2 with H as predictor	H	-0.074	0.576	0.236	0.266	0.226	0.185
Model 3 with P as predictor	P	0.293	**0.023**	-0.201	0.344	0.070	0.683
Model 4 with N as predictor	N	-0.069	0.601	0.284	0.179	-0.119	0.489
Model 5 with T as predictor	T	-0.022	0.867	-0.441	**0.031**	-0.086	0.617
		R_{part}	p	R_{part}	p	R_{part}	p
Model 6 with Sc and H as predictors	Sc	-0.018	0.892	-0.421	**0.045**	-0.287	0.094
	H	-0.063	0.637	0.171	0.436	0.278	0.106
Model 7 with Sc and P as predictors	Sc	-0.005	0.973	-0.433	**0.039**	-0.230	0.183
	P	0.290	**0.026**	-0.160	0.465	-0.032	0.856
Model 8 with Sc and N as predictors	Sc	-0.062	0.641	-0.384	0.071	-0.314	0.066
	N	-0.082	0.536	0.141	0.521	-0.241	0.164
Model 9 with Sc and T as predictors	Sc	-0.042	0.755	-0.502	**0.015**	-0.223	0.198
	T	-0.020	0.879	-0.497	**0.016**	-0.005	0.978

tween density of grazers and mass of periphyton is noted only in group II, we consider the influence of nutrients, turbidity and diversity on this correlation only in that group. Total phosphorus decreases the influence of scrapers on periphyton biomass, but this effect still remains statistically significant (from p=0.028 Table 4. Model 1; to p=0.039 Table 4. Model 7). On the other hand, total nitrogen decreases the influence of scrapers on periphyton biomass to statistically insignificant level (from p=0.028 Table 4. Model 1; to p=0.071 Table 4 Model 8). Diversity also decreases influence of scrapers on periphyton biomass (from p=0.028 Table 4. Model 1; to p=0.045 Table 4 Model 6). On the contrary, turbidity increases the effect (from p=0.028 Table 4. model 1; to p=0.015 Table 4. Model 9) that scrapers have on periphyton biomass.

Discussion

Our research has confirmed that grazer density has an effect on periphyton biomass. We consider the terms periphyton, litophyton, biofilm and aufwuchs as synonyms, all referring to the algal, bacterial and fungal species complex that inhibit stream benthic substrates (Feminella and Hawkins 1995). Moreover, we consider only macrozoobenthos consumers widely recognized as periphyton grazers. The results of our study is consistent with the results observed in many other studies (e.g., Feminella and Hawkins 1995; Steinman 1996; Liess and Hillebrand 2004; Gjerlov and Richardson 2010; Mahdy et al. 2015; Braccia et al. 2014) which showed that grazers in their natural compartment usually reduce periphyton biomass. Most of those studies were restricted to certain taxonomic groups (Liess and Kahlert 2009) or even individual species (Villanueva et al. 2004, Moulton et al. 2015).

Our study showed that the grazing pressure is significant only with higher density of scraper organisms. Statistically significant influence of grazer density on periphyton biomass was recorded only in the site group II, characterized by the highest scraper average density. On the other hand, it

seems that grazer effects decreased with increasing algal biomass, as the scraper density was lowest in the site group I characterized with the highest periphyton biomass. This is not consistent with Hillebrand (2009), who showed that grazer effects increased with increasing algal biomass. However, some studies (e.g., Hillebrand and Kahlert 2001) showed no relation between grazer impact and grazer density, i.e. grazer impact was low in some experiments, although grazer densities were high.

The results of SIMPER analysis have shown that the scrapers assemblage at the localities of the group II was mostly determined by the species *Stagnicola palustris*. Moreover, we have concluded based on SIMPER analysis that harvesting effect of scrapers was stronger in assemblage more dominated by *Stagnicola palustris* (81.8% in site group II vs. 53.23% in group III). Our study shows that Shannon diversity index negatively corresponds with the impact of scrapers on the periphyton biomass, indicating that more diverse assemblages decrease the negative impact exerted by grazers on periphyton biomass.

The results of our study also showed that increasing scraper density did not affect the quantitative variation of periphyton biomass. The mass of periphyton happens to be most stable at the localities of group II.

Many studies demonstrated that the negative effect of scrapers densities on periphyton varies in relation with nutrients (McCormick and Stevenson 1991; Liess and Kahlert 2009). Hillebrand and Kahlert (2001) showed in laboratory conditions that nutrient addition can greatly reduce the direct negative effect that grazers have on primary producers. This corresponds with our results obtained in *in situ* conditions.

Our study revealed that high level of total nitrogen, and to a lesser extent of total phosphorus, reduce the negative impact that scrapers have on periphyton. Some studies have shown that when density of grazers is controlled by intense predation, nutrient addition can increase algal stock (Biggs 2000; Liess and Hillebrand 2004). In our case, an increase in nutrients lead to increasing of periphyton biomass and decreasing the predation pressure on periphyton by scrapers. It is likely that at the studied localities scrapers were controlled more by predation then by food resources. That finding corresponds to the results of Winkelman et al. (2007), who showed that grazers are generally more vulnerable to fish predation because they are visible to the predators during their feeding on stone surfaces.

As regarding nutrients, turbidity has played an antagonistic role. Turbidity increased the impact of scrapers on the periphyton biomass to the more significant level. The model with scrapers as the dingle predictor had a significance of p=0.028. The model with turbidity added as predictor had a significance of p=0.015. This might be partially explained by the cascading effect of predators on prey populations, owing to the reduced efficiency of predators in turbid water (Van de Meutter et al. 2005), or an increase in periphyton mass caused by the control of herbivores by their predator (Hairston et al. 1960; Hillebrand and Kahlert 2001; Liess and Hillebrand 2004).

Table 4 shows slight differences between correlation coefficients of periphyton and nutrients (P-Model 3 and N-Model 4) and turbidity (Model 5), on one side, and their partial correlation coefficients when abundance of scrapers is involved (Models 7, 8 and 9), on the other. It means that scrapers density has poor influence on the relations between periphyton biomass and nutrients (P and N) and turbidity.

Liess and Hillebrand (2004) found that in most studies grazing reduced periphyton biomass, implying that the direct negative effect was stronger than the indirect positive effect through habitat facilitation. Studies where the indirect positive effects outweigh the direct negative effects were mostly restricted to situations with low grazer density (Steinman 1996; Liess and Hillebrand 2004). Our study showed that scrapers density has poor influence on the relations between periphyton biomass and nutrients (P and N) and turbidity (Table 4). This implies that the indirect positive influence through nutrient regeneration by grazers was not pronounced.

Analysing the ratio of the whole grazer assemblage and mass of periphyton (most previous studies were restricted to a certain taxonomic group or even individual species) under *in situ* conditions (most previous studies were restricted to laboratory research), we have concluded that this ratio was caused neither by season nor by river order. The abundance of grazers was important for the intensity of pressure on periphyton. Only under a certain density of grazers there is a statistically significant negative impact. The pressure by grazers may be modified both by abiotic (concentration of total N, total P, turbidity) and biotic factors (diversity). Increase in amount of nutrients leads to decrease in this type of pressure, while total N has the more significant impact. Turbidity has the opposite effect to nutrients on grazer pressure of periphyton. The increased diversity leads to decrease in negative impact.

This topic should definitively get more attention as increase in amount of periphyton is one of the recognized signs of eutrophication, which has been a common problem in aquatic ecosystems throughout the world in the last decades.

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