Aquatic Macroinvertebrates of the Sava River

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Abstract The objective of this chapter is to present the data on aquatic macroinvertebrate communities along the Sava River, based on investigation performed during 2011 and 2012 at 12 sampling sites within the sector between Vrhovo (Slovenia) and Belgrade (confluence to the Danube). During our study 227 macroinvertebrate taxa were recorded in the Sava River. Having in mind that upper stretch of the Sava River was not covered by this work (alpine and subalpine stretch), as well as based on the review of previous works on the macroinvertebrate fauna of the Sava River, more than 300 species will be confirmed for the Sava River. The data on the distribution of aquatic macroinvertebrates revealed five different stretches—alpine, subalpine, Upper Sava plain, Middle Sava and Lower Sava. Physical habitat degradation, pollution and pressure caused by biological

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invasions were found to be the main factors of endangerment of aquatic macroinvertebrate fauna diversity. There is an obvious need for further investigation of the Sava River in order to complete the data on aquatic macroinvertebrates and to provide the basis for accurate assessment of environmental status of the river.

Keywords Aquatic macroinvertebrates • Sava River • Community structure • Species richness

1 Introduction

Aquatic macroinvertebrates are diverse group of organisms that spent their entire (e.g. aquatic worms, leeches, molluscs or crustaceans) or a part of life cycle (e.g. some orders of insects, such mayflies or caddis flies) in water. The term macroinvertebrates describes animals that have no backbone and can be seen with the naked eye. In general, the group comprises species larger that than 0.5 mm (could be collected by mesh with opening size of 0.5 mm). Smaller animals that pass through such a sieve are called meiozoobenthos. In regard to size, aquatic macroinvertebrates include small organisms such as tiny aquatic worms (Oligochaeta) or different insect larvae, but also some species that could be larger than 10 cm, such as freshwater mussels (Bivalvia: Unionidae) or crayfish species (Crustacea: Decapoda).

Other names are also commonly used for this group of animals, such as macrozoobenthos or macrozoobenthon. We prefer to use the formulation aquatic macroinvertebrates rather than other mentioned terms which denote that organisms live on the bottom of water bodies, which is not the case. The group also includes animals that live on the aquatic vegetation, submerged objects or water surface.

Aquatic macroinvertebrates comprise different taxonomic assemblages and it is not taxonomic, but ecological group. In some habitats aquatic macroinvertebrates occur in a great variety of species and in large quantities, and thus, this group plays an important role in energy cycling and mass balance in aquatic ecosystems and is represented with wide scale of functional feeding guilds. Macroinvertebrates inhabit all types of waters, from fast-flowing mountain streams of different sizes to large lowland rivers, lakes and ponds. They play an important role in maintaining ecosystem health, as they are consumers of organic matter, and thus help to remove nutrients from water systems. They also provide a food source for a variety of predators such as invertebrates, fish, amphibians and birds.

The aim of this paper is to present the diversity of macroinvertebrate communities of the Sava River. Also, attention was focussed to nonindigenous taxa, since mass occurrence of invasive alien species could significantly influence native biodiversity and could disturb the functionality of aquatic ecosystems.

2 Previous Investigations

Despite importance of the Sava as large transboundary river, macroinvertebrate communities of its main course have not been systematically studied recently. The most comprehensive research of macroinvertebrates of the Sava River was carried out by Matoničkin et al. [1]. The investigation was performed in period 1966–1975 on 41 sampling sites covering the entire length of the Sava River, including the Sava Dolinka and Sava Bohinjka (the Sava River is formed on the place of confluence of those two rivers). The authors [1] provided extensive biocenological and saprobiological analyses. Also, Matoničkin et al. [1] presented the literature review on the investigation of the Sava River and main tributaries up to 1970s and concluded that only the results of taxonomical investigations limited to individual taxa groups are available. Since the comprehensive study of Matoničkin et al. [1], published results concerning macroinvertebrates of the Sava were mostly restricted to limited stretches of the river [2–11]. Recently, Paunović et al. [12] presented the results of investigation on macroinvertebrate community along 622 km of the Sava River, between Martinska Ves (downstream Zagreb) and confluence to the Danube. The most comprehensive study of macroinvertebrates that involved the Sava River Basin in Slovenia was provided by Urbanič [13].

Based on the review of previous investigation, we can conclude that still limited information is available on aquatic macroinvertebrate communities along the Sava River. The comparable high-quality data is necessary not only for research purposes but also for design of proper management of water resources within the basin area.

3 Study Area

The detailed description of the Sava River Basin is provided in Simić et al. [14] of this volume. The Sava flows from the mountain region in Slovenia to the lowlands of Croatia, Bosnia and Herzegovina and Serbia and confluences of the Danube in Belgrade (river km 1171). It is the largest tributary of the Danube. Due to the different influences along the course caused by diverse surroundings (relief, geological substrate, altitude, bad slope and climate), this mighty river is heterogeneous concerning overall environmental conditions. Due to the geographic position, diverse climate, petrographic and pedological variety and orographic characteristics, the Sava River Basin is one of the most complex regions in Europe concerning the distribution of plants and animals [15]. Consequently, the investigation on the distribution of aquatic macroinvertebrates along the Sava River is complex issue.

4 Material and Methods

The overview of aquatic macroinvertebrates of the Sava River was performed based on recent investigations in 2011 and 2012. In addition, the literature data were used to complement our survey data.

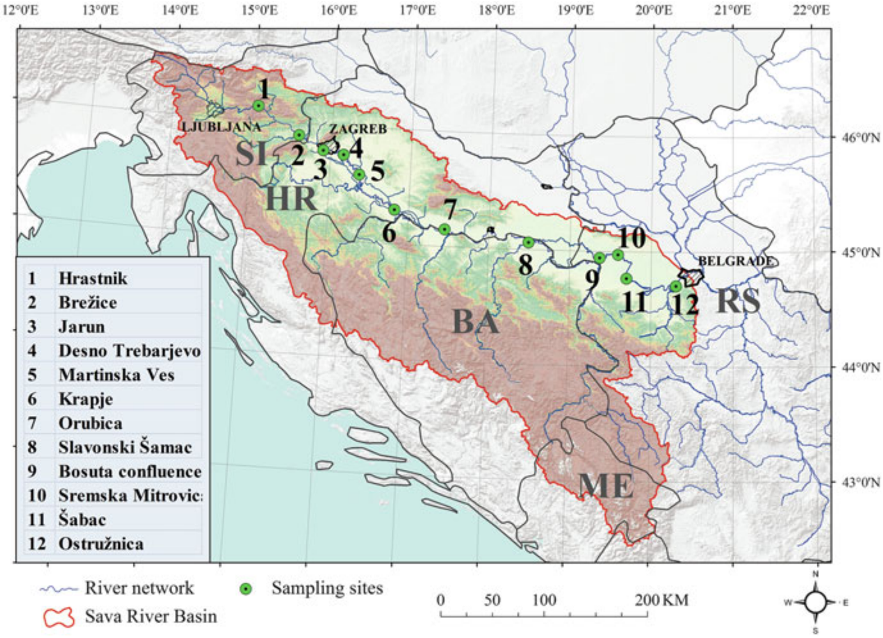


Fig. 1 Sampling sites along the Sava River—2011 and 2012 surveys

Macroinvertebrate sampling was performed during September (low water conditions) 2011 and 2012 at 12 sampling sites (Fig. 1). Low-water condition period was selected since most microhabitats on river bank are available for sampling in that period and in order to get comparable data with recent investigations on the Danube that were performed in same period of the year (Joint Danube Survey 1, 2 and 3 [16–18], and AquaTerra Danube Survey—[19–22]).

Samples were collected using hand nets (mesh size 500 μm) on the area of 0.0625 m^2, in a shallow bank region (up to the depth of 1.5 m), from all available types of substrate (stones, gravel, sand, mud, as well as from artificial structures—groynes, longitudinal dykes and revetments). During the material collection, the relative contribution to each microhabitat was taken into the consideration and the number of samples collected from particular microhabitat within each reach corresponds to the relative contribution of this microhabitat to the substrate of the assessed river reach (10 % = 1 sample). The fauna attached to stone surfaces was collected with tweezers and, if necessary, scraped with a brush. Freediving was also performed to collect mussels.

Approximate length of investigated reach at each sampling site was 100 m of the shore region.

Qualitative (number of taxa) composition and quantitative composition (relative abundance) of macroinvertebrate community were discussed. Relative abundance was analysed as the mean number of taxa in ten replicate samples and expressed as percentage participation of each taxa group.

Asterics software Version 3.3.1. [23] was applied for calculating community structure in regard to saprobic preference, substrate type, river zonation and feeding-type composition, while the autecological data are used from AQEM [23].

5 Results and Discussion

5.1 Qualitative, Quantitative and Functional Analyses of Macroinvertebrate Community

Based on the examined material collected during 2011 and 2012 survey, 227 macroinvertebrate taxa were recorded in the Sava River, within the sector of investigation (Tables 1 and 2).

Aquatic insects were found to be the principal component of the community with 157 recorded species. Among insects, order Diptera (true flies) was characterised by larger number of identified species (70) with 52 recorded taxa belonging to family Chironomidae (chironomids or nonbiting midges). Insect's orders Trichoptera (caddis flies), Coleoptera (beetles) and Ephemeroptera (mayflies) were also found to be important element of the macroinvertebrate community in regard to taxa richness with 35, 23 and 15 identified species, respectively.

Considerable taxa richness was recorded among molluscs (27—Gastropoda 19 and Bivalvia 8) and annelids (24—Oligochaeta 18, Hirudinea 5 and Polychaeta 1). Based on our results, other macroinvertebrate groups of the investigated stretch of the Sava River contain less species.

Analysis of the molluscs fauna along the Sava in regard to relative abundance are *Theodoxus danubialis* (33.82 %) and *Lithoglyphus naticoides* (33.12 %), followed by *Bithynia tentaculata* (8.05 %) and *Esperiana daudebartii acicularis* (7.59 %), while percentage participation of the other taxa in the mollusc community was significantly lower.

Bivalves *Corbicula fluminea* and *Unio pictorum*, together with two snail species *Lithoglyphus naticoides* and *Bithynia tentaculata*, were the most frequent representatives of molluscs on investigated stretch.

It is important to emphasise that stable population of freshwater mussel *Unio crassus* (Fig. 2) was found in the middle and part of the lower stretch of the Sava River—sites 5–10. The species is included in Annexes 2 and 4 of the EU Habitat Directive and is considered as rare and endangered species in many European countries according to IUCN classification [24, 25] This fact indicates the importance of the Sava River in respect to protection of *U. crassus*.

The number of recorded taxa per locality (Fig. 3) varied between 28 (Brežice, sampling site 2) and 106 (Martinska Ves, sampling site 5). Considerable taxa richness was detected for sites: Orubica (site 7, 86 taxa) and Jarun (site 3, 81 taxa).

During our investigations, the change of macroinvertebrate community related to alter of general river type is recorded. Beside the above-mentioned change in the total number of recorded taxa, the change along the river continuum is also illustrated by other community patterns. Thus, the decrease of the number of mayflies (ordo

Table 1 The list of recorded macroinvertebrate taxa

Spongillidae Gen. sp.
Nematoda
Turbellaria
Dugesia lugubris (Schmidt, 1861)
Dugesia tigrina (Girard, 1850)*
Planaria torva (Müller, 1774)
Polycelis tenuis (Ijima, 1884)
Oligochaeta
Branchiura sowerbyi (Beddard, 1892)*
Eiseniella tetraedra (Savigny, 1826)
Embolocephalus velutinus (Grube, 1879)
Enchytraeidae
Isochaetides michaelseni (Lastockin, 1936)
Limnodrilus claparedeanus (Ratzel, 1868)
Limnodrilus hoffmeisteri (Claparède, 1862)
Limnodrilus udekemianus (Claparède, 1862)
Nais bretscheri (Michaelsen, 1899)
Nais communis (Piguet, 1906)
Nais elinguis (Müller, 1774)
Ophidonais serpentina (O.F. Müller, 1773)
Potamothrix hammoniensis (Michaelsen, 1901)
Propappus volki (Michaelsen, 1916)
Psammoryctides barbatus (Grube, 1861)
Stylaria lacustris (Linnaeus, 1767)
Stylodrilus heringianus (Claparède, 1862)
Tubifex tubifex (Müller, 1774)
Hirudinea
Glossiphonia complanata (Linnaeus, 1758)
Erpobdella octoculata (Linnaeus, 1758)
Erpobdella lineata (O. F. Müller, 1774)
Helobdella stagnalis (Linnaeus, 1758)
Piscicola geometra (Linnaeus, 1761)
Polychaeta
Hypania invalida (Grube, 1860)*
Gastropoda
Acroloxus lacustris (Linnaeus, 1758)
Borysthenia naticina (Menke, 1845)
Bithynia tentaculata (Linnaeus, 1758)
Esperiana daudebartii acicularis (A. Ferussac, 1823)
Esperiana esperi (A. Ferussac, 1823)
Ferrissia clessiniana (Jickeli, 1882)
Gyraulus albus (Müller, 1774)
Gyraulus laevis (Alder, 1838)

(continued)

Table 1 (continued)

Gyraulus crista (Linnaeus, 1758)
Holandriana holandrii (Pfeiffer, 1828)
Lithoglyphus naticoides (Pfeiffer, 1828)
Physella acuta (Draparnaud, 1805)*
Planorbis planorbis (Linnaeus, 1758)
Radix auricularia (Linnaeus, 1758)
Radix labiata (Rossmässler, 1835)
Theodoxus danubialis (C. Pfeiffer, 1828)
Theodoxus fluviatilis (Linnaeus, 1758)
Viviparus acerosus (Bourguignat, 1862)
Valvata cristata (O. F. Müller, 1774)
Bivalvia
Corbicula fluminea (O. F. Müller, 1774)*
Dreissena polymorpha (Pallas, 1771)*
Sinanodonta woodiana (Rea, 1834)*
Sphaerium rivicola (Lamarck, 1818)
Pisidium sp.
Unio crassus (Philipsson, 1788)
Unio pictorum (Linnaeus, 1758)
Unio tumidus (Philipsson, 1788)
Crustacea
Isopoda
Asellus aquaticus (Linnaeus, 1758)
Amphipoda
Corophium curvispinum (Sars, 1895)*
Dikerogammarus haemobaphes (Eichwald, 1841)*
Dikerogammarus villosus (Sowinsky, 1894)*
Gammaridae
Mysidae
Decapoda
Astacus leptodactylus (Eschscholtz, 1823)
Orconectes limosus (Rafinesque, 1817)
Odonata
Calopteryx splendens (Harris, 1782)
Coenagrionidae Gen. sp.
Cercion lindeni (Sélys, 1840)
Coenagrion mercuriale (Charpentier, 1840)
Gomphus flavipes (Charpentier, 1825)
Gomphus vulgatissimus (Linnaeus, 1758)
Ischnura elegans (Vander Linden 1820)
Onychogomphus forcipatus (Linnaeus, 1758)
Platycnemis pennipes (Pallas, 1771)
Pyrrhosoma nymphula (Sulzer, 1776)

(continued)

Table 1 (continued)

Ephemeroptera
Baetis fuscatus (Linnaeus, 1761)
Baetis lutheri (Müller-Liebenau, 1967)
Baetis rhodani (Pictet, 1843)
Baetis vernus (Curtis, 1834)
Brachycentrus subnubilus (Curtis, 1834)
Caenis luctuosa (Burmeister, 1838)
Cloeon dipterum (Linnaeus, 1761)
Cloeon simile (Eaton, 1870)
Cloeon sp.
Ephemera danica (Müller, 1764)
Ephemerella sp.
Heptageniidae
Heptagenia sulphurea (Müller, 1776)
Heptagenia sp.
Torleya major (Klapálek, 1905)
Neuroptera
Sisyra fuscata (Fabricius, 1793)
Trichoptera
Athripsodes albifrons (Linnaeus, 1758)
Athripsodes sp.
Ceraclea fulva (Rambur, 1842)
Ceraclea sp.
Cheumatopsyche lepida (Pictet, 1834)
Cyrnus trimaculatus (Curtis, 1834)
Ecnomus tenellus (Rambur, 1842)
Ecnomus sp.
Holocentropus stagnalis (Albadra, 1864)
Holocentropus sp.
Hydropsyche angustipennis (Curtis, 1834)
Hydropsyche bulgaromanorum (Malicky, 1977)
Hydropsyche contubernalis (McLachlan, 1865)
Hydropsyche exocellata (Dufour, 1841)
Hydropsyche fulvipes (Curtis, 1834)
Hydropsyche pellucidula (Curtis, 1834)
Hydropsychidae spp.
Hydropsyche sp.
Hydroptila vectis (Curtis, 1834)
Hydroptila sp.
Leptoceridae
Lepidostoma hirtum (Fabricius, 1775)
Mystacides sp.
Neureclipsis bimaculata (Linnaeus, 1758)

(continued)

Table 1 (continued)

Oecetis notata (Rambur, 1842)
Oecetis sp.
Polycentropodidae
Polycentropus flavomaculatus (Pictet, 1834)
Psychomyia pusilla (Fabricius, 1781)
Psychomyia sp.
Rhyacophila sp.
Setodes punctatus (Fabricius, 1793)
Trichoptera Gen. sp.
Tinodes pallidulus (McLachlan, 1878)
Tinodes sp.
Collembola
Collembola
Coleoptera
Dytiscidae
Dryopidae Gen. sp. Lv.
Elmidae
Elmis aenea (Müller, 1806)
Esolus angustatus (Müller, 1821)
Hydrophilidae
Hydrophilus sp.
Hydroporus sp. Lv.
Hemerodromia unilineata Zetterstedt, 1842
Limnius volckmari (Panzer, 1793)
Oulimnius troglodytes (Gyllenhal, 1827)
Oulimnius tuberculatus (Müller, 1806)
Oulimnius sp.
Orectochirus villosus (Müller, 1776)
Macronychus sp. Ad.
Normandia nitens (Müller, 1817)
Noterus sp.
Patambus sp.
Pomatinus substriatus Ad. (Müller, 1806)
Potamophilus acuminatus (Fabricius, 1772)
Polycentropodidae Gen. sp.
Riolus cupreus (Müller, 1806)
Stenelmis canaliculata (Gyllenhal, 1808)
Diptera
Athericidae
Atherix ibis (Fabricius, 1789)

(continued)

Table 1 (continued)

Antocha sp.
Ceratopogonidae
Chaoboridae
Chelifera sp.
Ephydridae
Hemerodromia unilineata (Zetterstedt, 1842)
Ibisia marginata (Fabricius, 1781)
Micronecta sp.
Micronecta scholtzi (Fieber, 1860)
Oxycera sp.
Stratiomyidae
Scatella sp.
Chironomidae
Ablabesmyia longistyla (Fittkau, 1962)
Beckidia zabolotzkyi (Goetghebuer, 1938)
Dicrotendipes nervosus (Staeger, 1839)
Demicryptochironomus vulneratus (Zetterstedt, 1838)
Cricotopus gr. sylvestris sensu (Hirvenoja, 1973)
Cricotopus trifascia (Edwards, 1929)
Cricotopus triannulatus agg. sensu (Moller Pillot, 1984)
Cricotopus bicinctus (Meigen, 1818)
Cryptochironomus sp.
Cryptotendipes sp.
Conchapelopia melanops (Meigen, 1818)
Cladotanytarsus spp.
Cladopelma gr. laccophila
Chironomus spp.
Harnischia sp.
Lipiniella araenicola (Shilova, 1961)
Microchironomus tener (Kieffer, 1918)
Micropsectra bidentata (Goetghebuer, 1921)
Microtendipes pedellus agg. sensu (Moller Pillot, 1984)
Nanocladius dichromus (Kieffer, 1906)
Nanocladius bicolor agg.
Orthocladius (*Orthocladius*) spp.
Parametriocnemus stylatus (Spaerck, 1923)
Paratanytarsus dissimilis (Johannsen, 1905)
Paratanytarsus austriacus (Kieffer, 1924)
Paratendipes nubilus (Meigen, 1830)
Procladius sp.

(continued)

Table 1 (continued)

Parachironomus frequens (Johannsen, 1905)
Parachironomus gr. arcuatus
Paralauterborniella nigrohalteralis (Malloch, 1915)
Paratendipes albimanus (Meigen, 1818)
Paratrichocladius rufiventris (Meigen, 1830)
Phaenopsectra sp.
Polypedilum nubeculosum (Meigen, 1804)
Polypedilum cultellatum (Goetghebuer, 1931)
Polypedilum convictum (Walker, 1856)
Polypedilum scalaenum (Schrank, 1803)
Polypedilum albicorne (Meigen, 1838)
Potthastia gaedii (Meigen, 1838)
Pseudochironomus prasinatus (Staeger, 1839)
Rheotanytarsus spp.
Rheopelopia sp.
Rheocricotopus chalybeatus (Edwards, 1929)
Rheocricotopus effusus (Walker, 1856)
Stictochironomus maculipennis (Meigen, 1818)
Synorthocladius semivirens (Kieffer, 1909)
Thienemanniella majuscula (Edwards, 1924)
Tvetenia discoloripes (Goetghebuer and Thienemann, 1936)
Tanypus punctipennis (Meigen, 1818)
Tanytarsus spp.
Thienemanniella majuscula (Edwards, 1924)
Xenochironomus xenolabis (Kieffer, 1916)
Empididae
Hexatoma sp.
Simuliidae
Tipula sp.
Heteroptera
Aphelocheirus aestivalis (Fabricius, 1794)
Micronecta sp.
Neuroptera
Sisyra fuscata (Fabricius, 1793)
Hydracarina
Hydrachnidia Gen. sp.
Bryozoa
Plumatellidae

Table 2 Number of species per taxa group

Group	No. of taxa
Phylum Porifera192978_Talapatra	1
Phylum Bryozoa	1
Phylum Nematoda	1
Phylum Platyhelminthes	
Class Turbellaria	4
Phylum Annelida	24
Oligochaeta	18
Hirudinea	5
Polychaeta	1
Phylum Mollusca	27
Gastropoda	19
Bivalvia	8
Phylum Arthropoda	
Subphylum Crustacea	7
Class Arachnida	
Hydracarina	1
Class Collembola	1
Class Insecta	157
Odonata	10
Ephemeroptera	15
Neuroptera	1
Trichoptera	35
Coleoptera	23
Diptera	70
Diptera: other than Chironomidae	18
Diptera: Chironomidae	52
Heteroptera	2
Neuroptera	1

Fig. 2 *Unio crassus* collected from the Sava River in Sremska Mitrovica (site 10) (photo by Paunović 2012)

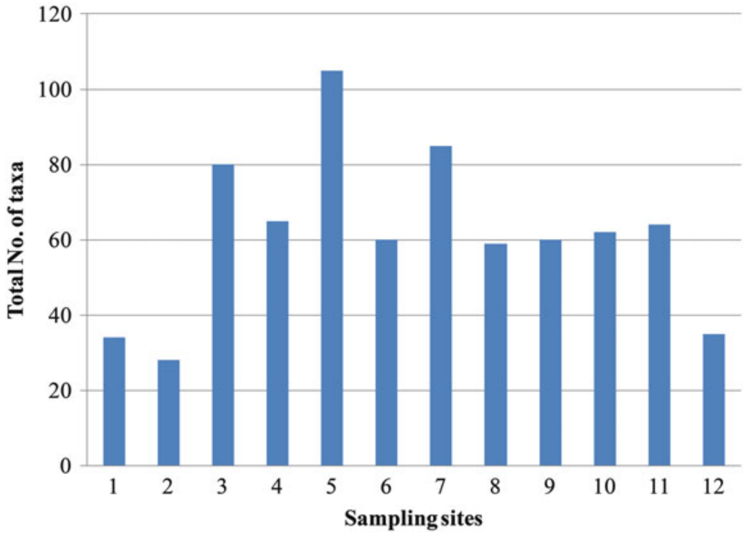


Fig. 3 Number of recorded taxa per locality

Ephemeroptera) and caddis flies (ordo Trichoptera) taxa along the watercourse (Fig. 4) clearly reflects change in the overall character of the river. Those insect orders are generally characterised by occurrence of higher number of species in the middle and upper stretches of the rivers in comparison to lower stretches [26]. Flat worms, Turbellaria, were detected on the sites 1–8. The number of taxa among the groups that are characteristic for large lowland rivers (aquatic worms, Oligochaeta; bivalves, Bivalvia; snails, Gastropoda; true flies, Diptera; and dragonflies and damselflies, Odonata) is larger at sites 3–12 in comparison to sites 1 and 2.

Lithoglyphus naticoides (Mollusca: Gastropoda) and *Limnodrilus hoffmeisteri* (Annelida: Oligochaeta) were found to be the most frequent and abundant species within the investigated stretch. Aquatic worms *Potamotrix hammoniensis* and *Psammoryctides barbatus* were also recorded along the entire sector of investigation.

In regard to quantitative composition of the macroinvertebrate community, gradual changes were also detected along the Sava River, with the similar pattern as detected for qualitative composition (Fig. 5). Thus, the general decline of percentage participation of caddis flies (Trichoptera) and Turbellaria in the total macroinvertebrate community was observed from upper to lower stretch. Further, the increase of percentage participation of aquatic worms (Oligochaeta) and molluscs (Gastropoda and Bivalvia) was recorded within the sites 4–12 in comparison to sites 1–3.

According to ecological classification of taxa in regard to saprobic valence of Moog [27], beta-mesosaprobic taxa are the most numerous with 23.75 % in respect to the total number of identified species. Almost 15 % of the recorded taxa could be characterised as typical for rivers with high organic load (alpha-mesosaprobic and polysaprobic indicators). Only 2.59 % of recorded taxa could be characterised as sensitive to organic pollution (xeno- and oligosaprobic indicators). For the rest of

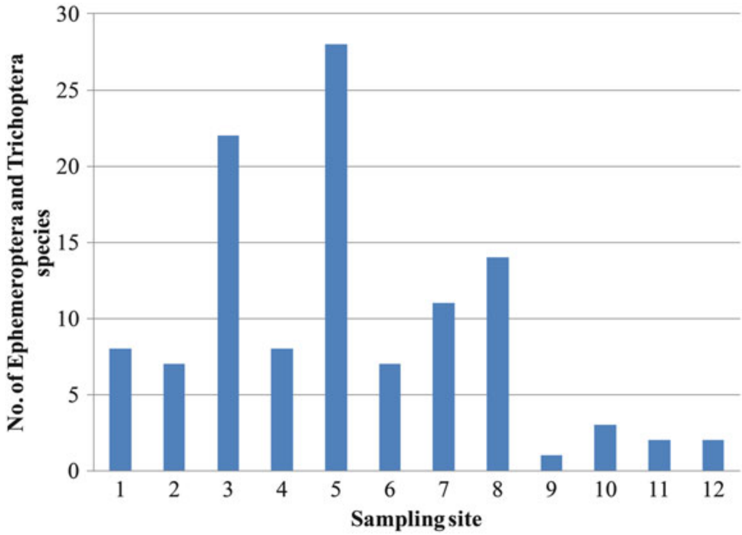


Fig. 4 Number of mayfly (Ephemeroptera) and caddis fly (Trichoptera) species at sampling sites

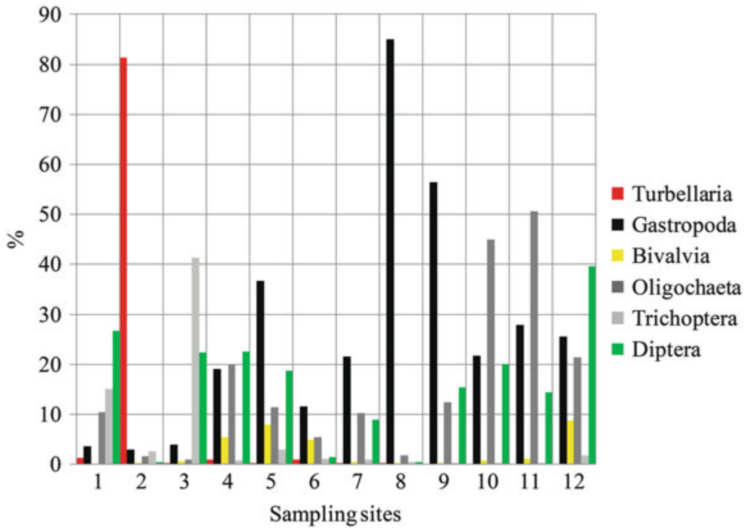
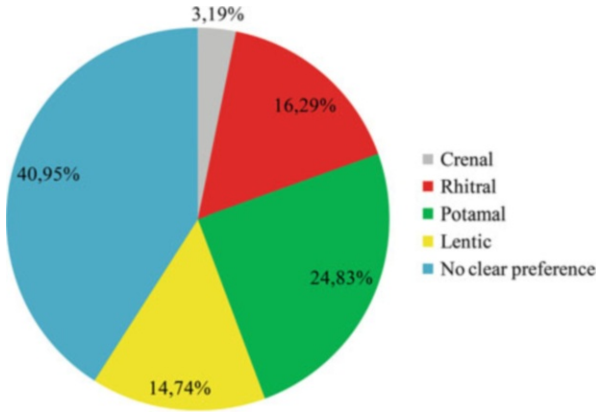


Fig. 5 Percentage participation of the main faunistic groups in the total macroinvertebrate community at sampling sites

the species (52.59 %), there is no data to classify them in regard to saprobic tolerance [23]. This finding indicates that organic pollution is a significant pressure that influences the macroinvertebrate community along the investigated stretch.

In regard to a preferred zone within the river continuum (longitudinal zonation), the greatest proportion of recorded species (24.83 %) is characteristic for the lower river stretches (hypopotamal, epipotamal, metapotamal)—potamal species [23, 26,

Fig. 6 Proportion of species with different preferences to particular zone of the river continuum



27] (Fig. 6). The rest of the taxa prefer lentic zones (standing water) (14.74 %) or fast-flowing stretches (rhitral zone—16.29 %). Small amount of taxa is characteristic for source region of the river (Crenal), while information about preferred zone for smaller number of registered species is not available (9.3 %).

The majority of the identified species (19.96 %) are adapted to the river bed consisted of gravel and stones [23, 27], while 16.90 % of the total number of taxa is characteristic for substrate types typical of large lowland rivers (substrate types pelal, psammal and argillal). For other identified species, there is not enough information to determine clear preference for particular substrate type [23].

In regard to functional feeding types, the greatest part of recorded species belongs to functional groups characteristic to be dominant in the lower stretches of the rivers (Fig. 7)—gatherers/collectors (25.40 %) and filtrators (11.10 %) [26]. Grazers/scrapers and shredders that are typically dominant in the middle and upper stretches of the rivers [26] are also characterised with significant proportion in the total number of recorded species—17.80 and 3.50 %, respectively.

For 13.40 % of the taxa, feeding preference is unknown [23].

Analyses of overall species composition in regard to saprobic, feeding and bottom preference, as well as specific zone within river continuum, illustrate that investigated stretch is diverse in respect to environmental conditions. The change of relative abundance of the main taxa groups and functional analyses provided the information on changes of the community along the watercourse.

The domination of organisms adapted to fine substrate (silt, sand and clay) was recorded for sites 4, 5 and 9–12 (Fig. 8), which indicates gradual change of the river type along the watercourse.

Gradual change of macroinvertebrate community along the watercourse was also identified by functional analyses of saprobic groups and feeding preference (Figs. 9 and 10).

Thus, percentage participation of organisms that are adapted to high organic load (species typical for polysaprobic conditions) increases in downstream direction,

Fig. 7 Proportion of species with characteristic feeding preference

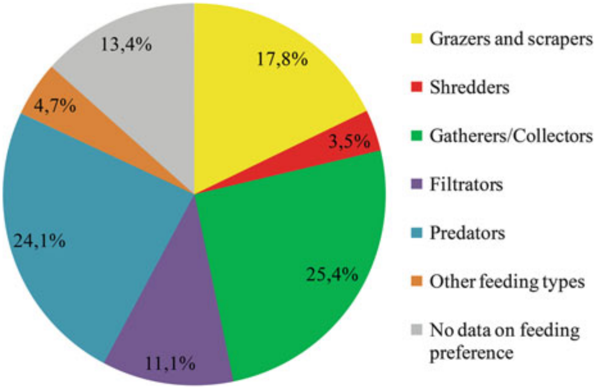
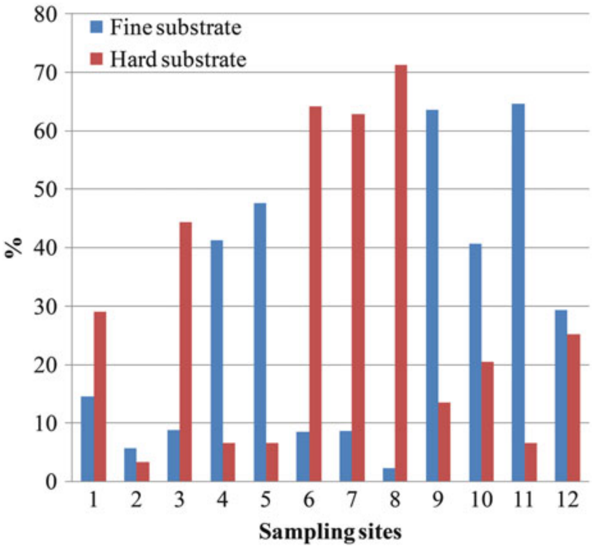


Fig. 8 Percentage participation of organisms that prefer fine (silt, clay and sand) and hard substrate (gravel and stone) type in the total macroinvertebrate density



while the share of beta-mesosaprobic organisms increases from site 2 to site 9 and then decreases (sites 10–12) (Fig. 9).

The change of functional feeding group percentage participation is presented at Fig. 10. In respect to feeding preference, gatherers/collectors and filter feeders (groups characteristic for the lower stretches of the rivers [26]) are dominant at sites 9–12, while the share of grazers/scrapers and shredders (groups characteristic for the middle and upper stretches of the river) is larger at the sites 1–8.

During our study, a significant number of species were detected (227), in comparison to previous investigations. Thus, Matoničkin et al. [1] reported 143 macroinvertebrate species for longer stretch of the Sava River, with domination of insects (69 species). Matoničkin et al. [1] also reported 27 species of aquatic worms (Oligochaeta), eight species of leeches (Hirudinea) and 21 species of

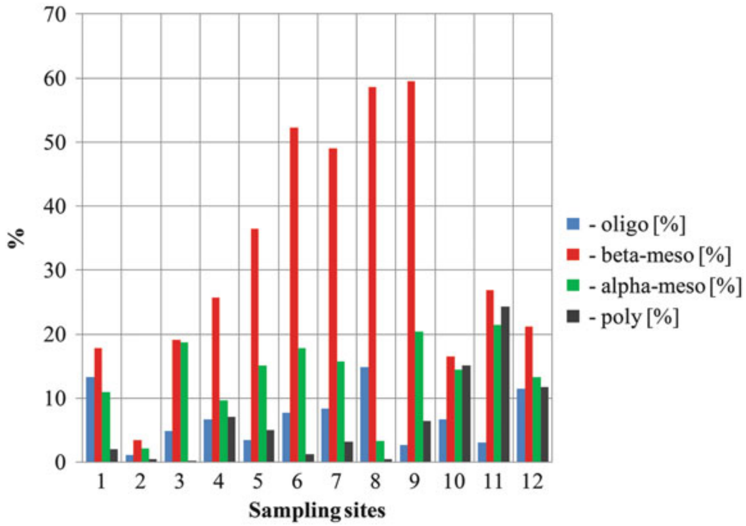


Fig. 9 Percentage participation of saprobic groups at sampling sites

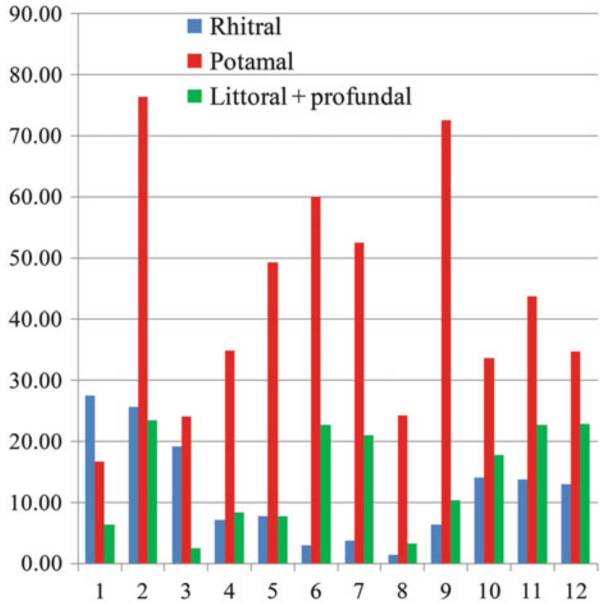


Fig. 10 Percentage participation of functional feeding groups at sampling sites

molluscs (15 snails and six bivalves). Having in mind that their research comprised the upper stretch of the Sava River, which was not covered by our investigation, it is expected that they identified 16 species of stoneflies (Plecoptera), while in the material collected during our study, those insects were not present. A total of 98 macroinvertebrate taxa were found during the investigation on a cobble substrate in the lower rhitron section of the Sava River at four different sampling sites [7]. Paunović et al. [10] reported 63 macroinvertebrate species for lower stretch of the Sava River, but this study did not comprise the analysis of nonbiting midges (Chironomidae).

Having in mind the above-mentioned investigations, and the fact that this study did not provide information on the diversity within the stretch upstream Hrastnik, which is different in respect to overall environmental conditions, the total number of macroinvertebrate taxa of the Sava River is much higher and we could expect more than 300 species to be found. The additional number of species is expected primarily among aquatic insects—stoneflies (Plecoptera), mayflies (Ephemeroptera) and caddis flies (Trichoptera)—but also within other macroinvertebrate groups that include species characteristic for fast water and hard bottom substrate.

During the 9-year study on the artificial substrates in the middle stretch of the Sava River, Mihaljević et al. [8] reported Chironomidae and Oligochaeta as the dominant groups, which is in accordance with the results of our study for the middle section of the Sava River.

High species richness of the Sava River could be revealed based on the comparison with the investigation of other large river within the Danube River Basin. Thus, during the AquaTerra Danube Survey (ADS) in the sector between Klosterneuburg (Austria, 1,942 river km) and Vidin-Calafat (Bulgaria-Romania, 795 river km), 89 macroinvertebrate taxa were detected [19] with molluscs as a dominant group in macroinvertebrate community with regard to species richness (35 taxa). Altogether 107 macroinvertebrate taxa were found during 2001 International Tisa Survey [28] that covered 744 km of the river.

Molluscs were also found to be one of the principal components of the macroinvertebrate community of the Sava River in its middle and lower stretch [1, 11, 12, 29], as well as in our study.

Molluscs and oligochaetes constitute two of the largest groups of invertebrates in regard to the number of identified species, as well as in regard to relative abundance, especially in large lowland rivers [20, 21, 30–32].

5.2 Sectioning of the Sava River Based on Aquatic Macroinvertebrates

Qualitative, quantitative and functional analyses clearly show the gradual changes along the watercourse.

For accurate discussion on the sectioning of the river, more research effort is needed. The proper typology, based on basic natural characteristics of water types, is an important activity which presents the basis for effective water management and monitoring of ecological status, as proposed by Water Framework Directive (WFD; WFD [33]). Grouping of similar rivers is a prerequisite to following the river-type-specific approach of the WFD. Thus, the classification of river types, as relatively homogeneous ecological systems, implies similar associated biological communities. The concept offered in the WFD in regard to typology is complex, because it demands the water classification in functional entities, characterised by the array of common features that could be described by biological traits from one side, but from the other side, the system should be simple enough to be applicable for an effective management, which includes monitoring, as well [22].

Based on the presented data on macroinvertebrate communities, the border between distinctive stretches of the Sava River could be between sites 8 (Slavonski Šamac) and 9 (the Bosut confluence). In a particular stretch, the Sava River became the typical large lowland river, after receiving several larger tributaries (the Bosna and Drina Rivers). The change occurs in the bottom substrate as well [34, 35] from substrate dominated by gravel and sand to this dominated by sand, with different proportion of silt and clay. Based on the preliminary study of macroinvertebrates along the longitudinal profile, the additional border between river types could be positioned upstream Zagreb, since the change of macroinvertebrate community structure is also observed at sites 3 and 4, in comparison to sites 1 and 2. Part of the recorded changes are consequence of anthropogenic pressures that are evident in the area (damming of the Sava River in Slovenian stretch, influence of settlements and water regulation structures), which makes the analyses in regard to river typology complex.

In regard to the upper stretch, Urbanič [13] identified the mouth of the Ljubljanica River (confluence of the Sava downstream Ljubljana) as the natural border between typical alpine watercourses belonging to ecoregion 4 (Alps [36]) and subalpine waters belonging to ecoregion 5 (Dinaric western Balkan [36]). Further, Urbanič [13] indicated that the border between ecoregions 5 (Dinaric western Balkan [36]) and ecoregion 11 (Pannonian plain [36]) is at elevation of about 200 m (Kraško-Brezinska Kotlina plain or between settlements Radeče and Zidani Most).

Based on the previous discussions on findings of Urbanič [13], as well as data presented in this work, the Sava River could be preliminarily divided into five distinct sectors—alpine, subalpine, Upper Sava plain, Middle Sava and Lower Sava (Fig. 11). For further divisions of sectors along the Sava River, additional material is needed.

Presented sectioning of the Sava River is in accordance with the general natural characteristics of the region. The Upper Sava course (upper reach or upper geomorphologic unit—hereby referred as alpine, subalpine, Upper Sava plain) is characterised by a steep slope, torrential tributaries and domination of coarse fractions in the bottom substrate [34, 35]. The hilly mountain terrain dominates. The reach is about 260 km long (together with the Sava Dolinka, longer headwater). The region is characterised by diverse environmental conditions and consequently

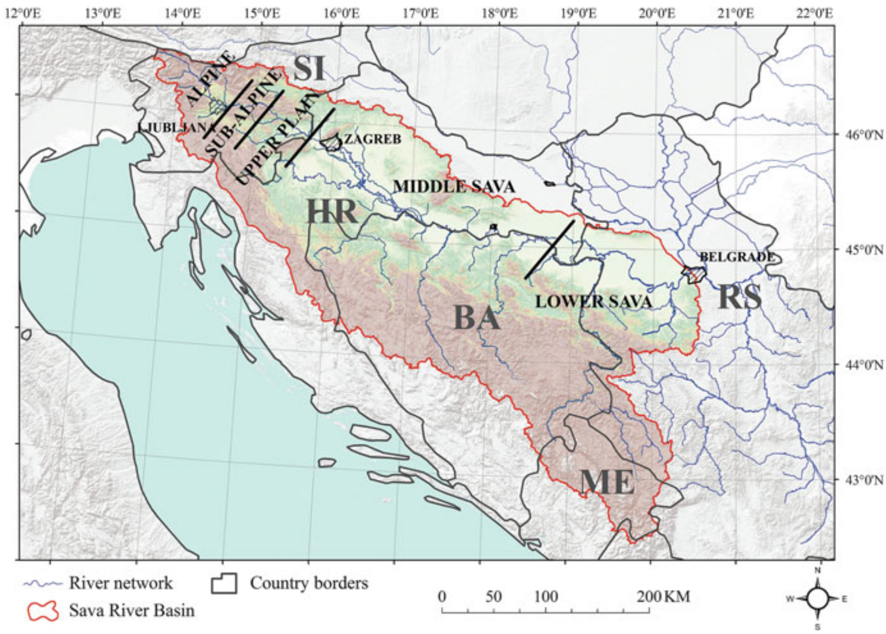


Fig. 11 Preliminary sectioning of the Sava River based on aquatic macroinvertebrates

complex biogeographical features, which are illustrated by division to ecoregions—three ecoregions are shared within a narrow area: 4 Alps, 5 Dinaric western Balkan and 11 Pannonian plain [36].

Further, general changes in bottom characteristics determine the border between the Middle and the Lower Sava River. According to available data, the gravel dominates down to the Una confluence and Sisak. In the stretch between Sisak and Slavonski Brod, the bottom is dominated by sand and gravel, while further downstream, the sand and silt dominate in bottom substrate. Since the bottom character is one of the dominant factors influencing the macroinvertebrate distribution [26], the changes in the community are expected.

5.3 *Nonindigenous Macroinvertebrate Taxa*

The last century has witnessed an increasing realisation of the role of humans in the dispersal of species beyond their natural range. Based on previous studies, the Sava River is also exposed to biological invasions [10–12, 37, 38]. Many of nonindigenous species recorded all over Europe are aquatic macroinvertebrates. In the following text, we provide short overview of nonindigenous aquatic macroinvertebrates recorded in the Sava River.

During our investigation, 11 nonindigenous aquatic macroinvertebrates were detected (marked with * in Table 1).

The dispersal of nonindigenous Ponto-Caspian amphipods (Crustacea: Amphipoda) in Croatian stretch of the Sava River was extensively discussed by Žganec et al. [37], and the details on the distribution of two species (*Chelicorophium curvispinum* and *Dikerogammarus haemobaphes*) were presented. Our investigation, as well as findings of Paunović et al. [12], confirmed the presence of one more amphipod invasive alien species, *D. villosus*, in the most downstream stretch of the Sava River (site 12). In addition, within the same stretch, the occurrence of spiny-cheek crayfish (*Orconectes limosus*; Crustacea: Decapoda), an invasive decapod species was confirmed during 2012, (site 12, Fig. 12). Further investigation will provide more details on the dispersal and abundance of nonindigenous crustaceans within the Sava River Basin. In that regard, the occurrence of the signal crayfish, *Pacifastacus leniusculus* (Dana 1852) (fast spreading nonindigenous invasive North American crayfish) could be expected in the Sava River, since the species was recently discovered in Korana River (Sava Basin) in Croatia [39]. Signal crayfish already successfully colonised many European freshwaters [39–42].

Besides crustaceans, several mollusc species were found to be successful invaders of the Sava River [1, 10–12]. Based on our study, as well as previous research [1, 10–12, 20, 21, 43], *C. fluminea*, *Dreissena polymorpha* and *Sinanodonta woodiana* are the most prominent mollusc invaders recorded in the Sava River. *C. fluminalis* was also recorded in the most downstream stretch of the Sava River [20, 21].

There are still a lot of efforts needed to properly assess the pressures caused by biological invasions within the Sava River, to identify the most prominent invaders, to recognise the most effective ways of introduction and to design appropriate, achievable measures for prevention of further introduction and spreading of aquatic invaders.

The general feeling is that there is a lack of systematised data on invasive aquatic macroinvertebrates within the Sava River Basin, i.e., there is no detailed list of invasive taxa, their abundance and influence on native biota and habitats.

Fig. 12 Specimen of spiny-cheek crayfish collected at site 12 (photo by Paunović 2012)

5.4 Basic Threats to the Biodiversity of Aquatic Macroinvertebrates of the Sava River

Based on the review of literature data (Paunović et al. 2008, 2012) [1–10, 34, 35], as well as based on our data, the following threats to aquatic macroinvertebrate diversity could be revealed:

- Physical habitat degradation—water regulation (flood protection and navigation), damming (electricity production, water supply and flood protection), change of bottom characteristics (sedimentation due to hydrological change and gravel and sand extraction), hydrological changes (damming and other regulative works), disruption of longitudinal and lateral connectivity (damming and other regulative works), drying out of riparian ecosystems (agriculture and regulative works), etc.
- Organic and nutrient pollution (untreated wastewaters from settlements and farms) and agriculture
- Pollution by hazardous and other harmful substances (different pressures caused by industrial production, as well as thermal power plants)
- Biological invasions (presented in the previous subchapter)

The consequences of the above-mentioned activities should be further elaborated in order to provide bases for effective water management practice. Some of the threats were already quantified, but for some of them, there is still need for further elaboration [34, 35].

6 Conclusions

The investigated section of the Sava River, despite anthropogenic impacts (organic pollution, impact of agricultural activity and damming in Slovenian stretch), has considerable habitat diversity and the resulting macroinvertebrate fauna diversity.

A total of 227 macroinvertebrate taxa were recorded in the Sava River based on the result of our study. Having in mind that the upper stretch of the river, which is different in overall environmental conditions, was not studied in detail, the taxa richness is certainly higher. Based on the review of previous works on the macroinvertebrate fauna of the Sava River, as well as based on the comparison with findings in other large rivers within the Danube Basin, it could be expected that more than 300 species will be confirmed for the Sava River.

There is an obvious need for further investigation of the Sava River in order to complete the data on aquatic macroinvertebrates and to the provide basis for accurate assessment of environmental status of the river. This work represents the contribution to the basic knowledge on the aquatic fauna of this large river, as the basis for future designs of more effective water resource management within the Sava River Basin.

Based on previous discussions provided in this work, the Sava River could be preliminarily divided into five distinct sectors—alpine, subalpine, Upper Sava plain, Middle Sava and Lower Sava. For further divisions of sectors along the Sava River, additional material is needed.

Different forms of physical habitat degradation; organic, nutrient and chemical pollution; as well as biological invasions were underlined as the major threats to the biological diversity of aquatic macroinvertebrates.

There is an obvious need for further work on aquatic macroinvertebrates of the Sava River that primarily includes research on diversity and distribution, identification of relation of distribution of taxa and environmental factors, study on nonindigenous aquatic macroinvertebrate distribution patterns, functional community and ecosystem analyses and the work on better involvement of know-how on aquatic macroinvertebrates in water management practice.

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