

# THE LONGITUDINAL IMPACT OF MULTIPLE STRESSORS ON THE BENTHIC INVERTEBRATE COMMUNITY IN THE BEDNJA RIVER

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University of Zagreb

FACULTY OF SCIENCE  
DEPARTMENT OF BIOLOGY

Iva Vidaković Maoduš

**THE LONGITUDINAL IMPACT OF  
MULTIPLE STRESSORS ON THE  
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IN THE BEDNJA RIVER**

DOCTORAL THESIS

Zagreb, 2022



Sveučilište u Zagrebu

PRIRODOSLOVNO-MATEMATIČKI FAKULTET  
BIOLOŠKI ODSJEK

Iva Vidaković Maoduš

**UTJECAJ VIŠESTRUKIH STRESORA  
NA ZAJEDNICU MAKROZOOBENTOSA  
DUŽ TOKA RIJEKE BEDNJE**

DOKTORSKI RAD

Zagreb, 2022.

This doctoral thesis was written at the University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, under the supervision of Prof. Zlatko Mihaljević, PhD as a part of the Doctoral study programme of the University of Zagreb, Faculty of Science, Department of Biology.

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## DISSERTATION SUPERVISOR

Professor Zlatko Mihaljević, PhD

Zlatko Mihaljević was born on January 21, 1966 in Varaždin. He enrolled in the biology studies (ecology) at the University of Zagreb, Faculty of Science Department of Biology in 1986. He graduated in January 1991, received his master's degree in 1994, and defended his doctoral dissertation in 1999. Since May 1991, he has been working continuously at the University of Zagreb, Faculty of Science Department of Biology Division of Zoology as a teaching assistant, senior teaching assistant, assistant professor, associate professor, full professor and, since 2022, as a tenured full professor. Twenty-seven diploma theses, eight final theses, one thesis that was awarded the Rector's Award, two master's theses and five doctoral dissertations were prepared under his supervision.

Prof. Zlatko Mihaljević, PhD has been actively involved in the activities of both the Department of Biology and the Faculty of Science. He was the leader and coordinator of field courses at the Department of Biology from 2003 to 2015 and the field courses head assistant from 2005 to 2015. From 2005 to 2016, he was the student leader at the undergraduate and graduate studies of Environmental Sciences, and from 2015 to 2020 he held a position of the head of the Division of Zoology. Since 2017, he has been a member of the Scientific Field Committee for Natural Sciences in the Field of Biology, and since 2018 a member of the committee for the evaluation of teaching/learning materials at the Faculty of Science Department of Biology.

Prof. Zlatko Mihaljević has so far participated in the preparation of about 50 scientific and technical studies. He has been leading the research team for the development of classification systems for the ecological status and potential assessment of rivers and lakes in Croatia according to the requirements of the EU Water Framework Directive. He has published 6 popular (professional) articles, co-authored three high school (Biology 4, Living World 1 and Biology 1) and one university textbook (Field and Laboratory Exercises in Ecology). He is also the co-author of the chapter Ecology in the textbook Environmental Engineering - Basic Principles. He is the winner of the 2019 Annual Award of Hrvatske Vode as one of the co-authors of the university textbook Field and Laboratory Exercises in Ecology. Prof. Zlatko Mihaljević's field of scientific research focuses on ecological research of freshwater ecosystems. So far, he has published 89 scientific papers, of which 51 are cited in the WoS database, 12 in the Scopus database and 4 papers are cited in other databases. The remaining 22 scientific papers have been published in proceedings of international and domestic scientific and technical conferences.

**THE LONGITUDINAL IMPACT OF MULTIPLE STRESSORS ON THE  
BENTHIC INVERTEBRATE COMMUNITY IN THE BEDNJA RIVER**

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Rivers and their biological communities are often simultaneously exposed to multiple human stressors that alter environmental conditions. Benthic macroinvertebrates are components of river ecosystems and respond to environmental alterations through structural and functional changes in their assemblages. The objectives of this study were to determine the composition and structure of benthic macroinvertebrate fauna of the Bednja River and to investigate their response to natural factors and anthropogenic stressors. Benthic macroinvertebrate samples were collected in summer 2015 at 20 longitudinally distributed study sites using AQEM multihabitat sampling method. Water quality, landuse and hydromorphological alterations were quantified for each site. Several water quality parameters and nutrients followed a longitudinal gradient but hydromorphological alteration did not, indicating that degradation was present along the entire river reach. Of 193,638 macroinvertebrates identified, Chironomidae larvae were the dominant taxa group. Collector gatherers were the predominant feeding group. Benthic macroinvertebrate assemblages and diversity varied between study sites and microhabitats, and there was no grouping of assemblages based on river type. Assemblages on technolithal differed from those on natural lithal substrate. Benthic macroinvertebrates responded best to hydromorphological alteration by flow and sediment related functional metrics, while diversity and sensitivity metrics generally correlated poorly with hydromorphological scores. The results indicate that organic pollution is a greater problem than hydromorphological degradation of the Bednja River. The response gradient indicates an improvement in ecological status by lowering ammonium levels, improving riparian vegetation structure and increasing substrate size heterogeneity.

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**Keywords:** Multiple stressors / River Habitat Survey / EN 15843:2010 / water quality / landuse / benthic macroinvertebrates

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## **UTJECAJ VIŠESTRUKIH STRESORA NA ZAJEDNICU MAKROZOOBENTOSA DUŽ TOKA RIJEKE BEDNJE**

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Rijeke i njihove biološke zajednice često su pod utjecajem višestrukih ljudskih pritisaka koji mijenjaju okolišne uvjete. Bentički makroskopski beskralješnjaci (makrozoobentos) sastavni su dio riječnih ekosustava, gdje reagiraju na promjene u okolišu kroz strukturalne i funkcionalne promjene u svojoj zajednici. Ciljevi ovog istraživanja bili su utvrditi sastav i strukturu makrozoobentosa rijeke Bednje te identificirati odgovor zajednice prema prirodnim ekološkim čimbenicima i antropogenim pritiscima. Uzorci makrozoobentosa sakupljeni su tijekom ljeta 2015 na 20 longitudinalno raspoređenih istraživačkih postaja, korištenjem AQEM 'multihabitat' metode. Kakvoća vode, zemljišni pokrov u podslivu i hidromorfološke promjene kvantificirani su za svaku postaju. Pokazatelji kakvoće vode i hranjive tvari slijedili su longitudinalni gradijent. Hidromorfološko stanje nije pratilo longitudinalni gradijent, što ukazuje da je degradacija prisutna duž cijelog riječnog toka. Temeljem 193 638 identificiranih jedinki makrozoobentosa, ličinke trzalaca (Diptera-Chironomidae) su dominantna skupina. Probirači/sakupljači su najzastupljenija hranidbena skupina u makrozoobentosu. Sastav zajednice i raznolikost varirala je među postajama i ovisno o mikrostaništu. Zajednice se nisu grupirale prema riječnom tipu. Zajednice na tehnotalalu razlikovale su se od zajednica na prirodnom staništu. Odgovor makrozoobentosa prema hidromorfološkim promjenama najbolje se pokazao kroz funkcionalne metrike vezane za preferenciju supstrata i brzine strujanja vode, dok indeksi raznolikosti i osjetljivosti nisu značajno korelirali sa hidromorfološkim ocjenama. Rezultati ukazuju kako je opterećenje organskim tvarima značajniji pritisak od hidromorfološke degradacije rijeke Bednje. Gradijent pritisaka pokazuje kako se ekološko stanje može poboljšati smanjivanjem razine amonijaka, poboljšanjem vegetacijske strukture na obali rijeke te povećanjem raznolikost supstrata.

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Ključne riječi: višestruki stresori / River Habitat Survey / EN 15843:2010 / hidromorfologija / kakvoća vode / korištenje zemljišta / makrozoobentos

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### ***Prošireni sažetak***

Rijeke i njihove biološke zajednice često su pod utjecajem višestrukih ljudskih pritisaka koji mijenjaju okolišne uvjete. Bentički makroskopski beskralješnjaci (makrozoobentos) sastavni su dio riječnih ekosustava gdje reagiraju na promjene u okolišu kroz strukturalne i funkcionalne promjene u svojoj zajednici. Rijeka Bednja, ukupne duljine 106 km, desna je pritoka rijeke Drave u Panonskoj ekoregiji. Sustavno ispitivanje rijeke Bednje provedeno je sa ciljevima da se:

- 1) Utvrdi sastav i struktura makrozoobentosa rijeke Bednje koji do sada nije nikada bio sustavno istraživani;
- 2) Identificiraju utjecaji prirodnih čimbenika i antropogenih pritisaka na zajednicu makrozoobentosa;
- 3) Odredi gradijent promjena u zajednici bentičkih beskralješnjaka uslijed utjecaja antropogenih pritisaka s naglaskom na hidromorfološke promjene;
- 4) Ispita poveznica između hidromorfološkog stanja i zajednice bentičkih beskralješnjaka.

Istraživanje je provedeno tijekom ljeta 2015. godine, kada su na 20 longitudinalno raspoređenih postaja sakupljeni uzorci makrozoobentosa korištenjem AQEM 'multihabitat' metode. Ukupno je sakupljeno i identificirano 193 638 jedinki makrozoobentosa. Fizikalno-kemijski pokazatelji i hranjive tvari mjereni su u tri navrata i obuhvatili su vegetativnu sezonu: proljeće, ljeto i jesen. Za svaku istraživanu postaju izračunat je udio površine zemljišnog pokrova na pripadajućem podslivu. Na svakoj postaji primijenjene su dvije različite metode za ocjenu hidromorfološkog stanja: europski standard EN 15843:2010 i standard koji je prvotno korišten u Velikoj Britaniji, „River Habitat Survey“ (RHS), a danas se koristi u više zemalja članica EU.

Ovim istraživanjem su dobiveni slijedeći rezultati vezano za strukturu i sastav zajednice makroskopskih beskralješnjaka rijeke Bednje:

- Ličinke Chironomidae (Diptera) najbrojnija su skupina makrozoobentosa;
- Rakušac *Gammarus fossarum* Koch in Panzer, 1836 najbrojnija je i najrasprostranjenija svojta;
- Probirači/sakupljači čine dominantnu hranidbenu skupinu;

- Brojnost i raznolikost makrozoobentosa značajno se razlikovala između mikrostaništa i istraživanih postaja;
- Svojte koje su najviše doprinijele sličnosti između svih postaja su pleme Chironomini (Diptera-Chironomidae), maločetinaš *Limnodrilus hoffmeisteri* Claparede, 1862, rakušac *Gammarus fossarum* i pleme Tanytarsini (Diptera-Chironomidae);
- Bray-Curtis indeks sličnosti nije grupirao zajednice rema riječnom tipu;
- Alohtona vrsta *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta) najviše doprinosi različitosti zajednica između dva riječna tipa rijeke Bednje.

Ovim istraživanjem dobiveni su slijedeći rezultati vezano za utjecaj prirodnih čimbenika i antropogenih pritisaka na makrozoobentos:

- Akal (sitni šljunak veličine od  $>0,2$  do 2 cm) je dominantan supstrat u rijeci Bednji;
- Zajednice na različitim mikrostaništima razlikuju se ovisno o tipu i veličini supstrata; *Gammarus fossarum* najviše doprinosi sličnosti između uzorka ksilala (drveni ostaci) i litala (makrolital, mezolital, mikrolital). Pleme Chironomini ima najveći doprinos u sličnosti između uzoraka sa sitnijih supstrata (pijesak, akal, argilal);
- Zajednice na prirodnom lital supstratu razlikovale su se od zajednica na umjetnom tehnolitalu. Potporodica Orthoclaadiinae (Diptera-Chironomidae) doprinosi najvećoj sličnosti između uzoraka sakupljenih na tehnolitalu;
- Koncentracija ukupnog dušika najznačajnije korelira s uzdužnim profilom rijeke;
- Udio urbanih površina značajnije korelira sa hranjivim tvarima nego udio poljoprivrednih površina u slivu Bednje;
- Ekstenzivna poljoprivreda pozitivno je utjecala na indekse raznolikosti makrozoobentosa;
- Modul saprobnost dao je nižu, odnosno konačnu ocjenu ekološkog stanja na onim postajama gdje dobro ekološko stanje nije postignuto, što ukazuje da opterećenje hranjivim tvarima na rijeci Bednji predstavlja značajniji pritisak od hidromorfološke degradacije;
- Koncentracije amonijaka značajnije su korelirale sa indeksom saprobnosti nego udjelom urbanih površina, što ukazuje kako urbana područja ne moraju predstavljati toliko negativan utjecaj na površinske vode ukoliko su otpadne vode odgovarajuće zbrinute;
- Nepročišćene otpadne vode grada Ivanca (postaja 9) odgovorne su za najnižu ocjenu ekološkog stanja na rijeci Bednji;

- Metrika ASPT (Prosječna ocjena po svojti) dala je najveći broj značajnih negativnih korelacija sa hranjivim tvarima i udjelom urbanih površina.

Ovim istraživanjem su dobiveni slijedeći rezultati vezano za odnos hidromorfoloških promjena i makrozoobentosa:

- Hidromorfološka degradacija prisutna je duž cijelog toka rijeke Bednje;
- Prisutnost tehnolitala i odsutnost ksilala ukazuje na veće hidromorfološke promjene;
- Hidromorfološke ocjene dobivene temeljem standarda EN 15843:2010 i „River Habitat Survey“ metode snažno međusobno koreliraju;
- Metrike osjetljivosti/tolerancije i raznolikosti nisu dobar pokazatelj hidromorfoloških promjena;
- Metrike temeljene na makrozoobentosu koje su najbolje reagirale na hidromorfološke promjene su udio svojti koje preferiraju šljunak (%) Akal, Rheoindex i udio reofilnih svojti (%) RP;
- RHS ocjena za strukturu obalne vegetacije (engl. Habitat Quality Assessment bank vegetation structure) je dala najveći broj značajnih korelacija sa svim testiranim metrikama temeljenih na makrozoobentosu;
- Hidromorfološke promjene povezane su s povećanjem udjela hranidbenih skupina predatora i bušaća u zajednici;
- Korelacija Hydrachnidia (vodengrinja) s hidromorfološkim promjenama povezana je prvenstveno s preferencijom ove skupine prema tehnolitalu;
- RHS ocjena za strukturu supstrata korita (engl. Habitat Quality Assessment channel substrate subscore) i indeks supstrata (engl. Channel Substrate Index) najviše utječu na zajednicu makrozoobentosa;
- Individualne ocjene hidromorfološke metode EN 15843:2010 za supstrat, količinu drvenih ostataka i vrstu/strukturu vegetacije na obalama najviše su korelirale sa pojedinim svojtima.

Gradijent pritisaka pokazuje kako se ekološko stanje može poboljšati smanjivanjem koncentracije amonijaka u vodi, poboljšanjem vegetacijske strukture na obali rijeke te povećanjem raznolikosti riječnog supstrata.

Dobiveni rezultati doprinose novim saznanjima o makrozoobentosu rijeke Bednje te predstavljaju doprinos u razumijevanju strukturiranja zajednice beskralježnjaka nizinskih rijeka kao odgovora na prirodne, ali i antropogeno izmijenjene okolišne uvjete.

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## 1. INTRODUCTION

Most freshwater organisms are restricted to either lentic (standing) or lotic (running) habitats (Illies, 1978). Lotic habitats, i.e., running waters, have a long history of being channelized for different purposes (e.g. land drainage or flood control) using conventional engineering methods which have led to undesirable consequences to the rivers' physical, biological but also downstream conditions (e.g. Brookes, 1988). Considering the wide range of additional threats, such as pollution (nutrients and emerging pollutants), alterations of the hydrologic regime, and climate change, running waters are amongst the most impacted ecosystems on earth (Malmqvist & Rundle, 2002; Sabater & Elosegi, 2014; Dudgeon, 2019). In degraded ecosystems, different stressors rarely act individually, so rivers are often exposed to multiple-stress situations (Ormerod et al., 2010; Hering et al., 2015; Urbanič et al., 2020). This is especially the case with European lowland rivers (Schinegger et al., 2012). Furthermore, multiple stressors are often correlated and closely related, and influence biological communities at different spatial scales (e.g. Feld & Hering 2007; Knehtl et al., 2021).

Lotic ecosystems, regardless of geographic area, are inhabited by benthic macroinvertebrates. Macroinvertebrates are generally considered organisms large enough to be seen, and arthropods, respectively insects, represent the greatest majority (Alba-Tercedor, 2006). In running waters, benthic macroinvertebrates occupy the bottom substrates such as sediments, debris, and macrophytes (Hauser & Resh, 2017; Moog et al., 2018). Apart from being an essential part of the river continuum food web as almost all macroinvertebrates are potential food sources for larger animals such as fish and birds (Vannote et al., 1980; McDowal, 1990; Johnson et al., 1993) they also play an important role in nutrient cycling in freshwater ecosystems (Merritt et al., 1984; Leslie & Lamp, 2019). Benthic macroinvertebrates integrate environmental conditions over longer periods due to their relatively long lifespans (De Pauw & Hawkes, 1994; Tachet et al., 2002). For this reason, different stressors affecting lotic systems can lead to structural and functional changes in the benthic macroinvertebrate communities (e.g. Schäfer, 2019; Leitner et al., 2021b). Owing to the good knowledge of their ecological requirements and responses to different environmental variables and stressors, benthic macroinvertebrates are considered one of the best indicators of habitat and water quality in rivers (Metcalf, 1989; Wright et al., 2000; Hering et al., 2004a; Ollis et al., 2006).

Benthic macroinvertebrates are widely used in the assessment of ecological integrity of rivers in Croatia (Mihaljević et al., 2020). They have also been the topic of several research studies covering both the Pannonian lowland (e.g. Vilenica et al., 2020) and Dinaric western Balkan ecoregion (e.g. Mičetić Stanković et al., 2018; Pozojević et al., 2021). However, most available research studies focus on individual taxa groups. This Thesis represent the first systematic analysis of benthic macroinvertebrate fauna of the Bednja River located in the Pannonian lowland ecoregion (ER 11) (Illies, 1978) in Croatia, and a rare example of longitudinal research of an entire river in Croatia. For example, previously longitudinal research was conducted on the Kupa River (Belinić, 1991), Cetina River (Vučković, 2011), and Sava River (Žganec et al., 2016), but these studies also focused specifically on a single taxa group and not the entire benthic macroinvertebrate community.

Results on benthic macroinvertebrate response to conditions under multiple-stress situations obtained by applying scientific methods and principles will have direct application in water management. Based on the qualitative and quantitative analysis of the structure of benthic macroinvertebrate communities collected on different substrates at different sites and based on the calculation of proposed indices and metrics, a response gradient will be determined for different stressors with special reference to hydromorphological degradation of mid-sized rivers.

In order to understand lotic system functioning, an interdisciplinary approach is required, which apart from biology incorporates knowledge of hydrology, water chemistry and environmental engineering (Giller & Malmqvist, 1998) all which will be presented in this Thesis. Furthermore, understanding which hydromorphological, habitat and environmental conditions support the taxonomic composition and structure of benthic macroinvertebrates representing good ecological status set by the EU Water Framework Directive (WFD) (European Commission, 2000) and how this composition changes along a degradation gradient can contribute to the design of more effective river restoration projects.

## 1.1 Objectives and hypotheses of research

The main objectives of the research were to:

- determine the composition and structure of benthic macroinvertebrates in the Bednja River,
- identify the effects of natural factors and anthropogenic stressors on the benthic macroinvertebrate community,
- establish the response gradient of the benthic macroinvertebrate community towards anthropogenic stressors and hydromorphological alterations,
- investigate the relationship between hydromorphological status and the benthic macroinvertebrate community longitudinally along the Bednja River.

The main hypotheses of this research are:

- The response of the benthic macroinvertebrate community to organic pollution will differ depending on the level of hydromorphological degradation.
- The composition of the benthic macroinvertebrate community is in direct correlation with the river habitat quality, riparian zone, substrate, and the physicochemical parameters of the water.
- Landuse on the catchment affects the composition of the benthic macroinvertebrate community.
- Greater hydromorphological degradation will cause a greater change in the reference benthic macroinvertebrate community.



## 2. LITERATURE OVERVIEW

### 2.1 General characteristics of benthic macroinvertebrates

The term ‘macroinvertebrate’ itself is not a category in taxonomy as it represents an “artificial delimitation of part of the groups of invertebrate animals” (Alba-Tercedor, 2006). Benthic macroinvertebrates groups used in the assessment of lotic systems include the insect orders: Ephemeroptera (mayflies); Trichoptera (caddisflies); Plecoptera (stoneflies); Diptera (flies, also encompassing the family Chironomidae (non-biting midges)); Odonata (dragonflies and damselflies); Coleoptera (beetles); Heteroptera (true bugs); Hydrachnidia (water mites); and Megaloptera (alderflies, dobsonflies); the phylum Mollusca (molluscs), Turbellaria, Bryozoa (moss animals), and Porifera (freshwater sponges); the subphylum Crustacea (which encompasses e.g. decapods, isopods and amphipods); the class Hydrozoa; and the subclasses Hirudinea (leeches) and Oligochaeta (worms) (Mihaljević et al., 2020). Despite fitting into the size category and being aquatic, some animal groups are not considered by assessment methodologies based on benthic macroinvertebrates (e.g. Nematoda, Nematomorpha, Cladocera, Copepoda) (Alba-Tercedor, 2006).

Benthic macroinvertebrate taxa have different ecological preferences based on which they can be categorised (see Schmidt-Kloiber & Hering, 2015). One of the categories is based on feeding preferences and according to Moog (2002), benthic macroinvertebrate taxa are assigned to functional feeding group as follows: grazers and scrapers - animals that feed on biofilm, endolithic and epilithic algal tissues and partially particulate organic matter (POM). Examples include some representatives of the class Gastropoda (snails), some larvae of the orders Ephemeroptera and Trichoptera etc.; miners - feed on algae, leaves, and cells of aquatic plants. Examples include some larvae from the genus *Hydroptila* (Trichoptera); xylophagous taxa - animals that feed from woody debris in water. Examples include some representatives of the family Elmidae (Coleoptera); shredders - feed from fallen leaves, coarse particulate organic matter (CPOM) and plant tissue. Examples of shredders include the order Amphipoda (Crustacea) and some Trichoptera; gatherers/collectors - feed from fine particulate organic matter (FPOM) in the sediment. Chironomidae and Oligochaeta are examples of gatherers/collectors; active filter feeders - animals that actively filter food from the water column. They feed from suspended FPOM, CPOM and micro prey. Examples are

representatives from the class Bivalvia; passive filter feeders - animals that also feed from suspended FPOM, CPOM and prey but unlike active filter feeders, they filter food in the water current by nets or specialised mouthparts. Example of passive filter feeders are representatives of the genus *Hydropsyche* (Trichoptera) and the family Simuliidae (Diptera); predators - feed from prey. Examples include Odonata, some Coleoptera, Hirudinea and Hydracarina; parasites - feed from host. Some Hirudinea are parasites; and the category 'other' feeding types. This category is for animals using other food sources which cannot be classified into this scheme.

Benthic macroinvertebrate assemblages can respond to environmental change through functional feeding groups as different stressors can alter the habitat conditions and consequently food availability (e.g. Sitati et al., 2021). Other benthic macroinvertebrate ecological preferences are described below, together with the review of natural and anthropogenic conditions in lotic systems.

## **2.2 Influence of natural conditions in lotic systems on benthic macroinvertebrates**

The conditions in lotic systems are generally the result of a combination of natural and anthropogenic factors. In order to distinguish which changes in a river are the result of human interventions/activities, it is important to give an overview of natural conditions affecting benthic macroinvertebrate communities longitudinally along a river system.

The main factor affecting water temperature in a river is air temperature (Arnell, 1996) but also solar radiation (Wetzel & Likens, 2000a; Benyahya et al., 2012), and factors such as cloud cover, and shade by riparian vegetation (e.g. Garner et al., 2014). Benthic macroinvertebrate assemblages are sensitive to changes in water temperature and are controlled by temperature gradients (e.g. Richards et al., 1997; Carlisle et al., 2008). For benthic macroinvertebrates, water temperature influences community structure (e.g. Daufresne et al., 2004), plays a dominant role in their growth and metabolism (e.g. Briers et al., 2004), but also plays a role in regulating their life cycle, i.e. timing of emergence (e.g. Durance & Omerod, 2007; Chadwick & Feminella, 2001).

Rivers experience diurnal (or diel) fluctuations in water temperature (Yakuwa, 1960, Ferencz & Cardenas, 1997; Wetzel & Likens, 2000b), and variations in daily temperature tend to be higher in smaller rivers whereas large rivers are more thermally stable (e.g. Łaszewski, 2018). According to Vannote et al. (1980) species diversity is higher in river reaches where variations in daily water temperature are more significant. Pursuant to these variations, benthic invertebrates also exhibit diel patterns in locomotory activity and density (Statzner et al., 1988; Elliot, 2002).

The turnover of water in a river channel is driven by the hydrological cycle and the time required for a complete replacement of water within the system (mean residence time) is around 7 to 14 days (Giller & Malmqvist, 1998). Water chemistry condition in a river is greatly influenced by catchment geology. In natural conditions, a river flowing over sedimentary rocks is expected to be alkaline and have higher levels of dissolved salts while a catchment based on igneous rocks can be acidic with lower levels of dissolved salts (Meybeck, 1987; Panigrahy & Raymanashay, 2005; Krám et al., 2012; Harmon et al., 2013). The geo-hydromorphological conditions on the catchment also influence levels of total suspended solids in a river which are naturally highest during floods (Schmutz & Moog, 2018). Furthermore, the geology of a catchment is the source of sediment which is transported and deposited within in a river system. This sediment together with its interstices, represents the mineral substrate microhabitat for benthic macroinvertebrates. Substrate size is influenced by sediment supply, gradient, water depth, and the magnitude and frequency of flood flows (Jowett, 2003).

Substrate type and size is one of the most significant natural factors in explaining benthic macroinvertebrate community variations (e.g. Cummins & Lauff, 1969; Hynes, 1970; Reice, 1980; Douglas & Lake, 1994). In general, benthic macroinvertebrate diversity and abundance increases with substrate size, with lowest numbers associated with fine sediment and highest with the coarser mesolithal and macrolithal (Allan, 1995; Leitner et al., 2021b). In a study on benthic macroinvertebrate diversity and composition in different substrates performed by Duan et al. (2009), Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa dominated in cobble and gravel substrates, including bedrock covered in moss, while Chironomidae larvae were dominant in clay beds. However, they also found that taxa richness was highest when the substrate was covered in macrophytes, and lowest if bare. Graf et al. (2016) found significant differences in assemblages between sand and lithal substrates. They also report significantly higher EPT taxa abundances in lithal substrates but also taxa richness in relation to families. In

their study, even though Chironomidae were the most abundant in lithal in absolute vales, their dominance in psammal was higher.

Benthic macroinvertebrate preferences of different size substrate have also been linked to the ability of different substrate to trap detritus. Results of colonization experiments conducted by Parker (1989) showed that gravel (smaller substrate) accumulated the greatest quantity of fine detritus while cobble (largest substrate) trapped the smallest quantity of fine detritus and largest quantity of coarse detritus. Subsequently, abundance of benthic macroinvertebrates colonized on gravel in the experiment was significantly higher than on cobble substrate. Similar results were confirmed earlier by Rabeni & Minshall (1977) and Wise & Molles (1979). Colonization of benthic macroinvertebrates on substrate can also be influenced by presence and abundance of periphyton (algae) on the substrate surfaces which is a food source (e.g. Lamberti & Resh, 1983; Robinson et al., 1990). Abundance and distribution of macroinvertebrates is also influenced by substrate stability and greater instability and bedload movement have shown to decrease macroinvertebrate abundance (e.g. Death, 1996; Townsend et al., 1997). Pardo & Armitage (1997) identified indicator species of different mesohabitats groups (sand, silt, gravel and macrophytes) based on seasonal samples from a lowland chalk stream in England. Some of the indicators included: oligochaet *Limnodrilus hoffmeisteri* Claparede, 1862 and midge *Prodiamesa olivacea* (Meigen, 1818) for sand; several representatives of oligochaetes, bivalves and chironomids for silt; high number of indicators with strong representation of Oligochaeta, Chironomidae, Ephemeroptera and Plecoptera for gravel; and a high number of snails, *Baetis* (Ephemeroptera), Chironomidae and Simuliidae (Diptera) in the macrophyte group.

Knowledge of substrate preference of individual taxa enabled categorisation of benthic macroinvertebrates based on microhabitat/substrate preference. Pursuantly, the following microhabitat related functional metrics have been derived based on percentage of community preferring a certain microhabitat: % Type Pel (taxa with a preference for pelal – mud); % Type Psa (taxa associated with psammal - sand); % Type Aka (taxa preferring akal - fine to medium-sized gravel); % Type Lit (taxa with a preference for lithal - coarse gravel, stones, boulders); % Type Phy (taxa preferring phytal - algae, mosses and macrophytes); % Type POM (taxa with a preference for particulate organic matter), and % Type Oth (other habitats) (Schmedtje & Colling, 1996; Moog et al., 1999; AQEM consortium, 2002).

Beisel et al. (2000) found benthic macroinvertebrate fauna richness to be higher at sites with heterogenous substrates i.e. composed of numerous substrate types, which can be explained by availability of more niches and shelters for the macroinvertebrates whereas homogenous environments offer the opposite. Overall, the favourable role of habitat heterogeneity and complexity, i.e. availability of diverse substrates and natural environmental conditions for benthic macroinvertebrates is unquestionable (Erman & Erman, 1984; O'Connor, 1991; Mackay, 1992).

Because the nature of lotic systems as habitats is characterized by the flow of water within a river channel which can be very diverse (Giller & Malmqvist, 1998), the hydraulic gradient also plays a significant role in organizing benthic macroinvertebrate communities within a river (Rempel et al., 2000) and different macroinvertebrates have evolved to be positively related to river flow by having precise requirements for specific flow regime and velocities (Statzner et al., 1988; Collier, 1993). In a river, the maximum flow velocity is achieved just below the surface while near the bottom the layer the velocity is low, pursuant to the phenomenon of laminar flow (Scotton et al., 2006) (Figure 2.1.1.).

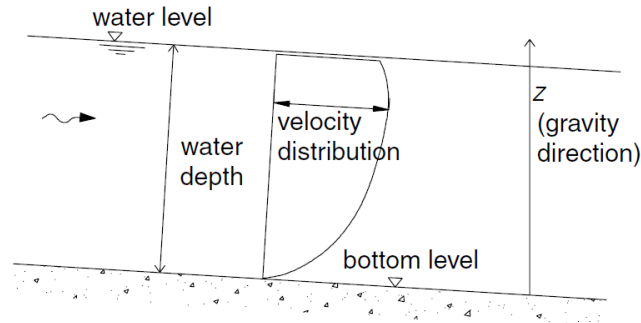


Figure 2.1.1. Velocity distribution along the depth of a river (Scotton et al., 2006).

In adapting to different current velocity habitats, benthic macroinvertebrates developed special anatomical strategies such as flat bodies, hydrodynamic shapes, reduced projecting structures, small body size and fixative/anchorage structures (Alba-Tercedor, 2006). The influence of flow velocity on respiration and metabolism of benthic macroinvertebrates has historically been established (e.g. Phillipson, 1956; Feldmeth, 1970), and the influence of flow velocity on benthic macroinvertebrates also extends to feeding biology especially of filter-feeders (e.g. LaBarbera, 1984; Chance & Craig, 1986). Furthermore, flow velocity has been linked to several behavioural characteristics of benthic macroinvertebrates such as case and net building of

Trichoptera (e.g. Edington, 1968; Philipson & Moorhouse, 1974), and assortative mating of *Gammarus pulex* (Linnaeus, 1758) (e.g. Elwood et al., 1987). However, it is possible for benthic invertebrates to respond differently to flow velocity depending on substrate and water depth (Statzner et al., 1988). Gore et al. (2001) in their instream flow evaluations found preferred velocities for EPT taxa to be 0.1 – 0.3 m/s at depths of around 0.45 – 0.8 m. Rempel et al. (2000) found highest macroinvertebrate density at samples from 0.2 and 0.5 m river depth while highest taxonomic richness in samples from 1.5 m depth. Banning (1990) developed the Rheoindex which describes the affinity of a taxon towards current velocity. A high value represents taxa typically found in river sections with high current velocity.

The following categories based on current velocity preference are assigned to taxa according to Schmedtje & Colling (1996): limnobiont (taxa occurring only in standing waters. Examples include some Coleoptera and Odonata taxa); limnophil (taxa preferably occurring in standing waters, rarely found in slowly flowing streams. Examples include some Ephemeroptera taxa e.g. *Cloeon dipterum* (Linnaeus, 1761), and some Trichoptera taxa from the family Limnephilidae); limno- to rheophile (taxa preferably occurring in standing waters, but regularly occurring in slowly flowing streams. Several Oligochaeta and gastropod taxa belong to this category); rheo- to limnophil (taxa usually found in streams, prefers slowly flowing streams and lentic zones but also found in standing waters. Several Ephemeroptera taxa belong to this category e.g. *Baetis fuscatus* (Linnaeus, 1761)); rheophil (taxa occurring in streams, prefers zones with moderate to high current. The greatest number of lotic taxa belong to this category including several Coleoptera and Plecoptera taxa, representatives of the family Simuliidae (Diptera), Gammaridae (Crustacea), Heptagenidae (Ephemeroptera), and Hydropsychidae (Trichoptera)); rheobiont (taxa occurring in streams, bound to zones with high current. Examples are the family Elmidae (Coleoptera) and several taxa belonging to the family Rhyacophilidae (Trichoptera)); and indifferent taxa with no preference for a certain current velocity.

Ways in which ecological processes in rivers are affected by flow is given in an extensive by review by Hart & Finelli (1999). In summary, key components of ecological process (dispersal, habitat use, resource acquisition, competition, and predator-prey interactions) can be modified by flow which consequently affects distribution, performance, and abundance of benthic macroinvertebrates.

In a natural river, the alternating riffle-pool sequence can be predicted (Leopold et al., 1964; Richards, 1976; Carling & Orr, 2000). Riffles are characterised by shallow, high velocity flow over unconsolidated gravel or cobble (Environment Agency, 2003), and they are considered harsh environments as the structure of biotic communities are strongly regulated by flow related substrate movement (e.g. Resh et al., 1988; Lake, 2000). Pools are deeper parts of the riverbed sustained by scouring (Environment Agency, 2003). As riffles and pools represent differing physical characteristics and habitats (velocity, depth, substrate etc.), there is also a clear and distinct difference in benthic macroinvertebrate assemblages between them, with riffles supporting higher benthic macroinvertebrate densities (e.g. Scullion et al., 1982; Brown & Brussock, 1991; Wang et al., 2012).

Finally, a natural river should respect the River Continuum Concept (RCC) proposed by Vannote et al. (1980) which can be used to explain the longitudinal progressive shift of benthic invertebrate structural and functional communities in harmony with the changing dynamics of the physical conditions. According to RCC the relative dominance (biomass) of shredders should be greatest in headwaters due to large amounts of allochthonous detritus from riparian vegetation which at the same time limits primary production through shading. As stream size increases primary production increases and detritus particle size decreases as it is transported from upstream reaches. The increased algae primary production is followed by a shift in dominance of scrapers and is most expressed in mid-sized streams. In large rivers the abundance and increase of fine and ultra-fine particulate organic matters increases the dominance of collectors and gatherers in benthic macroinvertebrate assemblages.

Pursuantly, benthic macroinvertebrates display zonation or longitudinal preferences and the following categories have been assigned according to Moog (2002): eucrenal (preference for the spring region); hypocrenal (preference for the spring-brook region); epirhithral (preference for the upper-trout region); metarhithral (preference for the lower-trout region); hyporhithral (preference for the grayling region); epipotamal (preference for the barbel region); metapotamal (preference for the bream region); hypopotamal (preference for the brackish water region); littoral (taxa inhabiting the lake and stream shorelines, lentic sites, ponds etc.); profundal (taxa inhabiting bottom of stratified lakes). The Rhithron Type Index was developed by Biss et al. (2002) to describe the affinity of a taxon towards the rhithral region of a river. A higher value indicates stronger association to the rhithral zone.

### 2.3 Benthic macroinvertebrate response to organic pollution

The main source of organic pollution in rivers is discharge of untreated urban wastewater which is composed of primarily proteins, carbohydrates, and lipids (Raunkjær et al., 1994). The decomposition of organic matter in rivers by microorganisms is reflected by depletion of available oxygen (e.g. Apoteker & Thevenot, 1983; Daniel et al., 2002). Changes in water quality as a result of wastewater discharge can also influence water temperature of a river (Durance & Omerod, 2009).

The impact of organic pollution on benthic macroinvertebrates is the longest studied and best documented stressor. Historical studies of the relationship between water quality and freshwater organisms date to mid-19<sup>th</sup> century (e.g. Kolenati, 1848; Cohn, 1853) with their beginnings in attempts to categorize certain indicator organisms into classes of water pollution (e.g. Cohn, 1870; Mez, 1898; Kolkwitz & Marrson, 1909; Liebmann, 1951; Zelinka & Marvan, 1961). Further development of indices to detect organic pollution continued to be based on the absence/presence of pollution-scored taxa, (e.g. Chandler, 1970; Armitage et al., 1983; De Pauw & Vanhooren, 1983). Two widely used metrics based on sensitivity of macroinvertebrates to pollution are the Biological Monitoring Working Party score (BMWP) and the Average Score Per Taxon (ASPT) (Armitage et al., 1983). In the BMWP system, families are assigned a score from 1 to 10, where Oligochaeta as a class receive the lowest score, and families such as Perlidae (Plecoptera) and Heptageniidae (Ephemeroptera) receive the highest score. The BMWP score represents the sum of values for all the scored families present. The ASPT metric is calculated by dividing the BMWP score by the number of taxa present (Armitage et al., 1983). Another suitable method to assess organic pollution, the Belgian Biotic Index (BBI) (De Pauw & Vanhooren, 1983), requires identification to family and even genus level. In testing different metrics focused on the detection of organic pollution in several stream types in Europe, Sandin & Hering (2004) report that the metric ASPT correlates best with the organic pollution gradient.

Today, the response of benthic macroinvertebrates communities to conditions under organic load in rivers is well known. In general, increased organic pollution leads to a decrease in macroinvertebrate taxonomic richness and diversity in which the nutrient sensitive or non-tolerant EPT taxa are adversely affected while tolerant taxa such as Oligochaeta and some Chironomidae flourish (Rosenberg & Resh, 1993; Wright et al., 1995; Shieh et al., 1999; Ortiz & Puig, 2007; Acre et al., 2014; Burdon et al., 2016). However, using indicator taxa in the



assessment of organic pollution is a limited approach as some taxa groups which are considered non-tolerant i.e. Ephemeroptera, also include species whose nymphs can inhabit very polluted waters (Alba-Tercedor et al., 1995) while some Chironomidae are typical of non-polluted sites (Molineri et al., 2020).

At sites under impact of urban wastewater discharge collector – gatherers have been reported as the dominant group, while densities of scrapers and shredders are reduced (e.g. Olive et al., 1998; Shieh et al., 1999). For studying the impact of organic pollution in rivers on benthic macroinvertebrates Charvet et al. (1998) propose biomonitoring through biological traits (e.g. reproduction and life duration) rather than using standard biotic indices. Their functional approach linked organic pollution to presence of organisms using the strategy of resistance. Sites impacted by wastewater discharge also showed an increase in occurrence of plurivoltine taxa, and taxa with tegument respiration while also supporting an increase in abundance of benthic macroinvertebrates larger than 2 cm. Although, the impact wastewater discharge will have on benthic macroinvertebrates, according to Mor et al. (2019), will be also be dependent on stream characteristics such as substrate size and hydrologic condition.

#### **2.4 Impact of landuse on the river ecosystem and benthic macroinvertebrates**

The boundaries of rivers extend beyond the river itself and today it is known that there are dynamic interactions between a river channel and the surrounding landscape (Hynes, 1975; Ward, 1989; Giller & Malmqvist, 1998). In addition to the River Continuum Concept, Junk et al. (1989) proposed the Flood pulse concept (FPC) which views rivers and their associated floodplains as a single dynamic system related through ecological and hydrological processes. On river catchments, natural and anthropogenic factors covary because natural gradients influence the suitability of areas for agricultural and urban development (Allan, 2004). Considering land cover across catchments can be very diverse, it can be concluded that the influence land cover imposes on river ecosystems can be both natural and anthropogenic.

To date, great research effort has been made in quantifying effects of different landuse types on fish (e.g. Roth et al., 1996; Wang et al., 2001; Meador & Goldstein, 2003; Sutherland et al., 2002; Trautwein et al., 2012; Filgueira et al., 2016; Tóth et al., 2019). Despite a different

indicator group was used, all these studies have in common the recognition that anthropogenic landuse accounted for changes in river ecosystem conditions, and subsequently the biological diversity. An overview of how landuse impacts benthic macroinvertebrates is given below:

#### *Agriculture landuse*

The impact of agricultural landuse of aquatic ecosystems, specifically agricultural drainage, can be summarised as having direct effects such as direct habitat loss due to channelization, and indirect effects ranging from water quality and habitat impacts of nitrogen, phosphorous or sediment to hydrological alterations (Blann et al., 2009). Although significant input of nitrogen in surface waters comes from atmospheric deposition (Howarth et al., 1996) agricultural landuse is good predictor of total nutrient loading in a river. This was confirmed by several studies. Johnson et al. (1997) studied the relationship between landscape factors (landuse and geology) and seasonal stream water chemistry. They found agricultural landuse to account for highest nutrient, total dissolved solids and alkalinity in summer while in autumn when fertilizer application is reduced and there is less surface runoff, catchment geology dominates over landuse in influencing water chemistry. Strayer et al. (2003) in testing of different landuse types with ecological response variables found percentage of cultivated land to be the best predictor for nitrate flux in rivers. Liu et al. (2000) also report an increase in Nitrate-N and total dissolved N in rivers as percentage of cropland increased. Boyer et al. (2002) in a study encompassing 16 catchments in the northeast USA quantified inputs of nitrogen from different sources found agriculture (combined effect of fertilizer use, fixation in crop lands and agricultural animal feed) to be the overall largest input source of nitrogen in rivers. Increased diffuse source nutrient concentrations from agricultural land can also result in high periphyton chlorophyll along a river continuum especially in combination with removed shading from large woody riparian vegetation (DeLong & Brusven, 1992). Furthermore, nutrients entering aquatic ecosystems by means of runoff and soil erosion can lead to eutrophication (Ngatia & Taylor, 2018; Bennet et al., 2001).

Landuse also influences river ecosystems through mechanisms related to sedimentation (Quinn, 2000). Sedimentation, primarily called river siltation, is the increase in suspended solids in a river leading to the clogging of pore-space by fine substrates of diameter  $<63 \mu$  (Graham, 1990). Although siltation can be a natural process, agriculture is the primary anthropogenic cause of siltation in rivers (Walling, 1990; Richards et al., 1993). Increase of agricultural landuse origin sediment in streams can manifest through reducing stream depth variability and substrate

complexity/heterogeneity (Walser & Bart, 1999), and increased turbidity (Herringshaw et al., 2011). The adverse effects of river siltation on benthic macroinvertebrates has been well documented by several research studies (e.g. Quinn, 2000; Kaller & Hartman, 2004; Bo et al., 2007; Davis et al., 2021; Leitner et al., 2021b). Jones et al. (2012) reviewed the existing knowledge on mechanisms by which fine sediment loading impacts macroinvertebrates. They elaborate physical effects (abrasion, clogging, burial listing abrasion, clogging, substrate composition), chemical effects (oxygen concentration), and indirect effects (habitat availability, food availability and quality, and food web changes).

Results of an investigation of 24 tributary watersheds belonging to two river basins in western North Carolina, USA, conducted by Harding et al. (1998) showed significant differences in both benthic invertebrate diversity and composition between agricultural and forested streams but showed no significant difference in invertebrate density between landuse types. Furthermore, for both basins the Margalef's Index and number of EPT taxa were significantly higher in forested streams than in agricultural streams.

Agricultural landuse on a catchment can leave long term effects on stream ecosystems. Harding et al. (1998) in their study also indicate that present day forest streams can support reduced benthic invertebrate diversity if there was historical agricultural landuse on the watershed, leading to the conclusion that even historical landuse can result in long-term modifications to benthic invertebrate communities despite latter reforestation of riparian zones i.e. a streams past landuse can be a predictor of stream benthic invertebrate diversity.

#### *Urban landuse*

The surface area of land occupied by urban development on a catchment is usually much smaller compared to percentage of catchment area used for agriculture, but the impact it imposes on benthic macroinvertebrates is disproportionately larger (Herringshaw et al., 2011). An extensive overview of mechanisms by which urbanized areas influence a rivers physical habitat and water quality is given by Paul & Meyer (2001). They explain how the extent of impervious surfaces associated with urban landuse leads to increased surface runoff and stormwater drainage, consequently, altering the hydrologic regime but also increasing input of different pollutants (nutrients, ions, metals, pesticides, and other organic contaminants). Furthermore, surface runoff from exposed surfaces in combination with loss of riparian

vegetation increases stream water temperature, while altered sediment supply, and channelization associated with urbanization affects channel geomorphology.

Extensive studies demonstrating the negative impact of urban landuse on streams and benthic macroinvertebrates assemblages are available reporting the following influences: decrease in total taxa and density, and significant increase in heavy metals in periphyton from substrate (Garie & McIntosh, 1984); degradation of the community composition (high abundances of a few tolerant taxa) with intensive urban drainage severely increasing degradation even at low urban densities (Walsh et al., 2001); low species richness for most groups and low abundance values, shift of dominant benthic macroinvertebrate groups from Ephemeroptera (forest stream) to Chironomidae (agricultural stream), and Oligochaeta (urban stream) (Lenat & Crawford, 1994); changes in functional feeding groups with gatherers dominating and shredders decreasing (Stepenuck et al., 2002; Roy et al., 2003); decreased sediment size, increased total suspended solids, increased specific conductance, increased nitrogen and phosphorous concentrations and turbidity, significant correlation with all macroinvertebrate variables to both high and low urban land cover (Roy et al., 2003); nonlinear and negative correlation with % EPT, EPT taxa, filterers and scrapers with increased urbanization, positive correlation with Hilsenhoff biotic index (HBI) (Wang & Kanehl, 2003); differing response to percent catchment urbanization dependant on sampled habitat, metrics derived from wood debris samples showed less EPT and lower diversity index value than riffle samples (Stepenuck et al., 2008); decrease in EPT, richness and diversity, strong positive linear relationship to % Oligochaeta and HBI (Bazinet et al., 2010). Single catchment studies (such as is the subject of this Thesis) dealing with the gradient of increasing urbanization (e.g. Whiting & Clifford, 1983; Thorne et al., 2000) report a decrease in benthic invertebrate diversity with increased urban landuse, regardless of catchment size.

#### *Landuse at different spatial scales*

Biological diversity of streams is influenced by landuse at multiple scales (Allan et al., 1997; Allan, 2004). Studies comparing the influence of landuse at different spatial scales on benthic macroinvertebrate composition report varying/mixed significance between the scales.

The following authors report a stronger influence of smaller, reach scale landuse on benthic macroinvertebrate assemblages:

Stewart et al. (2000) based on their study encompassing 21 sites on three watersheds in northwest Indiana, USA, found local scale landuse and instream habitat quality to be a stronger determinant of benthic macroinvertebrate assemblages than landuse at watershed scale. Sponseller et al. (2001) tested macroinvertebrate indices and percentage of non-forest landuse (which included both agricultural and urban/suburban areas) at five spatial scales: catchment, entire riparian corridor, and three riparian sub-corridors extending 200 m, 1000 m, and 2000 m upstream of sampling sites. Based on 9 study sites, they found the strongest relation between percentage of non-forest land at the 200 m sub-corridor scale to rank-abundance slope, taxon richness, diversity, percentage of five dominant taxa and EPT taxa richness. Macroinvertebrate density was the only indicator only slightly more closely related to percentage of non-forest land at the 2000 m sub-corridor scale than the 200 m scale. In their study, EPT taxa richness was the only index related to percentage of non-forest landuse at all scales including catchment scale while all other indices showed no significant relation to non-forest landuse at catchment scale. Sandin & Johnson (2004) using data from 428 non impacted Swedish stream showed that local scale physical (in-stream substrate, and in-stream and riparian vegetation) and some chemical variables most strongly explained among-site variability of community assemblages as opposed to landscape and large scale factors. Rios & Bailey (2006) tested the extent and nature of relationship between different riparian vegetation and benthic macroinvertebrate assemblage structure at three spatial scales: outflow reach (study site), stream network buffer, and sub-catchment. They quantified landuse as forest or agricultural at stream network buffer scale (30 m wide corridor), and at  $\mu$ -basin (sub-catchment) scale for 33 sites. Their results showed no relationship between benthic macroinvertebrate community and agricultural landuse at sub-catchment scale but report a lower Simpson's equitability index with increased agricultural land cover at stream network buffer scale. The strongest reported influence was related to tree cover in the riparian zone at outflow reach scale where taxon richness including total number of EPT taxa and Simpson's diversity all increased with increased tree cover.

The following research concluded a stronger influence of landuse at catchment scale on benthic macroinvertebrate assemblages:

Richards et al. (1997) connected catchment scale variables; landuse, surficial geology, elevation and hydrography to stream reach-scale physical habitat variables influencing benthic macroinvertebrate metrics at 46 study sites on the Saginaw Bay Basin, Michigan, USA. Their results showed that surficial geology variable and landuse variables (intensive agriculture and

presence of wetlands) at catchment scale had the strongest influence determining stream physical habitats accounting for strongest variation in benthic macroinvertebrate assemblage structure. Landscape variables at the 100 m stream buffer scale were not positively related to stream physical habitat i.e. channel morphology, but were strong predictors of sediment related habitat variables: percent fine sediment and percent bank erosion. Townsend et al. (2003) researched four scales of landscape variables; geographical position, catchment, reach, and bedform at 55 sites. They found natural variables (relief ratio, diameter of basin and drainage area) at catchment scale to account for most variation in benthic macroinvertebrate assemblage composition.

In the Upper Mississippi River basin in central Iowa, USA, Herringshaw et al. (2011) evaluated landuse impact on benthic macroinvertebrate assemblages at four spatial scales: 100 m buffer riparian zone extending 100 m upstream from sample site, 200 m riparian buffer zone extending 1 km upstream, 200 m riparian buffer zone extending the entire upstream length, and sub-catchment scale encompassing entire upstream catchment area of sample site. Based on 29 study sites they found strong relationships between land cover and benthic macroinvertebrate assemblages at both local and sub-catchment scale. Their results also showed that urban landuse had greater negative impact on stream conditions than intensive agriculture land cover in the catchment. Although in their results taxa richness and % EPT were positively related to agriculture landuse and negatively to urban, macroinvertebrate abundance and diversity was highest at sights with coarse substrate, abundant CPOM, and plants.

Knehtl et al. (2021) on 6 large rivers in Slovenia (Drava, Mura, Sava, Ljubljana, Krka, and Kolpa) tested the effects of hydromorphology and riparian landuse on benthic macroinvertebrates and fish at different scales: river length of 500 m, 1000 m, 2000 m, and 5000 m and land cover buffers up to 150 m wide. Their results imply that in large rivers, riparian land cover and hydromorphological conditions affect benthic invertebrates at generally longer segments (the 5000 m reach length and 50 m buffer riparian width best explained benthic invertebrate assemblages).

## 2.5 Impact of hydromorphological degradation on benthic macroinvertebrates

The previously discussed landuse is closely related to hydromorphological degradation since in agricultural areas there is a long history of stream channelization and surface drainage system development to facilitate crop production (Blann et al., 2009). Hydromorphological degradation encompasses a variety of impacts to the hydrological regime, morphology, sediment, and landuse/riparian features of a stream e.g. bank modification, floodplain landuse, flow modifications, removal of riparian vegetation (Feld, 2004; DIN, 2010). The first legislative recognition of hydromorphological condition as a precondition for supporting biological communities was introduced with the EU Water Framework Directive (WFD) (European Commission, 2000), where hydromorphological status is considered through three elements: morphology, longitudinal continuity, and hydrology. Alterations to each of these elements, impacts ecosystem functioning, and in turn, the macroinvertebrate community structure and functioning.

Recognizing that the physical alterations are closely related to aquatic communities has initiated the development of different methods in different countries for assessing the level of hydromorphological habitat modification. For example, Raven et al. (1997) developed the River Habitat Survey (RHS) method used in the UK for the classification of river habitat quality through a Habitat Quality Assessment (HQA) score which refers to quantification of variety of natural features, and a Habitat Modification Score (HMS) which quantifies the type and extent of artificial features. In Germany, there is the German 'Strukturgütekartierung' for small and mid-sized rivers (LAWA, 2000) and Ecomorphological Survey for large rivers (Fleischhacker & Kern, 2002). Austria uses the Austrian Habitat Survey (Werth, 1987; Muhar et al., 1996, 1998). Tavzes & Urbanič (2009) developed the Slovenian hydromorphological assessment methodology (SIHM) which is based on the River Habitat Survey (RHS), and through which five hydromorphological indices are derived. In Croatia, the European Standard EN 15843:2010 has been adapted for the purpose of hydromorphological monitoring of rivers (Vučković et al., 2018). Wiatkowski & Tomczyk (2018) tested the methodologies RHS, LAWA, QBR (assessment of bank habitats, Spain), and HEM (comprehensive morphological assessment, the Czech Republic) on Polish Rivers and conclude that all the methods meet requirements of WFD.

As a further step to assessing the physical habitat, some authors succeeded in developing multimeric indices based on benthic macroinvertebrates for assessing hydromorphological modification. Lorenz et al. (2004) developed the German Fauna Index for five German stream types which is based on taxa dominantly occurring at sites of a certain level of morphological degradation. Urbanič (2014) constructed a Slovenian multimetric index for assessing the hydromorphological impact on benthic invertebrates in large rivers (SMEIH<sub>VR</sub>) from the River Fauna Index for large rivers (RFI<sub>VR</sub>) and the metric % akal + lithal + psammal taxa (scored taxa = 100%). The River Fauna Index (RFI), which is based on indicator responses to hydromorphological degradation, has been integrated into the General Degradation module as a basis for ecological status assessment of rivers in Croatia (Mihaljević et al., 2020).

The impact of hydromorphological degradation on benthic macroinvertebrates has been a topic of research interest in the past 20 years and a review of some of the finding is given below:

Fleituch (2003) examined the change in benthic macroinvertebrate community under the influence of different hydro-technical structures (weirs, sills, and channelization) in a mountain stream in Poland. His results suggest that most types of regulations negatively influence the functional organization of benthic macroinvertebrates (scrapers, collector-gatherers, and predators). Horsák et al. (2009) suggest that the biggest threat to benthic macroinvertebrate diversity of lowland rivers are morphological modifications of the river channel. Their study showed that channelization has a greater effect on benthic macroinvertebrate communities than organic pollution or flow alterations from reservoirs. Armitage & Pardo (1995) examined the impact of stream regulation with sluice gates using conventional biological techniques and tested the applicability of the mesohabitat method in describing changes resulting from regulation. They found that indices based on family richness were unable to demonstrate the impact of regulations, but the proportions of mesohabitats and their faunal community were a good indicator of regulation and the altered physical habitat. Brooker (1985) in a time of limited studies on the impact of channelization on in-stream ecology gives a review of available literature on effects on different vertebrates, vegetation, and macroinvertebrates. Regarding macroinvertebrates, he concludes that recovery is often rapid following dredging, but also that channelization changes the distribution and diversity of available habitats, leading to loss in taxa richness.



Tavez et al. (2006) applied River Habitat Survey to a small urban stream where organic pollution could be excluded as a factor affecting the macroinvertebrate assemblages due to overall moderate pollution. Based on five sites, they observed a longitudinal downstream physical habitat degradation which was not followed by the macroinvertebrate taxonomic composition and functional feeding groups (i.e. both the pristine and the most altered sites were the most diverse). They did however find strong correlations between changes in the physical habitat quality (the RHS scores: HMS, HMS class and HQA) and % of detritivores, % of *Caenis luctuosa* (Burmeister, 1839), number of individuals, and % of EPT individuals. Erba et al. (2006) correlated the River Habitat Survey scores against different biological metrics from 79 sites within the EU STAR project. The metrics ASPT, EPT taxa and ICMi (Inter-calibration Common Metric index) in general gave the best correlations with RHS scores. But the highest correlation was achieved for the number of families and the number of EPT taxa in relation to the presence of artificial substrates and the HQA index respectively. RHS scored artificial structures affecting flow character and lateral / longitudinal connectivity gave the lowest correlation values with all biological metrics. Furthermore, diversity indices were the worst performing metrics. Buffagni et al. (2004) based on samples collected from depositional (pool) areas of small rivers in south Italy found strong effects of hydromorphological degradation on benthic macroinvertebrates. Petkovska & Urbanič (2015) tested the relationship between river morphological variables and benthic macroinvertebrates among three ecoregions. Their results show that RHQ variables of the Slovenian Hydromorphology assessment method are more important for structuring benthic macroinvertebrate assemblages than the RHM variables. There are also studies dealing with the impact of hydromorphological modification on individual benthic macroinvertebrate groups e.g. Coleoptera (Pakulnicka et al., 2016); Ephemeroptera (Vilenica et al., 2022), and Odonata (Vidaković Maoduš et al., 2022).

Friberg et al. (2009) in an extensive study encompassing 1049 sites in three countries (Denmark, Slovakia, and Sweden) found only relatively weak relationships between hydromorphological degradation and standard macroinvertebrate indices. The RHS HQA score did not correlate with the Shannon-Wiener diversity index while the RHS HMS did not correlate with any of the tested diversity metrics. They attribute their results to multiple stressors at the majority of the reaches, scaling issues, and the scope of commonly used macroinvertebrate assessment systems. Dahm et al. (2013) using a large dataset from Germany and Austria found the functional metric (%) microhabitat preference pelal, the best responding metric to hydromorphological condition for lowland streams, while the metric (%) zonal preference

metarhithral responded best to hydromorphological condition for mountain streams. Furthermore, in their study, local hydromorphological variables such as artificial embankment or instream habitat modification, had a small, but measurable effect on metric variability for all organism groups. The impact of hydromorphological degradation (along with other stressors) on benthic macroinvertebrates at different scales was also investigated by Feld & Hering (2007). Their study shows the importance of using benthic macroinvertebrate functional measures for detecting the impact of hydromorphological degradation at different spatial scales. At meso (reach) scale, they found the highest relation between hydromorphological degradation and the molluscs *Bithynia tentaculata* (Linnaeus, 1758) and *Potamopyrgus antipodarum* (Gray, 1843), and the amphipod *Gammarus roeselii* (Gervais, 1835). At micro (site) scale, the proportion of artificial macrolithal and mesolithal used for bank enforcement (rip-rap) determined degradation, and these sites were also characterised by a high share of Oligochaeta. Graf et al. (2016) demonstrate how upstream channelization can impact macroinvertebrate diversity and functioning in the downstream morphologically natural stretch of a lowland river in Austria. They report degradation of the meandering reach through siltation as a result of the upstream local channelization.

The placement of large immobile rocks (rip-rap) along the river banks to stabilize channel banks from erosion can have both direct impact, such as change in bank structure or prevention of lateral channel movement, and indirect consequences such as reduced wood and sediment input (Reid & Church, 2015). Studies on the biological impacts of rip-rap report both positive and negative effects on fish and invertebrates. Older dated studies have already shown that rip-rap as a microhabitat supports higher benthic macroinvertebrate abundance in comparison to other microhabitats (e.g. Wolf et al., 1972), especially if the rip-rap is covered in bryophytes (Linhart et al., 2002). On the other hand, bank reinforcement structures in rivers can act as stepping stones of invasion for alien Peracarida (Crustacea) (Žganec et al., 2018). Artificial material placed as rip-rap has also been associated with invasive invertebrate species such as the Zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) (Jude & DeBoe, 1996), and invasive fish species. Studies from both the USA (e.g. Moyle & Light, 1996) and Europe (e.g. Roche et al., 2013; Janáč et al., 2018) have reported great abundances of the Ponto-Caspian gobiid (*Neogobius melanostomus* (Pallas, 1814)) near rip-rap but rarely in other unaltered channel segments. The presence of invasive species could influence benthic macroinvertebrates by altering trophic relationships in the ecosystem adjacent to the rip-rap.

The importance of natural riparian vegetation as an integral element of river hydromorphology can be observed through several functions. Through shading, riparian vegetation regulates water temperature (e.g. Rutherford et al., 1997; Roth et al., 2010) but also light levels, and therefore influences primary production (Gregory et al., 1991). Broadmeadow et al. (2011) showed that even a low level of shading by riparian trees of 20% canopy over a 500 m reach is already effective in regulating maximum summer temperatures from exceeding lethal limits for salmonid fish. Rios & Bailey (2006) found that taxon richness (including total number of EPT taxa) as well as Simpson's diversity of the macroinvertebrate community all increased with increased tree cover in the riparian zone at the outflow reach scale.

The riparian zone plays a significant role in the amount, timing and form of nutrient input into the river because not only does it directly supply organic matter like leaf litter which is a food and energy source in rivers but also the rooting zone intercepts soil solution from the watershed before entering the channel (Gregory et al., 1991). It is furthermore the source of large woody debris which contributes to morphological complexity by providing water depth variations caused by flow obstruction (Buffington et al., 2002). Hydromorphologically degraded rivers and streams often lack large woody debris which is removed during channelization and river management. Since several benthic macroinvertebrate taxa are associated with wood debris (e.g. Hoffmann & Hering, 2000; Dossi et al., 2017), hydromorphological modification especially affects this microhabitat and associated benthic macroinvertebrates.

Zerega et al. (2021) give an extensive review of available literature on effects of hydromorphological rehabilitation measures on the benthic invertebrate communities. They conclude that the most commonly applied measures aimed at increasing habitat heterogeneity (such as addition of meanders and artificial riffles), are not sufficient to improve the benthic invertebrate communities. However, they find that re-establishment of natural hydrological patterns and water quality improvement are most effective measures.

## 2.6 Multiple stressor research

The previous overview of impacts to river systems shows that stressors rarely act individually and that rivers can simultaneously be exposed to multiple stressors which act at different spatial scales. Rivers of agricultural catchments for example are exposed to nutrient enrichment, altered flow, increased sediment input, pesticide fluxes and degradation of the riparian zone (Allan, 2004). One of the main issues with multiple-stressor combinations is that their effect on freshwater communities can be antagonistic (reduced total effects), additive (cumulative total effects) or synergistic (multiplied total effects) (e.g. Folt et al., 1999; Piggott et al., 2015; Jackson et al., 2016; Galic et al., 2018).

The most extensive analysis of multiple pressures affecting European rivers encompassing 9330 sampling sites (3100 rivers) and 14 countries was performed by Schinegger et al. (2012). They categorized pressures into four groups: water quality, morphology, hydrology, and connectivity. Only 21% of the sites were unaffected while 31% of the sites were affected by one pressure group, 29% by two, and 28 by a combination of three pressure groups. Furthermore, 12% of the sites were affected by all four pressure groups. Regarding European lowland rivers, approximately 90% are impacted by a combination of all four pressure groups. Out of the 10 encompassed ecoregions within the study, the Hungarian lowlands ecoregion (11) fell high on the list for having worst conditions in terms of water quality and morphological habitat degradation.

Nõges et al. (2016) reviewed existing scientific literature and reports of EU Member States concerning pressures acting on surface water bodies and conclude excess nutrients to be the main physicochemical stressor in multiple-stress situations. Similarly to Schinegger et al. (2012) they report that the majority of multiple stress situations in rivers accounted for a combination of nutrient and hydromorphological stress. This gains additional significance as Früh et al. (2012) showed that streams with morphological and physicochemical degradation are more prone to invasion by non-indigenous macroinvertebrate species.

Despite present-day knowledge on the influence of individual natural and anthropogenic stressors on benthic macroinvertebrates, the influence of multiple-stressor situations on species populations, communities and ecosystems is not fully understood and is a relatively new interest of research in freshwater ecology. An overview of some studies is given below:

Feld & Hering (2007) tested 130 environmental parameters (characterising hydromorphology and landuse) of 75 river sections of Central European lowland river basins against 244 benthic macroinvertebrate taxa and 84 community metrics and indices at ecoregion, catchment, reach, and site scale. At all spatial scales, they report proportion of variance explained by environmental variables to be greater for metrics than for taxa. Townsend et al. (2008) investigated the individual and combined effects of nutrient concentrations and streambed fine sediment cover on benthic macroinvertebrates. Their result suggests that an anthropogenic increase in sediment has a greater impact than increased nutrient concentrations.

Gieswein et al. (2017) used 12 stressor variables encompassing three stressor groups (riparian landuse, physical habitat quality, and nutrients) and studied stressor interactions and the impact on benthic macroinvertebrates, fish and macrophytes. Based on 1095 sites within the mountainous River Ruhr catchment in Western Germany, Europe, they found that additive stressor effects dominated while stressor interactions were generally weak and rare, implying they act independently. Their results further showed that habitat degradation (bed physical habitat structure) was the dominant stressor group for the river fauna, and that general integrative metrics such as % EPT taxa performed better than ecological traits e.g. % feeding types.

The first attempt in partitioning changes in the benthic macroinvertebrate community between different stressors groups in Slovenia was conducted by Pavlin et al. (2011), using partial Canonical Correspondence Analysis (pCCA). Their results show that the pure effect of stressor-groups (70%) are more than twice as high as the sum of their combined effects (30%). Villeneuve et al. (2018) studied the direct and indirect effects of multiple stressors on benthic macroinvertebrates from the French monitoring network encompassing 1200 sites. They conclude that landuse influences both hydromorphology and the physico-chemical condition which consequently indirectly influences the biology. Furthermore, their results imply hydromorphological alterations have a major indirect effect on macroinvertebrate assemblages. Schäfer et al. (2020) conclude that in a multiple stress environment, fine sediment load is the key factor shaping the macroinvertebrate community structure in a Eurasian steppe river.

Urbanič et al. (2020) developed a pCCA model using 292 benthic macroinvertebrate taxa from large rivers incorporating the effects of hydromorphology, landuse and water quality. Stressors and not the natural characteristics were the dominant factors shaping benthic macroinvertebrate

assemblages. The pCCA showed that unique effects of stressor groups dominated over joint effects. Leitner et al. (2021a) evaluated benthic macroinvertebrate response to multiple stressors in very large European rivers based on 1197 samples. Out of the eight studied pressures, they found alteration of the riparian vegetation to be the most frequent pressure at five out of seven river types. They also found that different metrics correlated with pressures depending on the river type. The pressures 'navigation' and 'channelization' significantly correlated with all tested metrics, while the pressure 'riparian vegetation alteration' significantly correlated with all metrics except for total abundance.

## 2.7 Research of the Bednja River

Earlier studies of the Bednja River were mainly limited to geographic and hydrologic research while ecological research has started in recent years. Petrić (2010) based on published and unpublished sources and literature gives a historical overview of villages from source to mouth of the Bednja River and writes that land along the river has been used primarily for agriculture already in the Middle Ages. Počakal (1982) calculated the hydrographical elements of the Bednja River catchment. According to methodologies he used, the Bednja catchment size is 596 km<sup>2</sup>, with a maximum width of 29 km and minimum of 4 km (average 5.78 km). The highest catchment elevation point is 1061 m. The total number of tributaries is 281, out of which 81 are first order tributaries (35 first order tributaries on the left catchment size and 47 on the right). Počakal (1982) calculated the length of the Bednja River to be 103 km. The Croatian encyclopaedia states the length of the Bednja River to be 133 km (Hrvatska enciklopedija, 2000). According to the most recent and most accurate measurements performed by Čanjevac et al. (2022) the total length of the Bednja River amounts to 106.26 km. This value was obtained by vectorising the river channel from the marked source of the longest headstream near the settlement of Bednjica to the mouth into the Drava River using a topographic map with a scale of 1:25 000 (Čanjevac et al., 2022).

Using the Pardé coefficient (Pardé, 1933), which represents the ratio of mean monthly flow and mean annual flow, Čanjevac (2013) defines the flow regime type of the Bednja River. Based on calculations from the stations Tuhovec, Željeznica, Lepoglava, and Ludbreg, he classifies the Bednja River as a Peripannonian pluvial-nival discharge regime river. This means that

rainfall / snowmelt are responsible for the two maximum and two minimum discharges during the year.

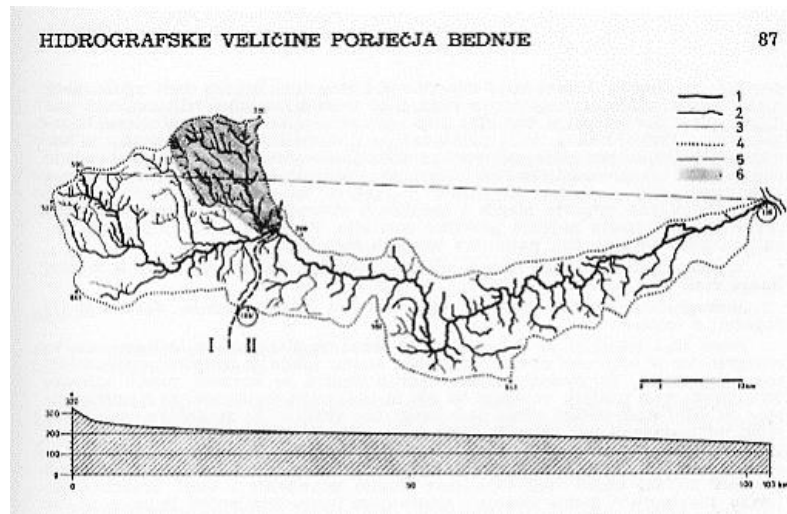


Figure 2.6.1. Map of the Bednja River catchment from the study by Počakal (1982).

The Bednja River, represented by sampling station “Bednja Slanje 09//12” is investigated within the project “Testing of Biological Methods for Ecological Status Assessment (Water Framework Directive 2000/60/EC) in Representative River Basins of the Pannonian and Dinaric Ecoregions” (Mihaljević et al., 2011), and its course is classified into two types: lowland mid-sized river on a silicate substrate and lowland mid-sized river on a carbonate substrate. The sampling station “Bednja Mali Bukovec” was sampled for the purpose of the project „Intercalibration of methods for ecological status assessment using biological elements phytobenthos, macrophytes and macroinvertebrates in rivers of the Pannonian ecoregion” which resulted in the „Report on fitting macroinvertebrate classification method with the results of the completed intercalibration of the EC GIG (R-E2 and R-E3)” (Mihaljević et al., 2020).

Odonata specimens collected from the sampling station “Bednja Stažnjevec” have been included in a study on anthropogenically impacted lotic habitats in Croatia by Vilenica et al. (2020). They conclude that degraded lowland rivers support a relatively low number of Odonata species with a broad ecological tolerance.

Finally, several Bednja tributaries streams (Striper, Slugovina, Ljubelj, Belski potok, Očura, Ivanečka Železnica) were part of a large-scale study on the cryptic diversity of *Gammarus fossarum* Koch in Panzer, 1836 conducted by Wattier et al., (2020). Their results showed a high level of cryptic diversity among the *G. fossarum* specimens collected at the Bednja River catchment.

### 3. MATERIALS AND METHODS

#### 3.1 Study area

##### 3.1.1 Geographic attributes

The Bednja River located in northern Croatia is a tributary of the Drava River and belongs to the Danube River Basin, respectively the Black Sea Basin. The Bednja River catchment belongs to the Pannonian lowland ecoregion (ER 11) (Hungarian lowlands according to Illies (1978); pursuant to the WFD). With a total length of 106 km (Čanjevac et al., 2022), from its source at the foothills of Ravna Gora mountain at 311 m a.s.l. (Fig. 3.1.1a) to its mouth into the Drava River at 136 m a.s.l. (Fig. 3.1.1b), the Bednja River changes typology from category: HR-R\_1 – small mid-altitude running waters (study sites 1 – 9) to HR-R\_4A - medium lowland running waters (study sites 10 – 20)<sup>1</sup> (Mihaljević et al., 2020). The boundary for this typology change is marked by the altitude drop below 200 m a.s.l. which occurs around Stažnjevec (Fig. 3.1.2.).

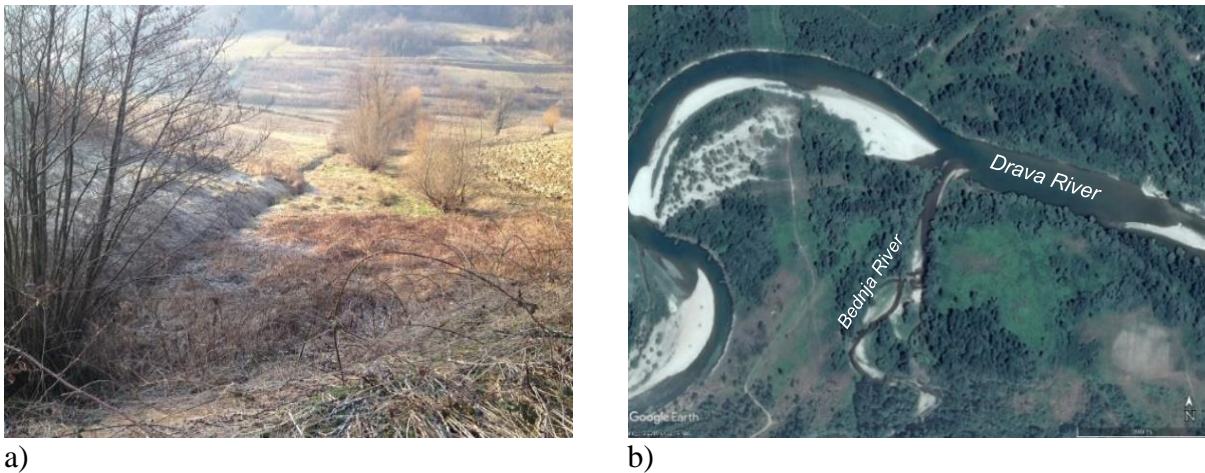


Figure 3.1.1. a) The rheohelocrene type source of the Bednja River at Bednjica, 20.03.2015; b) mouth of the Bednja River into the Drava River (Google Earth Pro, Image © 2019 CNES / Airbus).

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<sup>1</sup> Croatian types HR-R\_1 and HR-R\_4A are intercalibration types R-EX6, respectively R-E2, pursuant to the Eastern – Continental (EC) Geographic Intercalibration Group (GIG) (Mihaljević et al., 2020).



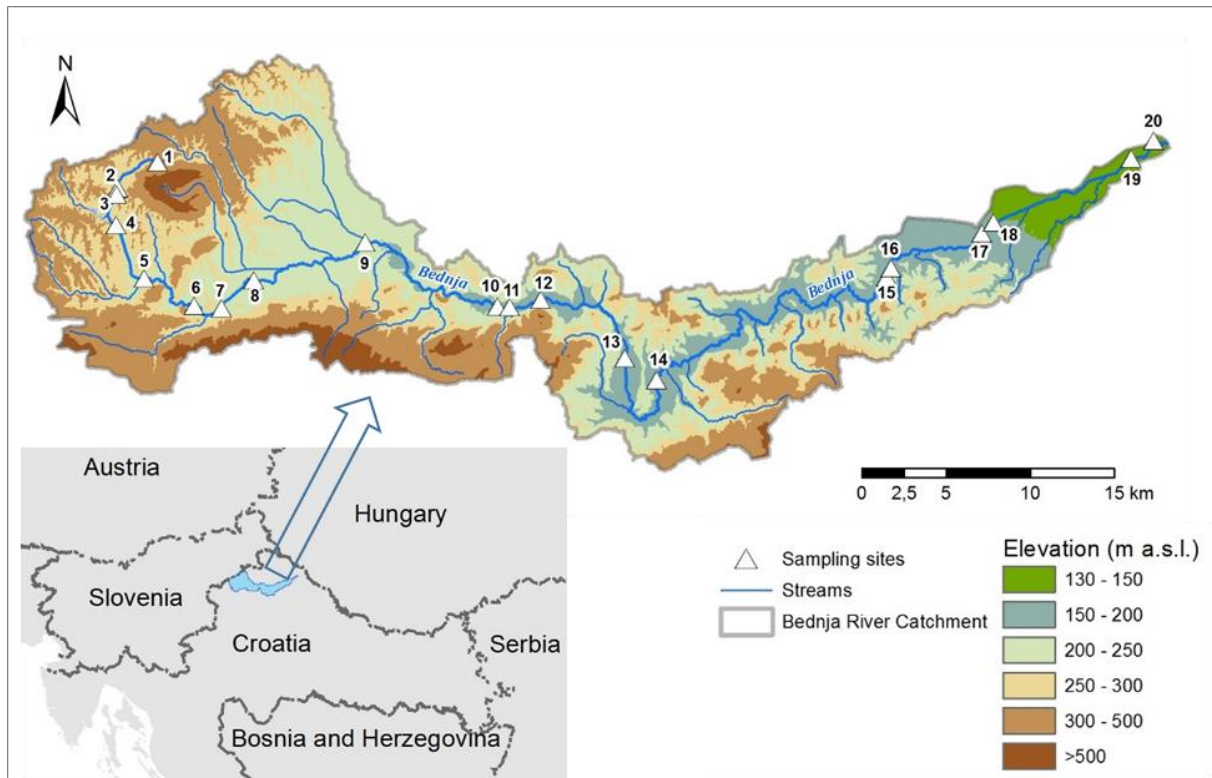


Figure 3.1.2. Position of the Bednja River catchment in Croatia and study sites in relation to altitude. Names of study sites are listed in Tab. 3.2.1.

### 3.1.2 Bednja catchment geology, hydrogeology and hydrology

Geology of the Bednja River catchment is quite complex (Fig. 3.1.3.). It is situated in the Western Pannonian Basin which started forming in early Miocene (Royden et al., 1983) while the major extensional processes in Early and Middle Miocene resulted in the formation of the Drava Basins and minor sub-basins (Prelogović et al., 1998). Regarding geomorphological processes, the Bednja catchment area within the Pannonian Basin falls under the macro-geomorphological region 1.4: Mountainous-basin region of North Western Croatia (Bognar, 2001). Fluvial and fluvio-denudational processes are responsible for formation of the relief meaning hydrogeological characteristics had a greater influence in developing of the valley network and fluvial outflow than hydrometeorological factors (Bognar et al., 2012). The hydrogeological map of Croatia positions the Bednja catchment on sandy and gravely deposits occasionally clayey with medium to low yielding aquifers (Majer & Prelogović, 1993). Based on the Geologic Map of Croatia 1:300 000 (HGI, 2009) Holocene alluvial deposits, the youngest deposits on the catchment are present along most of the Bednja River course and its floodplains (corresponding to study sites 5 – 20). Clay, clayey silt and small grained sand prevail in the top layers of these deposits while the lower levels constitute of gravel mixed with clay or sand. The

upper reach is situated on clastic and volcanic rocks (corresponding to study sites 2 – 4) while the source (study site 1) lays on Middle Triassic carbonate deposits (Fig 3.1.3).

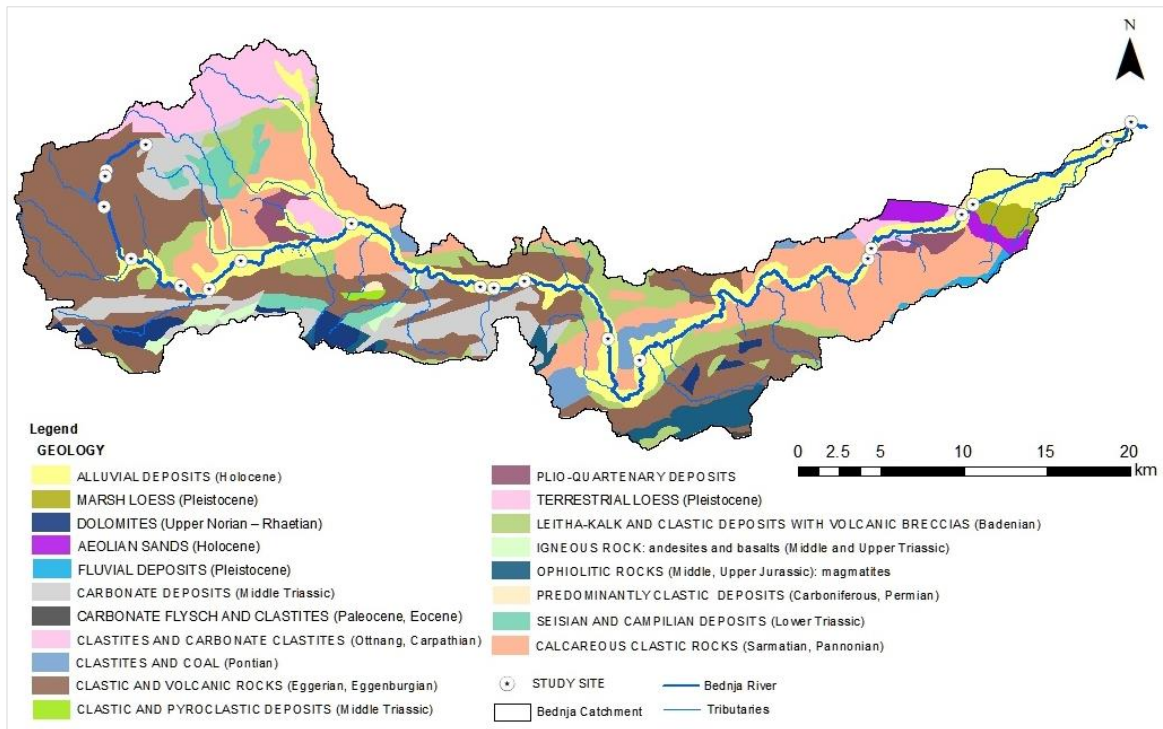


Figure 3.1.3. Geology composition of the Bednja catchment in relation to the Bednja River, Bednja tributaries, and study sites (Modified from HGI, 2009).

The Bednja River has a peripannonian pluvial-nival discharge regime (Čanjevac, 2013) with two maximum and two minimum discharges during the year caused by rainfall/snowmelt. The first maximum occurs in March or April, while the second, usually heavier occurs in December. The minimum discharge periods are in August and February.

Daily measurements of water level and discharge are performed at five hydrological stations (HS) along the Bednja River (Tab. 3.1.1): HS Lepoglava corresponding to study site 7, HS Željeznica between study sites 9 and 10, HS Ključ downstream of study site 14, HS Tuhovec, upstream of study site 15 and HS Ludbreg corresponding to study site 17. Data collected from the hydrological stations in 2015 show that research was conducted during periods of low water level (Fig. 3.1.4.) and low discharge (Fig. 3.1.5.).

Table 3.1.1. Hydrological stations along the Bednja River showing water level (cm) and discharge ( $\text{m}^3\text{s}^{-1}$ ) on 30.06.2015, the beginning of sampling (source: <https://hidro.dhz.hr>).

	Hydrological station (HS)	Code	Catchment area ( $\text{km}^2$ )	Water level (daily average) 30.06.2015.	Discharge (daily average) 30.06.2015.
1	LEPOGLAVA	5140	89.80	25 cm	$0.530 \text{ m}^3\text{s}^{-1}$
2	ŽELJEZNICA	5075	307.95	18 cm	$1.054 \text{ m}^3\text{s}^{-1}$
3	KLJUČ	5143	415.67	- 23 cm	$1.793 \text{ m}^3\text{s}^{-1}$
4	TUHOVEC	5065	469.54	35 cm	$2.278 \text{ m}^3\text{s}^{-1}$
5	LUDBREG	5089	546.98	- 12 cm	$2.613 \text{ m}^3\text{s}^{-1}$

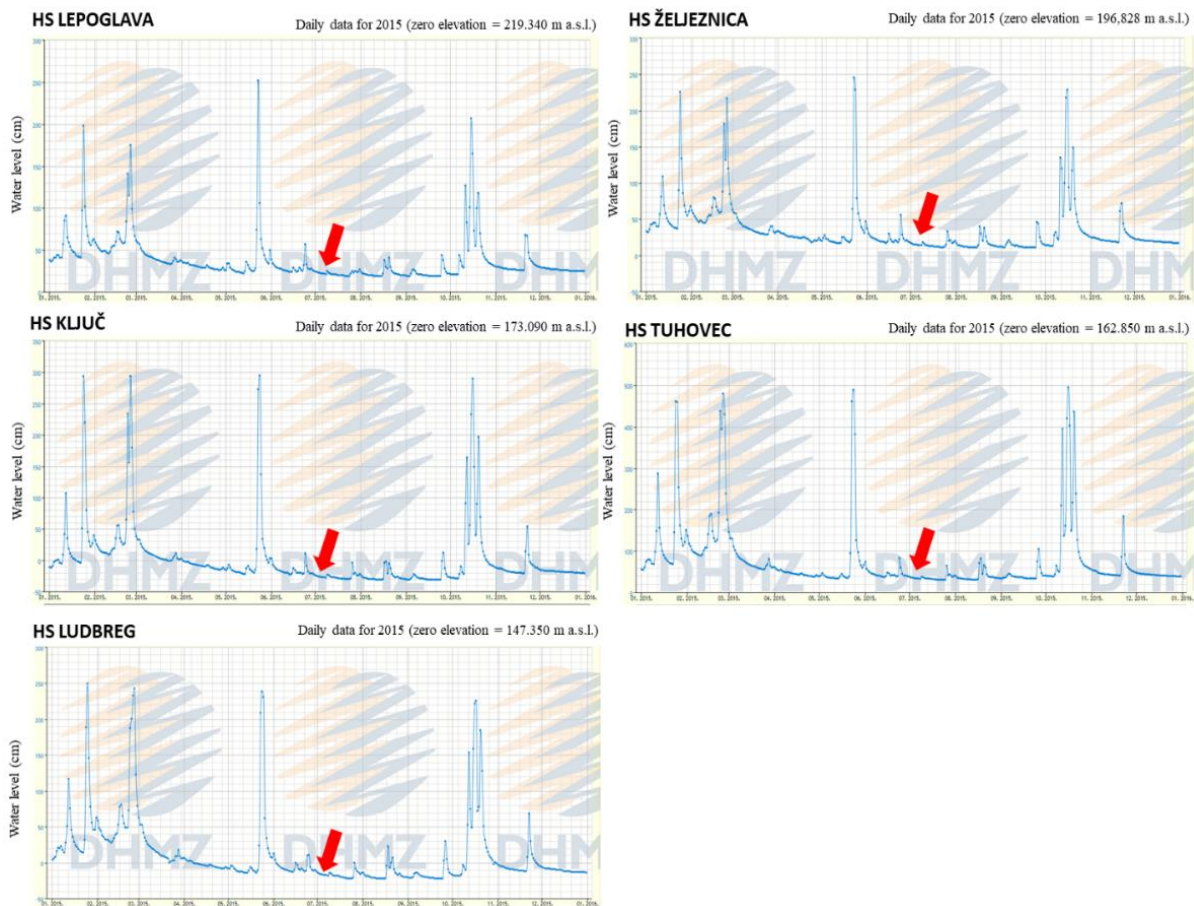


Figure 3.1.4. Daily average water level values for 2015 at five hydrological stations along the Bednja River. Red arrow points to research period (source: <https://hidro.dhz.hr>).

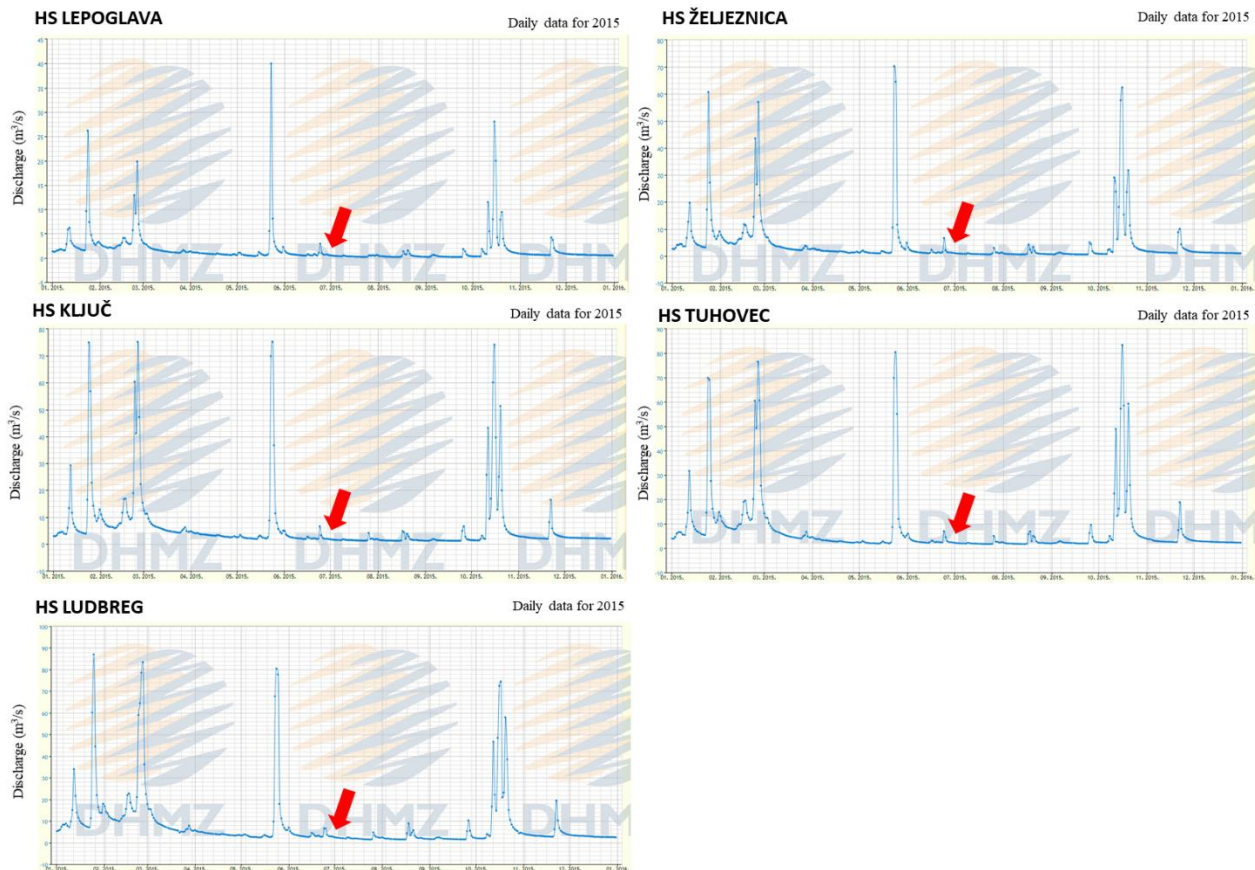


Figure 3.1.5. Daily average discharge in 2015 at five hydrological stations along the Bednja River. Red arrow points to research period (source: <https://hidro.dhz.hr>).

### 3.1.3 Bednja catchment climate and climate change

According to the Köppen climate classification, the Bednja catchment area falls under climate type Cfb - temperate humid climate with warm summers (Filipčić, 1998). In Cfb type climate regions, the average temperature of the coldest month does not drop below  $-3^{\circ}\text{C}$  while average temperature of the warmest month does not exceed  $22^{\circ}\text{C}$  (Šegota & Filipčić, 2003). Furthermore, at least four months of the year have an average temperature above  $10^{\circ}\text{C}$  (Kottek et al., 2006).

There are three meteorological stations (MS) along the Bednja River: MS Bednja corresponding to study site 5, MS Novi Marof in the vicinity of study site 13 and MS Ludbreg around study sites 17 and 18. The main meteorological station Varaždin is located outside the boundaries of the Bednja catchment (10 – 25 km from the study sites) but because it has the longest history of records it can be used for result comparison. Comparing the monthly results from

meteorological stations along the Bednja River during 2015, the research year, it can be observed that meteorological conditions over the Bednja catchment are not uniform (Tab 3.1.2).

The most upstream MS Bednja recorded cooler average monthly air temperatures (from 0.9°C to 22.0°C) and a cooler average yearly air temperature (10.8°C) while receiving the most total precipitation (995.7 mm). The mid catchment MS Novi Marof recorded the highest average monthly air temperatures (from 1.8°C to 23.6°C) and average yearly air temperature (12.2°C) and received the least amount of rainfall in the study year (771.4 mm). The most downstream MS Ludbreg recorded slightly lower average air temperatures to MS Novi Marof with monthly average air temperature values from 1.6°C to 23.3°C and the average yearly temperature of 11.9°C but received more precipitation (924.4 mm in total). MS Varaždin recorded similar values to MS Ludbreg with 11.7°C as the average temperature in 2015 and a total of 965.4 mm rainfall received (Tab. 3.1.2).

Table 3.1.2. Monthly and yearly average air temperature (°C) and total monthly and yearly precipitation values recorded at MS Bednja, MS Novi Marof, MS Ludbreg and MS Varaždin in 2015 (source: raw data from Croatian Meteorological and Hydrological Service (DHMZ) by request).

<b>AVERAGE AIR TEMPERATURE (°C) 2015</b>													
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	<b>Average</b>
<b>MS BEDNJA</b>	2.5	0.9	5.6	10.8	15.9	19.0	22.0	20.5	15.7	9.8	5.6	1.4	<b>10.8</b>
<b>MS NOVI MAROF</b>	3.1	1.8	7.1	12.0	16.8	20.4	23.6	22.8	16.8	10.6	8.2	3.3	<b>12.2</b>
<b>MS LUDBREG</b>	3.0	1.6	7.0	11.7	16.8	20.3	23.2	22.4	16.6	10.3	7.5	2.2	<b>11.9</b>
<b>MS VARAŽDIN</b>	3.2	1.7	6.8	11.4	16.4	19.8	23.0	21.9	16.4	10.2	7.3	2.2	<b>11.7</b>

<b>TOTAL PRECIPITATION (mm) 2015</b>													
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	<b>Total</b>
<b>MS BEDNJA</b>	99.2	83.7	23.4	21.7	165.8	74.4	77.7	74.7	102.7	226.6	44.8	1.0	<b>995.7</b>
<b>MS NOVI MAROF</b>	73.7	85.4	26.0	21.5	114.0	64.2	51.6	71.7	65.5	171.3	24.9	1.6	<b>771.4</b>
<b>MS LUDBREG</b>	94.4	118.1	25.0	18.0	150.9	60.6	67.4	80.5	91.0	180.6	34.8	3.1	<b>924.4</b>
<b>MS VARAŽDIN</b>	76.1	95	15.7	20.7	164.6	78.8	97.5	90.3	102	188.4	35.1	1.2	<b>965.4</b>

Continuous historical records based on which a trend in average annual air temperatures can be observed are available for the meteorological stations: MS Bednja (2007 – 2015), MS Novi Marof (2005 – 2015), MS Ludbreg (1985 – 2015) and MS Varaždin (1965 – 2015). A trend in average annual air temperatures derived from meteorological stations located along the Bednja River for a relatively short period already demonstrate a trend in increase of average annual air temperatures by 0.07°C (MS Bednja and MS Ludbreg) to 0.14°C yearly (MS Novi Marof) (Fig. 3.1.6.). These records also show that 2014 (the year prior research) was the warmest compared to prior years for which data are available.

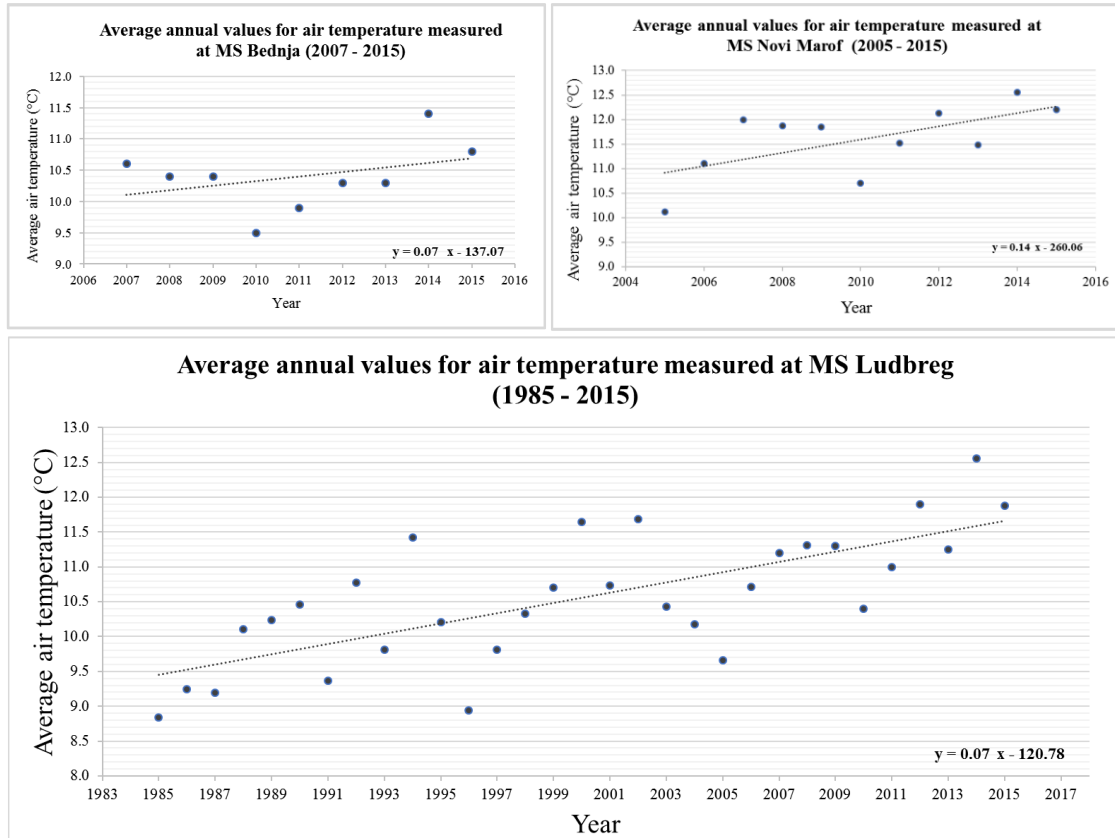


Figure 3.1.6. The trend of average annual air temperature (°C) measured at MS Bednja (2007 – 2015), MS Novi Marof (2005 – 2015) and MS Ludbreg (1985 – 2015) (source: raw data from Croatian Meteorological and Hydrological Service (DHMZ) by request).

However, to be able to comment about climate change, a much longer observed period is required so these results are checked against measurements from the main meteorological station Varaždin available for a 50-year period. A trend in increase of average annual air temperature by around 0.04 °C yearly is observed for the period 1965 – 2015 (Fig. 3.1.7.).

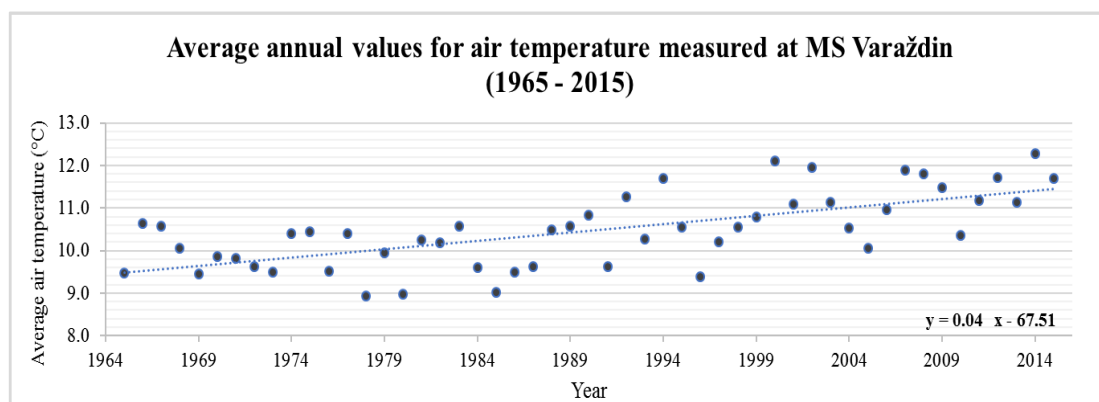


Figure 3.1.7. The trend of average annual air temperature (°C ) measured at meteorological station Varaždin for the period 1965 – 2015 (source: raw data from Croatian Meteorological and Hydrological Service (DHMZ) by request).

Total annual precipitation values measured at MS Bednja, MS Novi Marof and MS Ludbreg for the same given periods are shown in Fig. 3.1.8.

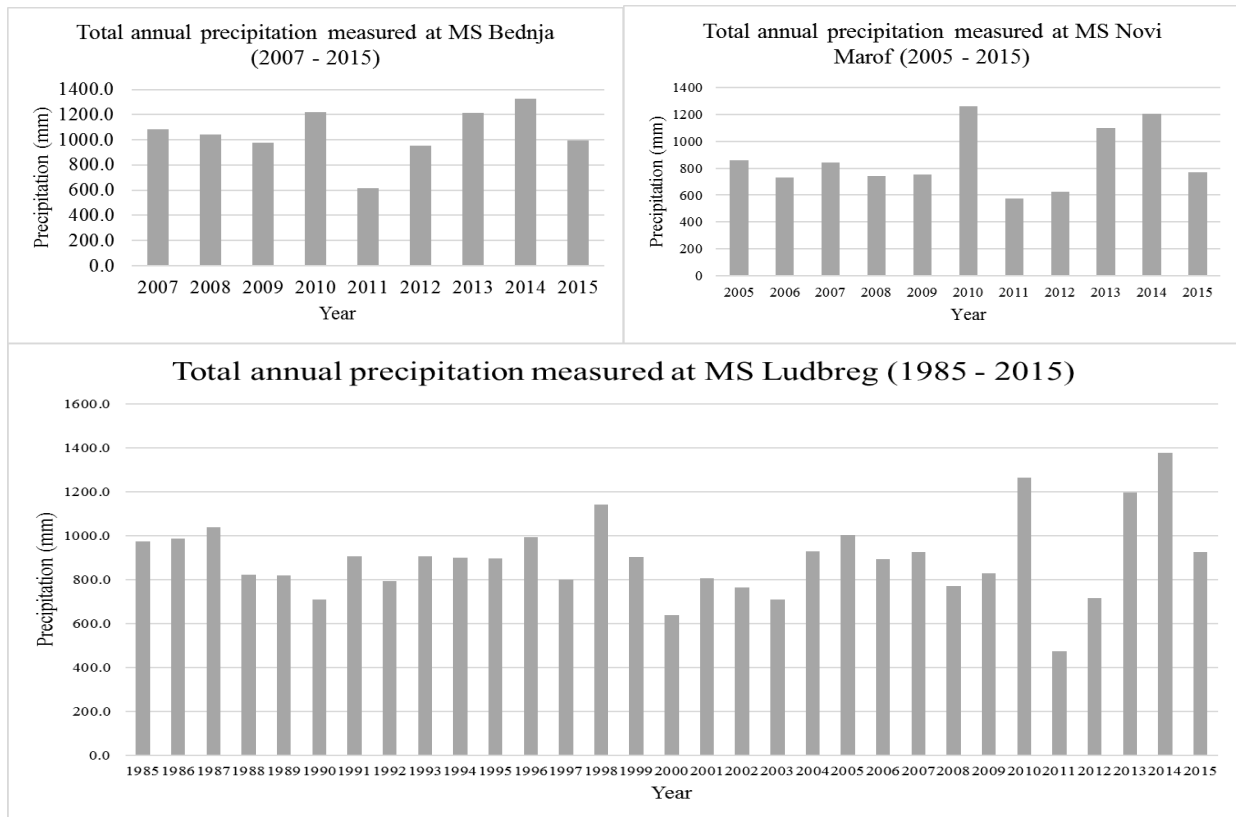


Figure 3.1.8. Total annual precipitation (mm) measured at MS Bednja (2007 – 2015), MS Novi Marof (2005 – 2015) and MS Ludbreg (1985 – 2015) (source: raw data from Croatian Meteorological and Hydrological Service (DHMZ) by request).

### 3.1.4 Water quality

Data from the National Surveillance Monitoring conducted by Hrvatske vode on the Bednja River are available for monitoring stations Bednja Stažnjevec (located downstream of study site 9) and Bednja Mali Bukovec (situated at study site 19) for physicochemical elements, nutrients and metals. Based on median values calculated from monthly measurements in 2014 and 2015 the Bednja River receives relatively low loading with only ammonium exceeding limit values for good status in the upper part of the catchment (Tab. 3.1.3.).

Table 3.1.3. Median values for parameters measured from January to December (12 months) in 2014 and 2015 for monitoring stations Bednja Stažnjevec and Bednja Mali Bukovec (source: Hrvatske vode by request, personal calculation of median). Values exceeding limit values for good status are bolded.

Parameter	Monitoring station (Hrvatske vode)			
	Bednja Stažnjevec (Downstream of study sites 1 – 9)		Bednja Mali Bukovec (Downstream of study sites 10 – 19)	
	2014	2015	2014	2015
Air temperature (°C)	15.0	8.0	16.5	13.0
Water temperature (°C)	12.5	9.0	14.5	10.9
Dissolved oxygen (mgO <sub>2</sub> L <sup>-1</sup> )	8.85	9.40	9.55	10.45
Oxygen saturation (%)	82.5	84.05	91.1	89.95
Alkalinity m-value (mgCaCO <sub>3</sub> L <sup>-1</sup> )	262	253.5	275.5	253
Conductivity at 25°C (µScm <sup>-1</sup> )	541	538	588.5	559.5
pH	8.05	7.9	8.1	8.0
Σ suspended solids (mgL <sup>-1</sup> )	10.5	16	16	20
NH <sub>4</sub> <sup>+</sup> (mgNL <sup>-1</sup> )	<b>0.1885</b>	<b>0.3275</b>	0.095	0.124
Inorganic N (mgNL <sup>-1</sup> )	1.0635	1.264	1.1075	1.2805
Unionised ammonia (mgNL <sup>-1</sup> )	0.00537	0.00556	0.00325	0.0024
NO <sub>3</sub> <sup>-</sup> (mgNL <sup>-1</sup> )	0.825	0.97	0.985	1.07
NO <sub>2</sub> <sup>-</sup> (mgNL <sup>-1</sup> )	0.036	0.029	0.0265	0.0205
Organic N (mgNL <sup>-1</sup> )	0.247	0.239	0.341	0.2815
Dissolved orthophosphates (mgPL <sup>-1</sup> )	0.0385	0.031	0.0365	0.0275
Σ N (mgNL <sup>-1</sup> )	1.335	1.515	1.425	1.525
Σ P (mgPL <sup>-1</sup> )	0.1095	0.1545	0.1025	0.1335
BOD <sub>5</sub> (mgO <sub>2</sub> L <sup>-1</sup> )	2.0	2.1	2.65	1.9
COD <sub>Mn</sub> (mgO <sub>2</sub> L <sup>-1</sup> )	4.0	3.25	4.0	3.7
Fl <sup>-</sup> (mgL <sup>-1</sup> )	0.0955	0.0675	0.1195	0.08
Ca (mgL <sup>-1</sup> )	80.6	77.85	84.95	80.45
K (mgL <sup>-1</sup> )	1.785	1.805	2.08	2.33
Chlorides (mgL <sup>-1</sup> )	7.055	7.515	11.385	11.28
Mg (mgL <sup>-1</sup> )	20.35	19.15	21.2	20.1
Na (mgL <sup>-1</sup> )	6.205	6.825	8.99	10.22
Σ Residual Cl (mgCl <sub>2</sub> L <sup>-1</sup> )	<0.03	<0.03	<0.03	<0.03
Σ Residual Cl (mgHOCIL <sup>-1</sup> )	<0.022182	<0.022182	<0.022182	<0.022182
Sulphates (mgL <sup>-1</sup> )	24.5	23.25	26.4	24.15
Dissolved Cu (µgL <sup>-1</sup> )	0.822	1.0945	1.0085	1.088
Σ Zn (µgL <sup>-1</sup> )	3.4355	5.5295	4.637	4.1025
Dissolved silicates (mgSiO <sub>2</sub> L <sup>-1</sup> )	-	9.305	-	8.53
Dissolved arsenic (µgL <sup>-1</sup> )	-	0.9235	1.332	1.026
Dissolved Zn (µgL <sup>-1</sup> )	-	2.6955	2.0485	0.9385
Dissolved Cd (µgL <sup>-1</sup> )	-	0.0115	-	<0.01
Dissolved Cr (µgL <sup>-1</sup> )	-	0.2555	0.3065	0.2
Dissolved Ni (µgL <sup>-1</sup> )	-	1.293	1.671	1.3295
Dissolved Pb (µgL <sup>-1</sup> )	-	0.23	0.1615	0.1385
Dissolved Hg (µgL <sup>-1</sup> )	-	0.0024	<0.002	0.0028
Hardness (mgCaCO <sub>3</sub> L <sup>-1</sup> )	-	276.4	300.65	281.15
DOC (mgCL <sup>-1</sup> )	-	2.65	-	2.485
TOC (mgCL <sup>-1</sup> )	-	2.82	3.025	2.79

Measured parameters exhibit monthly variations and certain nutrients exceed limit values for good ecological status during the year. In 2015, at the monitoring station Bednja Stažnjevac the following parameters exceed limit values for good ecological status: BOD<sub>5</sub> and COD<sub>Mn</sub> in October, ammonium concentrations in all months except for February and March with highest measured concentrations in September, total nitrogen and orthophosphate concentrations in August and September, total phosphorous in May, July and September.



At the downstream monitoring station Bednja Mali Bukovec the following parameters do not achieve good ecological status during the months of 2015: BOD<sub>5</sub> and COD<sub>Mn</sub> measured in October, ammonium concentrations only in November, nitrate concentrations in June, August and September, total nitrogen values in August, total phosphorous in May, July, August and October.

### 3.1.5 Human impact - existing pressures to the Bednja River

There are five towns (six defined agglomerations<sup>2</sup>) along the course of the Bednja river all having to some extent a constructed sewage system but no wastewater treatment, resulting in untreated wastewater being directly discharged as point source pollution either into the Bednja River (the case with towns Lepoglava, Novi Marof, Varaždinske Toplice and Ludbreg) or tributaries of the Bednja River (the case of town Ivanec<sup>3</sup>) (Varaždinska županija, 2009b). The households of smaller villages scattered along the Bednja River catchment deal with wastewater through individual septic tanks or by directly discharging their wastewater into the river (Varaždinska županija, 2014a) (Fig. 3.1.9.).



a) 22.3.2015



b) 22.3.2015

Figure 3.1.9. Absence of wastewater treatment on the Bednja River: a) direct discharge into the river from household, Rinkovec; b) downstream of Ludbreg sewage outlet, large amounts of sewage origin trash left trapped on branches after flooding especially evident during the non-vegetative period.

With the accession of Croatia to the EU in 2013 and the adoption of the Urban Waste Water Treatment Directive (European Commission, 1991) collection and treatment of wastewater is

<sup>2</sup> An agglomeration as per Directive 91/271/EEC is defined as „an area where the population and/or economic activities are sufficiently concentrated for urban wastewater to be collected and conducted to an urban waste water treatment plant or to a final discharge point”.

<sup>3</sup> Town Ivanec has 3 sewage collectors discharging wastewater into tributaries of Bednja River: Ivanuševac, Bistrica and Matačina.

required for all agglomerations >2000 population equivalent (p.e.)<sup>4</sup>. Furthermore, since the Danube River Basin is a designated sensitive area, the Directive requires at least secondary level of treatment for agglomerations >2000 p.e. and tertiary treatment for agglomerations >10,000 P.E. The six defined agglomerations situated along the Bednja River discharging untreated wastewater into the river at the time of the Study in 2015 and year prior are given in Tab. 3.1.4.

Table 3.1.4. Defined agglomerations along the Bednja River with total potential generated load, planned level of treatment and planned wastewater treatment plant (WWTP) construction date, given in order from most upstream to downstream (Hrvatske vode, 2015a).

	Agglomeration	Recipient	Total potential generated load P.E. (2014)	Existing level of treatment	Planned treatment level	WWTP construction planned date
1	Lepoglava	Bednja River	6,894	none	secondary	2023
2	Ivanec		11,806	none	tertiary	2020
3	Novi Marof		7,464	none	tertiary	2023
4	Varaždinske Toplice		5,423	none	secondary	2023
5	Ludbreg		8,260	none	secondary	2023
6	Veliki Bukovec		2,588	none	secondary	2023

The Bednja River along its course demonstrates a spectrum of degraded habitats but it is also possible to find semi-natural areas. In the Bednja catchment, most of the floodplain is used for intensive and extensive agriculture while the surrounding hills and mountains are mainly forest and near natural areas. Agricultural land represents a diffuse source of pollution in rivers (Novotny, 1999).



a) 30.6.2015



b) 20.05.2016

Figure 3.1.10. Typical landuse within the floodplain of the upper and mid-catchment:  
 a) management of agriculture land immediately by the Bednja River at Cvetlin;  
 b) tilled land with sprouting corn on the floodplains at Završje Podbelsko.

<sup>4</sup> 1 p.e. (population equivalent) is a term used in wastewater treatment defined by Directive 91/271/EEC as „the organic biodegradable load having a five-day biochemical oxygen demand (BOD<sub>5</sub>) of 60g of oxygen per day”.

Hydromorphological degradation as a result of river training however is the most evident pressure observed along the entire Bednja River. Large segments of the river have historically been over-deepened and channelized. According to digitalized maps of the Habsburg Empire (Timár et al., 2007) the first major regulation of the Bednja River was performed between the 18<sup>th</sup> and 19<sup>th</sup> century (downstream from Ludbreg) showing that human interventions to lowland rivers in Croatia date back to more than 200 years ago. During this time, a new river channel was dug out, cutting off the old Bednja riverbed which today no longer exists (Fig. 3.1.11.).

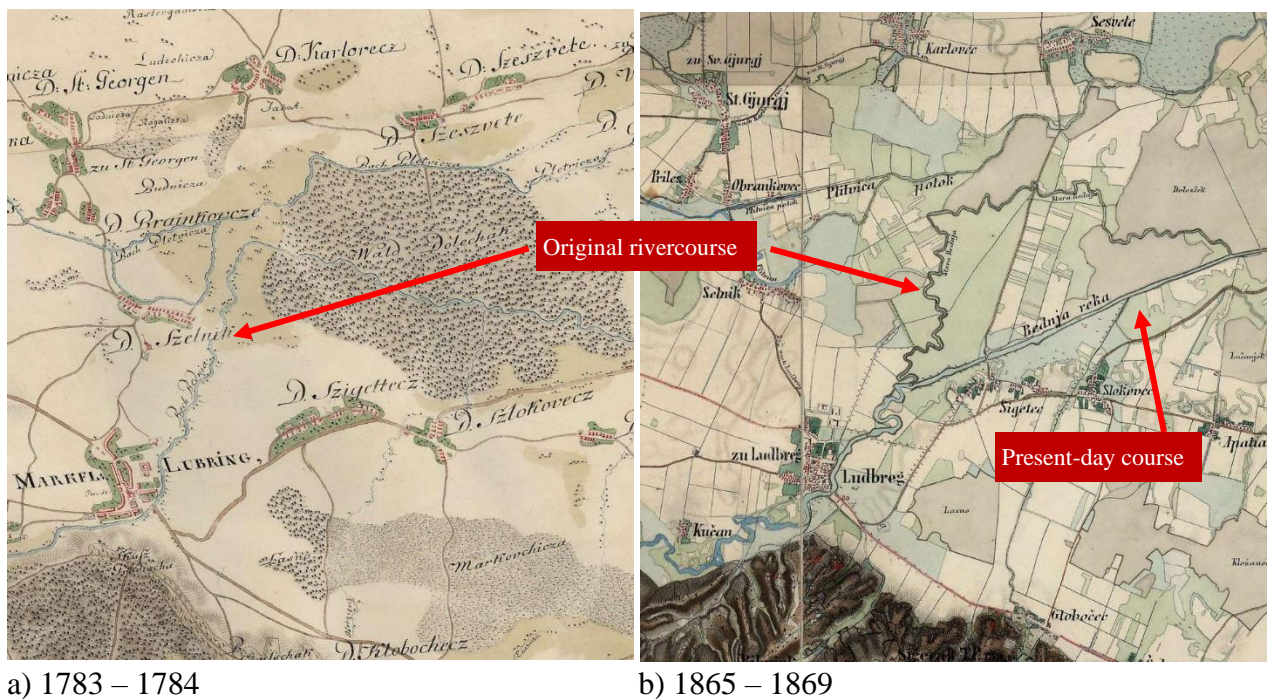


Figure 3.1.11. Comparison of Bednja riverbed based on the First and Second military survey maps of the Habsburg Empire: a) the original course of the Bednja River (“*Bach Bednja*”) downstream from Ludbreg, excerpt from map: Provinz Kroatien (1783–1784); b) the present-day course of the Bednja River (“*Bednja reka*”) showing the old riverbed (“*Stara Bednja*”), as drawn on map: Croatia (1865 – 1869).

Further regulation projects on the Bednja River were quite extensive during the 1960’s which can clearly be observed through satellite imagery dating 1968 (Fig. 3.1.12.). This imagery also shows that up until 1968, great lengths of the Bednja River were still in reference condition as per the Water Framework Directive.

Past and present interventions to the river channel altering the river morphology are the main hydromorphological pressure on the Bednja River. There are no hydropower related alterations to the flow regime on the catchment to date.



Figure. 3.1.12. An example of Bednja River training underway at Veliki Bukovec as seen on satellite imagery from 1968 (scale 1:2000). Image shows river width restriction works and meander cutting off (source: <https://ispu.mgipu.hr/> accessed 21.03.2019).

Regular management and regulations of the Bednja River and its tributaries, which are the main reason for instream habitat loss, continue to be planned and executed to date (Hrvatske Vode, 2015b). These management practices are designed primarily for conveyance of water by removal of riparian vegetation (Fig 3.1.13a. and 3.1.13b.). Subsequently, the riverbanks need to be stabilized and reinforced, often with “rip-rap” (Fig 3.1.13c.) due to increased erosion resulting from a river which now possesses higher kinetic energy but lacks natural bank stabilization provided by riparian vegetation and tree roots (e.g. Abernethy & Rutherford, 2000; 2001).



a) 25.03.2015

b) 11.07.2017

c) 18.05.2017

Figure 3.1.13. Regular management practices on the Bednja River: a) example where both banks are resectioned, Jerovec; b) example of single bank management, Novi Marof; c) bank reinforcement with “rip-rap”, Bednja downstream from Ludbreg.

### 3.2 Study site selection

Preliminary selection of study sites was performed using Google Earth Pro for general conception on landuse, river morphology and site accessibility. Physical plans of Varaždin County, and of the towns Lepoglava, Ivanec, Novi Marof, Varaždinske Toplice, and Ludbreg, were reviewed for information regarding status of sewage systems, wastewater treatment and location of wastewater outlets (Varaždinska županija, 2009a; 2009b; 2010; 2012; 2014b; 2015). After examination of possible study sites along the entire course of the Bednja River, and verification in the field, 20 sites suitable for sampling were singled out, each representative of different stressor level conditions (Fig 3.2.1.).

Following study site selection, GIS tools Arc Map version 10.0 (ESRI, 2010) and the extensions Spatial Analyst and ArcHydro were used to calculate main geographical attributes of the sites (Tab. 3.2.1.). The Bednja river and its tributaries were identified and delineated through topographic maps. Boundaries of the Bednja River catchment and sub-catchments of the study site were identified and calculated through the digital elevation model and topographic maps scale 1:25 000.

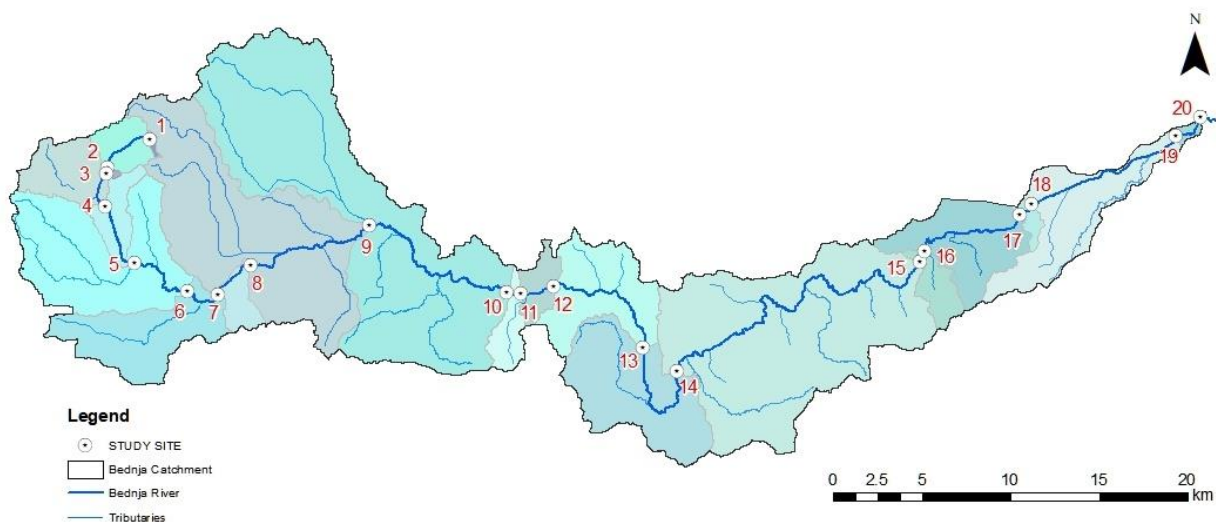


Figure 3.2.1. Longitudinal distribution of the study sites along the Bednja River and associated sub-catchments between study sites. For more details see Tab. 3.2.1.

Current morphological status of chosen sites was compared with the following historical maps: The First Military Survey of the Habsburg Empire - Provinz Kroatien (1783-1784) and the Second Military Survey of the Habsburg Empire - Croatia (1865-1869) scaled 1:28:800 (Timár, 2009) (available at: <https://mapire.eu/>), 2011 Digital Orthophoto map and 1968 satellite imagery of Croatia (Physical Planning Information System Geoportal, Ministry of Construction and Physical Planning available at: [www.ispu.mgipu.hr](http://www.ispu.mgipu.hr)) and Google Earth Pro Timelapse feature for insight into river engineering works within the past 10 years.

Table 3.2.1. Main geographic attributes of the selected study sites on the Bednja River. River type HR-R\_1 = small mid-altitude running waters, and HR-R\_4A = medium lowland running waters.

Study site	Nearest settlement	River type	Distance from source (km)	Altitude (m a.s.l)	Sub-catchment area (km <sup>2</sup> )	Coordinates*
1	Bednjica	HR-R_1	0.4	290	0.4	15°59'27.369"E 46°17'17.53"N
2	Cvetlin	HR-R_1	3.7	250	8.3	15°57'34.893"E 46°16'27.089"N
3	Cvetlin	HR-R_1	4.1	248	8.6	15°57'30.751"E 46°16'15.801"N
4	Trakošćan	HR-R_1	6.5	245	23.2	15°57'24.309"E 46°15'18.471"N
5	Bednja	HR-R_1	10.6	236	31.5	15°58'48.436"E 46°13'34.163"N
6	Rinkovec	HR-R_1	15.5	228	80.3	16°1'10.551"E 46°12'41.024"N
7	Lepoglava	HR-R_1	18.0	225	108.9	16°2'26.113"E 46°12'36.308"N
8	Lepoglava	HR-R_1	20.9	215	114.9	16°3'57.28"E 46°13'30.18"N
9	Ivanec	HR-R_1	29.5	203	201.0	16°9'0.739"E 46°14'41.654"N
10	Završje Podbelsko	HR-R_4A	40.8	197	335.0	16°14'51.219"E 46°12'52.091"N
11	Završje Podbelsko	HR-R_4A	41.6	195	341.9	16°15'53.294"E 46°12'41.158"N
12	Završje Podbelsko	HR-R_4A	43.8	193	348.7	16°17'11.691"E 46°12'55.076"N
13	Novi Marof	HR-R_4A	51.8	189	376.5	16°21'5.914"E 46°11'2.651"N
14	Ključ	HR-R_4A	59.7	178	416.7	16°22'31.942"E 46°10'20.286"N
15	Slanje	HR-R_4A	83.5	160	529.3	16°33'14.585"E 46°13'40.193"N
16	Slanje	HR-R_4A	84.1	159	536.7	16°33'25.264"E 46°13'58.835"N
17	Ludbreg	HR-R_4A	91.5	152	563.1	16°37'37.856"E 46°15'5.913"N
18	Ludbreg	HR-R_4A	92.6	150	565.5	16°38'9.794"E 46°15'25.41"N
19	Mali Bukovec	HR-R_4A	102.2	139	597.2	16°44'34.203"E 46°17'26.424"N
20	Mali Bukovec	HR-R_4A	104.2	136	598.5	16°45'34.707"E 46°18'2.524"N

\* The coordinates represent mid-point of sampling site which corresponds to spot check 9 of River Habitat Survey (Environment Agency, 2003).

The 20 selected study sites along the course of the Bednja River, each representing different morphological condition and different expected impacts of point and diffuse pollution from urban areas and agricultural land are shown on Figs. 3.3.1. – 3.3.20.

### 3.3 Study site description

1) **Study site 1** (N 46°17'17.53", E 15°59'27.369") is situated at 290 m a.s.l. in the village Bednjica, immediately downstream from the rheohelocene type source of the Bednja River. It has a very small catchment area (only 0.408 km<sup>2</sup>) and although the morphology of the reach is near natural, the site cannot be considered in reference condition due to the vicinity of agriculture activities in valley. The entire study reach is relatively shallow but very diverse in terms of water velocity and microhabitats. The study site is dominantly shaded (Fig 3.3.1.).



a) 30.06.2015



b) 30.06.2015



c) 20.03.2015



d) 20.03.2015

Figure 3.3.1. Study site 1: a) natural cascades created by tree roots; b) adjacent landuse and valley form (arrow points to river); c) natural erosion of riverbank and coarse substrate; d) coarse substrate.

**2) Study site 2** (N 46°16'27.089", E 15°57'34.893") represents a channelized reach at 250 m a.s.l., located at the village Cvetlin, 3.7 km downstream from the source. The site is evidently over-deepened and straightened and is subject to regular management activities primarily designed for conveyance of water, including in-channel and bank vegetation removal. The absence of channel shading facilitates growth of in channel reeds which during summer decelerate water velocity and contributes to large quantities of decaying organic matter (CPOM) in the substrate. Floodplain use is dominantly extensive agriculture which stretches all the way to the riverbank. The associated catchment size is 8.3 km<sup>2</sup>. Instream microhabitats comprise of sand with large quantities of decaying organic matter (reed origin), small-coarse substrate “akal” and macrophytes both submerged broad-leaved and emergent (Fig 3.3.2.).



a) 14.4.2016



b) 14.4.2016



c) 15.04.2016



d) 30.06.2015

Figure 3.3.2. Study site 2: a) and b) channel morphology and adjacent landuse; c) substrate with organic matter, CPOM; d) channel choked with vegetation during sampling.



**3) Study site 3** (N 46°16'15.801", E 15°57'30.751") is located immediately downstream of study site 2, and only 4.1 km from the source. The altitude at the study site is 248 m a.s.l. and catchment size 8.6 km<sup>2</sup>. The course of the river at the study site runs closer to the foot of the hill in a narrowing valley. The study site is characterized by a high level of shading from riparian trees and diverse microhabitats. The flow of water is obstructed by large quantities of woody debris which traps trash and forms impoundments creating areas of no perceptible flow (Fig. 3.3.3.).



a) 30.6.2015



b) 30.6.2015



c) 30.06.2015



d) 30.06.2015

Figure 3.3.3. Study site 3: a) coarse substrate at the foot of the hill; b) adjacent land use in the floodplain (arrow points to river); c) low water velocity area downstream of debris dam; d) clay (argyllal) in the riverbed.

**4) Study site 4** (N 46°15'18.471", E 15°57'24.309") is located 6.5 km from the source and downstream of Lake Trakošćan outlet. The altitude at the site is 245 m a.s.l. and the associated catchment area is still small (23.2 km<sup>2</sup>). The entire site is characterised by large quantities of woody debris covering the riverbed but also creating debris dams which obstruct water flow. The dominant substrate is small grained but containing significant amounts of coarse particulate organic matter (decaying leaves). Water is turbid at deeper sections. The site is not subject to any recent flood protection management activities and the natural riparian zone creates a high level of shading over the channel. There is evidence of recent beaver activity at the study site (Fig 3.3.4.).



a) 14.4.2017



b) 16.04.2016



c) 01.07.2015



d) 01.07.2015

Figure 3.3.4. Study site 4: a) representative section with low velocity flow; b) adjacent floodplain landuse (arrow points to river); c) typical substrate with large quantities of wood and CPOM; d) turbid water at deeper sections with sand substrate.

**5) Study site 5** (N 46°13'34.163", E 15°58'48.436") at the town Bednja represents a visibly over-deepened reach with high embankments flowing parallel to a road. The study site is located 10.6 km from the source at 236 m a.s.l. with a catchment size of 31.5 km<sup>2</sup>. The substrate comprises of sand mixed with large quantities of CPOM, argyllal (clay) and some woody debris. There is no coarse sized substrate present. The site is under impact of untreated wastewater from individual households. Characteristic of the site is smooth low velocity flow. Young willow trees along the banks offer some level of shading (Fig. 3.3.5.).



a) 1.7.2015



b) 24.05.2016.



c) 1.7.2015



d) 33.03.2015

Figure 3.3.5. Study site 5: a) the site, high turbidity; b) adjacent landscape, road is directly on bank top (arrow points to river); c) height of embankment; d) clay in the riverbed and banks visible in non-vegetative period.

**6) Study site 6** (N 46°12'41.024", E 16°1'10.551") at Rinkovec lays at 228 m a.s.l., 15.5 km from the source with a catchment size of 80.3 km<sup>2</sup>. Differing from previous sites, this site is dominated by coarse sized substrate and faster and more diverse flow velocities. Mesolithal, macrolithal and microlithal together comprise 95% of the riverbed. The left riverbank has been resectioned while the right bank running along the foot of a hill has been left natural. The surrounding households of the village directly discharge their wastewater into the river. Only the 100 m where sampling took place is shaded while the remaining upstream reach is heavily modified with bare and reinforced banks (Fig 3.3.6.).



a) 01.07.2015



b) 01.07.2015



c) 28.6.2015



d) 28.06.2015

Figure 3.3.6. Study site 6: a) the left resectioned bank and right natural bank; b) adjacent landuse (arrow points to river); c) and d) diversity of high velocity flow over coarse sized substrate.

**7) Study site 7** (N 46°12'36.308", E 16°2'26.113") in town Lepoglava is 18 km from the source at 225 m a.s.l. The catchment size at this site has increased to 108.9 km<sup>2</sup>. Both the riverbed and banks are reinforced with rip rap as the river here flows through an urban area. The technolital is loosely scattered over the gravel riverbed composed of microlithal mixed with a large share of akal. The instream vegetation is comprised of algae and submerged angiosperms. There is no channel shading and the flow at the site is impacted by the immediate upstream bridge. The study site is located upstream of the town's wastewater discharge outlet (Fig. 3.3.7.).



a) 07.07.2015



b) 22.03.2015



c) 02.05.2016



d) 07.07.2015

Figure 3.3.7. Study site 7: a) the site; b) adjacent landuse and bank enforcement (rip-rap) visible only during non vegetative season; c) the microlithal and akal sediment mix; d) technolital on the banks, submerged blocks are covered in moss.

**8) Study site 8** (N 46°13'30.18", E 16°3'57.28") still administratively in Lepoglava town, this site is 20.9 km from the source at 215 m a.s.l. and has an associated catchment area of 114.9 km<sup>2</sup>. Although the Bednja River at this site is situated away from urban areas and the surrounding land is not cultivated, historically it has been distinctively over-deepened during which the excavated material was left on the banks as levees. The riparian vegetation (dominantly willows) has been left to succession and offers some level of shading. There are no regular in-channel management activities. The substrate size is homogenous with akal dominating but the water velocity is more diverse. This site is located downstream of the wastewater outlet with no treatment for 7,334 population equivalent (Fig 3.3.8.).



a) 07.07.2015



b) 16.04.2016



c) 28.06.2015



d) 30.04.2016

Figure 3.3.8. Study site 8: a) the site with willow trees as riparian vegetation; b) adjacent landuse (arrow points to river); c) evidence of historical over deepening, the riverbed cannot be seen from the height of the banks; d) dominant substrate "akal".

**9) Study site 9** (N 46°14'41.654", E16°9'0.739") is downstream of town Ivanec, 29.5 km from the source and has catchment area of around 201 km<sup>2</sup>. The altitude at this site is 203 m a.s.l.. The site is morphologically very similar to the previous study site 8, also over-deepened with high riverbanks and with riparian vegetation left to succession. This site is subjected to untreated wastewater from two upstream agglomerations Lepoglava (7,334 population equivalent) and Ivanec (13.373 population equivalent). The entire studied reach comprises small grained sediment (akal and sand) but the submerged branches from riparian vegetation also provides some habitats (Fig. 3.3.9.).



a) 07.07.2015



b) 28.06.2015



c) 30.04.2016



d) 28.06.2015

Figure 3.3.9. Study site 9: a) the site; b) adjacent landuse (arrow points to river); c) woody debris microhabitats along the banks; d) bank height in relation to river.

**10) Study site 10** (N 46°12'52.091", E 16°14'51.219") located 40.8 km from the source at Završje Podbelsko is the first site where the Bednja River is classified as a lowland mid-sized river. The corresponding catchment size is 335 km<sup>2</sup> and altitude 197 m a.s.l. The study site is characterized by very low water velocity as the river reach is impounded by a downstream weir constructed to protect a bridge from erosion. The substrate is very homogenous, composed of akal with finer interstitial sediment. Due to historical regulations, the site is uniformly deep and lacks natural dynamics such as riffles. Most of the river reach is bare of trees with not shading (Fig. 3.3.10.).



a) 03.07.2015



b) 03.07.2015



c) 03.07.2015



d) 03.07.2015

Figure 3.3.10. Study site 10: a) the site, smooth water surface; b) eroding banks and depth of site; c) dominant substrate; d) sampled wood in water.



**11) Study site 11** (N 46°12'41.158", E 16°15'53.294") is 41.6 km from the source at 195 m a.s.l. with a catchment area of 341.9 km<sup>2</sup>. It is located directly downstream of the weir spillway significantly influencing flow conditions. The artificial technolithal which has been placed on the riverbed to prevent erosion is covered in moss and amounts to 55% of the present substrate. The remaining microhabitats are fine leaved macrophytes (*Myriophyllum*) and microlithal sized gravel. The site is characterized by high velocity flows up to 1.29 ms<sup>-1</sup> (Fig. 3.3.11.).



a) 03.07.2015



b) 03.07.2015



c) 03.07.2015



d) 03.07.2015

Figure 3.3.11. Study site 11: a) the site downstream of the bridge; b) River Habitat Survey chaotic flow (high energy) over technolithal covered in moss; c) partially submerged technolithal; d) fine leaved macrophytes.

**12) Study site 12** (N 46°12'55.076", E 16°17'11.691") was selected due to its vicinity to sites 10 and 11 (all within 3 km of each other) and for having different morphological characteristics to the previous. The site is situated 43.8 km from the source at 193 m a.s.l. and has a catchment size of 348.7 km<sup>2</sup>. This site, although natural looking, is regulated (morphology has been significantly altered) with the old cut-off meanders still present and used as illegal waste dumping sites. Akal is the dominant substrate, followed by sand, and microlithal, distributed across varying depth and water velocity areas. Majority of the floodplain is used for extensive agriculture (Fig. 3.3.12.).



a) 03.07.2015



b) 22.04.2016



c) 03.07.2017



d) 10.07.2017

Figure 3.3.12. Study site 12: a) downstream view of the site, shallow area; b) upstream view of the site showing deeper area and Čevo mountain peak; c) akal and sand substrate; d) adjacent landuse and upstream bridge.

**13) Study site 13** (N 46°11'2.651", E 16°21'5.914") at Novi Marof is located mid-way along the Bednja River course at 189 m a.s.l. The site is 51.8 km from the source and has a corresponding catchment size of 376.5 km<sup>2</sup>. Satellite imagery from 1968 shows that present-day course at this study site has been channelized in comparison to the originally highly meandering Bednja River at this location. However, the age of the riparian trees shows that it has since been left to succession. Dominant substrate is akal, followed by sand and large woody debris from fallen over trees, and there is some microlithal size substrate present as well. The Bednja River at Novi Marof passes next to the towns industrial zone but upstream of wastewater outlets (Fig. 3.3.13.).



a) 03.07.2015



b) 03.07.2015



c) 03.07.2015



d) 03.07.2015

Figure 3.3.13. Study site 13: a) upstream view of the site; b) downstream view of the site with woody debris; c) access to the site via degraded riverbank showing sand substrate; d) akal substrate.

**14) Study site 14** (N 46°10'20.286", E 16°22'31.942") is also positioned mid-way on the Bednja River 59.7 km from the source at 178 m a.s.l. and with a catchment size 416.7 km<sup>2</sup>. The site has been regulated but it still supports erosion and sedimentation processes and succession allowed for good development of the riparian vegetation and trees. The site is characterised by small-grained sediment (akal and sand) and a smooth surface flow. Adjacent landuse beyond the riparian zone includes extensive agriculture, wetland meadows and a nearby highway (right bank side) (Fig. 3.3.14.).



a) 03.07.2015



b) 14.10.2015



a) 10.07.2017



d) 03.07.2015

Figure 3.3.14. Study site 14: a) the site at the riverbend; b) adjacent landuse (arrow points to river); c) smooth flow and riparian vegetation; d) dominant substrate.

**15) Study site 15** (N 46°13'40.193", E 16°33'14.585") at Slanje is 83.5 km from the source at an altitude of 160 m a.s.l. The Bednja River at this site has grown to a catchment of 529.3 km<sup>2</sup>. The instream microhabitats are relatively uniform. Akal with finer grained interstitial sediment is the dominant substrate but there is also sand. The morphology of the river and riparian zone are degraded with steep resectioned banks and minimal shading. The left floodplain is used for intensive agriculture while the right floodplain is narrow grassland confined between a hill and the river (Fig. 3.3.15.).



a) 04.07.2015



b) 11.05.2017



c) 04.07.2015



d) 04.07.2015

Figure 3.3.15. Study site 15: a) the site and smooth surface flow; b) degraded riverbank and landuse; c) sand substrate; d) sediment on side bar.

**16) Study site 16** (N 46°13'58.835", E 16°33'25.264") is only 600 m downstream from study site 15 and represents one of the most diverse sites on the Bednja River in terms of microhabitats. Distance from the source is 84.1 km and altitude drop only 1 m lower to the previous site (159 m a.s.l.). Corresponding catchment size is 536.7 km<sup>2</sup>. Large coarse mesolithal is the dominant substrate accompanied by high energy flows. The remaining microhabitats comprise of underwater roots and fallen trees, akal, microlithal and sand. The study site has a natural morphology and is shaded by trees (Fig. 3.3.16.).



a) 04.07.2015



b) 04.07.2015



c) 04.07.2015



d) 04.07.2015

Figure 3.3.16. Study site 16: a) the study site, mid channel bar present comprised of mesolithal; b) natural riparian zone with tree roots; c) fallen over tree; d) mid channel bar with mesolithal and rippled flow.

**17) Study site 17** (N 46°15'5.913", E 16°37'37.856") in the town of Ludbreg is a heavily modified reach 91.5 km from the source at 152 m a.s.l. The associated catchment area is 563.1 km<sup>2</sup> and adjacent landuse is urban. The study site is characterised by no perceptible flow within an over deepened channel reinforced with technolithal (rip rap). The entire riverbed is comprised of artificial technolithal blocks and there is no channel shading. Reeds, grasses, and amphibious vegetation make up the riparian vegetation. Part of the towns untreated wastewater is discharged at the study site with outlets present on both riverbanks (Fig. 3.3.17.).



a) 20.04.2016



b) 11.05.2017



c) 21.03.2015



d) 20.04.2016

Figure 3.3.17. Study site 17: a) the study site; b) bank height and adjacent landuse; c) riverbed substrate – technolithal; d) the substrate and water turbidity.

**18) Study site 18** (N 46°15'25.41", E 16°38'9.794") is 900 m downstream from study site 17, at 150 m a.s.l. and has a catchment size of 565.5 km<sup>2</sup>. The study site is morphologically in a good condition with diverse water velocity and sediment dynamics, but water odour indicates the impact of untreated wastewater discharge from Ludbreg town (8.260 population equivalent) and upstream landfill. Dominant substrates at the site are mesolithal and microlithal covered in algae. The remaining microhabitats are akal and large woody debris (Fig. 3.3.18.).



a) 20.04.2016



b) 05.07.2015



c) 28.06.2015



d) 05.07.2015

Figure 3.3.18. Study site 18: a) the study site; b) area of large woody debris and gravel bars; c) microlithal covered in algae; d) area of marginal dead water.



**19) Study site 19** (N 46°17'26.424", E 16°44'34.203") at Mali Bukovec is 102.2 km downstream from the source at 139 m a.s.l. and with a catchment size of 597.2 km<sup>2</sup>. The left bank of this study site has been regulated and reinforced with rip rap only a few months prior to research. The entire riverbed is composed of uniform microlithal substrate covered in algae while the remaining microhabitats are formed by the technolithal blocks. Landuse is urban with households extending immediately along the river (Fig. 3.3.19.).



a) 21.03.2015



b) 05.07.2015



c) 05.07.2015



d) 05.07.2015

Figure 3.3.19. Study site 19: a) the study site, upstream view with fresh rip rap; b) downstream view of the study site from left bank; c) microlithal substrate and right bank during low flow; d) submerged technolithal.

**20) Study site 20** (N 46°18'2.524", E 16°45'34.707") is the final and most downstream study site located near mouth of the Bednja River at 136 m a.s.l. The study site is 104.2 km from the source with an associated catchment area of 598.5 km<sup>2</sup>. The dominant substrate at the site is microlithal comprising 70% of the riverbed while the remaining substrates are sand, akal, and large woody debris. Morphologically the site is entirely natural with undisturbed erosion and sedimentation processes resulting in riverbed lateral movement after flooding. Due to the width of the river the site is naturally unshaded (Fig. 3.1.21.).



a) 05.07.2015



b) 05.07.2015



c) 05.07.2015

Figure 3.3.20. Study site 20. a) the study site and gravel bar; b) flow variations and natural banks; c) the substrate (microlithal and sand).

### 3.4 Benthic macroinvertebrate sampling

Benthic macroinvertebrates were collected in summer 2015 (the recommended sampling season for medium to large size rivers) during low discharge using the “multi-habitat” method as presented in AQEM manual (AQEM consortium, 2002). At each sampling site, a careful assessment of distribution and composition of microhabitats (mineral and biotic) within a 100 m river reach was made prior to sampling to avoid disturbance of the substrate and fauna (Tab 3.4.1.).

Table 3.4.1. Description of sampled mineral and biotic microhabitats.

Microhabitat	Grain size	Description
Macrolithal	>20 cm - 40 cm	Coarse blocks, head-sized cobbles, with a variable percentage of cobble, gravel and sand
Mesolithal	>6 cm - 20 cm	Fist to hand-sized cobbles with a variable percentage of gravel and sand
Microlithal	>2 cm - 6 cm	Coarse gravel, with variable percentages of medium to fine gravel
Akal	>0.2 cm - 2 cm	Fine to medium-sized gravel
Psammal	< 6 $\mu$ m - 0.2 cm	Sand
Argyllal	< 6 $\mu$ m	Clay (inorganic)
Technolithal	>2 cm - >40 cm	Artificial substrate
Phytal	-	Submerged and emergent macrophytes
Xylal	-	Wood - tree trunks, dead wood, branches and roots
CPOM	-	Deposits of coarse particulate organic matter, e.g. fallen leaves

A 100 m sampling reach length was used for all sites regardless of stream size, including both erosional (“riffle”) and depositing (“pool”) areas if present. Microhabitats with a share of less than 5% coverage were excluded from sampling. Within each selected sampling reach, 20 “single habitat samples“ (or „sampling units“) distributed according to share of microhabitat were taken (Fig 3.4.1.).

Benthic macroinvertebrates were collected using a standardized 500  $\mu$ m mesh size hand-net with a 25 x 25 cm frame resulting in the sampling of approximately 1.25 m<sup>2</sup> stream bottom area at each site through the 20 single habitat samples. Both kick-sampling, “jabbing” and “sweeping” were used as a method depending on site characteristics and water depth, starting at the downstream end of the reach and proceeding upstream.

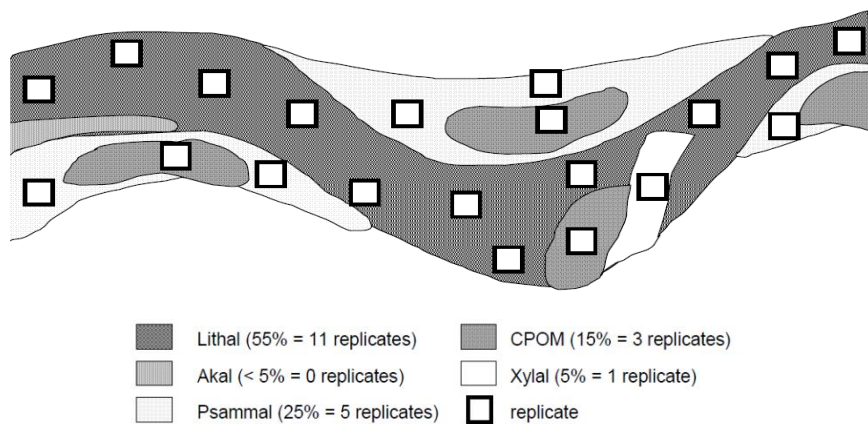


Figure 3.2.1. Example of distributing 20 sampling units (replicates) in relation to microhabitat distribution in a theoretical sampling site for „multi habitat sampling“ method applied in AQEM (AQEM consortium, 2002).

Individual collected sampling units were rinsed, decanted and transferred into separate sample containers according to microhabitat. The samples were preserved with 95% ethanol and sample containers labelled.

All 20 study sites from source to mouth of the Bednja River were sampled within a short time interval to minimize changes in communities resulting from natural conditions (30.06., 01.07., 03.07., 04.06., 05.07., 07.07.).



a) b) c)  
Figure 3.2.2.: a) Sampling by jabbing and sweeping, study site 14; b) sampling of submerged macrophytes by disturbing sample area and allowing macroinvertebrates to flow into the net with the current, study site 11; c) grouping of sampling units according to microhabitat, study site 2.

### *Sample processing*

Because the 20 sample units from each sampling site were not combined after sampling into one composite sample per site but were grouped and stored according to microhabitat/substrate, further division of the sample prior to sorting/isolation as foreseen by AQEM was not performed. **All** organisms in a given microhabitat subsample were isolated and separated according to taxonomic groups and counted.

### *Species identification*

Identification was done to the lowest possible level (species level for majority of the faunal groups) in cooperation with experts from the Faculty of Science and Faculty of Teacher Education (University of Zagreb), and the Croatian Natural History Museum. Genus was the lowest possible level for most Diptera, Heteroptera, juvenile stages of insects and Turbellaria. Identification was performed using the following literature: Ephemeroptera (Müller-Liebenau, 1969; Bauernfeind & Humpesch, 2001; Malzacher, 1984); Trichoptera (Malicky, 2004; Neu & Tobias, 2004; Waringer & Graf, 2011); Plecoptera (Ravizza, 2002; Graf & Schmidt-Kloiber, 2003; Zwick, 2004); Odonata (Askew, 2004; Brochard et al., 2012); Megaloptera (Nilsson,

1996; Hölzer, 2002), Diptera (Cranston, 1982; Nilsson, 1997; Brooks et al., 2007; Vallenduuk & Moller Pillot, 2007; Andersen et al., 2013; Vallenduuk 2017); Coleoptera (Berthélemy, 1979; Franciscolo, 1979; Jäch & Brojer, 2006; Janssens, 1965; Olmi, 1976; Pirisinu, 1981); Hemiptera (Nilsson, 1996); Crustacea (Goedmakers, 1972; Karaman and Pinkster, 1977; Argano, 1979; Karaman 1993); Mollusca (Bole, 1969; Glöer, 2002; Killeen et al., 2004); Oligochaeta (Hrabě 1981; Brinkhurst, 1986; Timm, 2009); Hirudinea (Nesemann & Neubert, 1999); Hydrachnidia (Davids et al., 2007; Di Sabatino et al., 2010; Gerecke et al., 2016); Turbellaria (Reslová, 2011).

The voucher specimens are deposited in the authors private collection and at the Department of Biology, Faculty of Science, University of Zagreb, Croatia.

### 3.5 Assessment of catchment landuse

Landuse was analysed at sub-catchment scale of each study site in Arc Map version 10.0 (ESRI, 2010) using CORINE Land Cover (CLC) as a layer (HAOP, 2012). Landuse categories were grouped as near natural area, intensive agriculture, extensive agriculture and urbanised and artificial land cover and quantified as percentage% area on corresponding sub-catchment of each study site (Tab. 3.5.1.).

Table 3.5.1. Grouping of CORINE Land Cover (CLC) categories present on the Bednja River catchment.

Landuse group	Quantification	CLC categories on the Bednja River catchment		
		Level 1	Level 2	Level 3
<b>Near natural area</b>	% Near natural		31 Forests	311 Broad-leaved forest
		3 Forest and semi natural areas		312 Coniferous forest
			32 Scrub and/or herbaceous vegetation associations	313 Mixed forest
		4 Wetlands	41 Inland wetlands	324 Transitional woodland-shrub
		5 Water bodies	51 Inland waters	411 Inland marshes
			511 Water courses	
			512 Water bodies	
<b>Intensive agriculture</b>	% Intensive agriculture	2 Agricultural areas	21 Arable land	211 Non-irrigated arable land
			22 Permanent crops	221 Vineyards
			24 Heterogeneous agricultural areas	242 Complex cultivation patterns
<b>Extensive agriculture</b>	% Extensive agriculture	2 Agricultural areas	23 Pastures	231 Pastures
			24 Heterogeneous agricultural areas	243 Land principally occupied by agriculture, with significant areas of natural vegetation
<b>Urbanised and artificial land cover</b>	% Urban	1 Artificial surfaces	11 Urban fabric	112 Discontinuous urban fabric
			12 Industrial, commercial and transport units	121 Industrial or commercial units
				122 Road and rail networks and associated land
			13 Mine, dump and construction sites	131 Mineral extraction sites
			14 Artificial, non-agricultural vegetated areas	142 Sport and leisure facilities

### 3.6 Measurement of environmental variables

Measurements of physicochemical elements were conducted during vegetative seasons (spring, summer and autumn) prior to benthic macroinvertebrate sampling (in March 2015), during sampling (end of June/beginning of July 2015), and after sampling (October 2015).

The following physicochemical elements were measured at each study site:

- Water temperature (using the oximeter WTW Oxi 330/SET)
- pH value (using the pH-meter WTW ph 330)
- Conductivity (using the conductometer WTW LF 330)
- Oxygen regime:
  - Dissolved oxygen (HRN EN 25813:2003 method)
  - Oxygen saturation (calculated through dissolved oxygen and water temperature)
  - COD<sub>Mn</sub> (HRN EN ISO 8467:2001 method)
  - BOD<sub>5</sub> (HRN EN 1899-1:2004 method).
- Alkalinity (titration with 0.1 chlorovodic acid with methyl-orange as indicator) - only in spring 2015.

Additionally, the following nutrients were measured:

- Nitrates (HRN ISO 7890-3:2001 method)
- Ammonium ions (HRN ISO 70-3:1998 method)
- Orthophosphates (HRN ISO 6878:2001 method).

Due to the close proximity of study sites 2 and 3 (400 m difference), study sites 10, 11 and 12 (3000 m in total) and study sites 15 and 16 (600 m difference) a single water sample for nutrient determination was taken for those study sites.

Parallel to benthic macroinvertebrate sampling, the following abiotic parameters were recorded above each sample unit (20 measurements per study site):

- Water velocity (using the water current meter P-670-M)
- Water depth (using a handheld ruler).

### 3.7 Assessment of hydromorphological modification

For the assessment of hydromorphological modification of each study site, two separate assessment systems were chosen. The European Standard EN 15843:2010 “Water quality – Guidance standard on determining of modification of river hydromorphology” (DIN, 2010) developed for the purpose of WFD waterbody monitoring and the River Habitat Survey (RHS) manual (Environment Agency, 2003) through which several habitat quality and modification scores are derived.

#### *European Standard EN 15843:2010 methodology*

The degree of hydromorphological modification at each study site was assessed using the European Standard EN 15843:2010 “Water quality – Guidance standard on determining of modification of river hydromorphology” which evaluates the “*departure from naturalness as a result of human pressures on river hydromorphology*” (DIN, 2010). The assessment was performed on river reaches of different length: 100 m reach corresponding to the reach where benthic macroinvertebrates were sampled and a 500 m long reach encompassing the 100 m sampled reach and extending upstream to match the River Habitat Survey reach for comparability.

Scoring for hydromorphological modification was given as follows:

- A single mean score for the assessed reach (study site) derived by calculating the mean value from all 16 features assessed (the 16 features are given in Annex III).
- Three separate scores derived by grouping of the features according to WFD hydromorphological quality elements (morphology, hydrological regime and longitudinal continuity). Calculation of the mean score for morphology encompasses features 1a, 1b, 2a, 2b, 7, 8, 9, 10a, 10b, hydrological regime (category 5) and longitudinal continuity (category 6).
- Three separate scores derived by grouping of the categories according to zone and producing three separate mean values for the channel (category 1a, 1b, 2a, 2b, 5a, 5b, 5c, 6), bank/riparian zone (category 7, 8) and floodplain (category 9, 10a, 10b)
- Individual score for each of the 16 assessed features (Annex III).

Hydromorphological modification scores are grouped into five classes representing different degrees of hydromorphological modification, from near-natural to severely modified (Tab. 3.7.1.).

Table 3.7.1. Scoring system and five classes of hydromorphological modification according to European Standard EN 15843:2010 methodology.

Score	Class	Description
1 to < 1.5	1	Near-natural
1.5 to < 2.5	2	Slightly modified
2.5 to < 3.5	3	Moderately modified
3.5 to < 4.5	4	Extensively modified
4.5 to 5.0	5	Severely modified

*River Habitat Survey (RHS) methodology*

At each study site a River Habitat Survey (RHS) was performed on a 500 m reach pursuant Raven et al. (1997) using the Field Survey Guidance Manual: 2003 Version (Environment Agency, 2003). The assessed reach was first aligned with the position where sampling of benthic macroinvertebrates took place (Fig 3.7.1.). During the assessment, attributes stated in the Manual were recorded at 10 spot-checks placed 50 m apart encompassing the river channel, riverbanks and adjacent landuse.

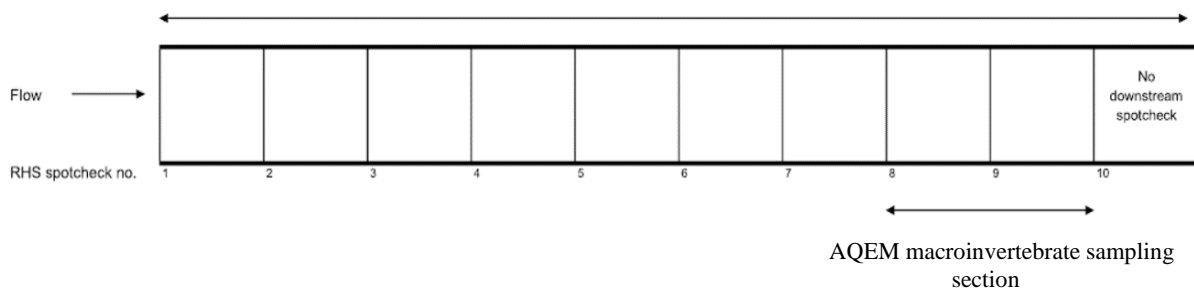


Figure 3.7.1. Schematic overview of the 500 m River Habitat Survey (RHS) assessment reach showing the position of 10 RHS spot check (50 m apart) in respect to direction of flow and site of macroinvertebrate sampling section. The AQEM macroinvertebrate sampling section is between RHS spot-checks 8 and 10 (modified from Furse et al., 2006).

Features recorded in the field protocol were entered into the RHS Toolbox 1.4 software (available at: [www.riverhabitatsurvey.org/river-habitat-survey-toolbox-software](http://www.riverhabitatsurvey.org/river-habitat-survey-toolbox-software)) for calculation of the following indices:



- Habitat Modification Score (HMS)
- Habitat Quality Assessment (HQA)
- Riparian Quality Index (RQI)
- Hydromorphological indices (CSI, FRI, CVI).

The RHS Habitat Modification Score (HMS) represents the extent and severity of human modification to the river banks and channel (Raven et al., 1998b). It is derived through a sum of sub-scores for recorded modifications including bank and channel resectioning, bank and channel reinforcement, embankments, artificial berms, dams, weirs, bridges, fords, flow deflectors and drainage outfalls (Environment Agency, 2003). A higher score represents a more heavily modified site (Tab. 3.7.2.).

Table 3.7.2. Habitat Modification Score (HMS) categories for describing the physical state of the assessed site.

<b>HMS Score</b>	<b>Descriptive category of channel</b>
0 – 16	Pristine / semi – natural
17 – 199	Predominantly unmodified
200 – 499	Obviously modified
500 – 1399	Significantly modified
> 1400	Severely modified

The RHS Habitat Quality Assessment (HQA) is derived through a sum of sub-scores for channel substrate and flow type diversity, channel vegetation diversity and bank vegetation structure, occurrence of natural landuse, number of pools and riffles and occurrence of special features and tree features (Environment Agency, 2003). HQA scores total between 0 – 100 where a higher score represents higher habitat quality.

The Riparian Quality Index (RQI) scores total between 0 and 120 and encompass sub-scores for riparian vegetation complexity, naturalness and continuity where a higher score represents higher quality of riparian vegetation.

RHS Channel Substrate Index represents the gradient in average substrate size. An increase in score represents a gradual increase in average sediment size present at study site. The Flow Regime Index represents a gradient between slow tranquil (lower scores) and fast turbulent flow types (higher scores). Channel Vegetation Index represents a gradient in channel vegetation in relation to flow where the lower scores represent domination of floating vegetation and mosses being at the top of the scale.

### 3.8 Benthic macroinvertebrate metric calculation

Biological diversity and functional metrics based on benthic macroinvertebrate communities at each study site were calculated using the software ASTERICS Version 4.0.4. (Furse et al., 2006) with exemption for functional feeding groups. Functional feeding groups (% grazers/scrapers, % miners, % xylophagous taxa, % shredders, % gatherers/collectors, % active filter feeders, % passive filter feeders, % predators, % parasites, and % other feeding) were assigned according to Moog (2002), and their share was calculated manually. The feeding group was modified for the order Trombidiformes (Hydrachnidia) by assigning all taxa 10 points for predation, as all collected specimens were predators. Moog (2002) assigns Hydrachnidia with 7 points for predation and 3 points for parasitism, which originally interfered with results by giving significant correlations for environmental variables and parasites.

The Ecological Quality Ratio (EQR) was calculated pursuant to Mihaljević et al. (2020) through the Saprobity module and General Degradation module. The Saprobity module is based on the EQR of the Croatian Saprobity Index ( $SI_{HR}$ ), which is based on the Pantle Buck index, but with adapted indicator values. The General Degradation module is the normalized multimetric index (General Degradation<sub>MI</sub>) consisting of metrics: Rhithron Type Index, Diversity (Margalef Index), EPT (%) abundance classes, and River Fauna Index (RFI) (applied for study sites 1 – 9; river type HR-R\_1) and the metrics: ASPT (Average Score per Taxon), Diversity (Margalef Index), EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata), and the River Fauna Index (RFI) (applied for study sites 10 – 20: river type HR-R\_4A). The General Degradation<sub>MI</sub> equals the average EQR of all four metrics. The final EQR equals the lower value of the two modules (Mihaljević et al., 2020).

$$SI_{HR} = \frac{\sum SIu_i}{\sum u_i}$$

where:

$SI_{HR}$  = Croatian saprobity index

SI = individual species/taxa indicator value

$u_i$  = number of individuals calculated per 1 m<sup>2</sup>

$$RFI_{VR_j} = \frac{\sum_{i=1}^n ac_i \times Rf_i \times HW_i}{\sum_{i=1}^n ac_i \times HW_i}$$

where:

$ac_i$  is the log5 abundance class of the  $i^{th}$  taxon,

$Rf_i$  is the river fauna value of the  $i^{th}$  taxon,

$HW_i$  is the hydromorphological indicative weight of the  $i^{th}$  taxon

$n$  is the number of indicative taxa

Figure 3.8.1. Formulas for calculating the Croatian Saprobity Index ( $SI_{HR}$ ), and the River Fauna Index (RFI) (Mihaljević et al., 2020).

The list of calculated benthic macroinvertebrate metrics is given in Tab. 3.8.1.

Table 3.8.1. List of biological metrics of benthic macroinvertebrates tested within this study (selection from Hering et al., 2004b and Mihaljević et al., 2020).

<b>Diversity</b>	<b>Microhabitat preference</b>	<b>Dominant representatives of taxa groups in the Bednja River</b>
Total individuals (N)	(%) Type Pelal (scored taxa = 100%)	<i>Baetis fuscatus</i>
Total species (S)	(%) Type Psammal (scored taxa = 100%)	<i>Baetis buceratus</i>
Simpson-Index	(%) Type Akal (scored taxa = 100%)	<i>Hydropsyche angustipennis angustipennis</i>
Shannon-Wiener-Index	(%) Type Lithal (scored taxa = 100%)	<i>Hydropsyche incognita/pellucidula</i>
Margalef Index	(%) Type Phytal (scored taxa = 100%)	<i>Psychomyia pusilla</i>
Evenness/Pielou's evenness: $J' = H'/\log(S)$	(%) Type Akal + Lithal + Psammal (scored taxa = 100%)	<i>Onychogomphus forcipatus forcipatus</i> <i>Platycnemis pennipes</i>
<b>Ecological status &amp; saprobity</b>	<b>Taxonomic group (%)</b>	
Croatian Saprobity Index (HR <sub>SI</sub> )	Coelenterata (%)	Empididae Gen. sp.
River Fauna Index (RFI)	Turbellaria (%)	Simuliidae Gen. sp.
Average score per Taxon (ASPT)	Gastropoda (%)	Chironomini Gen. sp.
Biological Monitoring Working Party (BMWP Score)	Bivalvia (%)	Orthocladiinae Gen. sp.
Belgian Biotic Index (BBI)	Oligochaeta (%)	<i>Esolus pygmaeus</i> Ad.
Altered Indice Biotico Estesio (IBE AQEM)	Hirudinea (%)	<i>Oulimnius tuberculatus</i> Ad.
Mayfly Total Score (MTS)	Crustacea (%)	<i>Oulimnius tuberculatus</i> Lv.
r-Dominance	Ephemeroptera (%)	<i>Pisidium</i> sp.
	Odonata (%)	<i>Sphaerium rivicola</i> <i>Holandriana holandrii</i>
<b>Feeding types</b>	Plecoptera (%)	<i>Bythinella opaca opaca</i>
Grazers and scrapers (%)	Heteroptera (%)	<i>Limnodrilus hoffmeisteri</i>
Miners (%)	Megaloptera (%)	<i>Potamothenix hammoniensis</i>
Xylophagous Taxa (%)	Trichoptera (%)	Erpobdellidae Gen. sp.
Shredders (%)	Coleoptera (%)	<i>Helobdella stagnalis</i>
Gatherers/Collectors (%)	Diptera (%)	<i>Hygrobates fluviatilis</i>
Active filter feeders (%)	Hydrachnidia (%)	<i>Lebertia</i> sp.
Passive filter feeders (%)	(%) EPT-Taxa	<i>Asellus aquaticus</i>
Predators (%)	EPT (%) (abundance classes)	<i>Gammarus fossarum</i>
Parasites (%)	EPT/OL taxa	<i>Gammarus roeselii</i>
Other feeding types (%)	EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata)	
<b>Zonation</b>	<b>Current preference</b>	
(%) Hypocrenal (scored taxa = 100%)	(%) Type RP (rheophile) (scored taxa = 100%)	
(%) Epirhithral (scored taxa = 100%)	Rheoindex (Banning, with abundance)	
(%) Metarhithral (scored taxa = 100%)	Rhithron Type Index	
(%) Hyporhithral (scored taxa = 100%)		
(%) Epipotamal (scored taxa = 100%)		
(%) Metapotamal (scored taxa = 100%)		
(%) Littoral (scored taxa = 100%)		

### 3.9 Data analysis

#### *General benthic macroinvertebrate community analyses*

Benthic macroinvertebrate community structure between study sites was assessed as cluster and non-metric Multi-Dimensional Scaling analysis based on Bray-Curtis resemblance. SIMPER (similarity percentages) analysis to determine which species / taxa contribute most to Bray-Curtis similarity and dissimilarity was also performed in Primer 6 (Clarke & Gorley, 2006). Data were log-transformed prior to analysis.

Table and graphic figures were developed in Microsoft Excel 2010.

#### *Stressor and natural factor interactions analysis*

In the first step, Spearman's rank correlation coefficient (R) was used to test the statistical relationship between natural factors (distance from source, altitude, and sub-catchment size) and anthropogenic stressors from the three main stressor groups (water quality/nutrients, landuse, and hydromorphological quality and modification) (Tab. 3.9.1.). Spearman's rank correlation coefficient (R) was also used to analyse the correlations between stressors groups against each other. The following interactions were explored:

- Water quality vs landuse
- Water quality vs hydromorphology
- Hydromorphology vs landuse and microhabitats
- EN 15843:2010 scores vs River Habitat Survey scores.

These correlations were performed in Statistica version 13.0 (TIBCO Software Inc., 2017).

#### *Stressor and biological interactions analysis*

Spearman's rank correlation coefficient (R) was also used to test the statistical relationship between the benthic macroinvertebrate metrics and anthropogenic stressors from the three main stressor groups (water quality/nutrients, landuse, and hydromorphological quality and modification) (Tab. 3.9.1.). In addition to using biological metrics, the two most dominant species per taxa group were included in the analyses. These correlations were also performed in Statistica version 13.0 (TIBCO Software Inc., 2017).

The identified statistically significant positive and negative correlations ( $p < 0.05$ ) were further ordinated using Redundancy Analysis (RDA) in CANOCO 5.0 (Ter Braak and Smilauer, 1998).

All 259 taxa were analysed in a Canonical correlation analysis (CCA) against the EN 15843:2010 and RHS hydromorphological scores using 499 permutations and the downweight rare species option. These analyses were also performed in CANOCO 5.0 (Ter Braak and Smilauer, 1998).

For describing the significance of a correlation, Spearman's rank correlation coefficient ( $R$ ) of 0.40 to 0.59 is considered moderate, 0.60 – 0.79 is considered strong, and 0.80 – 1.0 a very strong correlation.

For descriptive purposes, study sites 1 – 9 are considered as upper reach, study sites 10 – 14 as the middle reach, and study sites 15 – 20 as belonging to the lower reach of the Bednja River (Fig. 3.9.1.).

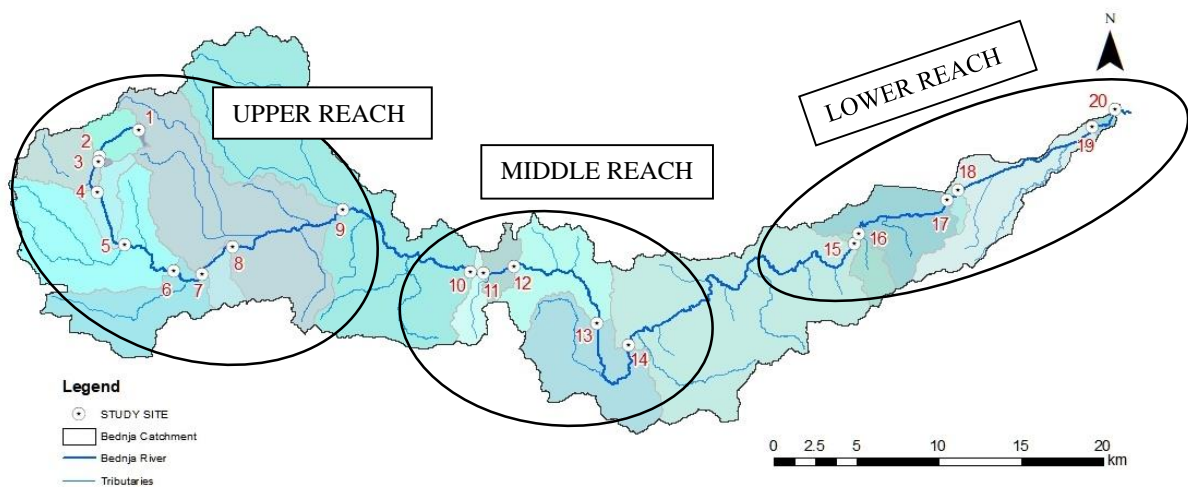


Figure 3.9.1. Grouping of the study sites according to corresponding Bednja River reach.

Table 3.9.1. List of anthropogenic stressors and natural factors included in the analyses.

<b>STRESSOR GROUP</b>	<b>Stressors</b>
<b>Water quality / nutrients</b>	Water temperature (°C) Dissolved oxygen (mgL <sup>-1</sup> ) Oxygen saturation (%) Conductivity (µScm <sup>-1</sup> ) pH Biological oxygen demand (BOD <sub>5</sub> , mgO <sub>2</sub> L <sup>-1</sup> ) Chemical Oxygen Demand (COD <sub>Mn</sub> , mgO <sub>2</sub> L <sup>-1</sup> ) Ammonium concentration (NH <sub>4</sub> <sup>+</sup> , mgNL <sup>-1</sup> ) Nitrites (NO <sub>2</sub> <sup>-</sup> , mgNL <sup>-1</sup> ) Nitrates (NO <sub>3</sub> <sup>-</sup> , mgNL <sup>-1</sup> ) Kjeldahl Nitrogen (mgNL <sup>-1</sup> ) Organic Nitrogen (mgNL <sup>-1</sup> ) Total nitrogen (Σ N, mgNL <sup>-1</sup> ) Orthophosphates (PO <sub>4</sub> <sup>3-</sup> , mgPL <sup>-1</sup> ) Total phosphorous (Σ P, mgPL <sup>-1</sup> )
<b>Landuse</b>	% Near natural % Intensive agriculture % Extensive agriculture % Total agriculture % Urban
<b>Hydromorphological quality and modification</b>	<b>EN 15843:2010 - 100 m scale</b> AVERAGE SCORE 100 m Morphology Flow Continuity Channel Riparian zone Floodplain <b>EN 15843:2010 - 500 m scale</b> AVERAGE SCORE 500 m Morphology Flow Continuity Channel Riparian zone Floodplain
	<b>Habitat Modification Score (HMS)</b> Resectioned Bank Bed subscore Reinforced Bank Bed subscore Realigned subscore <b>Habitat Quality Assessment Score (HQA)</b> HQA flow type HQA channel substrate HQA channel features HQA bank vegetation structure HQA channel vegetation 95-97 HQA landuse HQA trees HQA special features 95-97 <b>Riparian Quality Index score (RQI)</b> Complexity subscore Naturalness subscore Continuity subscore Channel Substrate Index (CSI) Flow Regime Index (FRI) Channel Vegetation Index (CVI)
<b>Natural factors</b>	Distance from source (km) Altitude (m a.s.l.) Sub-catchment size above study site (km <sup>2</sup> )
<b>Other variables</b>	% Microhabitat (macrolithal, mesolithal, akal, psammal, xylal, technolithal) Depth (average sampling depth, cm) Water velocity (average water velocity, ms <sup>-1</sup> )

## 4. RESULTS

### 4.1 Environmental variables and stressors

#### 4.1.1 Microhabitat characteristics at study sites

The most frequent and abundant substrate was fine to medium-sized gravel “akal” (>0.2 – 2 cm) which was present at 15 out of 20 study sites and amounted to 29.5% of all sampled microhabitats, followed by coarse gravel “microlithal” (>2 – 6 cm) present at 12 study sites and representing 16% of the total sampled microhabitats. Sand “psammal” (< 6  $\mu\text{m}$  – 0.2 cm) was present at 13 study sites and amounted to 14.5% of total sampled microhabitats. Natural coarse blocks “macrolithal” (>20 – 40 cm) were present at only two study sites representing 1% of total sampled microhabitats while equal sized artificial blocks “technolithal” was sampled at four study sites and made up 11.5% of total sampled microhabitats. Instream wood “xylal” comprising dead wood, branches and roots was sampled at 12 study sites amounting to 9% of total sampled microhabitats. Inorganic clay “argyllal” (< 6  $\mu\text{m}$ ) was present at three study sites and the samples totalled 4% of all sampled substrate. Microhabitat “phytal” which encompasses both submerged and emergent macrophytes was sampled at four study sites and amounted to 3.8% of the total sampled substrate. The least represented substrate was a single sample of deposit of coarse particulate organic matter “CPOM” present at only one site amounting to 0.3% of total sampled substrate in the Bednja River (Tab. 4.1.1. and Fig. 4.1.1.).

Table 4.1.1. Microhabitat characteristics at study sites. Microhabitat “macrophytes” comprises both submerged and emergent macrophytes. \*Technolithal covered in moss.

Study site / microhabitat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	<i>% Microhabitat at study site</i>																			
MACROLITHAL	10%					10%														
MESOLITHAL	35%		20%			75%										50%		30%		
MICROLITHAL	15%		30%	5%		10%	55%				20%	15%	10%			10%		30%	50%	70%
AKAL	5%	20%	10%			5%	10%	80%	80%	95%		55%	50%	55%	85%	15%		20%		5%
PSAMMAL	25%	40%	5%	50%	45%			5%	10%			20%	20%	40%	10%	5%				15%
ARGYLLAL	10%		30%		40%															
PHYTAL		40%					5%				25%							5%		
XYLAL				45%	15%			15%	10%	5%		10%	20%	5%	5%	20%		20%		10%
CPOM			5%																	
TECHNOLITHAL							30%				55%*						95%		50%	

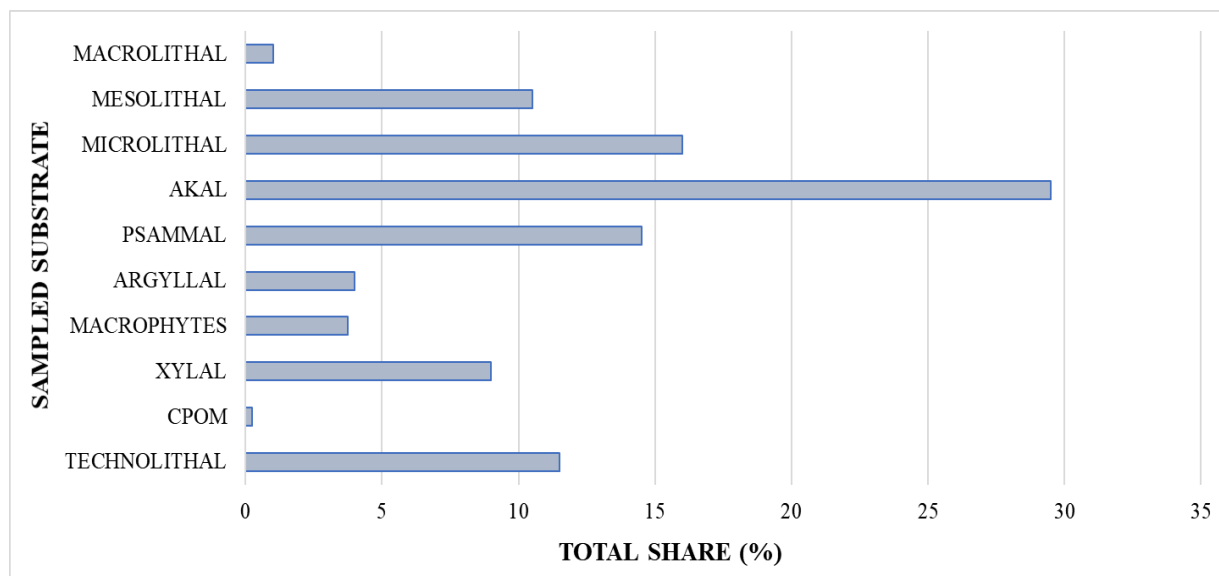


Figure 4.1.1. Total share of sampled substrate from 20 study sites in the Bednja River.

#### 4.1.1 Depth and water velocity

Sampling depth varied between study sites and although sampling was done during a low water level period, some study sites possessed greater depths due to channel over-deepening and regulations. The maximum depth was recorded at study site 10 at 110 cm on akal substrate. The highest flow velocities were recorded at study sites 11 ( $1.29 \text{ ms}^{-1}$ ) and 13 ( $3.20 \text{ ms}^{-1}$ ) (Tab. 4.1.2.). More detailed recordings for individual microhabitats within study sites is given in Annex II.

Table 4.1.2. Minimum, maximum and average values of abiotic characteristics derived from 20 individual measurements above subsamples at each study site: sampling depth and water velocity.

Study site	Sampling depth (cm)			Velocity ( $\text{ms}^{-1}$ )		
	Min	Max	Average	Min	Max	Average
1	2	14	6.30	0.01	0.70	0.168
2	16	44	23.60	0.01	0.35	0.074
3	5	26	11.95	0.01	0.30	0.080
4	15	53	26.80	0.00	0.25	0.066
5	10	41	24.55	0.01	0.25	0.091
6	6	35	17.20	0.01	0.70	0.311
7	5	28	17.90	0.00	0.57	0.182
8	1	43	16.00	0.00	0.41	0.171
9	4	38	19.85	0.00	0.50	0.237
10	5	110	87.75	0.00*	0.01	0.006
11	4	35	14.60	0.06	1.29	0.533
12	2	90	46.35	0.01	0.70	0.116



Table 4.1.2. (Continued). Minimum, maximum and average values of abiotic characteristics derived from 20 individual measurements above subsamples at each study site: sampling depth and water velocity.

Study site	Sampling depth (cm)			Velocity (ms <sup>-1</sup> )		
	Min	Max	Average	Min	Max	Average
13	5	55	29.25	0.01	3.20**	0.770
14	8	70	40.20	0.01	0.30	0.101
15	11	80	47.28	0.00	0.35	0.178
16	5	62	20.50	0.01	0.83	0.451
17	20	60	41.65	0.00	0.02	0.009
18	6	50	24.65	0.02	0.86	0.260
19	20	42	26.40	0.01	0.53	0.192
20	9	47	24.90	0.00	1.19	0.420

\*Water impounded by weir \*\*Sampled branch near water surface not riverbed.

#### 4.1.2 Nutrient concentrations and physicochemical water properties

Results of nutrient concentrations and physicochemical water properties measures at the study sites in spring, summer and autumn are given in ANNEX IV. The seasonal difference in measured water quality parameters across study sites is shown in Figs. 4.1.2. – 4.1.9.

Average, minimum and maximum values of physicochemical water properties and nutrients measured at study sites are given in Tab. 4.1.3.

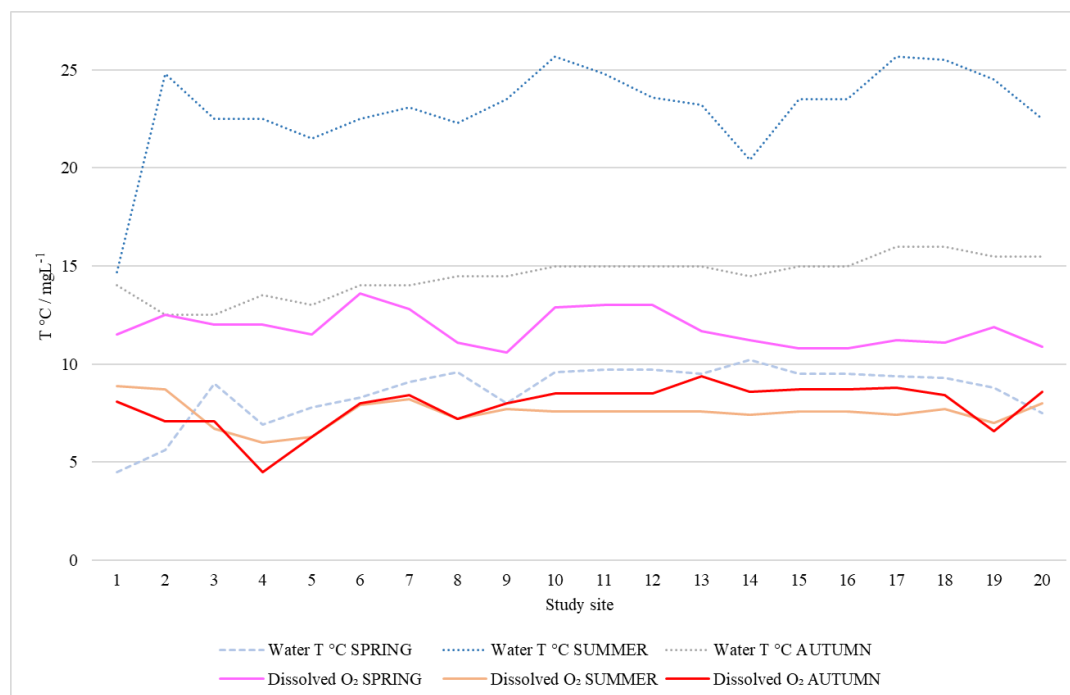


Figure 4.1.2. Seasonal range in water temperature (°C) and dissolved oxygen (O<sub>2</sub>, mgL<sup>-1</sup>) at the study sites.

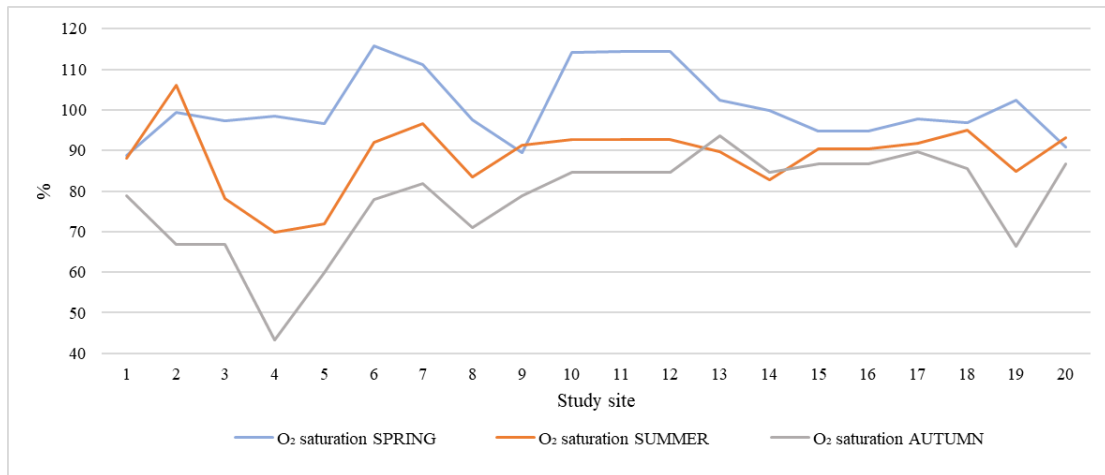


Figure 4.1.3. Seasonal range in oxygen saturation (%) at the study sites.

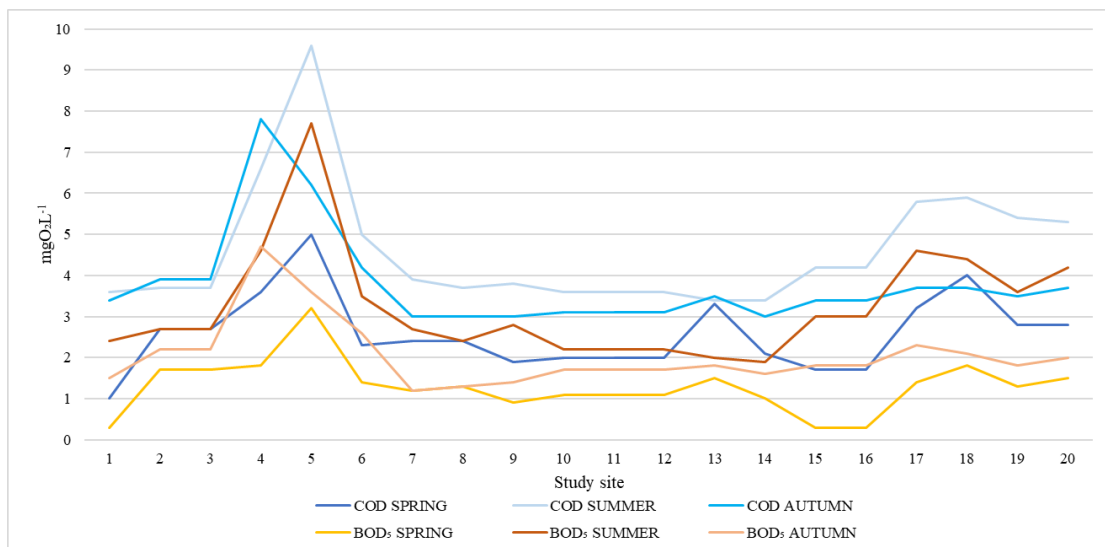


Figure 4.1.4. Seasonal range in chemical oxygen demand (COD<sub>Mn</sub>, mgO<sub>2</sub>L<sup>-1</sup>) and biological oxygen demand (BOD<sub>5</sub>, mgO<sub>2</sub>L<sup>-1</sup>) at the study sites.

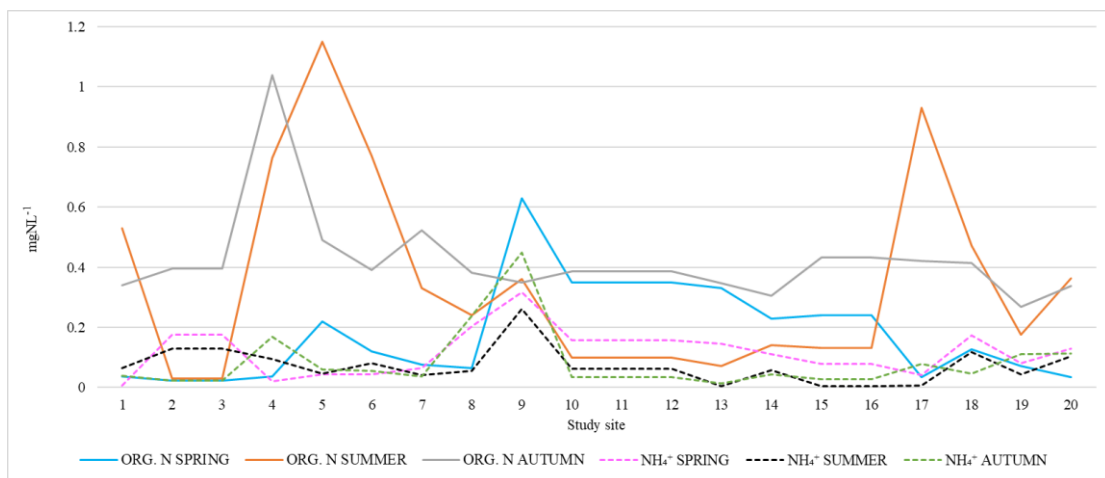


Figure 4.1.5. Seasonal range in organic nitrogen (ORG N, mgNL<sup>-1</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>, mgNL<sup>-1</sup>) at the study sites.

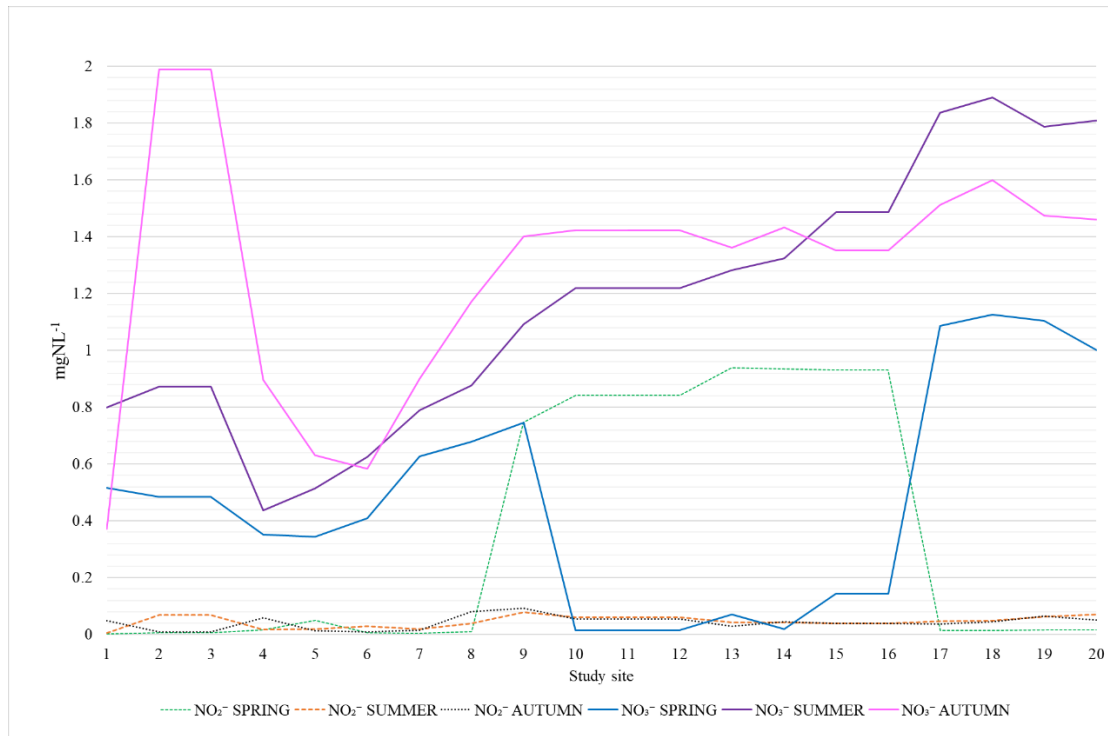


Figure 4.1.6. Seasonal range in nitrite (NO<sub>2</sub><sup>-</sup>, mgNL<sup>-1</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>, mgNL<sup>-1</sup>) at the study sites.

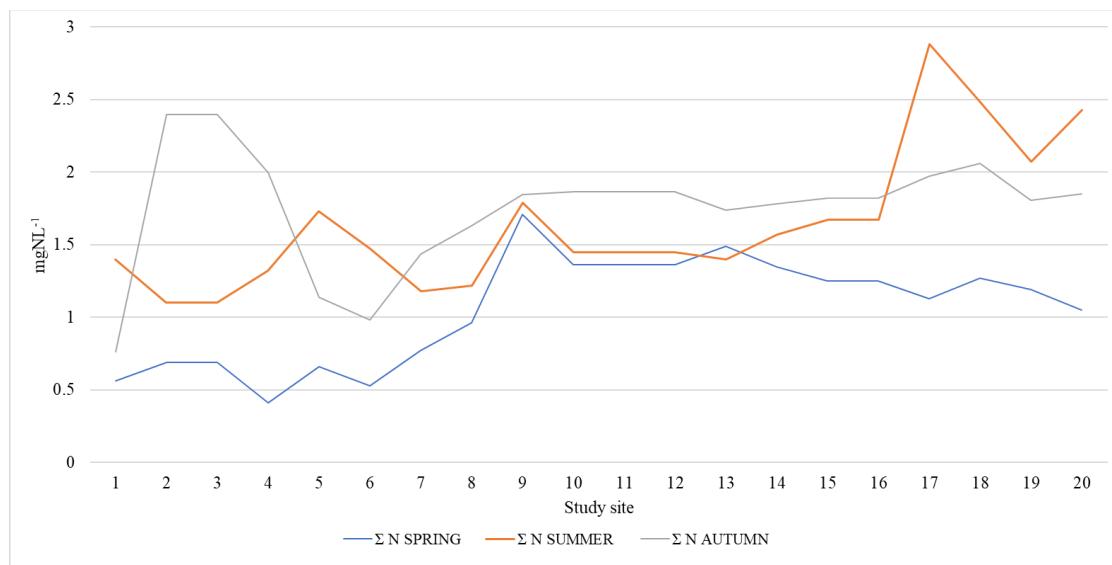


Figure 4.1.7. Seasonal range in total nitrogen (Σ N, mgNL<sup>-1</sup>) at the study sites.

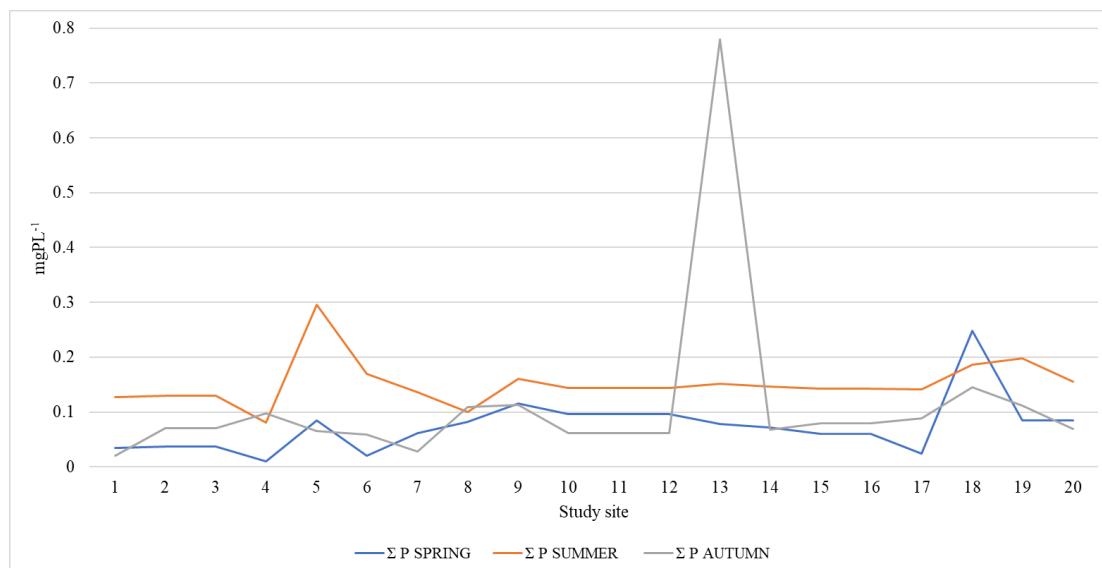


Figure 4.1.8. Seasonal range in total phosphorous ( $\Sigma P$ ,  $\text{mgPL}^{-1}$ ) at the study sites.

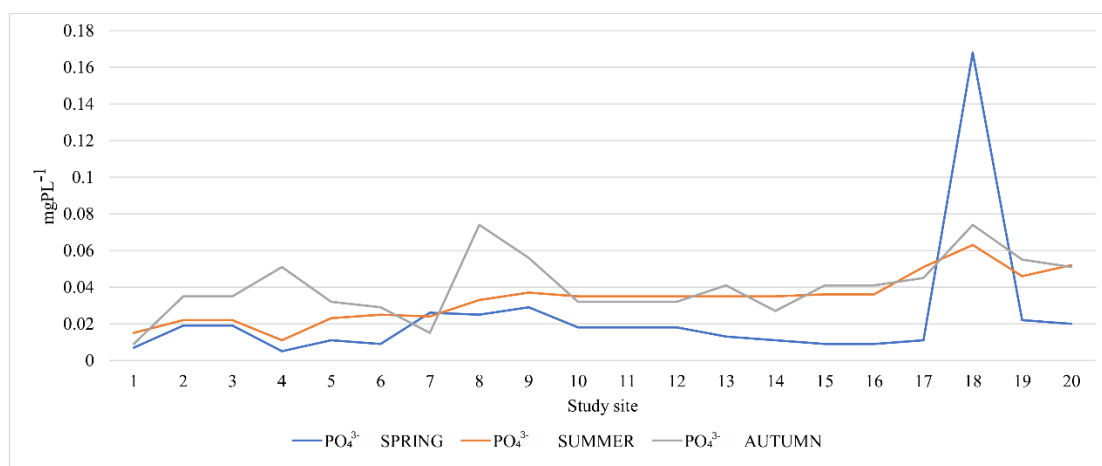


Figure 4.1.9. Seasonal range in orthophosphates ( $\text{PO}_4^{3-}$ ,  $\text{mgPL}^{-1}$ ) at the study sites.

Table 4.1.3. Average, minimum and maximum values of physicochemical water properties and nutrients measured at study sites. Measurements recorded during benthic macroinvertebrate sampling in summer 2015 are shaded grey. Abbreviations:  $\text{COD}_{\text{Mn}}$  = chemical oxygen demand,  $\text{BOD}_5$  = biological oxygen demand,  $\text{NH}_4^+$  = ammonium,  $\text{NO}_2^-$  = nitrite,  $\text{NO}_3^-$  = nitrate,  $\text{ORG N}$  = organic nitrogen,  $\Sigma \text{N}$  = total nitrogen,  $\Sigma \text{P}$  = total phosphorous,  $\text{PO}_4^{3-}$  = orthophosphates.

	Water temp. °C	Dissolved $\text{O}_2$ $\text{mgL}^{-1}$	Oxygen saturation %	Conductivity $\mu\text{Scm}^{-1}$	pH	$\text{COD}_{\text{Mn}}$ $\text{mgO}_2\text{L}^{-1}$	$\text{BOD}_5$ $\text{mgO}_2\text{L}^{-1}$	$\text{NH}_4^+$ $\text{mgNL}^{-1}$	$\text{NO}_2^-$ $\text{mgNL}^{-1}$	$\text{NO}_3^-$ $\text{mgNL}^{-1}$	KJELDAHL N $\text{mgNL}^{-1}$	ORG. N $\text{mgNL}^{-1}$	$\Sigma \text{N}$ $\text{mgNL}^{-1}$	$\text{PO}_4^{3-}$ $\text{mgPL}^{-1}$	$\Sigma \text{P}$ $\text{mgPL}^{-1}$	
1	AVE	11.1	9.5	85.2	588	8.16	2.7	1.4	0.0379	0.0186	0.5621	0.342	0.303	0.91	0.010	0.061
	MIN	4.5	8.1	78.9	556	8.10	1.0	<0.6	0.0077	0.0016	0.3716	0.046	0.038	0.56	0.007	0.020
	MAX	14.7	11.5	88.7	620	8.22	3.6	2.4	0.0659	0.0497	0.7991	0.600	0.530	1.40	0.015	0.127
2	AVE	14.3	9.4	90.7	585	8.10	3.4	2.2	0.1097	0.0279	1.1153	0.260	0.150	1.40	0.025	0.079
	MIN	5.6	7.1	66.9	562	8.03	2.7	1.7	0.0240	0.0057	0.4841	0.160	0.024	0.69	0.019	0.037
	MAX	24.8	12.5	106.0	608	8.17	3.9	2.7	0.1763	0.0688	1.9892	0.420	0.396	2.40	0.035	0.130
3	AVE	14.7	8.6	80.8	581	8.08	3.4	2.2	0.1097	0.0279	1.1153	0.260	0.150	1.40	0.025	0.079
	MIN	9.0	6.7	66.9	552	8.03	2.7	1.7	0.0240	0.0057	0.4841	0.160	0.024	0.69	0.019	0.037
	MAX	22.5	12.0	97.3	609	8.13	3.9	2.7	0.1763	0.0688	1.9892	0.420	0.396	2.40	0.035	0.130

Table 4.1.3. (Continued). Average, minimum and maximum values of physicochemical water properties and nutrients measured at study sites. Measurements recorded during benthic macroinvertebrate sampling in summer 2015 are shaded grey. Abbreviations: COD<sub>Mn</sub> = chemical oxygen demand, BOD<sub>5</sub> = biological oxygen demand, NH<sub>4</sub><sup>+</sup> = ammonium, NO<sub>2</sub><sup>-</sup> = nitrite, NO<sub>3</sub><sup>-</sup> = nitrate, ORG N = organic nitrogen, Σ N = total nitrogen, Σ P = total phosphorous, PO<sub>4</sub><sup>3-</sup> = orthophosphates.

		Water temp. °C	Dissolved O <sub>2</sub> mgL <sup>-1</sup>	Oxygen saturation%	Conductivity µScm <sup>-1</sup>	pH	COD <sub>Mn</sub> mgO <sub>2</sub> L <sup>-1</sup>	BOD <sub>5</sub> mgO <sub>2</sub> L <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> mgNL <sup>-1</sup>	NO <sub>2</sub> <sup>-</sup> mgNL <sup>-1</sup>	NO <sub>3</sub> <sup>-</sup> mgNL <sup>-1</sup>	KJELDAHL N mgNL <sup>-1</sup>	ORG. N mgNL <sup>-1</sup>	Σ N mgNL <sup>-1</sup>	PO <sub>4</sub> <sup>3-</sup> mgPL <sup>-1</sup>	Σ P mgPL <sup>-1</sup>
4	AVE	14.3	7.5	70.6	419	8.03	6.0	3.7	0.0956	0.0309	0.5622	0.710	0.614	1.24	0.022	0.063
	MIN	6.9	4.5	43.4	419	7.90	3.6	1.8	0.0218	0.0170	0.3523	0.060	0.038	0.41	0.005	0.010
	MAX	22.5	12.0	98.5	419	8.16	7.8	4.7	0.1700	0.0585	0.8966	1.210	1.040	2.00	0.051	0.097
5	AVE	14.1	8.0	76.2	475	8.03	6.9	4.8	0.0506	0.0271	0.4963	0.671	0.620	1.18	0.022	0.148
	MIN	7.8	6.3	60.0	448	8.02	5.0	3.2	0.0450	0.0128	0.3447	0.264	0.219	0.66	0.011	0.065
	MAX	21.5	11.5	96.6	501	8.03	9.6	7.7	0.0600	0.0490	0.6308	1.200	1.150	1.73	0.032	0.296
6	AVE	14.9	9.8	95.3	512	8.30	3.8	2.5	0.0598	0.0150	0.5395	0.473	0.427	0.99	0.021	0.083
	MIN	8.3	7.9	78.0	510	8.24	2.3	1.4	0.0433	0.0072	0.4101	0.160	0.120	0.53	0.009	0.020
	MAX	22.5	13.6	115.7	513	8.36	5.0	3.5	0.0812	0.0283	0.6257	0.820	0.770	1.47	0.029	0.170
7	AVE	15.4	9.8	96.6	509	8.29	3.1	1.7	0.0475	0.0130	0.7726	0.357	0.310	1.13	0.022	0.075
	MIN	9.1	8.2	81.9	502	8.21	2.4	1.2	0.0380	0.0045	0.6271	0.141	0.077	0.77	0.015	0.028
	MAX	23.1	12.8	111.1	516	8.37	3.9	2.7	0.0639	0.0197	0.9006	0.560	0.522	1.44	0.026	0.136
8	AVE	15.5	8.5	84.0	519	8.17	3.0	1.7	0.1661	0.0427	0.9088	0.396	0.229	1.27	0.044	0.097
	MIN	9.6	7.2	70.9	509	8.01	2.4	1.3	0.0560	0.0099	0.6778	0.268	0.064	0.96	0.025	0.082
	MAX	22.3	11.1	97.5	529	8.32	3.7	2.4	0.2380	0.0798	1.1716	0.620	0.382	1.63	0.074	0.109
9	AVE	15.3	8.8	86.6	518	8.19	2.9	1.7	0.3424	0.3060	1.0803	0.791	0.447	1.78	0.041	0.130
	MIN	8.0	7.7	78.8	508	8.02	1.9	0.9	0.2612	0.0778	0.7468	0.622	0.350	1.71	0.029	0.113
	MAX	23.5	10.6	91.4	528	8.35	3.8	2.8	0.4500	0.7468	1.4010	0.950	0.630	1.84	0.056	0.161
10	AVE	16.8	9.7	97.2	504	8.44	2.9	1.7	0.0846	0.3192	0.8856	0.363	0.279	1.56	0.028	0.101
	MIN	9.6	7.6	84.7	495	8.39	2.0	1.1	0.0340	0.0544	0.0146	0.166	0.100	1.36	0.018	0.062
	MAX	25.7	12.9	114.2	512	8.48	3.6	2.2	0.1572	0.8422	1.4227	0.503	0.386	1.86	0.035	0.144
11	AVE	16.5	9.7	97.3	503	8.40	2.9	1.7	0.0846	0.3192	0.8856	0.363	0.279	1.56	0.028	0.101
	MIN	9.7	7.6	84.7	495	8.31	2.0	1.1	0.0340	0.0544	0.0146	0.166	0.100	1.36	0.018	0.062
	MAX	24.8	13.0	114.5	510	8.48	3.6	2.2	0.1572	0.8422	1.4227	0.503	0.386	1.86	0.035	0.144
12	AVE	16.1	9.7	97.3	503	8.38	2.9	1.7	0.0846	0.3192	0.8856	0.363	0.279	1.56	0.028	0.101
	MIN	9.7	7.6	84.7	495	8.28	2.0	1.1	0.0340	0.0544	0.0146	0.166	0.100	1.36	0.018	0.062
	MAX	23.6	13.0	114.5	511	8.48	3.6	2.2	0.1572	0.8422	1.4227	0.503	0.386	1.86	0.035	0.144
13	AVE	15.9	9.6	95.3	513	8.47	3.4	1.8	0.0546	0.3364	0.9047	0.306	0.249	1.54	0.030	0.337
	MIN	9.5	7.6	89.8	506	8.31	3.3	1.5	<0.008	0.0286	0.0709	0.080	0.072	1.40	0.013	0.078
	MAX	23.2	11.7	102.5	520	8.62	3.5	2.0	0.1459	0.9384	1.3611	0.477	0.346	1.74	0.041	0.780
14	AVE	15.0	9.1	89.1	524	8.18	2.8	1.5	0.0709	0.3411	0.9253	0.297	0.225	1.57	0.024	0.095
	MIN	10.2	7.4	82.7	517	8.08	2.1	1.0	0.0440	0.0436	0.0187	0.200	0.140	1.35	0.011	0.068
	MAX	20.4	11.2	99.8	531	8.28	3.4	1.9	0.1100	0.9342	1.4326	0.350	0.306	1.78	0.035	0.146
15	AVE	16.0	9.0	90.6	557	8.18	3.1	1.7	0.0366	0.3366	0.9941	0.303	0.268	1.58	0.029	0.094
	MIN	9.5	7.6	86.7	548	8.15	1.7	<0.6	<0.008	0.0390	0.1440	0.140	0.132	1.25	0.009	0.060
	MAX	23.5	10.8	94.7	566	8.21	4.2	3.0	0.0777	0.9309	1.4872	0.460	0.432	1.82	0.041	0.142
16	AVE	16.0	9.0	90.6	557	8.18	3.1	1.7	0.0366	0.3366	0.9941	0.303	0.268	1.58	0.029	0.094
	MIN	9.5	7.6	86.7	548	8.15	1.7	<0.6	<0.008	0.0390	0.1440	0.140	0.132	1.25	0.009	0.060
	MAX	23.5	10.8	94.7	566	8.21	4.2	3.0	0.0777	0.9309	1.4872	0.460	0.432	1.82	0.041	0.142
17	AVE	17.0	9.1	93.1	567	8.27	4.2	2.8	0.0419	0.0321	1.4786	0.525	0.462	1.99	0.036	0.084
	MIN	9.4	7.4	89.6	563	8.06	3.2	1.4	0.0068	0.0134	1.0859	0.076	0.035	1.13	0.011	0.024
	MAX	25.7	11.2	97.9	570	8.48	5.8	4.6	0.0780	0.0466	1.8374	1.000	0.930	2.88	0.051	0.141
18	AVE	16.9	9.1	92.5	569	8.25	4.5	2.8	0.1120	0.0366	1.5388	0.433	0.338	1.94	0.102	0.193
	MIN	9.3	7.7	85.5	564	8.08	3.7	1.8	0.0460	0.0142	1.1260	0.300	0.127	1.27	0.063	0.145
	MAX	25.5	11.1	96.8	574	8.41	5.9	4.4	0.1729	0.0496	1.8915	0.540	0.473	2.48	0.168	0.248
19	AVE	16.3	8.5	84.6	577	8.15	3.9	2.2	0.0795	0.0480	1.4549	0.251	0.172	1.69	0.041	0.132
	MIN	8.8	6.6	66.5	574	8.02	2.8	1.3	0.0444	0.0159	1.1038	0.154	0.072	1.19	0.022	0.085
	MAX	24.5	11.9	102.5	580	8.27	5.4	3.6	0.1120	0.0653	1.7868	0.380	0.268	2.07	0.055	0.198
20	AVE	15.2	9.2	90.3	578	8.21	3.9	2.6	0.1150	0.0461	1.4245	0.360	0.245	1.78	0.041	0.103
	MIN	7.5	8.0	86.7	575	8.17	2.8	1.5	0.1033	0.0155	1.0020	0.163	0.034	1.05	0.020	0.069
	MAX	22.5	10.9	93.2	580	8.24	5.3	4.2	0.1286	0.0710	1.8096	0.467	0.364	2.43	0.052	0.156

### 4.1.3 Hydromorphological modification of study sites

#### 4.1.3.1 Hydromorphological modification according to EN 15843:2010

Hydromorphological modification of the study sites was assessed using EN 15843:2010 for river reaches of different length: the 100 m reach where benthic macroinvertebrates were sampled and a 500 m length reach extending upstream and corresponding to the River Habitat Survey assessed reach. Four study sites (1, 4, 16 and 20) are reaches of the Bednja River not subject to recent management activities and have a class 1 mean score representing near natural conditions regardless of length of assessed reach. Study site 18 has a class 1 mean score for the 100 m length reach but due to upstream degradation the entire 500 m assessed reach received a class 2 mean score. Seven study sites (3, 8, 9, 12, 13, 14 and 15) have a class 2 mean score representing slightly modified conditions despite having some level of degradation. Three study sites (2, 5 and 10) are moderately modified (class 3), and two study sites (7 and 11) are extensively modified (class 4). Study site 6 received differing scores depending on length of assessed reach. The 100 m assessed reach falls borderline as slightly modified (class 2) while the entire 500 m reach extending upstream is assessed as extensively modified (class 4). No study site received a class 5 (severely modified) status.

Grouped and mean hydromorphological modification scores are given in Tab. 4.1.4. Individual scores for all 16 assessed features for the 100 m and 500 m assessed reach are given in Annex III.

Table 4.1.4. Hydromorphological modification of study sites according to EN 15843:2010 with assessed length of 100 m and 500 m river reach. Scoring is presented as follows: grouped score for the three main hydromorphological quality elements given in the WFD (morphology, flow regime, and longitudinal continuity), a single mean score for the reach assessed, and scores grouped according to the three main river zones (channel, bank/riparian zone and floodplain).

Study site	Length of assessed reach	WFD quality elements			MEAN SCORE	Scores for features grouped according to zone		
		Morphology	Flow	Longitudinal continuity		Channel	Banks, riparian zone	Floodplain
1	100 m	1.33	1.00	1.00	<b>1.19</b>	1.00	1.50	1.67
	500 m	1.78	1.00	1.00	<b>1.44</b>	1.25	2.00	2.00
2	100 m	3.00	1.00	1.00	<b>2.88</b>	2.25	3.00	2.33
	500 m	3.00	1.00	1.00	<b>2.75</b>	2.25	3.00	2.33
3	100 m	2.00	1.00	1.00	<b>1.69</b>	2.00	1.00	1.33
	500 m	2.22	1.00	1.00	<b>1.81</b>	1.88	1.50	2.00
4	100 m	1.44	1.00	1.00	<b>1.38</b>	1.38	1.00	1.33
	500 m	1.44	1.00	1.00	<b>1.38</b>	1.38	1.00	1.33
5	100 m	3.89	1.00	1.00	<b>2.88</b>	2.25	3.00	5.00
	500 m	3.89	1.00	1.00	<b>2.88</b>	2.25	3.00	5.00

Table 4.1.4. (Continued). Hydromorphological modification of study sites according to EN 15843:2010 with assessed length of 100 m and 500 m river reach. Scoring is presented as follows: grouped score for the three main hydromorphological quality elements given in the WFD (morphology, flow regime, and longitudinal continuity), a single mean score for the reach assessed, and scores grouped according to the three main river zones (channel, bank/riparian zone and floodplain).

Study site	Length of assessed reach	WFD quality elements			MEAN SCORE	Scores for features grouped according to zone		
		Morphology	Flow	Longitudinal continuity		Channel	Banks, riparian zone	Floodplain
6	100 m	3.33	1.00	1.00	2.44	1.75	3.50	4.33
	500 m	4.56	1.67	1.00	3.50	2.75	5.00	5.00
7	100 m	4.78	1.00	1.00	3.63	2.75	5.00	5.00
	500 m	4.22	1.00	1.00	3.19	2.50	4.50	4.33
8	100 m	2.67	1.00	1.00	2.06	2.00	2.50	2.33
	500 m	2.78	1.00	1.00	2.13	2.00	2.50	2.67
9	100 m	2.78	1.00	1.00	2.13	2.00	2.50	2.67
	500 m	2.78	1.00	1.00	2.13	2.00	2.50	2.67
10	100 m	3.22	1.67	3.00	2.75	2.75	3.00	3.00
	500 m	3.22	1.67	3.00	2.75	2.75	3.00	3.00
11	100 m	4.78	1.67	3.00	3.75	3.25	5.00	5.00
	500 m	4.11	2.33	3.00	3.50	3.38	4.00	4.00
12	100 m	3.33	1.00	1.00	2.31	2.00	3.00	4.00
	500 m	2.89	1.00	1.00	2.06	1.75	3.00	3.33
13	100 m	2.56	1.00	1.00	2.00	2.00	2.50	2.00
	500 m	2.89	1.00	1.00	2.19	2.00	2.50	3.00
14	100 m	1.89	1.00	1.00	1.50	1.63	2.00	1.33
	500 m	2.11	1.00	1.00	1.63	1.63	2.00	2.00
15	100 m	2.89	1.00	1.00	2.19	1.88	3.00	3.00
	500 m	2.89	1.00	1.00	2.19	1.88	3.00	3.00
16	100 m	1.67	1.00	1.00	1.38	1.13	1.50	2.33
	500 m	1.67	1.00	1.00	1.38	1.25	1.50	2.00
17	100 m	4.78	1.67	1.00	3.88	3.00	5.00	5.00
	500 m	5.00	1.67	1.00	4.00	3.25	5.00	5.00
18	100 m	1.22	1.00	1.00	1.13	1.25	1.00	1.00
	500 m	2.44	1.00	1.00	1.81	1.75	2.50	2.33
19	100 m	4.11	1.00	1.00	2.88	2.00	5.00	5.00
	500 m	3.67	1.00	1.00	2.63	1.88	4.00	4.67
20	100 m	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	500 m	1.22	1.00	1.00	1.13	1.00	1.50	1.33

Class	Score	Description
1	1 to < 1.5	Near natural
2	1.5 to < 2.5	Slightly modified
3	2.5 to < 3.5	Moderately modified
4	3.5 to < 4.5	Extensively modified
5	4.5 to 5.0	Severely modified

#### 4.1.3.2 River Habitat Survey (RHS) scores

##### *The Habitat Modification Score (HMS)*

The Habitat Modification Score (HMS) derived from River Habitat Survey represents the extent and severity of modification to the river banks and channel. Only one study site achieved the highest result for HMS (study site 20) and one study site achieved a class 2 HMS (study site 16). Study site 1 has a class 3 HMS while study sites 3, 4, 14 and 18 have a class 4 HMS. The

remaining 13 study sites (study sites 2, 5, 6, 7, 8, 9, 10, 11, 12,13, 15, 17 and 19) have achieved a class 5 HMS score (Tab. 4.1.5.).

Table 4.1.5. Results for River Habitat Survey – Habitat Modification Score (HMS).

Study site	Habitat Modification Class (HMC)	Habitat Modification Score (HMS)	Resectioned Bank Bed subscore	HMS Reinforced Bank Bed subscore	HMS Realigned subscore
1	3	340	280	0	0
2	5	3300	2800	0	400
3	4	760	560	0	100
4	4	940	880	0	0
5	5	4280	2800	0	400
6	5	3725	2760	160	400
7	5	3967	2720	460	400
8	5	3660	2800	0	400
9	5	3120	2320	0	400
10	5	3380	2680	0	400
11	5	3085	1880	180	400
12	5	1770	1480	40	100
13	5	3275	2800	0	400
14	4	780	600	60	100
15	5	2675	2200	0	400
16	2	40	40	0	0
17	5	5484	2800	1560	400
18	4	668	360	0	100
19	5	3735	2800	180	400
20	1	0	0	0	0

HMC	HMS Score	Description
1	0–16	Pristine/semi-natural
2	17–199	Predominantly unmodified
3	200–499	Obviously modified
4	500–1399	Significantly modified
5	> 1400	Severely modified

### *Habitat Quality Assessment (HQA)*

Habitat Quality Assessment (HQA) scores derived through RHS are given in Tab. 4.1.6. Higher HQA values indicate higher habitat quality. Study site 20 received the highest HQA score (75) while study site 17 received the lowest HQA score (28).

Table 4.1.6. Results for River Habitat Survey – Habitat Quality Assessment (HQA).

Study site	HQA Score	HQA flow type	HQA channel substrate	HQA channel features	HQA bank vegetation structure	HQA channel vegetation	HQA landuse	HQA trees	HQA special features
1	49	8	9	2	10	5	2	7	4
2	30	8	7	0	2	8	0	0	2
3	60	8	8	2	12	2	4	15	5
4	63	10	6	2	12	0	4	16	7
5	29	7	7	1	6	2	0	6	0
6	37	8	7	3	10	4	1	3	0
7	47	10	6	3	12	5	0	4	2
8	46	11	5	2	9	1	3	11	3
9	42	8	5	2	6	5	0	10	4
10	35	5	7	1	6	3	0	8	0



Table 4.1.6. (Continued). Results for River Habitat Survey – Habitat Quality Assessment (HQA).

Study site	HQA Score	HQA flow type	HQA channel substrate	HQA channel features	HQA bank vegetation structure	HQA channel vegetation	HQA landuse	HQA trees	HQA special features
11	46	8	6	2	11	9	0	7	1
12	51	9	7	3	9	7	1	11	2
13	51	9	8	1	12	7	0	12	0
14	49	6	5	2	12	3	1	12	2
15	45	9	7	1	12	4	2	8	0
16	61	9	7	3	12	2	3	15	3
17	28	7	4	0	7	9	0	1	0
18	54	8	5	4	12	4	3	15	0
19	37	8	5	0	12	2	1	6	0
20	75	9	5	3	12	7	5	16	9

#### *Riparian Quality Index (RQI) and hydromorphological indices*

Riparian Quality Index (RQI) values place study sites 4, 14, 16, 18 and 20 into very high RQI class. Study sites 3, 8, 13 and 15 received a high RQI while study sites 1, 7, 9, 11, 12 and 19 have moderate RQI. Study sites 5, 6, 10 and 17 have low RQI and study site 2 has very low RQI (Tab. 4.1.7.).

RHS Channel Substrate Index values range from -2.188 (study site 4) to 0.512 (study site 16). The Flow Regime Index scores range from -1.198 (study site 10) to 0.347 (study site 16). The Channel Vegetation Index ranges from -0.844 (study site 11) to 0.974 (study site 6) (Tab. 4.1.7.).

Table 4.1.7. Results for River Habitat Survey – Riparian Quality Index (RQI) and Hydromorphological indices.

Study site	Riparian Quality Index (RQI)					Hydromorphological indices		
	RQI class	RQI score	Complexity subscore	Naturalness subscore	Continuity subscore	Channel Substrate Index (CSI)	Flow Regime Index (FRI)	Channel Vegetation Index (CVI)
1	3	61	16	39	6	-1.502	-0.042	-0.309
2	5	2	2	0	0	-1.664	-0.506	-0.729
3	2	95	39	37	19	-0.976	-0.522	-0.194
4	1	101	46	35	20	-2.188	-0.868	0.71
5	4	33	17	0	16	-1.767	-0.537	-0.378
6	4	41	21	9	11	0.249	-0.207	0.974
7	3	58	36	3	19	-0.626	-0.537	0.295
8	2	79	40	19	20	-0.604	-0.372	0.71
9	3	60	20	20	20	-0.706	-0.042	-0.302
10	4	25	11	4	10	-1.121	-1.198	-0.525
11	3	69	32	18	19	-0.68	-0.763	-0.844
12	3	65	30	16	19	-0.808	-0.868	-0.374
13	2	91	52	19	20	-1.115	-0.207	-0.809
14	1	106	54	32	20	-1.32	-1.033	-0.395

Table 4.1.7. (Continued). Results for River Habitat Survey – Riparian Quality Index (RQI) and Hydromorphological indices.

Study site	Riparian Quality Index (RQI)					Hydromorphological indices		
	RQI class	RQI score	Complexity subscore	Naturalness subscore	Continuity subscore	Channel Substrate Index	Flow Regime Index	Channel Vegetation Index
15	2	83	46	18	19	-0.814	-0.372	0.069
16	1	109	52	37	20	0.512	0.347	0.34
17	4	28	15	0	13	0.502	-0.372	-0.076
18	1	110	54	36	20	-0.604	0.288	0.071
19	3	64	38	10	16	-0.604	-0.537	0.468
20	1	108	50	38	20	-0.604	0.220	-0.325

RQI Class	RQI Description
1	Very high
2	High
3	Moderate
4	Low
5	Very low

Table 4.1.8. Summary and comparison of River Habitat Survey Habitat Modification Class (HMC), Habitat Quality Assessment (HQA), Riparian Quality Index class (RQI class), and EN 15843:2010 scores for morphological modification and mean scores. Included is also the individual score for feature 1a – Channel geometry / planform assessed for the 500 m long reach. The remaining EN 15843:2010 individual feature scores are given in Annex III.

Study site	River Habitat Survey (RHS)			1a -channel geometry / planform 500 m	EN 15843:2010			
	Habitat Modification Class (HMC)	Habitat Quality Assessment (HQA)	Riparian Quality Index class (RQI class)		Morphology 100 m	Morphology 500 m	Mean score 100 m	Mean score 500 m
1	3	49	3	2	1.33	1.78	1	1
2	5	30	5	5	3.00	3.00	3	3
3	4	60	2	4	2.00	2.22	2	2
4	4	63	1	2	1.44	1.44	1	1
5	5	29	4	5	3.89	3.89	3	3
6	5	37	4	5	3.33	4.56	2	4
7	5	47	3	5	4.78	4.22	4	3
8	5	46	2	5	2.67	2.78	2	2
9	5	42	3	5	2.78	2.78	2	2
10	5	35	4	5	3.22	3.22	3	3
11	5	46	3	5	4.78	4.11	4	4
12	5	51	3	4	3.33	2.89	2	2
13	5	51	2	5	2.56	2.89	2	2
14	4	49	1	4	1.89	2.11	2	2
15	5	45	2	5	2.89	2.89	2	2
16	2	61	1	2	1.67	1.67	1	1
17	5	28	4	5	4.78	5.00	4	4
18	4	54	1	4	1.22	2.44	1	2
19	5	37	3	5	4.11	3.67	3	3
20	1	75	1	1	1	1.22	1	1

Class	HMC description	HQA description	RQI class description	EN 15843:2010 scores description
1	Pristine/semi-natural	Top 20%	Very high	Near natural
2	Predominantly unmodified	Top 40%	High	Slightly modified
3	Obviously modified	40%–60%	Moderate	Moderately modified
4	Significantly modified	Bottom 40%	Low	Extensively modified
5	Severely modified	Bottom 20%	Very low	Severely modified

#### 4.1.4 Sub-catchment landuse

Near natural areas are the most represented land cover on the Bednja catchment amounting to 53.5% of the total land cover (CLC category 311 Broad-leaved forest make up 45% of the total landuse within the catchment). 24.5% of the landuse falls under extensive agriculture which comprises of categories 231 Pastures and 243 Land principally occupied by agriculture, with significant areas of natural vegetation, with significant areas of natural vegetation. Intensive agriculture is practiced on 19.7% of the Bednja catchment as CLC category 242 Complex cultivation patterns, while categories 211 Non-irrigated arable land and 221 Vineyards occupy <1%. Urbanised and artificial land cover is the least represented landuse on the catchment and covers only 2.5% of the total catchment area (Fig. 4.1.10.).

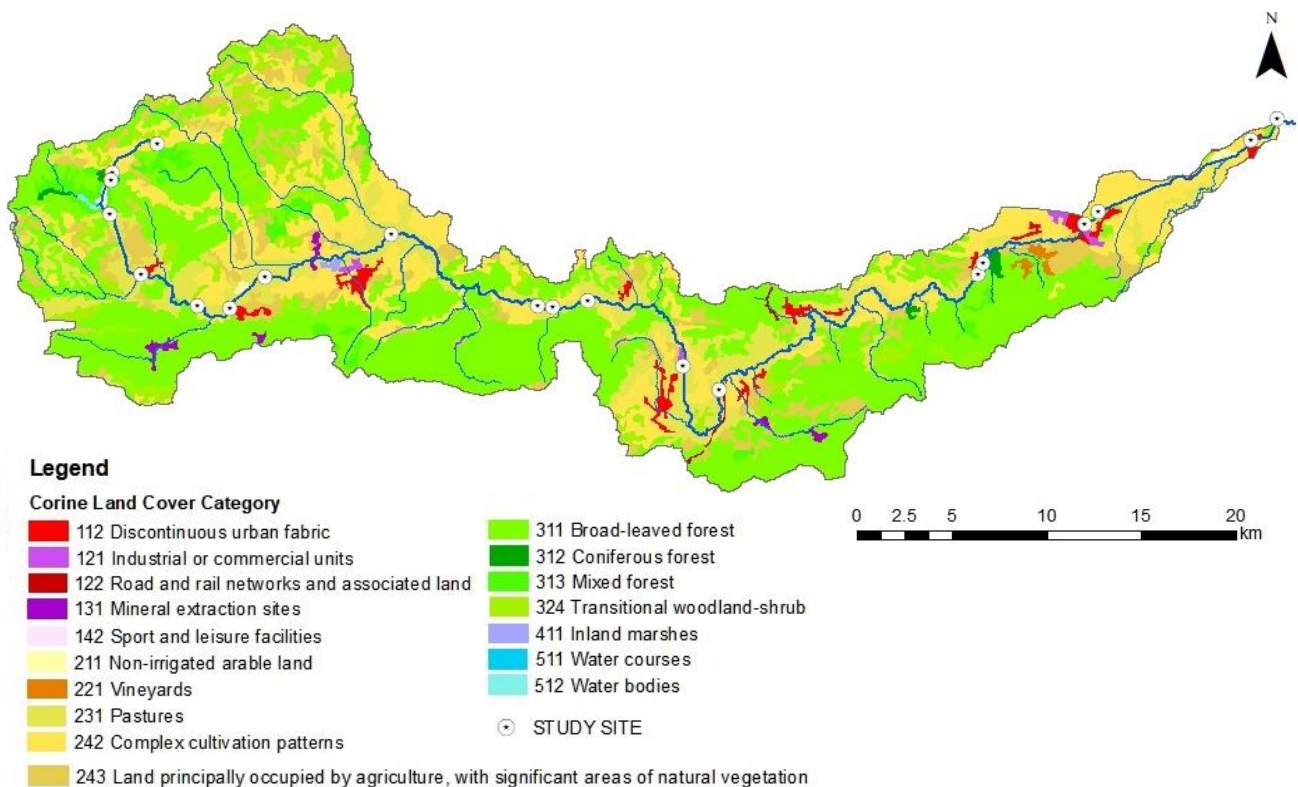


Figure 4.1.10. CORINE Land Cover (CLC) landuse on the Bednja River catchment (HAOP, 2012).

Sub-catchment size of study sites ranges from 0.4 km<sup>2</sup> to 598.5 km<sup>2</sup> with near natural land cover on sub-catchments ranging from 53.2% to 70.9%, intensive agriculture covering from 7.8% to 42.3%, extensive agriculture ranging from 0% to 32.3% and urban and artificial land cover share ranging from 0% to 2.7% (Tab. 4.1.9., Fig. 4.1.11.).

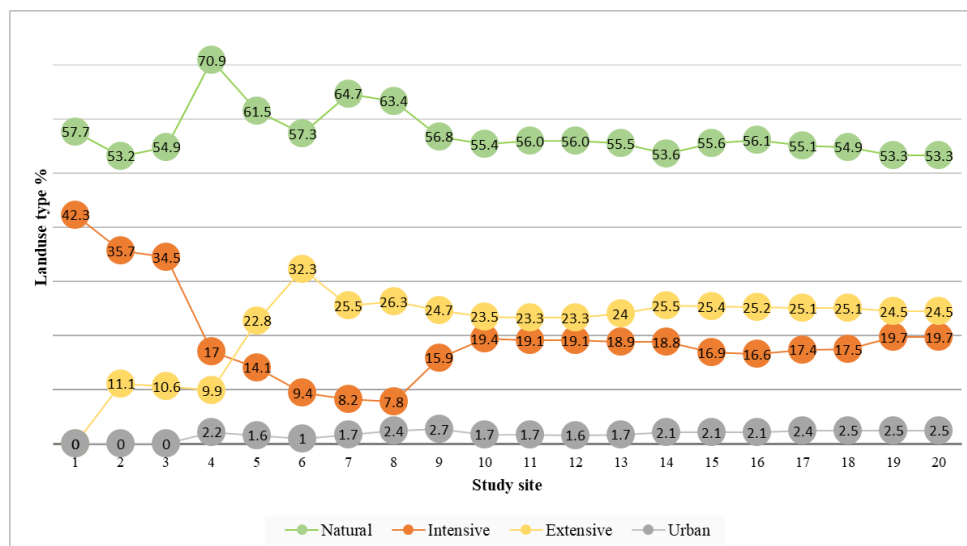


Figure 4.1.11. Variation of landuse share across study sites.

Table 4.1.9. Landuse share on study site associated sub-catchment. Total sub-catchment of each study site represents the entire drainage area upstream from each study site.

Study site	Total catchment size above study site (km <sup>2</sup> )	% Cover and surface area of landuse category on sub-catchment							
		% Near natural area	Area (km <sup>2</sup> )	% Intensive agriculture	Area (km <sup>2</sup> )	% Extensive agriculture	Area (km <sup>2</sup> )	% Urbanised and artificial land cover	Area (km <sup>2</sup> )
		CLC categories present: 311, 312, 313, 324, 411, 511, 512		CLC categories present: 211, 221, 242		CLC categories present: 231, 243		CLC categories present: 112, 121, 122, 131, 142	
1	0.4	57.7%	0.2	42.3%	0.2	0.0%	0.0	0.0%	0.0
2	8.3	53.2%	4.4	35.7%	3.0	11.1%	0.9	0.0%	0.0
3	8.6	54.9%	4.7	34.5%	3.0	10.6%	0.9	0.0%	0.0
4	23.2	70.9%	16.4	17.0%	3.9	9.9%	2.3	2.2%	0.5
5	31.5	61.5%	19.4	14.1%	4.4	22.8%	7.2	1.6%	0.5
6	80.3	57.3%	46.0	9.4%	7.5	32.3%	25.9	1.0%	0.8
7	108.9	64.7%	70.5	8.2%	8.9	25.5%	27.8	1.7%	1.9
8	114.9	63.4%	72.8	7.8%	9.0	26.3%	30.2	2.4%	2.8
9	201.0	56.8%	114.2	15.9%	32.0	24.7%	49.6	2.7%	5.4
10	335.0	55.4%	185.6	19.4%	65.0	23.5%	78.7	1.7%	5.7
11	341.9	56.0%	191.5	19.1%	65.3	23.3%	79.7	1.7%	5.8
12	348.7	56.0%	195.3	19.1%	66.6	23.3%	81.2	1.6%	5.6
13	376.5	55.5%	209.0	18.9%	71.2	24.0%	90.4	1.7%	6.4
14	416.7	53.6%	223.4	18.8%	78.3	25.5%	106.3	2.1%	8.8
15	529.3	55.6%	294.3	16.9%	89.5	25.4%	134.4	2.1%	11.1
16	536.7	56.1%	301.1	16.6%	89.1	25.2%	135.2	2.1%	11.3
17	563.1	55.1%	310.3	17.4%	98.0	25.1%	141.3	2.4%	13.5
18	565.5	54.9%	310.5	17.5%	99.0	25.1%	141.9	2.5%	14.1
19	597.2	53.3%	318.3	19.7%	117.6	24.5%	146.3	2.5%	14.9
20	598.5	53.3%	319.0	19.7%	117.9	24.5%	146.6	2.5%	15.0

Legend for CLC categories present:

- |                                 |   |  |
|---------------------------------|---|--|
| 311 Broad-leaved forest         | 211 Non-irrigated arable land   | 112 Discontinuous urban fabric                 |
| 312 Coniferous forest           | 221 Vineyards   | 121 Industrial or commercial units             |
| 313 Mixed forest                | 242 Complex cultivation patterns  | 122 Road and rail networks and associated land |
| 324 Transitional woodland-shrub |   | 131 Mineral extraction sites                   |
| 411 Inland marshes              |   | 142 Sport and leisure facilities               |
| 511 Water courses               | 231 Pastures  |  |
| 512 Water bodies                | 243 Land principally occupied by agriculture, with significant areas of natural vegetation. |  |

#### 4.1.5 Correlations between natural factors and anthropogenic stressors

Pursuant to the set methodology, Spearman's rank correlation coefficient (R) was used to test the statistical relationship between natural factors (distance from source, altitude, and sub-catchment size) and anthropogenic stressors (water quality/nutrients, landuse, and hydromorphological quality and modification) on the Bednja River catchment. Results of the correlations are given in Tab. 4.1.10.

Significant positive correlations are found between distance from source and water temperature, nitrite, nitrate, total nitrogen, orthophosphates and total phosphorous concentration. Pursuantly, the same parameters exhibit significant negative correlations with increase in altitude. For water quality parameters, the strongest correlation is found for total nitrogen and the longitudinal gradient ( $R = 0.844, p < 0.05$ ).

Significant correlations are also found for share of near natural land cover which decreases with distance from source and sub-catchment size and increases with altitude. The share of land used for extensive agriculture also increases with distance from source and decreases with altitude. For landuse, the strongest correlation is found between share of urban landuse and distance from source ( $R = 0.702, p < 0.05$ ).

Hydromorphological modification scores derived from the EN 15843:2010 methodology give no significant correlations to distance from source, altitude or sub-catchment size. River Habitat Survey scores for HQA channel substrate and RQI riparian vegetation complexity subscores give significant moderate correlation to distance from source, altitude and sub-catchment size. The strongest correlation for hydromorphological scores and the longitudinal gradient is found for the RHS Chanel Substrate Index ( $R = 0.603, p < 0.05$ ). Remaining RHS scores representing habitat modification, habitat quality and riparian quality give no significant correlation to the longitudinal gradient (Tab. 4.1.10.).

Table 4.1.10. Results of Spearman's correlation coefficient (R) for the relationship between natural parameters and stressors: water quality, landuse and hydromorphological quality and modification on the Bednja River catchment. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded.

	Distance from source / Sub-catchment area	Altitude
	r	r
<b>Water Quality (Average values)</b>		
Water temperature (°C)	<b>0.726</b>	<b>-0.726</b>
Dissolved oxygen (mgL <sup>-1</sup> )	-0.023	0.023
Oxygen saturation (%)	0.255	-0.255
Conductivity (µScm <sup>-1</sup> )	0.141	-0.141
pH	0.379	-0.379
Biological oxygen demand (BOD <sub>5</sub> , mg O <sub>2</sub> L <sup>-1</sup> )	0.158	-0.158
Chemical Oxygen Demand (COD <sub>Mn</sub> , mgO <sub>2</sub> L <sup>-1</sup> )	0.077	-0.077
Ammonium concentration (NH <sub>4</sub> <sup>+</sup> , mgNL <sup>-1</sup> )	-0.060	0.060
Nitrites (NO <sub>2</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	<b>0.610</b>	<b>-0.610</b>
Nitrates (NO <sub>3</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	<b>0.609</b>	<b>-0.609</b>
Kjeldahl Nitrogen (mgNL <sup>-1</sup> )	-0.115	0.115
Organic Nitrogen (mgNL <sup>-1</sup> )	-0.143	0.143
Total nitrogen (Σ N, mgNL <sup>-1</sup> )	<b>0.844</b>	<b>-0.844</b>
Orthophosphates (PO <sub>4</sub> <sup>3-</sup> , mgPL <sup>-1</sup> )	<b>0.720</b>	<b>-0.720</b>
Total phosphorous (Σ P, mgPL <sup>-1</sup> )	<b>0.576</b>	<b>-0.576</b>
<b>Landuse</b>		
% Near natural	<b>-0.484</b>	<b>0.484</b>
% Intensive agriculture	-0.012	0.012
% Extensive agriculture	<b>0.455</b>	<b>-0.455</b>
% Total agriculture	0.286	-0.286
% Urban	<b>0.702</b>	<b>-0.702</b>
<b>EN 15843:2010 - 100 m scale</b>		
AVERAGE SCORE 100 m	-0.101	0.101
Morphology 100 m	-0.047	0.047
Flow 100 m	0.158	-0.158
Continuity 100 m	0.000	0.000
Channel 100 m	-0.156	0.156
Riparian zone 100 m	0.055	-0.055
Floodplain 100 m	-0.002	0.002
<b>EN 15843:2010 - 500 m scale</b>		
AVERAGE SCORE 500 m	-0.106	0.106
Morphology 500 m	-0.043	0.043
Flow 500 m	0.043	-0.043
Continuity 500 m	0.000	0.000
Channel 500 m	-0.199	0.199
Riparian zone 500 m	0.073	-0.073
Floodplain 500 m	0.078	-0.078
<b>River Habitat Survey</b>		
<b>Habitat Modification Score (HMS)</b>		
Resectioned Bank Bed subscore	-0.134	0.134
Reinforced Bank Bed subscore	-0.140	0.140
Reinforced Bank Bed subscore	0.238	-0.238
Realigned subscore	-0.048	0.048
<b>Habitat Quality Assessment Score (HQA)</b>		
HQA flow type	0.149	-0.149
HQA flow type	0.000	0.000
HQA channel substrate	<b>-0.553</b>	<b>0.553</b>
HQA channel features	0.085	-0.085
HQA bank vegetation structure	0.408	-0.408
HQA channel vegetation	0.154	-0.154
HQA landuse	0.127	-0.127
HQA trees	0.263	-0.263
HQA special features	-0.308	0.308
<b>Riparian Quality Index score (RQI)</b>		
Riparian Quality Index score (RQI)	0.432	-0.432
Complexity subscore	<b>0.503</b>	<b>-0.503</b>
Naturalness subscore	0.103	-0.103
Continuity subscore	0.403	-0.403

Table 4.1.10. (Continued). Results of Spearman's correlation coefficient (R) for the relationship between natural parameters and stressors: water quality, landuse and hydromorphological quality and modification on the Bednja River catchment. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded.

	Distance from source / Sub-catchment area	Altitude
	r	r
<b>River Habitat Survey</b>		
Channel Substrate Index (CSI)	<b>0.603</b>	<b>-0.603</b>
Flow Regime Index (FRI)	0.227	-0.227
Channel Vegetation Index (CVI)	0.047	-0.047
Depth (average)	<b>0.523</b>	<b>-0.523</b>
Water velocity (average)	0.409	-0.409
% Macrolithal	-0.405	0.405
% Mesolithal	-0.160	0.160
% Microlithal	0.215	-0.215
% Akal	0.062	-0.062
% Psammal	-0.371	0.371
% Phytal	-0.141	0.141
% Xylal	0.223	-0.223
% Technolithal	0.282	-0.282

The 13 parameters that had significant correlations with distance from source, sub-catchment size and altitude (Tab.4.1.10.) were further ordinated in a Redundancy Analysis (RDA) (Fig. 4.1.12.).

The explanatory variables (distance from source, sub-catchment size and altitude) account for 50.2% of the total stressor variation. The eigenvalues of the first two axes are 0.375 and 0.087.

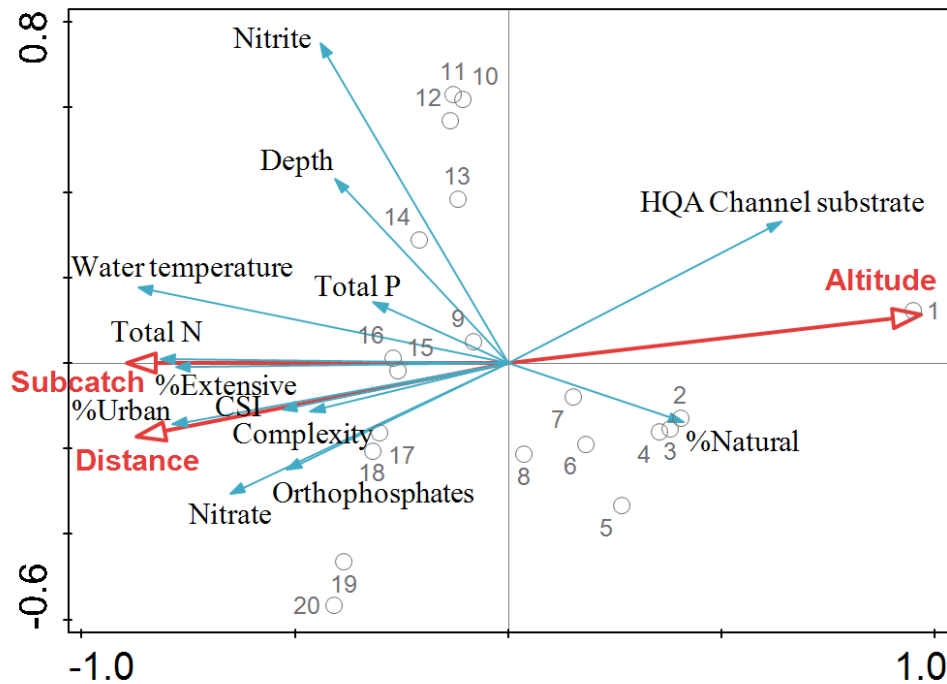


Figure 4.1.12. Redundancy analysis of significant stressors with explanatory variables: distance from source, sub-catchment size, and altitude. Arrow lengths on the ordination show the relative importance of explanatory variables, and their direction relative to each other, stressors, and to the study sites. Abbreviations: %Natural = natural land cover, %Extensive = extensive agriculture, %Urban = urban landuse, CSI = Channel Substrate Index, Complexity = Riparian Quality Index complexity subscore, Total N = total nitrogen, Total P = total phosphorous, Water temp = water temperature. Position of study sites are given as hollow circles.

#### 4.1.5.1 Relationship between water quality, landuse, and hydromorphological modification

Results of Spearman's rank correlation coefficient ( $R$ ) for the statistical relationship between water quality, landuse, and hydromorphological modification on the Bednja River are given in Tab. 4.1.11.

The strongest significant correlation for landuse is found for the share of urban areas and orthophosphate concentrations ( $R = 0.766$ ,  $p < 0.05$ ). The strongest significant correlation for hydromorphological scores is found for HQA channel vegetation subscore and oxygen saturation ( $R = 0.675$ ,  $p < 0.05$ ) (Tab. 4.1.11).



Table 4.1.11. Results of Spearman's correlation coefficient for the relationship between landuse and water quality and hydromorphological quality and modification. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Abbreviations: % Intensive = intensive agriculture, % Extensive = extensive agriculture, HMS = Habitat Modification Score, HQA = Habitat Quality Assessment, RQI = Riparian Quality Index, CSI = Channel Substrate Index, FRI = Flow Regime Index, CVI = Channel Vegetation Index.

	Water temperature	Dissolved O <sub>2</sub>	O <sub>2</sub> saturation	Conductivity	pH	Chemical oxygen demand	Biological oxygen demand	Ammonium	Nitrite	Nitrate	Kjeldahl nitrogen	Organic nitrogen	Total nitrogen	Orthophosphates	Total phosphorous
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<b>Landuse</b>															
% Near natural	-0.282	-0.058	-0.137	<b>-0.604</b>	-0.058	-0.096	-0.123	-0.212	-0.326	<b>-0.752</b>	<b>0.565</b>	<b>0.624</b>	<b>-0.598</b>	-0.392	-0.291
% Intensive	-0.051	0.161	0.040	<b>0.532</b>	-0.071	-0.156	-0.082	0.139	0.075	0.293	<b>-0.544</b>	<b>-0.498</b>	0.118	-0.044	-0.042
% Extensive	0.352	0.136	0.228	-0.037	0.336	-0.065	-0.173	-0.137	0.206	0.162	0.053	-0.018	0.245	0.317	0.111
% Total agriculture	0.160	0.149	0.147	<b>0.580</b>	0.066	-0.044	0.008	0.187	0.278	<b>0.589</b>	<b>-0.641</b>	<b>-0.688</b>	0.394	0.207	0.191
% Urban	<b>0.467</b>	-0.418	-0.183	0.031	0.044	0.215	0.156	0.314	0.366	<b>0.548</b>	0.281	0.112	<b>0.728</b>	<b>0.766</b>	<b>0.449</b>
<b>EN 15843:2010 - 100 m scale</b>															
AVERAGE SCORE	0.283	0.245	0.396	-0.323	0.216	0.032	0.063	-0.227	-0.168	-0.133	0.071	0.151	-0.080	-0.133	-0.013
Morphology	0.322	0.273	0.424	-0.395	0.278	0.005	0.025	-0.268	-0.146	-0.193	0.099	0.193	-0.090	-0.136	0.027
Flow	<b>0.571</b>	0.305	<b>0.486</b>	-0.219	<b>0.474</b>	-0.135	-0.025	-0.085	0.158	0.037	0.231	0.207	0.256	0.085	0.037
Continuity	0.405	0.377	<b>0.477</b>	-0.362	<b>0.463</b>	-0.349	-0.239	0.087	0.261	-0.203	0.087	0.029	0.029	-0.029	0.145
Channel	0.323	0.206	0.386	-0.361	0.271	-0.032	0.040	-0.003	-0.096	-0.070	0.109	0.080	-0.047	-0.016	0.091
Riparian zone	0.364	0.377	<b>0.499</b>	-0.291	0.354	-0.059	-0.068	-0.352	-0.082	-0.164	0.015	0.136	-0.050	-0.096	0.028
Floodplain	0.308	0.207	0.354	-0.409	0.235	-0.002	-0.014	-0.404	-0.122	-0.272	0.156	0.304	-0.092	-0.132	0.053
<b>EN 15843:2010 - 500 m scale</b>															
AVERAGE SCORE	0.288	0.357	<b>0.466</b>	-0.296	0.352	0.110	0.146	-0.236	-0.255	-0.166	0.177	0.245	-0.127	-0.119	0.055
Morphology	0.332	0.385	<b>0.500</b>	-0.310	0.379	0.124	0.148	-0.282	-0.255	-0.170	0.156	0.257	-0.111	-0.120	0.079
Flow	0.418	<b>0.471</b>	<b>0.552</b>	-0.287	<b>0.546</b>	-0.067	0.056	-0.117	-0.022	-0.159	0.315	0.290	0.043	-0.105	-0.051
Continuity	0.405	0.377	<b>0.477</b>	-0.362	<b>0.463</b>	-0.349	-0.239	0.087	0.261	-0.203	0.087	0.029	0.029	-0.029	0.145
Channel	0.277	0.344	<b>0.457</b>	-0.361	0.390	0.035	0.115	-0.051	-0.226	-0.174	0.269	0.235	-0.143	-0.083	0.051
Riparian zone	0.401	<b>0.450</b>	<b>0.561</b>	-0.227	0.421	0.060	0.071	-0.285	-0.210	-0.103	0.122	0.232	-0.022	-0.054	0.070
Floodplain	0.366	0.272	0.417	-0.384	0.369	0.156	0.157	-0.344	-0.171	-0.215	0.235	0.341	-0.061	-0.036	0.246
<b>River Habitat Survey</b>															
<b>HMS</b>	0.156	0.054	0.148	-0.350	0.113	0.239	0.236	-0.162	-0.317	-0.189	0.251	0.263	-0.188	-0.060	0.084
Resectioned Bank Bed	0.101	-0.032	0.078	-0.233	0.048	0.234	0.227	-0.063	-0.187	-0.090	0.114	0.049	-0.171	0.065	0.183
Reinforced Bank Bed	0.365	0.388	0.439	-0.178	0.360	-0.040	-0.052	-0.290	-0.093	-0.019	-0.017	0.119	0.077	-0.129	-0.120
Realigned	0.274	0.155	0.299	-0.250	0.278	0.024	0.032	-0.045	-0.052	-0.045	0.106	0.052	-0.034	0.134	0.258
<b>HQA</b>	-0.121	-0.065	-0.141	0.087	-0.029	-0.028	-0.071	0.181	0.192	0.070	-0.158	-0.193	0.036	0.072	-0.063
HQA flow type	-0.072	-0.069	-0.060	-0.120	-0.027	0.053	-0.060	0.079	-0.012	-0.097	-0.016	-0.101	-0.198	0.155	-0.170
HQA channel substrate	-0.406	0.257	0.075	-0.005	-0.034	-0.202	-0.198	-0.370	-0.121	<b>-0.499</b>	-0.318	-0.122	<b>-0.603</b>	<b>-0.564</b>	-0.259
HQA channel features	-0.023	0.283	0.186	-0.119	0.245	-0.113	-0.110	0.178	-0.091	-0.139	0.213	0.179	-0.061	-0.032	-0.054
HQA bank vegetation structure	0.081	-0.111	-0.125	0.158	-0.029	0.165	0.001	-0.184	0.232	0.201	-0.416	-0.272	0.146	0.096	-0.021
HQA channel vegetation	0.246	<b>0.662</b>	<b>0.675</b>	0.142	<b>0.577</b>	-0.188	-0.099	-0.053	0.024	0.168	0.006	0.043	0.236	0.078	0.063
HQA landuse	-0.191	-0.400	<b>-0.488</b>	0.342	-0.408	0.175	0.119	0.216	0.007	0.208	-0.138	-0.243	0.050	0.139	-0.207
HQA trees	-0.014	-0.332	-0.292	-0.006	-0.081	0.024	0.014	0.352	0.411	0.183	-0.004	-0.171	0.246	0.296	0.199
HQA special features	<b>-0.512</b>	-0.234	-0.440	0.197	-0.394	-0.226	-0.187	0.402	-0.123	0.001	-0.029	-0.177	-0.152	-0.108	-0.417
<b>RQI</b>	0.076	-0.293	-0.235	0.145	-0.086	0.063	-0.023	0.142	0.433	0.289	-0.188	-0.256	0.308	0.327	0.175
Complexity	0.127	-0.246	-0.167	0.051	-0.001	0.121	-0.011	0.045	<b>0.459</b>	0.259	-0.229	-0.275	0.282	0.338	0.257
Naturalness	-0.203	-0.226	-0.369	0.359	-0.181	-0.200	-0.226	0.204	0.213	0.163	-0.145	-0.205	0.118	0.146	-0.053
Continuity	0.110	-0.341	-0.208	-0.162	0.040	0.031	-0.026	0.329	<b>0.462</b>	0.224	0.143	-0.057	0.346	<b>0.474</b>	0.348
CSI	<b>0.579</b>	0.142	0.298	0.196	0.377	0.122	0.060	-0.039	0.080	0.429	0.064	-0.008	<b>0.462</b>	<b>0.539</b>	0.157
FRI	-0.019	-0.048	-0.093	<b>0.529</b>	0.015	0.171	0.106	-0.022	-0.088	0.354	0.054	0.047	0.247	0.389	0.111
CVI	-0.006	-0.348	-0.349	0.050	-0.294	0.342	0.222	-0.050	-0.321	0.043	0.187	0.203	-0.075	0.082	-0.314
Depth (average)	0.442	-0.008	0.242	-0.223	0.258	0.139	0.125	-0.161	<b>0.537</b>	0.194	0.005	0.038	<b>0.476</b>	0.237	0.315
Water velocity (average)	0.193	0.243	0.260	0.023	0.402	-0.055	-0.132	-0.032	0.261	0.066	-0.084	-0.092	0.171	0.333	0.422

Stressors that had significant Spearman's correlations with each other (see Tab. 4.1.11.) were ordinated in a Redundancy Analysis (RDA) (Fig. 4.1.13.). Landuse variables had the greatest number of significant correlations with water quality, so they were used as explanatory variables in the analysis. Landuse (explanatory variables) account for 39% of the total variation. The eigenvalues of the first two axes are 0.167 and 0.115 (Fig. 4.1.13.).

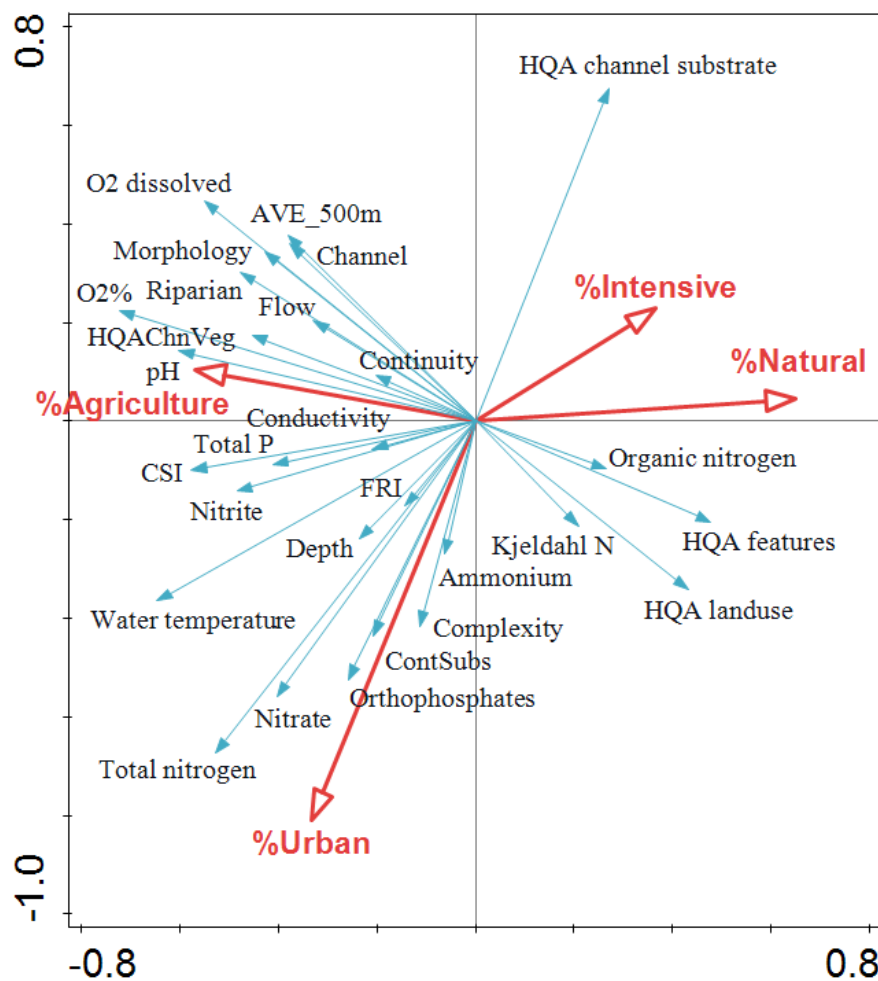


Figure 4.1.13. Redundancy analysis of significant stressors. Arrow lengths on the ordination show the relative importance of explanatory variables (landuse), and their direction relative to each other and to the stressors. Abbreviations: %Agriculture = total agriculture (extensive + intensive), %Intensive = intensive landuse, %Natural = natural land cover, %Urban = urban landuse, HQA features = Habitat Quality Assessment special features subscore, Kjeldahl N = Kjeldahl nitrogen, Complexity = Riparian Quality Index complexity subscore, ContSubs = Riparian Quality Index continuity subscore, FRI = Flow Regime Index, CSI = Channel Substrate Index, Total P = total phosphorous, HQAChnVeg = Habitat Quality Assessment channel vegetation subscore, Flow = EN 15843:2010 flow modification, Riparian = EN 15843:2010 riparian zone modification, Channel = EN 15843:2010 channel modification, Continuity = EN 15843:2010 longitudinal continuity modification, AVE\_500m = EN 15843:2010 average score. EN 15843:2010 100 m scores not shown in Figure due to close correlation with 500 m scores.

#### 4.1.5.2 Relationship between hydromorphological modification, landuse, and microhabitats

Results of Spearman's rank correlation coefficient (R) for the statistical relationship between hydromorphological quality and modification, landuse and share of microhabitats on the Bednja River are given in Tab. 4.1.12.

The highest number of significant correlations is found between hydromorphological modification and share of technolithal. The strongest correlation for EN 15843:2010 scores is found between degradation of the riparian zone and share of technolithal ( $R = 0.700$ ,  $p < 0.05$ ). The strongest correlation for RHS scores is found between HMS Reinforced Bank Bed subscore and share of technolithal ( $R = 0.812$ ,  $p < 0.05$ ) (Tab. 4.1.12.).

Table 4.1.12. Results of Spearman's rank correlation coefficient (R) for the relationship between hydromorphological quality and modification, landuse, and microhabitats. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Abbreviations: HMS = Habitat Modification Score, HQA = Habitat Quality Assessment, RQI = Riparian Quality Index, CSI = Channel Substrate Index, FRI = Flow Regime Index, CVI = Channel Vegetation Index.

	% Near natural	% Intensive agriculture	% Extensive agriculture	% Total agriculture	% Urban	% Macrolithal	% Mesolithal	% Microlithal	% Akal	% Psammal	% Phytal	% Xylal	% Technolithal
	r	r	r	r	r	r	r	r	r	r	r	r	r
<b>EN 15843:2010 - 100 m scale</b>													
AVERAGE SCORE	0.038	-0.160	0.072	-0.060	-0.202	-0.145	-0.427	-0.217	-0.206	-0.372	<b>0.659</b>	<b>-0.553</b>	<b>0.678</b>
Morphology	0.113	-0.227	0.137	-0.136	-0.182	-0.101	-0.380	-0.121	-0.217	-0.423	<b>0.560</b>	<b>-0.530</b>	<b>0.692</b>
Flow	-0.122	0.146	-0.085	0.146	0.025	-0.140	-0.239	-0.189	-0.135	<b>-0.485</b>	<b>0.469</b>	-0.315	<b>0.556</b>
Continuity	-0.043	0.217	-0.188	0.130	-0.117	-0.111	-0.190	-0.045	0.058	-0.385	0.269	-0.195	0.269
Channel	0.005	-0.084	-0.066	0.027	-0.190	-0.367	<b>-0.531</b>	-0.241	-0.051	-0.333	<b>0.648</b>	-0.403	<b>0.582</b>
Riparian zone	0.050	-0.191	0.244	-0.097	-0.130	0.015	-0.352	-0.116	-0.174	<b>-0.455</b>	<b>0.562</b>	<b>-0.572</b>	<b>0.700</b>
Floodplain	0.248	-0.312	0.166	-0.295	-0.124	0.000	-0.281	-0.123	-0.255	-0.410	0.428	-0.442	<b>0.653</b>
<b>EN 15843:2010 - 500 m scale</b>													
AVERAGE SCORE	0.058	-0.245	0.195	-0.079	-0.224	0.043	-0.248	-0.221	-0.173	<b>-0.479</b>	<b>0.591</b>	<b>-0.526</b>	<b>0.587</b>
Morphology	0.055	-0.244	0.220	-0.084	-0.217	0.058	-0.224	-0.137	-0.194	<b>-0.507</b>	<b>0.539</b>	<b>-0.527</b>	<b>0.612</b>
Flow	-0.006	-0.018	0.106	0.031	-0.122	0.228	0.040	-0.128	-0.219	<b>-0.575</b>	0.390	-0.421	<b>0.463</b>
Continuity	-0.043	0.217	-0.188	0.130	-0.117	-0.111	-0.190	-0.045	0.058	-0.385	0.269	-0.195	0.269
Channel	0.098	-0.261	0.157	-0.087	-0.212	-0.029	-0.262	-0.294	-0.067	<b>-0.483</b>	<b>0.588</b>	<b>-0.446</b>	<b>0.497</b>
Riparian zone	0.004	-0.216	0.307	-0.071	-0.131	0.117	-0.205	-0.073	-0.151	<b>-0.550</b>	<b>0.525</b>	<b>-0.565</b>	<b>0.627</b>
Floodplain	0.151	-0.349	0.271	-0.203	-0.114	0.073	-0.197	-0.109	-0.197	<b>-0.466</b>	0.301	-0.362	<b>0.550</b>
<b>River Habitat Survey</b>													
HMS	0.190	-0.402	0.233	-0.233	-0.047	-0.058	-0.405	-0.336	-0.155	-0.303	0.380	-0.320	<b>0.494</b>
Resectioned Bank Bed	0.063	-0.283	0.153	-0.095	-0.059	-0.117	<b>-0.459</b>	-0.425	-0.032	-0.126	0.328	-0.198	0.332
HMS Bank Bed	-0.038	-0.123	0.344	0.000	0.031	0.068	-0.155	0.233	-0.399	<b>-0.495</b>	<b>0.493</b>	<b>-0.580</b>	<b>0.812</b>
HMS Realigned	0.017	-0.319	0.299	-0.069	-0.021	-0.132	-0.423	-0.373	0.139	-0.379	0.393	-0.338	0.392

Table 4.1.12. (Continued). Results of Spearman's rank correlation coefficient (R) for the relationship between hydromorphological quality and modification, landuse, and microhabitats. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Abbreviations: HMS = Habitat Modification Score, HQA = Habitat Quality Assessment, RQI = Riparian Quality Index, CSI = Channel Substrate Index, FRI = Flow Regime Index, CVI = Channel Vegetation Index.

	% Near natural	% Intensive agriculture	% Extensive agriculture	% Total agriculture	% Urban	% Macrolithal	% Mesolithal	% Microlithal	% Akal	% Psammal	% Phytal	% Xylal	% Technolithal
	r	r	r	r	r	r	r	r	r	r	r	r	r
<b>HQA</b>	0.041	0.151	-0.124	0.007	0.125	-0.087	0.285	<b>0.554</b>	0.024	0.228	-0.383	<b>0.474</b>	-0.351
HQA flow type	0.424	-0.314	0.153	<b>-0.467</b>	0.159	-0.121	-0.059	0.315	0.091	0.114	-0.079	0.376	-0.118
HQA channel substrate	0.173	0.290	<b>-0.467</b>	0.042	<b>-0.829</b>	0.390	0.381	0.033	0.115	0.295	-0.166	-0.070	-0.435
HQA channel features	0.287	-0.263	0.285	-0.297	0.072	0.210	<b>0.499</b>	<b>0.521</b>	0.068	-0.145	-0.246	0.274	-0.263
HQA bank vegetation structure	-0.115	0.023	0.202	0.086	0.253	-0.124	0.203	<b>0.588</b>	-0.131	-0.083	-0.274	0.187	0.050
HQA channel vegetation	-0.303	0.310	-0.081	0.289	-0.130	0.058	-0.198	0.125	-0.044	-0.115	<b>0.617</b>	-0.380	0.352
HQA landuse	-0.020	0.097	-0.002	-0.001	0.204	0.090	0.419	0.355	-0.040	0.141	<b>-0.537</b>	0.324	-0.409
HQA trees	-0.040	0.072	-0.091	0.054	0.342	-0.305	0.123	0.240	0.213	0.269	<b>-0.596</b>	<b>0.697</b>	<b>-0.501</b>
HQA special features	0.177	0.162	-0.288	-0.110	0.035	0.000	0.078	0.214	-0.026	0.409	-0.110	0.132	-0.298
<b>RQI</b>	-0.114	0.012	0.127	0.076	0.381	-0.231	0.245	0.380	0.070	0.129	<b>-0.456</b>	<b>0.551</b>	-0.287
Complexity	-0.107	-0.169	0.353	0.042	0.428	-0.289	0.125	0.312	0.135	0.069	-0.425	<b>0.550</b>	-0.219
Naturalness	-0.011	0.242	-0.132	0.060	0.206	0.145	0.443	0.368	0.075	0.213	<b>-0.525</b>	0.353	-0.424
Continuity	0.106	-0.351	0.304	-0.191	<b>0.608</b>	<b>-0.452</b>	-0.097	0.120	0.230	0.156	-0.378	<b>0.724</b>	-0.248
CSI	-0.091	-0.319	<b>0.662</b>	-0.073	<b>0.457</b>	0.029	0.306	0.327	-0.088	<b>-0.738</b>	0.062	-0.091	0.394
FRI	-0.030	-0.113	0.243	-0.082	0.254	0.290	<b>0.550</b>	0.184	0.017	-0.115	-0.176	0.205	-0.224
CVI	0.386	<b>-0.519</b>	<b>0.453</b>	<b>-0.510</b>	0.336	0.231	0.386	0.149	-0.163	-0.303	-0.284	0.096	0.038
Depth (average)	-0.336	0.054	0.086	0.241	0.292	-0.434	<b>-0.498</b>	-0.324	0.299	0.120	-0.168	0.292	-0.040
Water velocity (average)	0.028	-0.172	0.355	-0.103	0.249	0.116	0.257	<b>0.483</b>	-0.036	-0.263	-0.104	0.178	0.060

#### 4.1.5.3 Comparison between assessment systems for hydromorphological modification.

Scores derived through application of both methodologies for assessing hydromorphological modification are highly correlated. The strongest positive correlation is found between the HMS realigned channel subscore and EN 15843:2010 500 m average score ( $R = 0.879$ ,  $p < 0.05$ ).

The strongest negative correlation is found between the RHS Riparian Quality Index naturalness subscore and the EN 15843:2010 100 m average score ( $R = -0.904$ ,  $p < 0.05$ ) (Tab. 4.1.13.).

Table 4.1.13. Results of Spearman's rank correlation coefficient (R) for the relationship between scores derived through River Habitat Survey and EN 15843:2010. Length of assessed reach for River Habitat Survey is 500 m and for EN 15843:2010 is 100 m and 500 m. Higher Habitat Modification Score (HMS) indicates more severely modified; Higher HQA values indicates higher/better habitat quality; Higher RQI score represents higher riparian vegetation quality. For all EN 15843:2010 scores, a higher score represents higher degree of modification. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded.

River Habitat Survey	EN 15843:2010 - 100 m scale							EN 15843:2010 - 500 m scale						
	AVERAGE SCORE	Morphology	Flow	Continuity	Channel	Riparian zone	Floodplain	AVERAGE SCORE	Morphology	Flow	Continuity	Channel	Riparian zone	Floodplain
	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<b>Habitat Modification Score (HMS)</b>	<b>0.847</b>	<b>0.833</b>	0.304	0.087	<b>0.757</b>	<b>0.805</b>	<b>0.789</b>	<b>0.867</b>	<b>0.871</b>	0.367	0.087	<b>0.810</b>	<b>0.798</b>	<b>0.845</b>
Resectioned Bank Bed subscore	<b>0.737</b>	<b>0.691</b>	0.172	0.000	<b>0.687</b>	<b>0.689</b>	<b>0.627</b>	<b>0.766</b>	<b>0.748</b>	0.212	0.000	<b>0.723</b>	<b>0.666</b>	<b>0.720</b>
HMS Reinforced Bank Bed subscore	<b>0.632</b>	<b>0.704</b>	0.371	0.119	0.437	<b>0.731</b>	<b>0.639</b>	<b>0.570</b>	<b>0.646</b>	<b>0.481</b>	0.119	<b>0.446</b>	<b>0.701</b>	<b>0.614</b>
HMS Realigned subscore	<b>0.804</b>	<b>0.769</b>	0.332	0.264	<b>0.772</b>	<b>0.781</b>	<b>0.704</b>	<b>0.879</b>	<b>0.838</b>	0.393	0.264	<b>0.868</b>	<b>0.782</b>	<b>0.781</b>
<b>Habitat Quality Assessment (HQA)</b>	<b>-0.796</b>	<b>-0.730</b>	-0.413	-0.217	<b>-0.664</b>	<b>-0.757</b>	<b>-0.718</b>	<b>-0.822</b>	<b>-0.793</b>	<b>-0.456</b>	-0.217	<b>-0.759</b>	<b>-0.767</b>	<b>-0.733</b>
HQA flow type	-0.280	-0.222	<b>-0.468</b>	-0.346	-0.278	-0.203	-0.175	-0.334	-0.313	<b>-0.447</b>	-0.346	-0.333	-0.262	-0.251
HQA channel substrate	-0.102	-0.100	-0.189	0.060	-0.078	-0.125	-0.065	-0.051	-0.058	-0.092	0.060	-0.054	-0.144	-0.068
HQA channel features	<b>-0.505</b>	-0.389	-0.327	-0.135	<b>-0.504</b>	-0.402	-0.341	-0.431	-0.371	-0.136	-0.135	-0.390	-0.287	-0.301
HQA bank vegetation structure	<b>-0.480</b>	-0.403	-0.351	-0.248	<b>-0.502</b>	-0.361	-0.393	<b>-0.463</b>	-0.419	-0.343	-0.248	<b>-0.528</b>	-0.382	-0.362
HQA channel vegetation	0.320	0.283	0.368	0.175	0.314	0.362	0.200	0.357	0.344	0.339	0.175	0.319	0.409	0.237
HQA landuse	<b>-0.779</b>	<b>-0.734</b>	<b>-0.455</b>	-0.361	<b>-0.780</b>	<b>-0.735</b>	<b>-0.677</b>	<b>-0.782</b>	<b>-0.759</b>	-0.411	-0.361	<b>-0.769</b>	<b>-0.688</b>	<b>-0.678</b>
HQA trees	<b>-0.828</b>	<b>-0.785</b>	-0.305	-0.116	<b>-0.612</b>	<b>-0.846</b>	<b>-0.772</b>	<b>-0.843</b>	<b>-0.843</b>	-0.425	-0.116	<b>-0.717</b>	<b>-0.855</b>	<b>-0.745</b>
HQA special features	<b>-0.538</b>	<b>-0.547</b>	-0.340	-0.225	-0.419	<b>-0.617</b>	<b>-0.544</b>	<b>-0.694</b>	<b>-0.726</b>	-0.422	-0.225	<b>-0.568</b>	<b>-0.720</b>	<b>-0.763</b>
<b>Riparian Quality Index (RQI)</b>	<b>-0.771</b>	<b>-0.710</b>	-0.376	-0.231	<b>-0.676</b>	<b>-0.706</b>	<b>-0.682</b>	<b>-0.756</b>	<b>-0.742</b>	-0.428	-0.231	<b>-0.723</b>	<b>-0.703</b>	<b>-0.637</b>
Complexity subscore	<b>-0.631</b>	<b>-0.565</b>	-0.425	-0.289	<b>-0.571</b>	<b>-0.533</b>	<b>-0.555</b>	<b>-0.582</b>	<b>-0.561</b>	-0.437	-0.289	<b>-0.581</b>	<b>-0.530</b>	<b>-0.454</b>
Naturalness subscore	<b>-0.904</b>	<b>-0.865</b>	-0.365	-0.188	<b>-0.792</b>	<b>-0.846</b>	<b>-0.793</b>	<b>-0.876</b>	<b>-0.889</b>	-0.405	-0.188	<b>-0.796</b>	<b>-0.848</b>	<b>-0.815</b>
Continuity subscore	<b>-0.578</b>	<b>-0.523</b>	-0.342	-0.241	-0.413	<b>-0.558</b>	<b>-0.510</b>	<b>-0.580</b>	<b>-0.589</b>	-0.429	-0.241	<b>-0.477</b>	<b>-0.592</b>	<b>-0.473</b>
Channel Substrate Index	0.070	0.150	0.134	-0.087	-0.060	0.218	0.218	0.140	0.195	0.274	-0.087	0.093	0.294	0.264
Flow Regime Index	<b>-0.466</b>	<b>-0.467</b>	-0.329	-0.435	<b>-0.521</b>	-0.366	-0.334	-0.279	-0.298	-0.219	-0.435	-0.303	-0.238	-0.227
Channel Vegetation Index	-0.165	-0.089	-0.352	<b>-0.463</b>	-0.360	-0.073	0.015	-0.130	-0.075	-0.144	<b>-0.463</b>	-0.190	-0.010	0.014

Individual scores of EN 15843:2010 are highly correlated with RHS scores and achieve several statistically significant Spearman's correlations coefficients (Tab. 4.1.14. and Tab. 4.1.15.).

The strongest statistically significant positive correlation is found for EN 15843:2010 Feature 1a – Channel planform assessed for a 500 m long reach, and the HMS Realigned subscore ( $R = 0.997$ ,  $p < 0.05$ ). The strongest negative correlation is found for HQA subscore for trees and EN 15843:2010 Feature 8 - modification of the vegetation type/structure on banks and adjacent

land ( $R = -0.876$ ,  $p < 0.05$ ) assessed for a 500 m long reach. Stronger correlations are found between EN 15843:2010 individual scores assessed when assessed on a 500 m long reach and RHS scores than EN 15843:2010 individual scores assessed on a 100 m long reach and RHS scores (Tab. 4.1.14. and 4.1.15.).

Table 4.1.14. Results of Spearman's rank correlation coefficient (R) for the relationship between EN 15843:2010 **100 m** individual scores and River Habitat Survey scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. EN 15843:2010 hydromorphological features assessed: 1a Planform, 1b Channel section (long-section and cross-section), 2a Extent of artificial material, 2b "Natural" substrate mix or character altered, 3a Aquatic vegetation management, 3b Extent of woody debris if expected, 4 Erosion/deposition character, 5a Impacts of artificial in-channel structures within the reach, 5b Effects of catchment-wide modifications to natural flow character, 6 Longitudinal continuity as affected by artificial structures, 7 Bank structure and modifications, 8 Vegetation type/structure on banks and adjacent land, 9 Adjacent landuse and associated features, 10a Degree of lateral connectivity of river and floodplain, 10b Degree of lateral movement of river channel.

River Habitat Survey	EN 15843:2010 - 100 m scale (individual features)													
	1a	1b	2a	2b	3a	3b	4	5b	6	7	8	9	10a	10b
	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<b>Habitat Modification Score (HMS)</b>	<b>0.767</b>	<b>0.792</b>	0.401	<b>0.464</b>	0.347	0.411	<b>0.784</b>	0.304	0.087	<b>0.464</b>	<b>0.776</b>	0.440	<b>0.831</b>	<b>0.620</b>
Resectioned Bank Bed subscore	<b>0.777</b>	<b>0.821</b>	0.197	0.332	0.410	0.336	<b>0.709</b>	0.172	0.000	0.272	<b>0.737</b>	0.305	<b>0.713</b>	0.407
HMS Reinforced Bank Bed subscore	0.327	0.332	<b>0.713</b>	0.235	0.204	<b>0.515</b>	0.269	0.371	0.119	<b>0.995</b>	0.401	<b>0.459</b>	<b>0.514</b>	<b>0.871</b>
HMS Realigned subscore	<b>0.880</b>	<b>0.810</b>	0.332	0.414	0.264	0.393	<b>0.765</b>	0.332	0.264	0.324	<b>0.830</b>	0.308	<b>0.815</b>	0.409
<b>Habitat Quality Assessment (HQA)</b>	<b>-0.669</b>	<b>-0.654</b>	-0.243	<b>-0.473</b>	<b>-0.492</b>	-0.370	<b>-0.706</b>	-0.413	-0.217	-0.307	<b>-0.816</b>	<b>-0.493</b>	<b>-0.663</b>	<b>-0.468</b>
HQA flow type	-0.144	-0.089	-0.025	-0.407	-0.271	-0.034	-0.239	<b>-0.468</b>	-0.346	-0.153	-0.226	-0.152	-0.062	-0.276
HQA channel substrate	0.066	-0.116	-0.315	0.113	-0.180	-0.172	-0.006	-0.189	0.060	-0.368	-0.038	0.139	-0.282	-0.250
HQA channel features	<b>-0.458</b>	<b>-0.560</b>	-0.038	-0.367	<b>-0.509</b>	-0.210	<b>-0.580</b>	-0.327	-0.135	-0.007	<b>-0.629</b>	-0.285	-0.264	-0.074
HQA bank vegetation structure	-0.429	<b>-0.539</b>	-0.026	-0.399	<b>-0.464</b>	-0.239	<b>-0.474</b>	-0.351	-0.248	0.067	<b>-0.477</b>	-0.335	-0.355	-0.107
HQA channel vegetation	0.161	0.244	<b>0.502</b>	0.238	<b>0.481</b>	<b>0.591</b>	0.169	0.368	0.175	0.324	0.300	0.223	0.100	0.191
HQA landuse	<b>-0.640</b>	<b>-0.773</b>	<b>-0.455</b>	<b>-0.529</b>	-0.361	<b>-0.538</b>	<b>-0.693</b>	<b>-0.455</b>	-0.361	-0.382	<b>-0.790</b>	<b>-0.484</b>	<b>-0.591</b>	<b>-0.474</b>
HQA trees	<b>-0.582</b>	<b>-0.556</b>	-0.439	<b>-0.493</b>	<b>-0.522</b>	<b>-0.604</b>	<b>-0.657</b>	-0.305	-0.116	<b>-0.511</b>	<b>-0.787</b>	<b>-0.651</b>	<b>-0.605</b>	<b>-0.606</b>
HQA special features	<b>-0.515</b>	-0.390	-0.164	-0.189	-0.150	-0.102	-0.384	-0.340	-0.225	-0.337	<b>-0.599</b>	-0.298	<b>-0.479</b>	<b>-0.458</b>
<b>Riparian Quality Index (RQI)</b>	<b>-0.612</b>	<b>-0.658</b>	-0.279	<b>-0.573</b>	<b>-0.491</b>	<b>-0.467</b>	<b>-0.728</b>	-0.376	-0.231	-0.273	<b>-0.735</b>	<b>-0.566</b>	<b>-0.552</b>	-0.408
Complexity subscore	<b>-0.450</b>	<b>-0.507</b>	-0.231	<b>-0.573</b>	<b>-0.492</b>	-0.412	<b>-0.611</b>	-0.425	-0.289	-0.153	<b>-0.573</b>	<b>-0.570</b>	-0.379	-0.274
Naturalness subscore	<b>-0.752</b>	<b>-0.774</b>	-0.390	<b>-0.574</b>	<b>-0.493</b>	<b>-0.541</b>	<b>-0.786</b>	-0.365	-0.188	<b>-0.458</b>	<b>-0.851</b>	<b>-0.628</b>	<b>-0.638</b>	<b>-0.612</b>
Continuity subscore	-0.360	-0.274	-0.164	<b>-0.530</b>	<b>-0.452</b>	-0.368	<b>-0.496</b>	-0.342	-0.241	-0.255	<b>-0.542</b>	<b>-0.604</b>	-0.207	-0.352
Channel Substrate Index	0.031	-0.114	0.305	-0.192	0.029	0.076	-0.169	0.134	-0.087	0.399	-0.029	0.081	0.324	0.246
Flow Regime Index	-0.364	<b>-0.487</b>	-0.183	-0.411	0.029	-0.173	-0.416	-0.329	-0.435	-0.368	-0.377	-0.287	-0.151	-0.387
Channel Vegetation Index	-0.140	-0.305	-0.085	-0.391	-0.173	-0.226	-0.202	-0.352	<b>-0.463</b>	0.093	-0.268	-0.070	0.143	0.045

Table 4.1.15. Results of Spearman's rank correlation coefficient (R) for the relationship between EN 15843:2010 **500 m** individual scores and River Habitat Survey scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. EN 15843:2010 hydromorphological features assessed: 1a Planform, 1b Channel section (long-section and cross-section), 2a Extent of artificial material, 2b "Natural" substrate mix or character altered, 3a Aquatic vegetation management, 3b Extent of woody debris if expected, 4 Erosion/deposition character, 5b Effects of catchment-wide modifications to natural flow character, 6 Longitudinal continuity as affected by artificial structures, 7 Bank structure and modifications, 8 Vegetation type/structure on banks and adjacent land, 9 Adjacent landuse and associated features, 10a Degree of lateral connectivity of river and floodplain, 10b Degree of lateral movement of river channel.

River Habitat Survey	EN 15843:2010 - 500 m scale (individual features)															
	1a	1b	2a	2b	3a	3b	4	5a	5b	6	7	8	9	10a	10b	
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	
<b>Habitat Modification Score (HMS)</b>	<b>0.862</b>	<b>0.801</b>	0.366	<b>0.580</b>	0.356	<b>0.487</b>	<b>0.761</b>	0.145	0.304	0.087	0.409	<b>0.810</b>	<b>0.568</b>	<b>0.830</b>	<b>0.578</b>	
Resectioned Bank Bed subscore	<b>0.874</b>	<b>0.848</b>	0.148	0.411	0.410	0.382	<b>0.720</b>	0.059	0.172	0.000	0.205	<b>0.737</b>	<b>0.469</b>	<b>0.761</b>	0.362	
HMS Reinforced Bank Bed subscore	0.321	0.204	<b>0.692</b>	0.399	0.225	<b>0.595</b>	0.230	0.424	0.371	0.119	<b>0.932</b>	<b>0.447</b>	<b>0.617</b>	0.378	<b>0.767</b>	
HMS Realigned subscore	<b>0.997</b>	<b>0.895</b>	0.307	<b>0.485</b>	0.263	<b>0.452</b>	<b>0.765</b>	0.264	0.332	0.264	0.272	<b>0.874</b>	<b>0.462</b>	<b>0.804</b>	0.347	
<b>Habitat Quality Assessment Score (HQA)</b>	<b>-0.820</b>	<b>-0.777</b>	-0.238	<b>-0.581</b>	<b>-0.494</b>	-0.437	<b>-0.768</b>	-0.174	-0.413	-0.217	-0.267	<b>-0.861</b>	<b>-0.553</b>	<b>-0.709</b>	-0.441	
HQA flow type	-0.209	-0.251	-0.113	<b>-0.457</b>	-0.278	-0.062	-0.356	-0.121	<b>-0.468</b>	-0.346	-0.195	-0.283	-0.271	-0.124	-0.331	
HQA channel substrate	-0.084	-0.040	-0.333	0.095	-0.199	-0.092	0.052	0.060	-0.189	0.060	-0.414	-0.002	0.251	-0.265	-0.308	
HQA channel features	<b>-0.549</b>	<b>-0.501</b>	0.217	-0.285	<b>-0.508</b>	-0.075	<b>-0.580</b>	0.210	-0.327	-0.135	0.163	<b>-0.484</b>	-0.278	-0.361	0.039	
HQA bank vegetation structure	<b>-0.453</b>	<b>-0.676</b>	0.007	-0.441	<b>-0.458</b>	-0.263	<b>-0.558</b>	-0.077	-0.351	-0.248	0.091	<b>-0.486</b>	-0.276	-0.417	-0.099	
HQA channel vegetation	0.185	0.248	0.440	0.272	<b>0.483</b>	<b>0.545</b>	0.136	0.248	0.368	0.175	0.316	0.337	0.369	0.004	0.150	
HQA landuse	<b>-0.757</b>	<b>-0.767</b>	-0.317	<b>-0.526</b>	-0.360	<b>-0.507</b>	<b>-0.623</b>	-0.180	<b>-0.455</b>	-0.361	-0.293	<b>-0.738</b>	<b>-0.578</b>	<b>-0.529</b>	-0.383	
HQA trees	<b>-0.716</b>	<b>-0.618</b>	-0.431	<b>-0.634</b>	<b>-0.520</b>	<b>-0.707</b>	<b>-0.669</b>	-0.305	-0.305	-0.116	<b>-0.460</b>	<b>-0.876</b>	<b>-0.773</b>	<b>-0.563</b>	<b>-0.540</b>	
HQA special features	<b>-0.655</b>	<b>-0.542</b>	-0.368	-0.319	-0.160	-0.203	<b>-0.465</b>	-0.225	-0.340	-0.225	-0.441	<b>-0.718</b>	<b>-0.548</b>	<b>-0.522</b>	<b>-0.571</b>	
<b>Riparian Quality Index score (RQI)</b>	<b>-0.672</b>	<b>-0.701</b>	-0.209	<b>-0.666</b>	<b>-0.488</b>	<b>-0.542</b>	<b>-0.739</b>	-0.145	-0.376	-0.231	-0.195	<b>-0.784</b>	<b>-0.611</b>	<b>-0.556</b>	-0.333	
Complexity subscore	<b>-0.448</b>	<b>-0.551</b>	-0.140	<b>-0.633</b>	<b>-0.488</b>	<b>-0.452</b>	<b>-0.644</b>	-0.145	-0.425	-0.289	-0.072	<b>-0.617</b>	<b>-0.530</b>	-0.384	-0.199	
Naturalness subscore	<b>-0.795</b>	<b>-0.762</b>	-0.351	<b>-0.658</b>	<b>-0.492</b>	<b>-0.593</b>	<b>-0.763</b>	-0.159	-0.365	-0.188	-0.410	<b>-0.874</b>	<b>-0.724</b>	<b>-0.619</b>	<b>-0.576</b>	
Continuity subscore	-0.373	-0.338	-0.179	<b>-0.636</b>	<b>-0.445</b>	<b>-0.470</b>	<b>-0.566</b>	-0.211	-0.342	-0.241	-0.214	<b>-0.661</b>	<b>-0.714</b>	-0.213	-0.309	
Channel Substrate Index	0.060	-0.032	<b>0.499</b>	0.010	0.052	0.199	-0.112	0.261	0.134	-0.087	<b>0.503</b>	0.105	0.087	0.304	0.317	
Flow Regime Index	-0.225	-0.242	0.055	-0.297	0.032	-0.092	-0.332	-0.044	-0.329	-0.435	-0.190	-0.239	-0.289	-0.081	-0.217	
Channel Vegetation Index	-0.110	-0.238	0.156	-0.189	-0.160	-0.042	-0.146	0.000	-0.352	<b>-0.463</b>	0.210	-0.135	-0.165	0.190	0.150	

#### 4.2 Composition and structure of benthic macroinvertebrates

A total of 20 study sites distributed longitudinally along the 106 km course of the Bednja River from source to mouth were sampled. Total sampled surface area encompassed 2 km of the Bednja River and covering 25 m<sup>2</sup> of the riverbed (100 m sampling reach x 20 study sites).

Based on the 193,638 individuals collected in summer 2015 and identified, the following composition and structure of benthic macroinvertebrates in the Bednja River is described:

Insects are the most numerous Class represented with a total share of 56.32%, followed by Malacostraca (22.61%) and Clitellata (19.10%) (Fig. 4.2.1.). The families Chironomidae and Gammaridae, subclass Oligochaeta and order Ephemeroptera alone make up 85.10% of all individuals collected. Chironomidae larvae are the most abundant taxa group, making up 33.31% of all sampled individuals (Tab. 4.2.1.).

The complete taxa list is given in Annex I.

Abundance (m<sup>2</sup>) of dominant taxonomic groups at study sites is shown in Fig. 4.2.2.

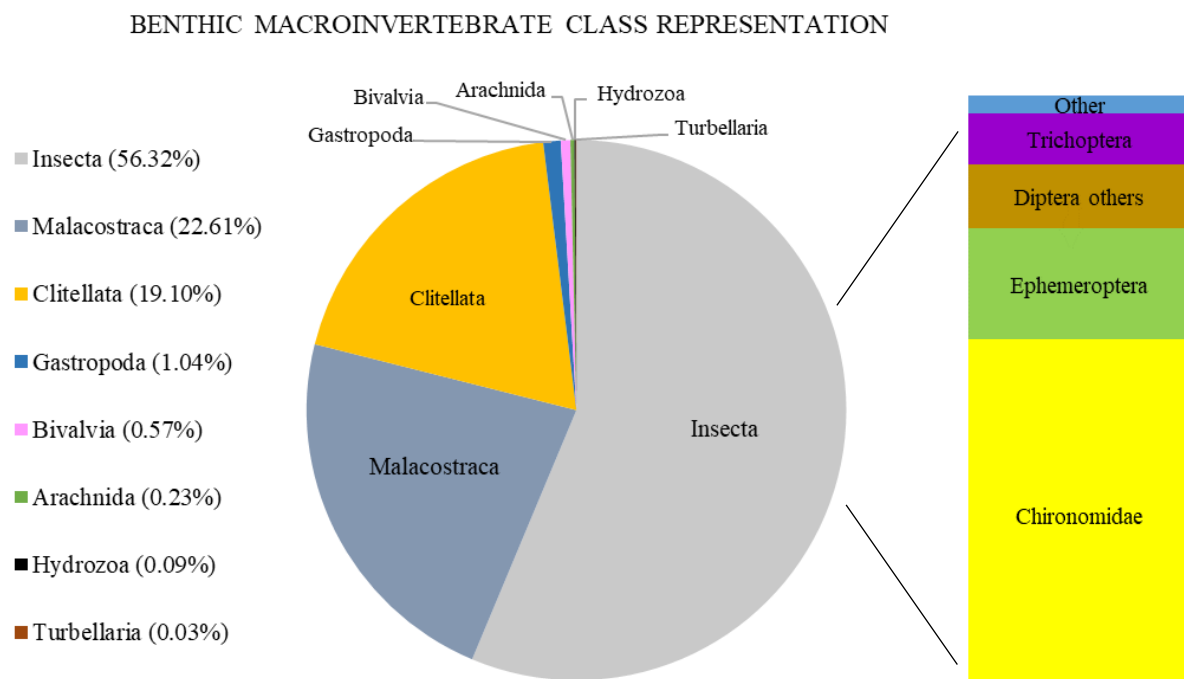


Figure 4.2.1. Representation of benthic macroinvertebrate classes in the Bednja River.



Table 4.2.1. Overview of identified benthic macroinvertebrates from the Bednja River with dominant taxa per taxonomic group, total taxa group share, number of identified individuals and distribution (presence at number of study sites).

<b>Taxonomic group</b>	Total share (%)	Total no. of identified individuals	No. of taxa	No. of families	Distribution (No. of sites present /20)
<b>1 Diptera (Chironomidae)</b>	<b>33.31</b>	<b>64,503</b>	<b>17</b>	<b>1</b>	<b>20/20</b>
Dominant species / taxa					
Chironomini Gen. sp.	15.12	29,277			20/20
Orthocladiinae Gen. sp.	9.91	19,191			20/20
<b>2 Crustacea</b>	<b>22.61</b>	<b>43,780</b>	<b>3</b>	<b>2</b>	<b>20/20</b>
<i>Gammarus fossarum</i>	18.90	36,588			20/20
<i>Gammarus roeselii</i>	3.54	6,854			19/20
<b>3 Oligochaeta</b>	<b>18.92</b>	<b>36,629</b>	<b>25</b>	<b>6</b>	<b>20/20</b>
<i>Limnodrilus hoffmeisteri</i>	10.84	20,998			20/20
<i>Potamothrix hammoniensis</i>	1.78	3,443			14/20
<b>4 Ephemeroptera</b>	<b>10.43</b>	<b>20,188</b>	<b>23</b>	<b>7</b>	<b>20/20</b>
<i>Baetis fuscatus</i>	5.31	10,275			18/20
<i>Baetis buceratus</i>	1.68	3,250			15/20
<b>5 Diptera (exl. Chironomidae)</b>	<b>6.09</b>	<b>11,792</b>	<b>26</b>	<b>13</b>	<b>20/20</b>
Simuliidae Gen. sp.	5.09	9,848			18/20
Empididae Gen. sp.	0.38	728			16/20
<b>6 Trichoptera</b>	<b>4.85</b>	<b>9,398</b>	<b>47</b>	<b>13</b>	<b>20/20</b>
<i>Hydropsyche angustipennis angustipennis</i>	1.24	2,403			9/20
<i>Hydropsyche incognita/pellucidula</i>	0.93	1,800			9/20
<i>Psychomyia pusilla</i>	0.91	1,767			14/20
<b>7 Coleoptera</b>	<b>1.25</b>	<b>2424</b>	<b>45</b>	<b>7</b>	<b>20/20</b>
<i>Oulimnius tuberculatus</i> Ad.+Lv.	0.51	990			17/20
<i>Esolus pygmaeus</i> Ad.	0.19	373			13/20
<b>8 Gastropoda</b>	<b>1.04</b>	<b>2020</b>	<b>15</b>	<b>9</b>	<b>17/20</b>
<i>Holandriana holandrii</i>	0.56	1084			13/20
<i>Bythinella opaca opaca</i>	0.25	487			1/20
<b>9 Bivalvia</b>	<b>0.57</b>	<b>1108</b>	<b>5</b>	<b>3</b>	<b>18/20</b>
<i>Pisidium</i> sp.	0.54	1055			15/20
<i>Sphaerium rivicola</i>	0.02	30			6/20
<b>10 Hydrachnidia</b>	<b>0.23</b>	<b>452</b>	<b>20</b>	<b>8</b>	<b>18/20</b>
<i>Hygrobates fluviatilis</i>	0.06	118			14/20
<i>Lebertia</i> sp.	0.05	97			17/20
<b>11 Hirudinea</b>	<b>0.19</b>	<b>359</b>	<b>10</b>	<b>4</b>	<b>17/20</b>
Erpobdellidae Gen. sp.	0.09	166			10/20
<i>Helobdella stagnalis</i>	0.04	86			8/20
<b>12 Heteroptera</b>	<b>0.18</b>	<b>342</b>	<b>9</b>	<b>7</b>	<b>20/20</b>
<i>Aphelocheirus</i> sp.	0.12	224			13/20
<b>13 Odonata</b>	<b>0.18</b>	<b>341</b>	<b>7</b>	<b>9</b>	<b>17/20</b>
<i>Onychogomphus forcipatus forcipatus</i>	0.06	118			15/20
Coenagrionidae Gen. sp. juv.	0.05	97			1/20
<b>14 Coelenterata</b>	<b>0.09</b>	<b>173</b>	<b>1</b>	<b>1</b>	<b>9/20</b>
<b>15 Turbellaria</b>	<b>0.03</b>	<b>64</b>	<b>3</b>	<b>3</b>	<b>5/20</b>
<i>Polycelis</i> sp.	0.02	46			2/20
<b>16 Plecoptera</b>	<b>0.03</b>	<b>57</b>	<b>8</b>	<b>4</b>	<b>12/20</b>
<i>Leuctra</i> sp.	0.02	37			9/20
<b>17 Megaloptera</b>	<b>0.004</b>	<b>8</b>	<b>3</b>	<b>1</b>	<b>4/20</b>
<i>Sialis fuliginosa</i>	0.003	5			2/20

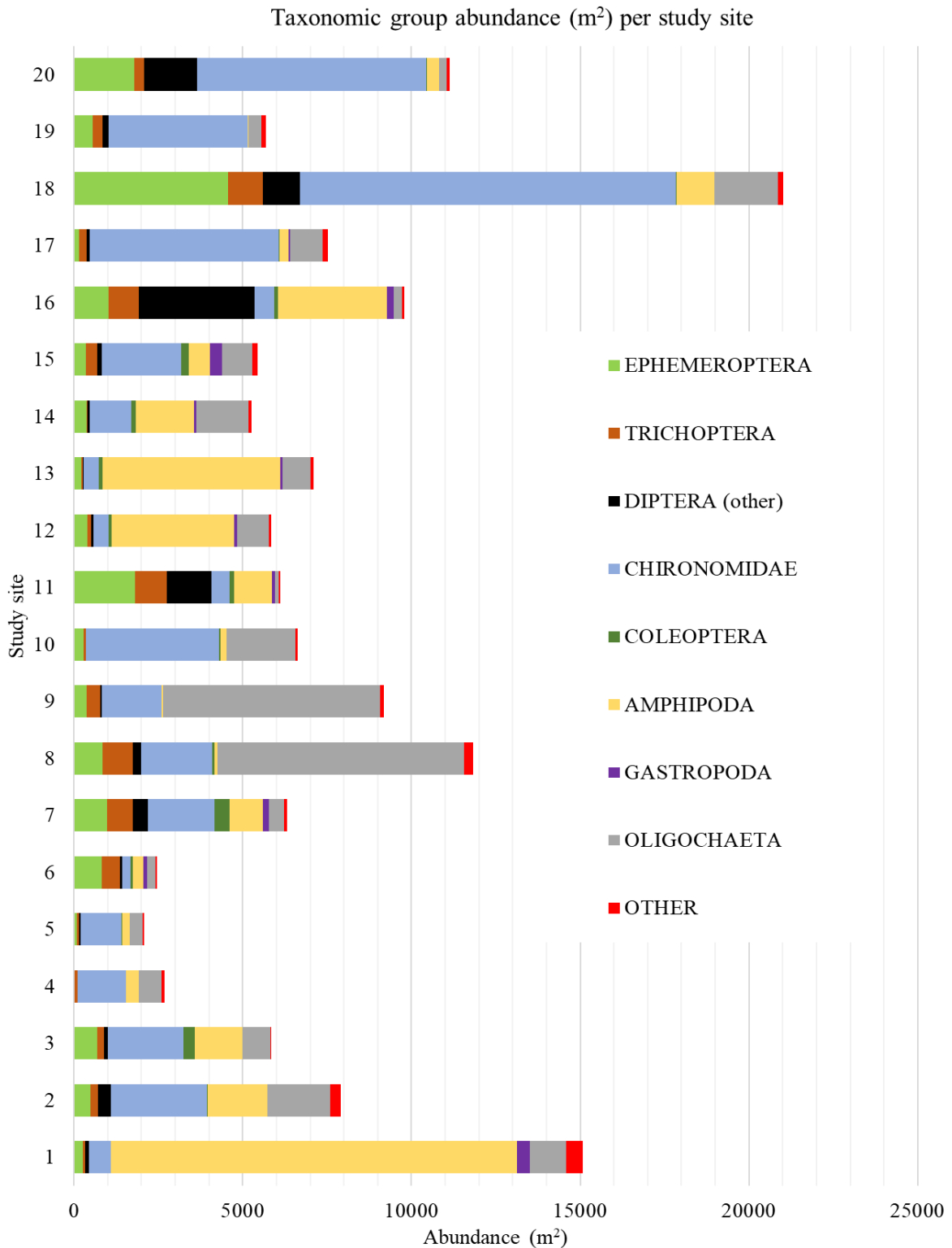


Figure 4.2.2. Abundance (m<sup>2</sup>) of dominant taxonomic groups at study sites. Category “other” includes Plecoptera, Odonata, Megaloptera, Hemiptera, Isopoda, Bivalvia, Hirudinea, Hydrachnidia, Turbellaria and Hydrozoa.

#### 4.2.1 Comparison of composition and structure of benthic macroinvertebrates between study sites

Macroinvertebrate abundance ranges from 2,078 ind/m<sup>2</sup> (study site 5) to 21,015 ind/m<sup>2</sup> (study site 18) with an average of 7,749 ind/m<sup>2</sup> per study site (Tab. 4.2.2.).

Table 4.2.2. Benthic macroinvertebrate abundance per m<sup>2</sup> at study sites.

Study site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Abundance (ind/m <sup>2</sup> )	15,090	7,900	5,853	2,696	<b>2,078</b>	2,458	6,313	11,817	9,191	6,632	6,115	5,858	7,095	5,256	5,436	9,805	7,527	<b>21,015</b>	5,697	11,142

Study site 1 located only 400 m from the rheohelocrene type source of the Bednja river possesses the lowest values for all diversity indices. Study site 6 has the highest values of Simpson Index (0.908) and Shannon-Wiener Index (2.909) while study site 7 has the highest values for Margalef Index (10.285) and Pielou's evenness (0.625). The greatest number of species is found at study site 18 (97 total species) (Tab. 4.2.3.).

Table 4.2.3. Values of benthic macroinvertebrate diversity metrics at study sites. Lowest value are bolded, highest value are given in bold and shaded.

Study site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Total species (S)	<b>53</b>	64	67	59	55	67	91	64	56	57	66	55	62	66	73	67	78	<b>97</b>	60	78
Simpson-Index	<b>0.361</b>	0.866	0.839	0.718	0.703	<b>0.908</b>	0.886	0.788	0.761	0.744	0.877	0.772	0.564	0.858	0.805	0.763	0.782	0.851	0.8	0.837
Shannon-Wiener-Index	<b>1.001</b>	2.376	2.498	1.93	1.995	<b>2.909</b>	2.817	2.308	2.048	1.872	2.464	2.029	1.528	2.406	2.46	2.018	2.082	2.411	2.14	2.232
Margalef Index	<b>5.404</b>	7.02	7.608	7.342	7.069	8.454	<b>10.285</b>	6.718	6.027	6.364	7.455	6.224	6.879	7.587	8.371	7.181	8.626	9.645	6.823	8.263
Evenness/ Pielou's evenness: $J' = \frac{H'}{H'_{max}}$	<b>0.252</b>	0.571	0.594	0.473	0.498	0.692	<b>0.625</b>	0.555	0.509	0.463	0.588	0.506	0.370	0.574	0.573	0.480	0.478	0.527	0.523	0.512

Variation in benthic macroinvertebrate diversity indices between sites is shown graphically on Fig. 4.2.3.

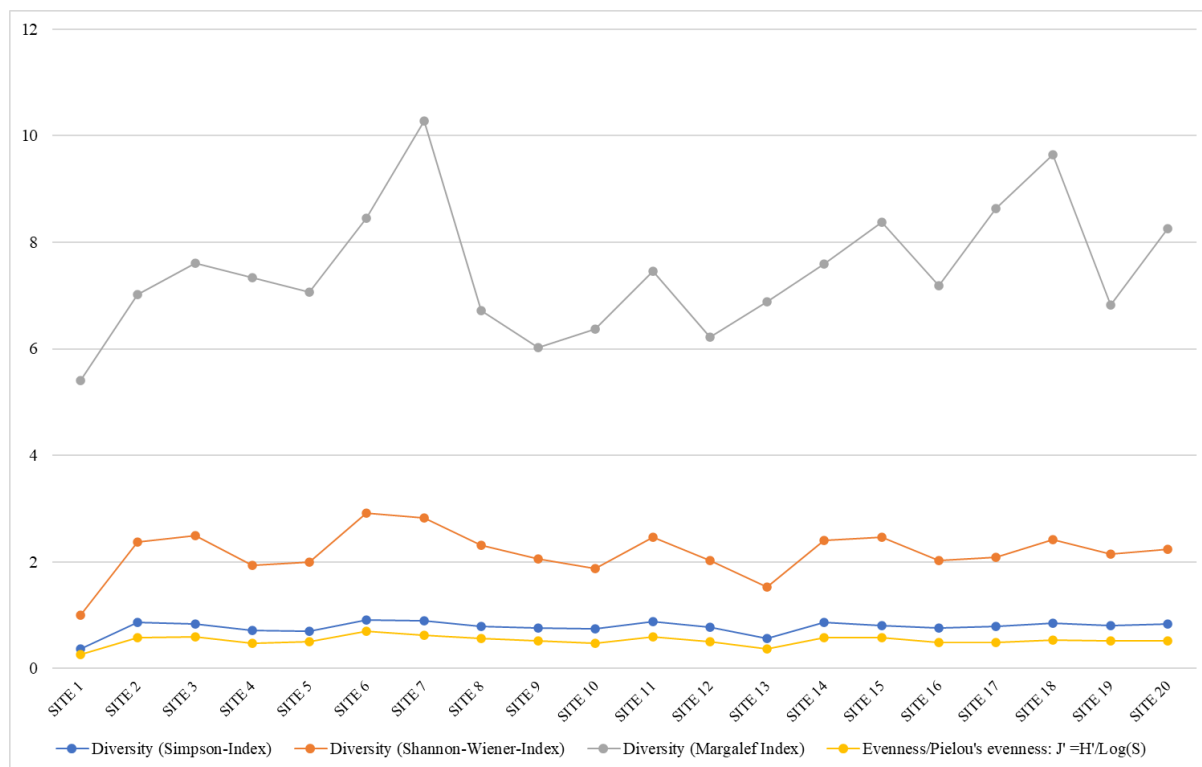


Figure 4.2.3. Values for diversity indices: Simpson Index, Shannon-Wiener Index, Margalef Index, Fisher's Alpha ( $\alpha$ ), Evenness/Pielou's evenness ( $J'$ ) at study sites.

Ecological status at study sites, given as ecological quality ratio (EQR), demonstrates a range from high (study sites 1, 11, 13 and 16) to poor (study sites 8 and 9). Five study sites achieve good status (study sites 6, 7, 12, 14 and 20) and the most study sites fall under moderate status (study sites 2, 3, 4, 5, 10, 15, 17, 18 and 19) (Tab. 4.2.4.).

Table 4.2.4. Ecological status at the study sites given as Ecological Quality Ratio (EQR) calculated through EQR of the General Degradation module and EQR of the Saprobity module.

Study site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EQR gen. deg. (transform)	0.84	0.93	0.99	0.89	0.94	1.02	1.09	0.89	0.77	0.86	1.08	1.05	1.03	1.11	1.11	1.22	1.12	1.26	0.95	1.16
EQR saprobity (transform)	0.86	0.45	0.57	0.49	0.47	0.67	0.70	0.28	0.20	0.45	0.83	0.69	0.84	0.69	0.57	0.82	0.52	0.55	0.45	0.62
EQR transform	0.84	0.45	0.57	0.49	0.47	0.67	0.70	0.28	0.20	0.45	0.83	0.69	0.84	0.69	0.57	0.82	0.52	0.55	0.45	0.62

High	0.80 – 1.00
Good	0.60 – 0.79
Moderate	0.40 – 0.59
Poor	0.20 – 0.39
Bad	< 0.20

Pursuant to Bray-Curtis similarity index, highest resemblance is displayed between study sites 13 and 14 (74.3%) followed by 8 and 9 (66.41%), 6 and 7 (63.9 2%), 19 and 20 (61.23%), 2 and 3 (55.27%) and 4 and 5 (52.46%) (Fig. 4.2.4. and 4.2.5.).

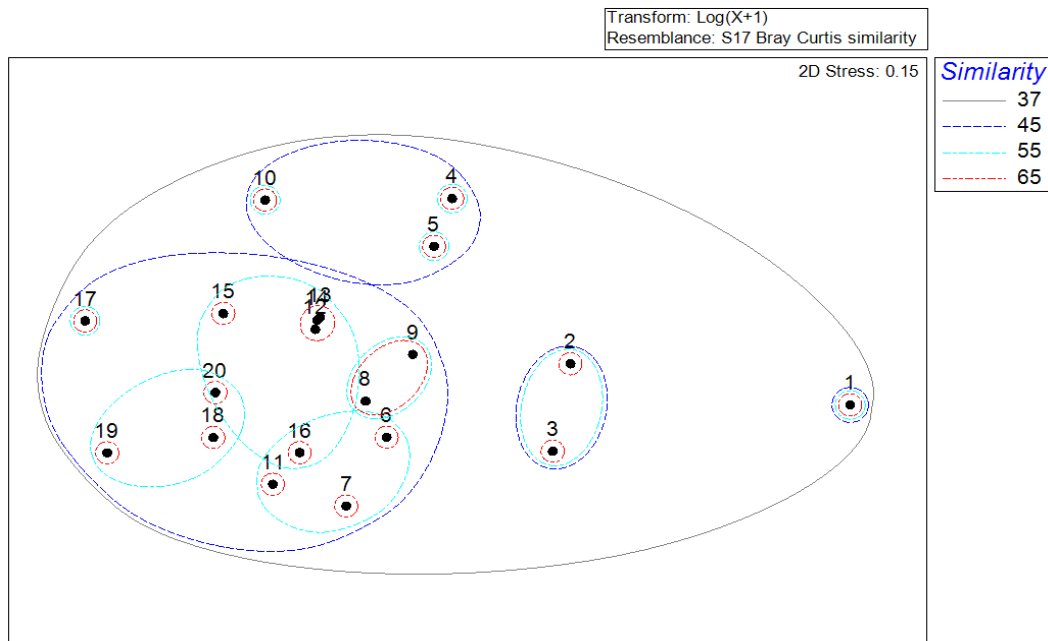


Figure 4.2.4. Non-metric Multi-Dimensional Scaling (NMDS) ordination of sampling sites based on Bray-Curtis similarity of benthic macroinvertebrate samples.

There is no clear distinction based on typology, respectively between communities inhabiting the river course belonging to river type HR-R\_1 – small mid-altitude running waters (study sites 1 – 9) and type HR-R\_4A – medium lowland running waters (study sites 10 – 20) (Fig. 4.2.5.).

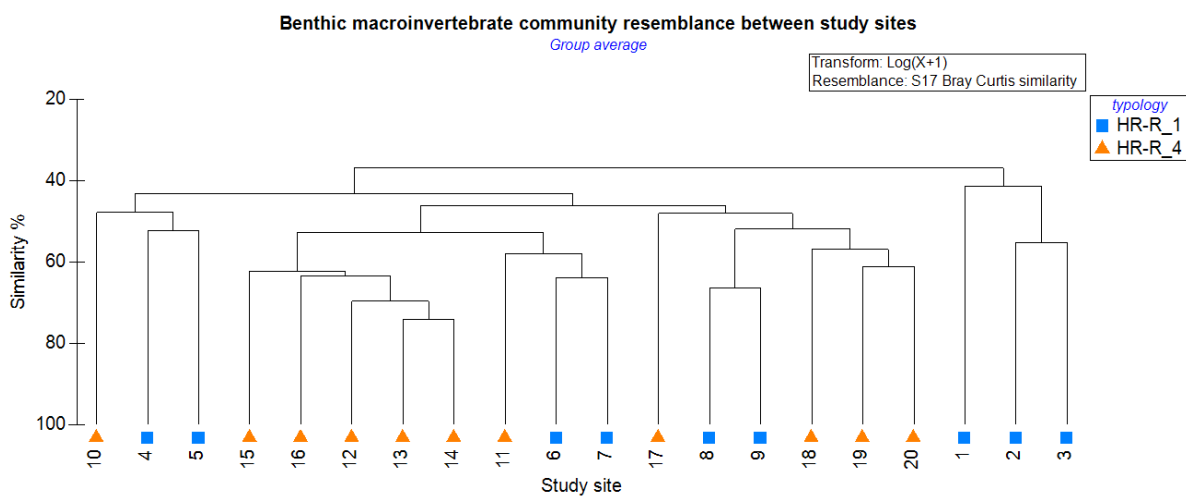


Figure 4.2.5. Hierarchical Cluster analysis based on Bray-Curtis resemblance of benthic macroinvertebrate samples. Cluster grouping displayed according to river type: Type HR-R\_1 (study sites 1 – 9) coloured blue, type HR-R\_4A (study sites 10 – 20) coloured orange.

SIMPER analysis identified species/taxa primarily contributing to community structural similarity between all study sites from the Bednja River. Average community similarity within the Bednja River amounts to 45.43%. The Tribus Chironomini, oligochaet *Limnodrilus hoffmeisteri* Claparede, 1862, amphipod *Gammarus fossarum* Koch in Panzer, 1836, and Tribus Tanytarsini contribute to community similarity with > 5%. (Tab. 4.2.5.).

Table 4.2.5. Results of SIMPER analysis for benthic macroinvertebrate species / taxa contributing to similarity between 20 study sites on the Bednja River (including contribution less than 5%).

<b>Bednja River</b>					
Average similarity: 45.43%					
Species / taxa	Average abundance	Average similarity	Sim/SD	Contribution %	Cumulative %
Chironomini Gen. sp.	6.61	3.78	4.54	<b>8.33</b>	8.33
<i>Limnodrilus hoffmeisteri</i>	5.92	3.26	4.04	<b>7.18</b>	15.5
<i>Gammarus fossarum</i>	5.9	2.97	2.68	<b>6.53</b>	22.03
Tanytarsini Gen. sp.	4.77	2.31	2.58	<b>5.08</b>	27.12
<i>Gammarus roeselii</i>	4.12	1.85	1.46	<b>4.08</b>	31.2
Tanypodinae Gen. sp.	3.42	1.78	3.62	<b>3.91</b>	35.11
Orthoclaadiinae Gen. sp.	4.37	1.77	1.96	<b>3.89</b>	39
<i>Baetis fuscatus</i>	4.3	1.76	1.33	<b>3.87</b>	42.87
<i>Serratella ignita</i>	3.6	1.6	1.67	<b>3.52</b>	46.38
Simuliidae Gen. sp.	3.84	1.47	1.53	<b>3.24</b>	49.62
<i>Prodiamesa olivacea</i>	2.71	1.10	1.22	<b>2.43</b>	52.05
<i>Potamothrix hammoniensis</i>	3.12	1.07	0.80	<b>2.35</b>	54.4
<i>Baetis buceratus</i>	3.00	0.96	0.90	<b>2.11</b>	56.51
<i>Oulimnius tuberculatus</i> Ad.	2.06	0.78	1.22	<b>1.73</b>	58.24
<i>Oulimnius tuberculatus</i> Lv.	2.07	0.78	1.05	<b>1.71</b>	59.94
<i>Baetis vernus</i>	2.42	0.76	0.78	<b>1.67</b>	61.62
<i>Limnodrilus claparedeanus</i>	2.57	0.74	0.74	<b>1.62</b>	63.24
<i>Baetis rhodani</i>	2.51	0.73	0.76	<b>1.6</b>	64.85
<i>Psychomyia pusilla</i>	2.48	0.72	0.81	<b>1.59</b>	66.43
Ceratopogonidae Gen. sp.	1.72	0.66	1.26	<b>1.45</b>	67.89
Empididae Gen. sp.	2.09	0.65	0.98	<b>1.42</b>	69.31
<i>Holandriana holandrii</i>	2.19	0.62	0.69	<b>1.37</b>	70.68
<i>Pisidium</i> sp.	2.00	0.60	0.79	<b>1.33</b>	72.01
<i>Hydroptila</i> sp.	1.55	0.60	1.24	<b>1.33</b>	73.34
<i>Lebertia</i> sp.	1.28	0.51	1.27	<b>1.12</b>	74.46
<i>Hydropsyche angustipennis angustipennis</i>	2.08	0.44	0.47	<b>0.97</b>	75.43
<i>Aphelocheirus</i> sp.	1.48	0.41	0.71	<b>0.91</b>	76.33
<i>Antocha</i> sp.	1.37	0.41	0.84	<b>0.89</b>	77.23
<i>Onychogomphus forcipatus forcipatus</i>	1.23	0.40	0.95	<b>0.88</b>	78.1
Limoniidae Gen. sp.	1.45	0.40	0.85	<b>0.88</b>	78.98
<i>Esolus</i> sp. Lv.	1.44	0.37	0.70	<b>0.82</b>	79.8
<i>Hygrobates fluviatilis</i>	1.19	0.37	0.78	<b>0.81</b>	80.61
<i>Esolus pygmaeus</i> Ad.	1.49	0.35	0.64	<b>0.77</b>	81.39
<i>Elmis</i> sp. Lv.	1.03	0.33	1.15	<b>0.74</b>	82.12
<i>Branchiura sowerbyi</i>	1.82	0.33	0.37	<b>0.73</b>	82.85
<i>Dicranota</i> sp.	1.24	0.33	0.76	<b>0.72</b>	83.57
<i>Hydropsyche incognita/pellucidula</i>	1.75	0.33	0.43	<b>0.72</b>	84.29
<i>Caenis luctuosa</i>	1.31	0.30	0.64	<b>0.66</b>	84.95
<i>Psammoryctides barbatus</i>	1.44	0.29	0.40	<b>0.63</b>	85.58
<i>Heptagenia flava</i>	1.06	0.27	0.51	<b>0.59</b>	86.17
<i>Hydraena riparia</i> Ad.	0.94	0.25	0.76	<b>0.54</b>	86.72
<i>Theodoxus danubialis danubialis</i>	1.25	0.24	0.41	<b>0.54</b>	87.25
<i>Limnius</i> sp. Lv.	1.05	0.24	0.71	<b>0.52</b>	87.77
Erpobdellidae Gen. sp.	1.13	0.23	0.47	<b>0.5</b>	88.28
Lumbricidae Gen. sp.	1.29	0.21	0.38	<b>0.47</b>	88.75
<i>Hygrobates calliger</i>	0.79	0.19	0.65	<b>0.41</b>	89.16
<i>Habrophlebia lauta</i>	0.99	0.18	0.54	<b>0.4</b>	89.56
Veliidae Gen. sp.	0.74	0.17	0.54	<b>0.38</b>	89.94
<i>Electrogena ujhelyii</i>	0.94	0.17	0.46	<b>0.37</b>	90.31

SIMPER analysis further identified taxa primarily contributing to structural similarity or dissimilarity within the two Bednja River types. Average community similarity within Type HR-R\_1 (study sites 1 – 9) amounts to 45.57% while average community similarity within Type HR-R\_4A (study sites 10 – 20) amounts to 51.39%. The Tribus Chironomini contributes most to community similarity for both river types, 8.6% similarity for sites belonging to Type HR-R\_1 and 7.11% for study sites belonging to Type HR-R\_4A. Taxa contributing with > 5% similarity for Type HR-R\_1 and Type HR-R\_4A are given in Tab. 4.2.6.

Table 4.2.6. Results of SIMPER analysis for benthic macroinvertebrate species / taxa contributing to average similarity between Bednja River types (excluding contribution less than 5%).

<b>Type HR-R_1: Small mid-altitude running waters (study sites 1 – 9)</b>		
Average similarity: 45.57%		
Species / taxa	Contribution %	Cumulative %
Chironomini Gen. sp.	<b>8.6</b>	8.6
<i>Limnodrilus hoffmeisteri</i>	<b>7.43</b>	16.02
<i>Gammarus fossarum</i>	<b>6.4</b>	22.43
Tanytarsini Gen. sp.	<b>5.39</b>	27.82

<b>Type HR-R_4A: Medium lowland running waters (study sites 10 – 20)</b>		
Average similarity: 51.39%		
Species / taxa	Contribution %	Cumulative %
Chironomini Gen. sp.	<b>7.11</b>	7.11
<i>Limnodrilus hoffmeisteri</i>	<b>6.11</b>	13.23
<i>Gammarus fossarum</i>	<b>5.82</b>	19.04

Average community dissimilarity between communities of Type HR-R\_1 and Type HR-R\_4A amount to 57.92%. The oligochaet *Branchiura sowerbyi* Beddard, 1892, contributes most to community dissimilarity between the two river types (1.84% contribution) (Tab. 4.2.7.).

Table 4.2.7. Results of SIMPER analysis for benthic macroinvertebrate species / taxa contributing to dissimilarity between river types on the Bednja River (excluding contribution less than 1%).

<b>River Type 1 and Type 4</b>		
Average dissimilarity = 57.92%		
Species / taxa	Contribution %	Cumulative %
<i>Branchiura sowerbyi</i>	1.84	1.84
<i>Hydropsyche incognita/pellucidula</i>	1.74	3.58
<i>Limnodrilus claparedeanus</i>	1.72	5.3
<i>Baetis fuscatus</i>	1.67	6.98
<i>Potamothenix hammoniensis</i>	1.66	8.64
<i>Psychomyia pusilla</i>	1.66	10.29
<i>Baetis rhodani</i>	1.65	11.94
<i>Hydropsyche angustipennis angustipennis</i>	1.58	13.52

Table 4.2.7. (Continued). Results of SIMPER analysis for benthic macroinvertebrate species / taxa contributing to dissimilarity between river types on the Bednja River (excluding contribution less than 1%).

<b>River Type 1 and Type 4</b>		
Average dissimilarity = 57.92%		
Species / taxa	Contribution %	Cumulative %
Orthoclaadiinae Gen. sp.	1.54	15.05
Simuliidae Gen. sp.	1.52	16.57
<i>Holandriana holandrii</i>	1.45	18.02
<i>Baetis buceratus</i>	1.44	19.46
<i>Serratella ignita</i>	1.36	20.82
<i>Baetis vernus</i>	1.34	22.15
<i>Gammarus roeselii</i>	1.31	23.47
<i>Gammarus fossarum</i>	1.29	24.76
<i>Hydropsyche contubernalis contubernalis</i>	1.23	25.99
Tanytarsini Gen. sp.	1.2	27.19
<i>Psammoryctides barbatus</i>	1.19	28.37
<i>Pisidium</i> sp.	1.17	29.54
<i>Esolus pygmaeus</i> Ad.	1.16	30.7
<i>Hydropsyche bulbifera</i>	1.11	31.81
<i>Prodiamesa olivacea</i>	1.09	32.91
Lumbricidae Gen. sp.	1.07	33.98
Empididae Gen. sp.	1.07	35.04
<i>Theodoxus danubialis danubialis</i>	1.07	36.11
<i>Propappus volki</i>	1.05	37.16

#### 4.2.1.1 Functional feeding groups

Based on taxon abundance within the benthic macroinvertebrate community at each study site the share of functional feeding groups was calculated (Tab. 4.2.8., Fig. 4.2.6.). Gatherers/collectors make up the overall dominant feeding group in the Bednja River being dominant at 17 study sites and having a share within communities ranging from 21.29% (study site 16) to 88.95% (study site 9), 58.50% average. Share of shredders ranges from 0.20% (study site 9) to 55.85% (study site 1), 14.48% average. Share of grazers ranges from 4.40% (study site 4) to 30.02% (study site 6), 14.03% average. Passive filter feeders follow with a share range from 0.04% (study site 1) to 38.47% (study site 16), 6.30% average. Less abundant are predators ranging from 1.22% (study site 1) to 8.01% (study site 6), 4.04% average, followed by active filter feeders ranging from 0.05% (study site 3) to 8.87% (study site 20), 2.47% average. Least abundant are miners, xylophagous taxa, parasites and “other” feeding groups, being absent from several study sites or having a maximum community share of 0.17% (miners), 0.20% (xylophagous taxa), 0.09% parasites and 0.63% (other feeding groups) (Tab. 4.2.8., Fig. 4.2.6.).



Table 4.2.8. Share of functional feeding groups at study sites. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types (Moog, 2002). Share (%) larger than 20% is shaded.

Study site	Functional feeding group%									
	GRA	MIN	XYL	SHR	DET	AFIL	PFIL	PRE	PAR	OTH
1	12.4	0.0	0.0	55.8	26.5	4.0	0.0	1.2	0.0	0.0
2	6.3	0.0	0.0	12.1	64.5	6.6	5.1	5.3	0.0	0.0
3	13.6	0.0	0.0	15.9	65.2	0.0	2.0	3.3	0.0	0.0
4	4.4	0.1	0.2	8.3	80.4	1.6	0.7	4.1	0.0	0.0
5	7.3	0.1	0.1	6.0	80.9	1.8	0.5	3.3	0.0	0.0
6	30.0	0.1	0.0	9.9	41.0	0.5	10.1	8.0	0.0	0.3
7	28.6	0.2	0.0	11.8	46.9	0.5	7.1	4.7	0.0	0.3
8	8.8	0.0	0.0	0.9	79.2	1.2	4.6	5.2	0.0	0.0
9	4.4	0.1	0.0	0.2	89.0	0.7	2.7	2.9	0.0	0.0
10	4.9	0.0	0.0	1.9	87.1	3.6	0.1	2.2	0.0	0.0
11	24.2	0.1	0.0	13.0	28.9	0.1	27.6	6.0	0.0	0.1
12	12.2	0.0	0.0	39.3	41.7	1.4	1.0	4.2	0.0	0.1
13	10.8	0.0	0.1	50.2	35.5	0.9	0.5	1.9	0.0	0.1
14	9.8	0.0	0.0	21.3	64.0	1.4	0.3	3.0	0.0	0.1
15	13.4	0.1	0.0	9.8	69.2	1.0	1.2	4.5	0.1	0.6
16	13.4	0.0	0.0	23.4	21.3	0.3	38.5	3.0	0.0	0.1
17	11.6	0.1	0.0	3.0	74.9	4.7	1.3	4.3	0.0	0.1
18	23.0	0.0	0.0	3.8	60.7	3.0	6.9	2.6	0.0	0.0
19	20.5	0.1	0.0	0.6	61.0	7.2	2.7	7.8	0.0	0.0
20	21.0	0.0	0.0	2.3	51.9	8.9	12.8	3.2	0.0	0.0
Average	14.0	0.1	0.0	14.5	58.5	2.5	6.3	4.0	0.0	0.1

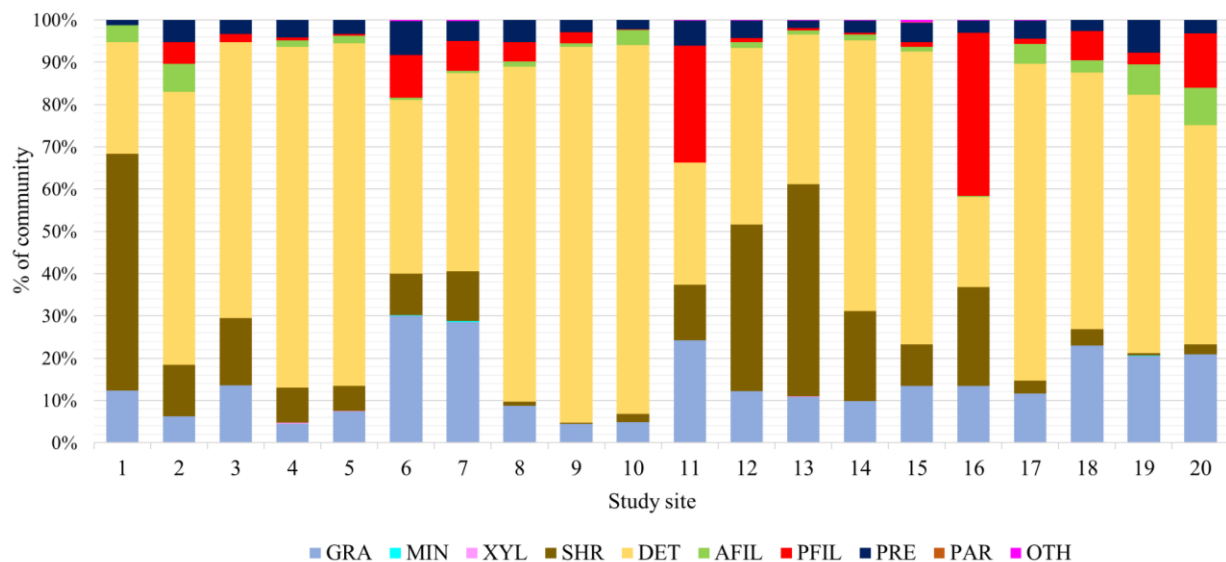


Figure 4.2.6. Share (%) of functional feeding groups at study sites. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types.

#### 4.2.1.2 Longitudinal zonation preference

Analysis of share of community preferring a certain stream zone (Moog, 2002) shows high share of mid and lower reach preferring taxa in the upper reach of the Bednja River. 9 study sites are dominated by crenal /rithral preferring taxa (sites 1, 3, 6, 7, 11, 12, 13, 14 and 16) while 11 study sites are dominated by potamal / littoral preferring taxa (site 2, 4, 5, 8, 9, 10, 15, 17, 18, 19 and 20) (Fig. 4.2.7.).

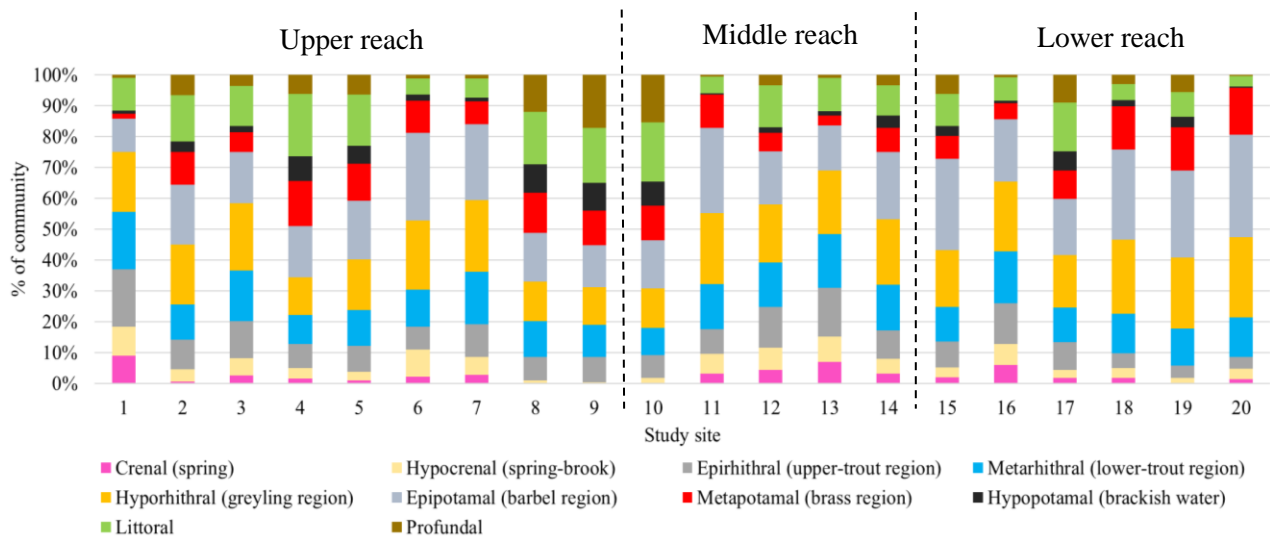


Figure 4.2.7. Zonation preference of benthic macroinvertebrates shown as share of community at study site preferring a certain zone.

#### 4.2.2 Comparison of composition and structure of benthic macroinvertebrates between microhabitats

A total of 71 microhabitat samples were collected from the 20 study sites. Macroinvertebrate abundance on individual microhabitats ranges from 1,584 ind/m<sup>2</sup> (macrolithal at study site 1) to 102,480 ind/m<sup>2</sup> (psammal substrate at study site 8). Psammal substrate at study site 8 possesses the lowest Margalef Index (1.3), while technolithal substrate from study site 17 has the highest Margalef Index (7.979). Shannon-Wiener Index ranges from 0.388 (xylal from study site 13) to 2.806 (akal at study site 7). Greatest evenness is found on mesolithal at study site 3 (0.808) and lowest at xylal at study site 13 (0.118). Lowest Simpson Index is on xylal at study site 13 (0.135) and highest on akal of study site 3 (0.913). The greatest number of species is found on technolithal of study site 19 (71 total species) (Tab. 4.2.9.).

On average, the highest total species and Margalef Index are found on technolithal substrate, the highest evenness and Shannon-Wiener Index on macrolithal substrate and the highest Simpson Index on akal substrate (Tab. 4.2.9.).

Table 4.2.9. Benthic macroinvertebrate diversity on individual microhabitats collected from the study sites. Highest and lowest values are bolded.

Study site - microhabitat	Number of subsamples / 20	Abundance (ind/m <sup>2</sup> )	Total species (S)	Margalef Index	Evenness/ Pielou's evenness	Shannon -Wiener Index	Simpson -Index
1-MACRO	2	<b>1,584</b>	14	1.764	0.628	1.656	0.693
1-MESO	7	2,371	30	3.732	0.515	1.751	0.731
1-MICRO	3	13,442	35	3.577	0.310	1.102	0.422
1-PSA	5	29,665	22	2.039	0.142	0.439	0.160
1-AKAL	1	49,792	18	1.572	0.424	1.226	0.601
1-ARG	2	21,752	21	2.003	0.204	0.620	0.213
2-PSA	8	4,174	29	3.359	0.537	1.808	0.762
2-MACROPHYTES	8	7,392	42	4.603	0.622	2.323	0.838
2-AKAL	4	16,364	43	4.329	0.565	2.126	0.818
3-ARG	6	6,088	28	3.098	0.482	1.604	0.621
3-MICRO	6	5,282	47	5.366	0.619	2.384	0.855
3-MESO	4	1,668	26	3.370	<b>0.808</b>	2.632	0.892
3-AKAL	2	12,784	41	4.230	0.753	2.798	0.913
3-DET	1	5,616	26	2.896	0.705	2.296	0.849
3-PSA	1	10,944	23	2.365	0.578	1.812	0.722
4-PSA	10	2,304	34	4.262	0.506	1.783	0.753
4-XYL	9	3,014	40	4.868	0.437	1.612	0.601
4-MICRO	1	3,760	24	2.794	0.628	1.996	0.784
5-ARG	8	1,964	30	3.824	0.537	1.825	0.673
5-PSA	9	2,175	31	3.904	0.458	1.571	0.646
5-XYL	3	2,082	29	3.664	0.660	2.223	0.823
6-AKAL	1	6,928	35	3.845	0.773	2.748	0.907
6-MICRO	2	4,008	38	4.460	0.734	2.670	0.893
6-MACRO	2	2,096	30	3.792	0.787	2.675	0.890
6-MESO	15	1,995	56	7.238	0.647	2.605	0.880
7-AKAL	2	4,040	36	4.215	0.783	<b>2.806</b>	0.912
7-MACROPHYTES	1	4,304	25	2.868	0.709	2.284	0.806
7-TEHNO	6	8,084	55	6.002	0.570	2.283	0.820
7-MICRO	11	5,928	64	7.252	0.656	2.728	0.892
8-XYL	3	4,804	28	3.185	0.717	2.389	0.858
8-AKAL	16	7,467	57	6.279	0.692	2.799	0.906
8-PSA	1	<b>102,480</b>	16	<b>1.300</b>	0.264	0.731	0.393
9-XYL	2	4,768	28	3.188	0.637	2.122	0.811
9-AKAL	16	9,548	47	5.020	0.517	1.992	0.756
9-PSA	2	10,720	16	1.616	0.503	1.393	0.629
10-AKAL	19	6,808	51	5.665	0.466	1.833	0.738
10-XYL	1	3,280	23	2.718	0.759	2.379	0.869
11-TECHNO	11	4,344	49	5.730	0.595	2.315	0.852
11-MACROPHYTES	5	10,157	23	2.385	0.559	1.753	0.732
11-MICRO	4	5,880	45	5.070	0.686	2.610	0.887
12-AKAL	11	8,243	37	3.992	0.468	1.689	0.709
12-PSA	4	2,940	24	2.880	0.644	2.048	0.809
12-MICRO	3	3,059	32	3.863	0.681	2.361	0.853
12-XYL	2	2,736	16	1.895	0.584	1.619	0.725
13-AKAL	10	5,337	46	5.243	0.546	2.089	0.795
13-PSA	4	2,440	20	2.436	0.592	1.773	0.755
13-MICRO	2	19,632	30	2.934	0.192	0.651	0.208
13-XYL	4	9,876	27	2.827	<b>0.118</b>	<b>0.388</b>	<b>0.135</b>
14-XYL	1	8,096	28	3.000	0.638	2.126	0.811
14-AKAL	11	6,761	44	4.876	0.601	2.274	0.847
14-PSA	8	2,824	34	4.153	0.530	1.870	0.716

Table 4.2.9. (Continued). Benthic macroinvertebrate diversity on individual microhabitats collected from the study sites.

Study site - microhabitat	Number of subsamples / 20	Abundance (ind/m <sup>2</sup> )	Total species (S)	Margalef Index	Evenness/Pielou's evenness	Shannon -Wiener Index	Simpson -Index
15-AKAL	17	4,046	58	6.863	0.558	2.266	0.766
15-XYL	1	9,440	32	3.387	0.646	2.237	0.767
15-PSA	2	15,256	41	4.153	0.603	2.241	0.789
16-MICRO	2	5,384	24	2.677	0.530	1.684	0.628
16-MESO	10	12,287	40	4.142	0.418	1.540	0.651
16-PSA	1	11,792	25	2.560	0.578	1.861	0.747
16-XYL	4	11,424	43	4.495	0.418	1.572	0.593
16-AKAL	3	1,627	24	3.110	0.709	2.255	0.809
17-MACROPHYTES	1	28,064	25	2.343	0.451	1.451	0.560
17-TECHNO	19	6,455	71	<b>7.979</b>	0.452	1.925	0.733
18-MICRO	6	19,606	50	4.958	0.580	2.270	0.827
18-MESO	6	24,646	54	5.241	0.548	2.185	0.804
18-XYL	4	16,976	45	4.518	0.596	2.270	0.845
18-AKAL	4	21,692	54	5.308	0.457	1.824	0.724
19-TECHNO	10	5,283	46	5.249	0.523	2.001	0.763
19-MICRO	10	6,115	37	4.129	0.521	1.882	0.725
20-MICRO	14	9,744	56	5.988	0.495	1.993	0.793
20-PSA	3	5,872	29	3.227	0.446	1.502	0.637
20-XYL	2	29,048	43	4.087	0.623	2.344	0.856
20-AKAL	1	10,592	20	2.050	0.567	1.698	0.748
<b>MIN</b>		<b>1584</b>	<b>14</b>	<b>1.300</b>	<b>0.118</b>	<b>0.388</b>	<b>0.135</b>
<b>MAX</b>		<b>102480</b>	<b>71</b>	<b>7.979</b>	<b>0.808</b>	<b>2.806</b>	<b>0.913</b>
			Total species (S)	Margalef Index	Evenness/Pielou's evenness	Shannon -Wiener Index	Simpson -Index
AVERAGE MACROLITHAL			22	2.778	<b>0.707</b>	<b>2.166</b>	0.791
AVERAGE PSAMMAL			26	2.943	0.491	1.602	0.655
AVERAGE ARGYLLAL			26	2.975	0.407	1.350	0.503
AVERAGE MACROPHYTES			29	3.050	0.585	1.953	0.734
AVERAGE XYLAL			32	3.486	0.569	1.940	0.724
AVERAGE MICROLITHAL			40	4.422	0.553	2.028	0.731
AVERAGE AKAL			41	4.440	0.592	2.162	<b>0.797</b>
AVERAGE MESOLITHAL			41	4.745	0.587	2.143	0.792
AVERAGE TECHNOLITHAL			<b>55</b>	<b>6.240</b>	0.535	2.131	0.792

Bray-Curtis similarity index shows 24.69% average resemblance between benthic macroinvertebrate communities of all sampled microhabitats. Highest resemblance is displayed between communities on akal substrates of study sites 13 and 14 (74.2%) followed by communities on microlithal and mesolithal substrate at study site 16 (72.07%) and macrolithal and mesolithal substrate at study site 6 (70.40%) (Fig. 4.2.8.).

Non-metric Multi-Dimensional Scaling (nMDS) analysis shows grouping of benthic macroinvertebrate communities occurs based on both substrate type and study site affiliation.

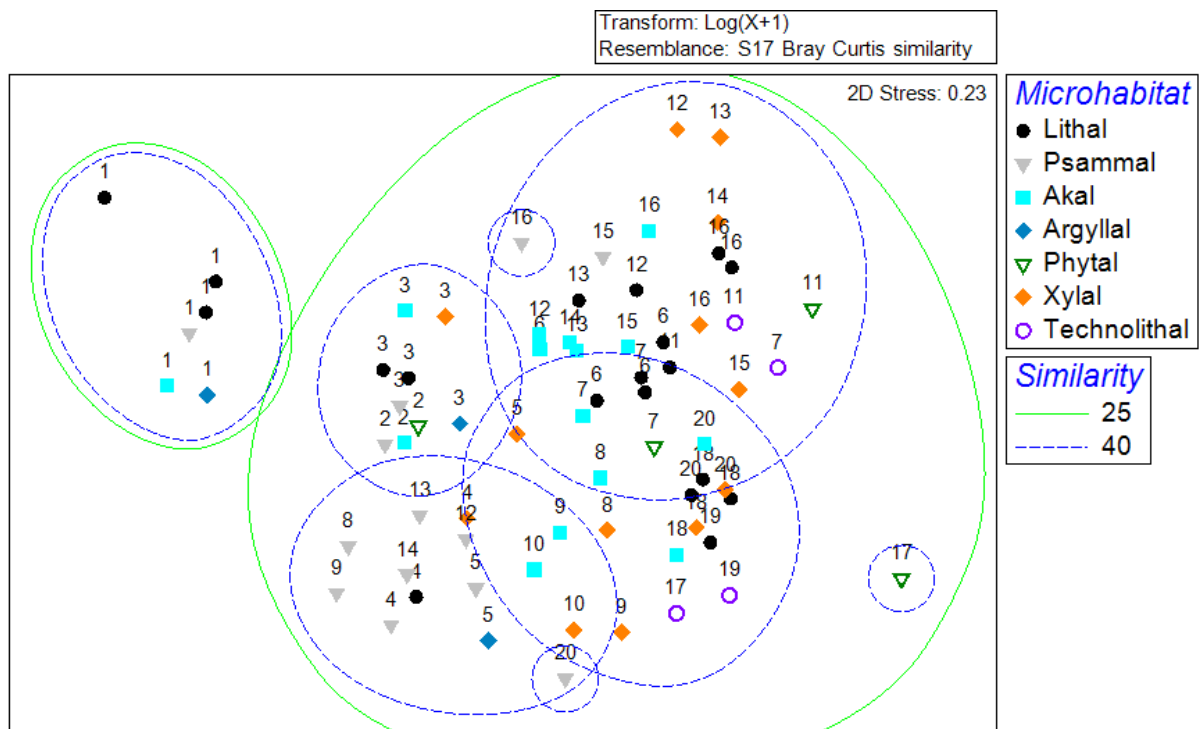


Figure 4.2.8. Non-metric Multi-Dimensional Scaling based on Bray-Curtis similarity of benthic macroinvertebrate communities between study site microhabitats in the Bednja River.

SIMPER analysis identified species / taxa primarily contributing to structural similarity or dissimilarity within sampled microhabitats. Species / taxa contributing to >5% similarity within microhabitats is given in Tab. 4.2.10.

Average community similarity within macrolithal, mesolithal and microlithal samples amounts to 18.84%, respectively 34.79% and 40.45%. Contributing most to similarities within all three microhabitats is the amphipod *Gammarus fossarum* with 36.56%, respectively 13.94% and 9.84%. Similarities within the smaller grained substrates psammal, akal and argyllal amount to 40.94%, respectively 43.00% and 35.33%. All three substrates have the Tribus Chironomini responsible for majority of these similarities with 16.72% contribution within psammal samples, 8.65% contribution within akal samples and 19.55% contribution within argyllal samples. Average community similarity within xylal samples amounts to 39.65% and *Gammarus fossarum* contributes most with 11.71%. Samples collected from artificial

technolithal substrate share average similarity of 39.02% with Orthoclaadiinae contributing most with 11.25% (Tab. 4.2.10).

Table 4.2.10. Results of SIMPER analysis for benthic macroinvertebrate species / taxa contributing to similarity within sampled microhabitats (excluding contribution less than 5%).

<b>Microhabitat: Macrolithal</b>		
Average similarity: 18.84%		
Species / taxa	Contribution %	Cumulative %
<b><i>Gammarus fossarum</i></b>	<b>36.56</b>	<b>36.56</b>
Chironomini Gen. sp.	29.92	66.47
Tanypodinae Gen. sp.	18.88	85.36
Tanytarsini Gen. sp.	14.64	100
<b>Microhabitat: Mesolithal</b>		
Average similarity: 34.79%		
Species / taxa	Contribution %	Cumulative %
<b><i>Gammarus fossarum</i></b>	<b>13.94</b>	<b>13.94</b>
Chironomini Gen. sp.	11.91	25.86
Orthoclaadiinae Gen. sp.	7.22	33.07
Tanytarsini Gen. sp.	5.72	38.8
<i>Baetis rhodani</i>	5.63	44.43
<b>Microhabitat: Microlithal</b>		
Average similarity: 40.45%		
Species / taxa	Contribution %	Cumulative %
<b><i>Gammarus fossarum</i></b>	<b>9.84</b>	<b>9.84</b>
Chironomini Gen. sp.	9.25	19.08
<i>Limnodrilus hoffmeisteri</i>	6.98	26.06
<i>Baetis fuscatus</i>	6.28	32.34
Tanypodinae Gen. sp.	5.55	37.9
Simuliidae Gen. sp.	5.46	43.36
<b>Microhabitat: Psammal</b>		
Average similarity: 40.94%		
Species / taxa	Contribution %	Cumulative %
<b>Chironomini Gen. sp.</b>	<b>16.72</b>	<b>16.72</b>
<i>Limnodrilus hoffmeisteri</i>	13.54	30.26
<i>Gammarus fossarum</i>	9.93	40.19
<i>Procladius olivacea</i>	8.65	48.84
Tanytarsini Gen. sp.	6.47	55.3
<i>Pisidium</i> sp.	5.23	60.53
<i>Gammarus roeselii</i>	5.04	65.57
<b>Microhabitat: Akal</b>		
Average similarity: 43.00%		
Species / taxa	Contribution %	Cumulative %
<b>Chironomini Gen. sp.</b>	<b>8.65</b>	<b>8.65</b>
<i>Gammarus fossarum</i>	7.96	16.61
Tanytarsini Gen. sp.	6.51	23.12
<i>Limnodrilus hoffmeisteri</i>	6.31	29.43
<i>Baetis fuscatus</i>	5.44	34.87
Tanypodinae Gen. sp.	5.14	40.01
<b>Microhabitat: Argyllal</b>		
Average similarity: 35.33%		
Species / taxa	Contribution %	Cumulative %
<b>Chironomini Gen. sp.</b>	<b>19.55</b>	<b>19.55</b>
<i>Limnodrilus hoffmeisteri</i>	16.18	35.73
Tanytarsini Gen. sp.	15.32	51.05
<i>Gammarus fossarum</i>	13.11	64.15
<i>Gammarus roeselii</i>	5.45	69.6

Table 4.2.10. (Continued). Results of SIMPER analysis for benthic macroinvertebrate species / taxa contributing to similarity within sampled microhabitats (excluding contribution less than 5%).

<b>Microhabitat: Xylal (wood)</b>		
Average similarity: 39.65%		
Species / taxa	Contribution%	Cumulative%
<b><i>Gammarus fossarum</i></b>	<b>11.71</b>	<b>11.71</b>
Chironomini Gen. sp.	10.18	21.89
<i>Gammarus roeselii</i>	10.18	32.08
<i>Baetis fuscatus</i>	6.6	38.67
<i>Serratella ignita</i>	5.2	43.87
Orthocladiinae Gen. sp.	5.16	49.03
Tanytarsini Gen. sp.	5.04	54.06
<b>Microhabitat: Technolihal</b>		
Average similarity: 39.02%		
Species / taxa	Contribution%	Cumulative%
<b>Orthocladiinae Gen. sp.</b>	<b>11.25</b>	<b>11.25</b>
<i>Gammarus fossarum</i>	7.23	18.47
<i>Serratella ignita</i>	6.22	24.69
Tanypodinae Gen. sp.	6.18	30.88
<i>Baetis buceratus</i>	5.89	36.76
<i>Baetis fuscatus</i>	5.81	42.57
Simuliidae Gen. sp.	5.58	48.15
Chironomini Gen. sp.	5.38	53.53

#### 4.2.2.1 Functional feeding groups present at microhabitats

Based on taxon abundance within the benthic macroinvertebrate community at individual microhabitats, the share of functional feeding groups was calculated (Tab. 4.2.11., Fig. 4.2.9.).

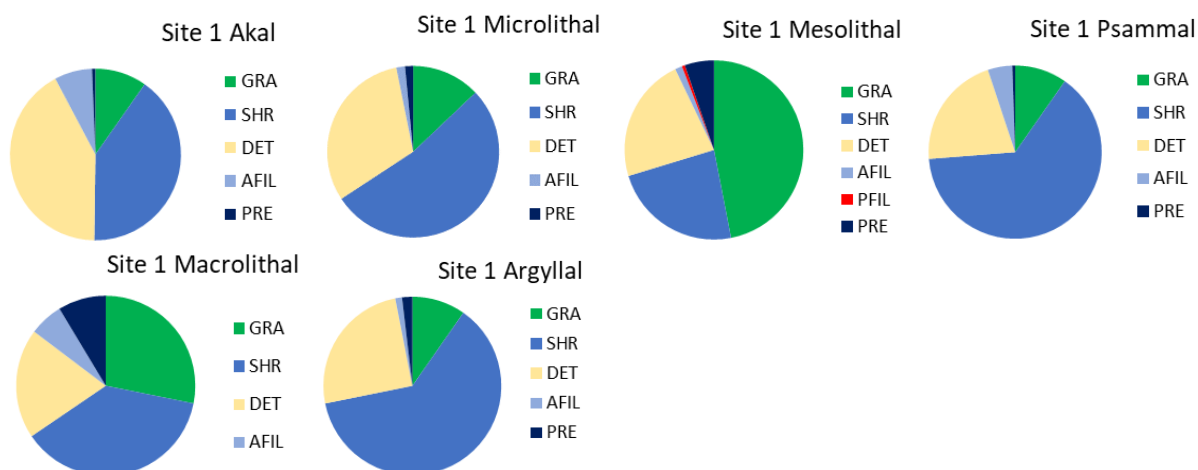
The highest share of gatherers/collectors (99.3%) is found on sand substrate at study site 8 while the highest share of shredders (67.3%) is present on xylal (wood) at study site 13. Passive filter feeders reach their maximum share of 58.0% on mesolihal substrate of study site 16. The highest share of grazers (46.9%) is found on mesolihal substrate of study site 1. Predators make up 15.8% of feeding groups on xylal (wood) of study site 8. Active filter feeders have the highest share on technolihal of study site 19. Less abundant are xylophagous taxa with a maximum share of 1.56% on xylal (wood) at study site 10, followed by “other” feeding groups with 1.22% on akal substrate of study site 6, parasites with 0.12% share on akal of study sites 13 and 15 and miners with a maximum share of 0.4% on xylal (wood) of study site 9.

On average, the highest share of gatherers/collectors is found on sand substrate (79.4% average) and smaller grained substrates akal and argyllal. The highest average share of grazers is found on macrolihal (30.3% average) and the larger substrates mesolihal, microlihal and technolihal.

Table 4.2.11. Share of functional feeding groups at microhabitats given as average based on the same microhabitat type at other study sites. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types (Moog, 2002). Highest values are bolded.

Microhabitat	Functional feeding group %									
	GRA	MIN	XYL	SHR	DET	AFIL	PFIL	PRE	PAR	OTH
AVERAGE										
MACROLITHAL	<b>30.3</b>	0.0	0.0	23.2	31.5	3.3	4.1	<b>7.5</b>	0.0	0.1
MESOLITHAL	27.8	0.0	0.0	13.8	36.0	0.9	<b>16.3</b>	5.1	0.0	0.0
MICROLITHAL	20.9	0.1	0.0	20.5	46.5	1.8	5.4	4.7	0.0	0.1
AKAL	14.3	0.0	0.0	14.2	62.4	3.0	1.8	4.0	0.0	<b>0.3</b>
PSAMMAL	4.1	0.0	0.0	9.5	<b>79.4</b>	4.3	0.3	2.2	0.0	0.1
ARGYLLAL	8.1	0.0	0.0	<b>27.1</b>	60.7	1.1	0.3	2.7	0.0	0.0
MACROPHYTES	18.1	0.0	0.0	8.5	48.3	1.6	19.0	4.4	0.0	0.0
XYLAL	16.4	0.1	<b>0.3</b>	23.0	46.0	1.5	6.6	6.1	0.0	0.1
TECHNOLITHAL	20.5	<b>0.2</b>	0.0	9.7	52.8	<b>4.5</b>	6.7	5.7	0.0	0.1

### Study site 1



### Study site 2

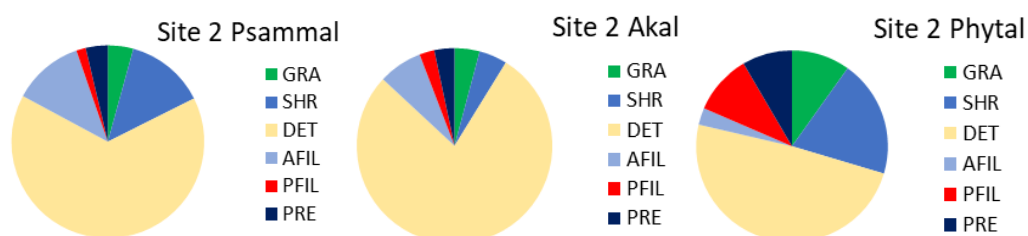
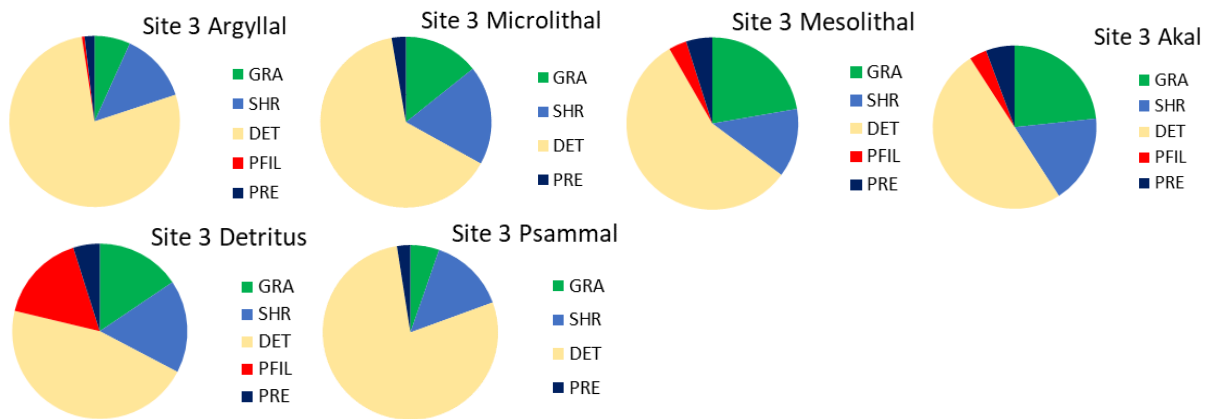


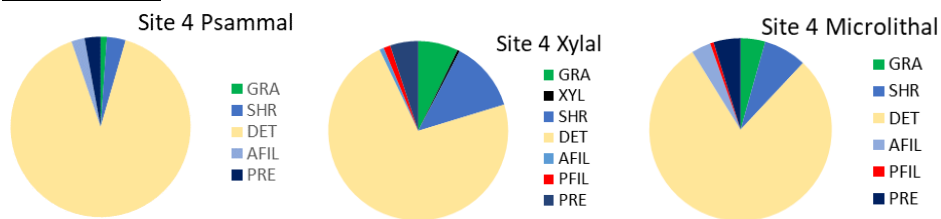
Figure 4.2.9. Share of functional feeding groups in the sampled microhabitats. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types.



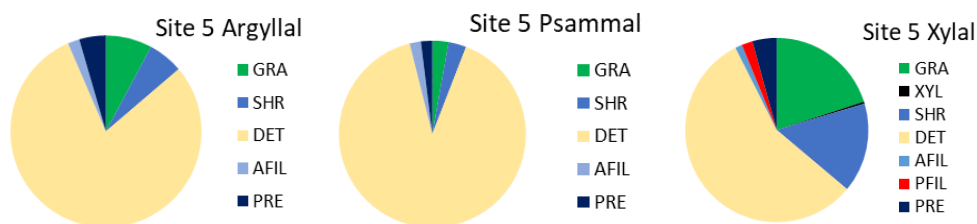
**Study site 3**



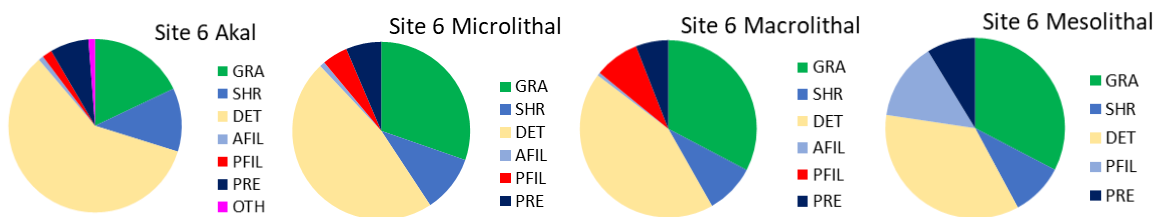
**Study site 4**



**Study site 5**



**Study site 6**



**Study site 7**

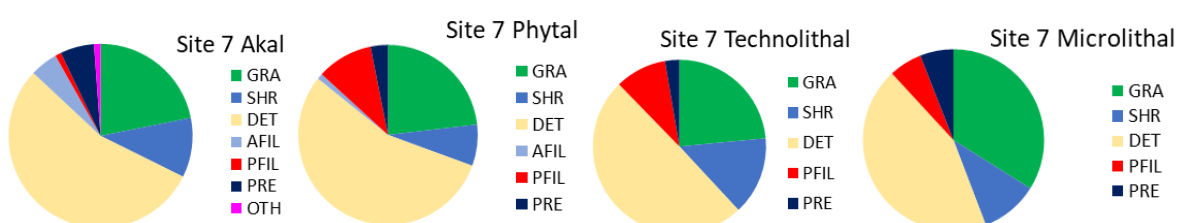
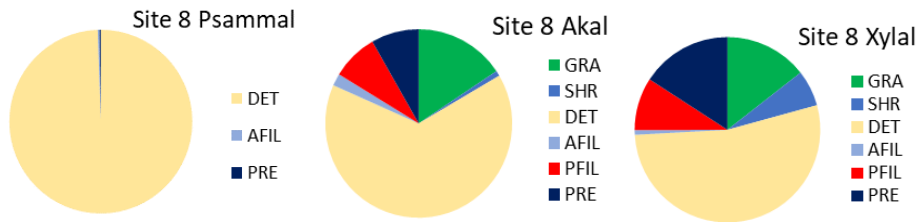
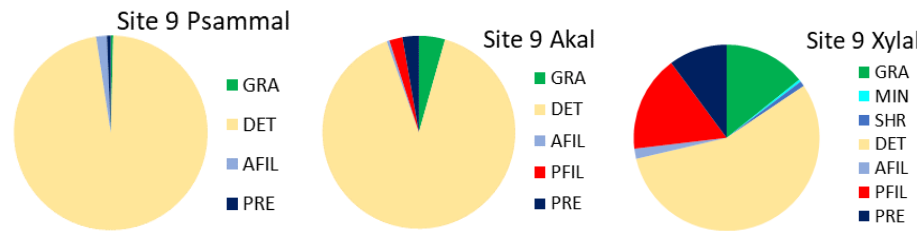


Figure 4.2.9. (Continued). Share of functional feeding groups in the sampled microhabitats. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types.

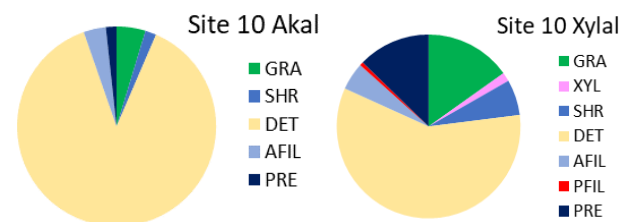
**Study site 8**



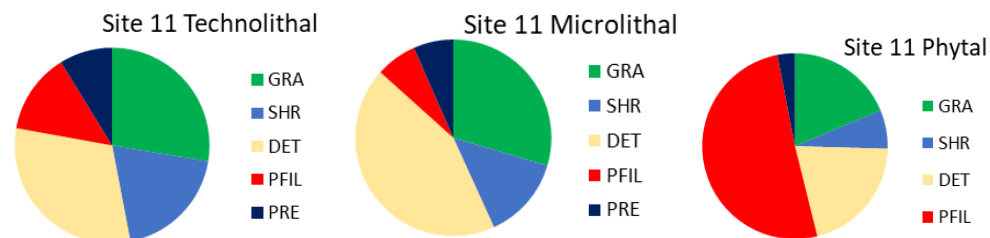
**Study site 9**



**Study site 10**



**Study site 11**



**Study site 12**

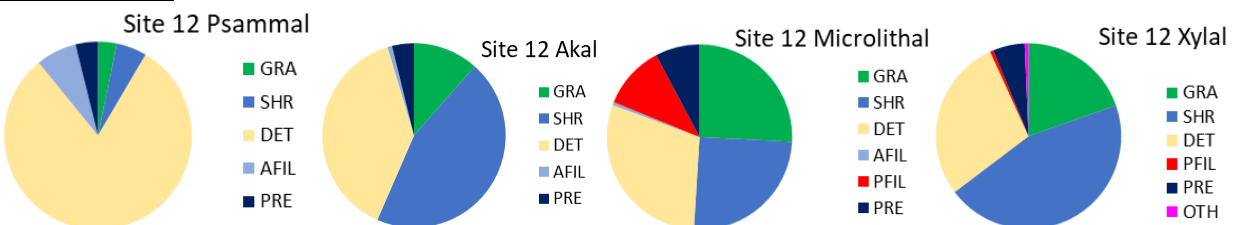
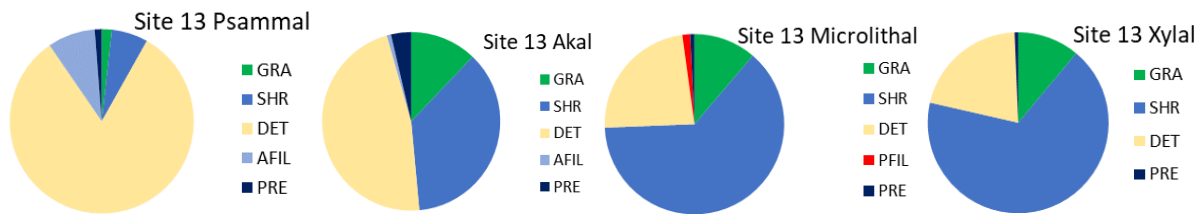
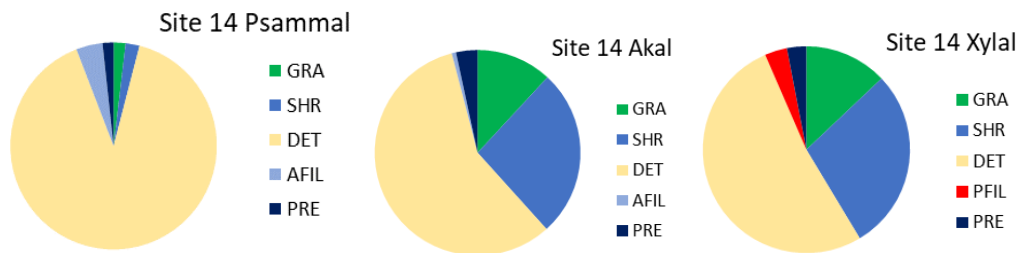


Figure 4.2.9. (Continued). Share of functional feeding groups in the sampled microhabitats. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types.

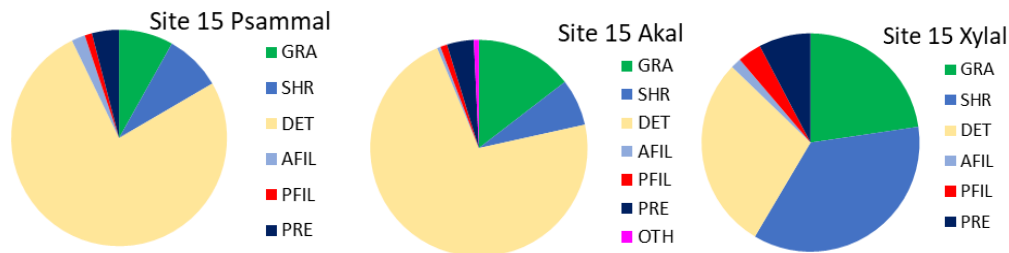
**Study site 13**



**Study site 14**



**Study site 15**



**Study site 16**

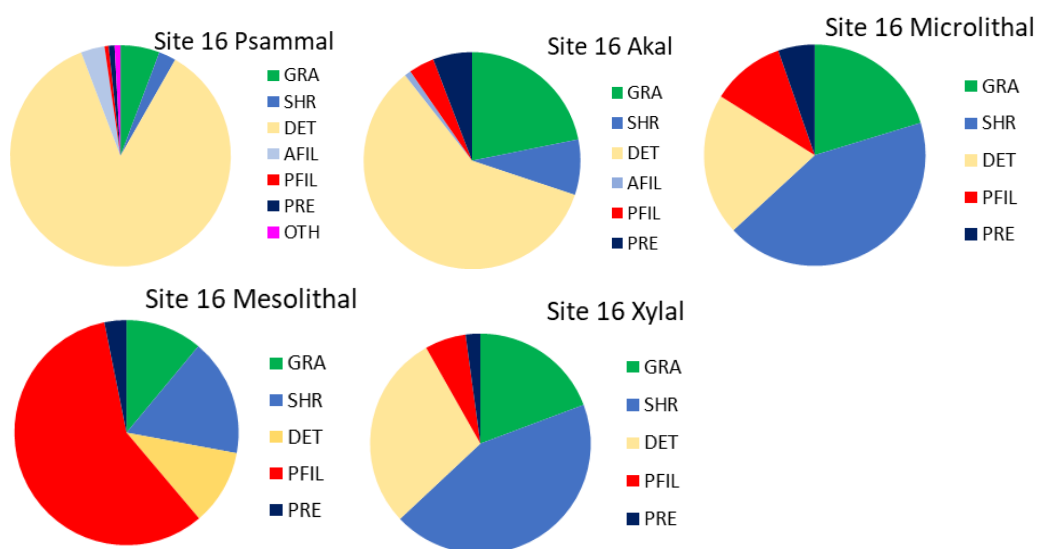
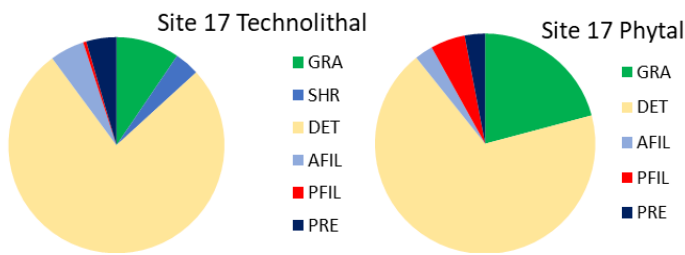
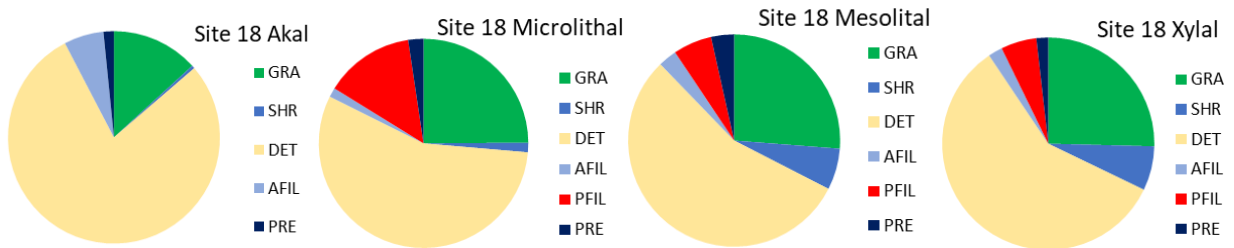


Figure 4.2.9. (Continued). Share of functional feeding groups in the sampled microhabitats. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types.

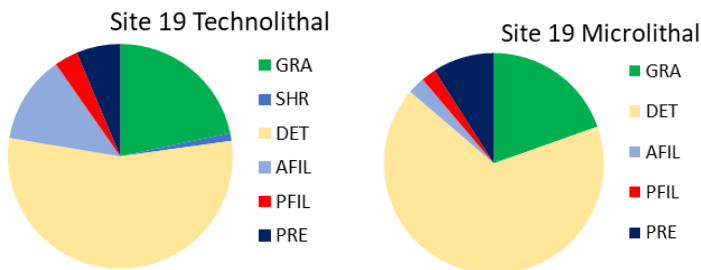
**Study site 17**



**Study site 18**



**Study site 19**



**Study site 20**

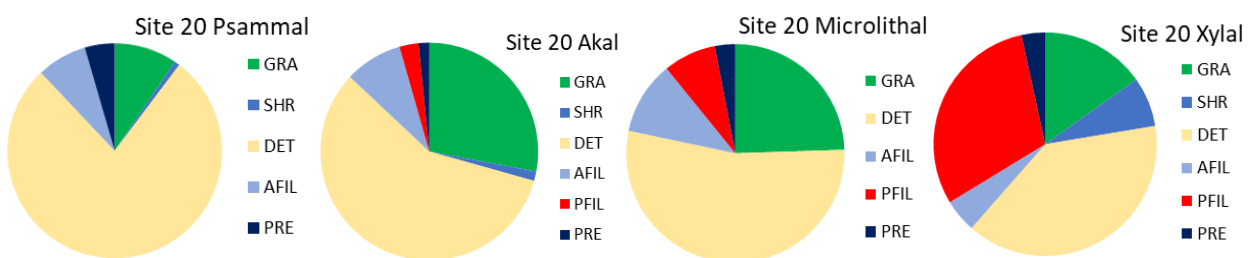


Figure 4.2.9. (Continued). Share of functional feeding groups in the sampled microhabitats. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types.

### 4.3 Effects of natural factors and anthropogenic stressors on the benthic macroinvertebrate community

#### 4.3.1 Correlations between natural parameters and benthic macroinvertebrate metrics

Pursuant to the set methodology, Spearman's rank correlation coefficient (R) was used to test the statistical relationship between natural gradients (distance from source, altitude, and sub-catchment size) and benthic macroinvertebrate metrics and dominantly occurring taxa per taxa group.

The River Fauna Index has the strongest significant correlation with the longitudinal gradient, ( $R = 0.739$ ,  $p < 0.05$ ) for distance from source. The analysis also shows that (%) littoral taxa ( $R = 0.451$ ,  $p < 0.05$ ) and the Rhithron Type Index ( $R = 0.602$ ,  $p < 0.05$ ) are significantly correlate with altitude (Tab. 4.3.1., Fig. 4.3.1.).

Table 4.3.1. Results of Spearman's correlation coefficient (R) for the relationship between natural parameters and benthic macroinvertebrate community metrics on the Bednja River catchment. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded.

	Distance from source / Sub-catchment size	Altitude
	r	r
Total individuals (N)	0.186	-0.186
Total species (S)	<b>0.450</b>	<b>-0.450</b>
Simpson-Index	0.102	-0.102
Shannon-Wiener-Index	0.045	-0.045
Margalef Index	0.280	-0.280
Evenness/Pielou's evenness: $J' = H'/\log(S)$	-0.059	0.059
Croatian Saprobity Index (HR <sub>SI</sub> )	0.027	-0.027
River Fauna Index (RFI)	<b>0.739</b>	<b>-0.739</b>
Average score per Taxon (ASPT)	-0.270	0.270
Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata (EPTCBO)	0.130	-0.130
Biological Monitoring Working Party (BMWP Score)	0.043	-0.043
Belgian Biotic Indeks (BBI)	0.193	-0.193
Altered Indices Biotico Estesio (IBE AQEM)	0.239	-0.239
Mayfly Total Score (MTS)	0.438	-0.438
r-Dominance	0.326	-0.326
(%) Hypocrenal (scored taxa = 100%)	-0.260	0.260
(%) Epirhithral (scored taxa = 100%)	-0.378	0.378
(%) Metarhithral (scored taxa = 100%)	0.017	-0.017
(%) Hyporhithral (scored taxa = 100%)	0.414	-0.414
(%) Epipotamal (scored taxa = 100%)	<b>0.529</b>	<b>-0.529</b>
(%) Metapotamal (scored taxa = 100%)	0.214	-0.214
(%) Littoral (scored taxa = 100%)	<b>-0.451</b>	<b>0.451</b>
(%) Type RP (scored taxa = 100%)	0.356	-0.356
Rheoindex (Banning, with abundance)	0.268	-0.268
Rhithron Type Index	<b>-0.602</b>	<b>0.602</b>
(%) Type Pelal (scored taxa = 100%)	-0.223	0.223
(%) Type Psammal (scored taxa = 100%)	-0.239	0.239
(%) Type Akal (scored taxa = 100%)	-0.006	0.006
(%) Type Lithal (scored taxa = 100%)	0.424	-0.424

Table 4.3.1. (Continued). Results of Spearman's correlation coefficient (R) for the relationship between natural parameters and benthic macroinvertebrate community metrics on the Bednja River catchment. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded.

	Distance from source / Sub-catchment size	Altitude
	r	r
(%) Type Phytal (scored taxa = 100%)	0.367	-0.367
(%) Type Akal + Lithal + Psammal (scored taxa = 100%)	0.253	-0.253
Grazers and scrapers (%)	0.305	-0.305
Miners (%)	-0.051	0.051
Xylophagous Taxa (%)	-0.242	0.242
Shredders (%)	-0.280	0.280
Gatherers/Collectors (%)	-0.152	0.152
Active filter feeders (%)	0.231	-0.231
Passive filter feeders (%)	0.266	-0.266
Predators (%)	-0.053	0.053
Parasites (%)	0.179	-0.179
Other feeding types (%)	0.223	-0.223
Coelenterata (%)	0.416	-0.416
Turbellaria (%)	-0.393	0.393
Gastropoda (%)	0.023	-0.023
Bivalvia (%)	-0.290	0.290
Oligochaeta (%)	-0.355	0.355
Hirudinea (%)	0.084	-0.084
Crustacea (%)	-0.313	0.313
Ephemeroptera (%)	0.280	-0.280
Odonata (%)	0.120	-0.120
Plecoptera (%)	-0.258	0.258
Heteroptera (%)	0.340	-0.340
Megaloptera (%)	<b>-0.498</b>	<b>0.498</b>
Trichoptera (%)	0.035	-0.035
Coleoptera (%)	-0.194	0.194
Diptera (%)	0.444	-0.444
Hydrachnidia (%)	0.127	-0.127
(%) EPT-Taxa	0.221	-0.221
EPT (%) (abundance classes)	0.081	-0.081
EPT/OL taxa	-0.117	0.117
<i>Baetis fuscatus</i>	<b>0.611</b>	<b>-0.611</b>
<i>Baetis buceratus</i>	0.261	-0.261
<i>Hydropsyche angustipennis angustipennis</i>	-0.026	0.026
<i>Hydropsyche incognita/pellucidula</i>	<b>0.657</b>	<b>-0.657</b>
<i>Psychomyia pusilla</i>	<b>0.537</b>	<b>-0.537</b>
<i>Onychogomphus forcipatus forcipatus</i>	<b>0.624</b>	<b>-0.624</b>
<i>Platycnemis pennipes</i>	0.360	-0.360
Empididae Gen. sp.	<b>0.577</b>	<b>-0.577</b>
Simuliidae Gen. sp.	0.353	-0.353
Chironomini Gen. sp.	0.210	-0.210
Orthocladiinae Gen. sp.	<b>0.595</b>	<b>-0.595</b>
<i>Esolus pygmaeus</i> Ad.	<b>0.596</b>	<b>-0.596</b>
<i>Oulimnius tuberculatus</i> Ad.	-0.186	0.186
<i>Oulimnius tuberculatus</i> Lv.	-0.137	0.137
<i>Pisidium</i> sp.	-0.057	0.057
<i>Sphaerium rivicola</i>	0.227	-0.227
<i>Bythinella opaca opaca</i>	-0.378	0.378
<i>Holandriana holandrii</i>	0.313	-0.313
<i>Limnodrilus hoffmeisteri</i>	-0.020	0.020
<i>Potamothenix hammoniensis</i>	<b>-0.537</b>	<b>0.537</b>
Erpobdellidae Gen. sp.	0.260	-0.260
<i>Helobdella stagnalis</i>	0.004	-0.004
<i>Hygrobates fluviatilis</i>	-0.213	0.213
<i>Lebertia</i> sp.	0.270	-0.270
<i>Asellus aquaticus</i>	0.436	-0.436
<i>Gammarus fossarum</i>	0.018	-0.018
<i>Gammarus roeselii</i>	-0.263	0.263

Significant correlations between the longitudinal gradient and macroinvertebrate metrics from Tab. 4.3.1. are displayed as RDA (Fig. 4.3.1.).

The RDA shows that explanatory variables (distance from source, sub-catchment size and altitude) account for 34.3% of the total benthic macroinvertebrate metric variation. The eigenvalues of the first two axes are 0.255 and 0.071 (Fig. 4.3.1.).

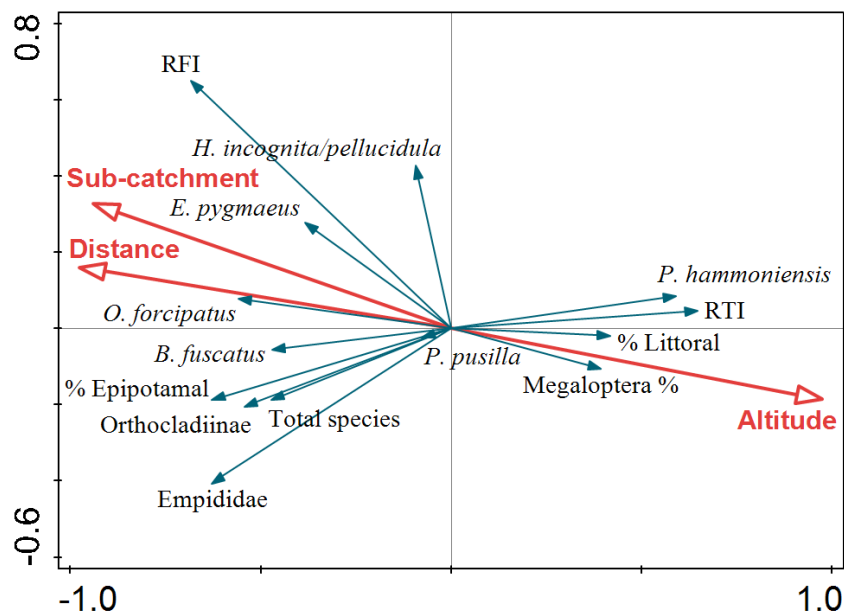


Figure 4.3.1. Redundancy analysis of benthic macroinvertebrate metrics significantly correlated to the longitudinal gradient. Arrow lengths on the ordination show the relative importance of variables, and their direction relative to each other. Abbreviations: RFI = River Fauna Index, RTI = Rhithron Type Index.

#### 4.3.2 Correlations between benthic macroinvertebrate metrics and anthropogenic stressors

Spearman's rank correlation coefficient (R) was further used to test the statistical relationship between benthic macroinvertebrate metrics and anthropogenic stressors from the three main stressor groups (water quality/nutrients, landuse, and hydromorphological quality modification (Tab. 4.3.2. – 4.3.5.).

Hydromorphological modification scores assessed by EN 15843:2010 give no significant correlations with diversity indices. Extensive landuse gives the greatest number of significant positive correlations with diversity indices, while urban landuse has a positive significant correlation with the Saprobity Index ( $R = 0.448$ ,  $p < 0.05$ ). However, the correlation between ammonium and the Saprobity Index is stronger ( $R = 0.515$ ,  $p < 0.05$ ) (Tab. 4.3.2.).

Table 4.3.2. Results of Spearman's rank correlation coefficient (R) for the relationship between benthic macroinvertebrate metrics and stressors. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Part 1 - diversity and Ecological Quality Ratio (EQR).

	Total individuals (N)	Total species (S)	Simpson Index	Shannon-Wiener Index	Margalef Index	Evenness	River Fauna Index	Saprobity Index	EQR
	r	r	r	r	r	r	r	r	r
<b>Water Quality</b>									
Water temperature (°C)	0.232	0.342	0.110	0.074	0.165	-0.055	<b>0.629</b>	0.098	-0.041
Dissolved oxygen (mgL <sup>-1</sup> )	0.119	0.162	0.356	0.202	0.131	0.168	0.107	<b>-0.569</b>	<b>0.566</b>
Oxygen saturation (%)	0.111	0.250	0.332	0.193	0.180	0.122	0.408	-0.397	0.425
Conductivity (µScm <sup>-1</sup> )	<b>0.519</b>	0.295	0.153	0.132	0.059	0.062	-0.251	0.019	-0.017
pH	0.170	0.213	0.157	0.070	0.116	-0.007	<b>0.466</b>	-0.371	0.402
Chemical Oxygen Demand (COD <sub>Mn</sub> , mgO <sub>2</sub> L <sup>-1</sup> )	-0.214	0.312	0.020	0.053	<b>0.476</b>	-0.036	-0.130	0.267	-0.286
Biological oxygen demand (BOD <sub>5</sub> , mg O <sub>2</sub> L <sup>-1</sup> )	-0.219	0.224	0.019	0.036	0.409	-0.051	-0.184	0.313	-0.333
Ammonium concentration (NH <sub>4</sub> <sup>+</sup> , mgNL <sup>-1</sup> )	0.270	-0.066	0.167	0.141	-0.187	0.171	-0.167	<b>0.515</b>	<b>-0.516</b>
Nitrites (NO <sub>2</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	0.026	-0.037	-0.154	-0.191	-0.213	-0.168	<b>0.827</b>	-0.044	0.114
Nitrates (NO <sub>3</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	0.436	<b>0.492</b>	0.234	0.212	0.231	0.096	0.186	0.362	-0.310
Kjeldahl Nitrogen (mgNL <sup>-1</sup> )	-0.005	-0.146	-0.249	-0.183	0.015	-0.236	-0.129	0.257	-0.286
Organic Nitrogen (mgNL <sup>-1</sup> )	-0.139	-0.121	-0.291	-0.227	0.134	-0.296	-0.151	0.008	-0.044
Total nitrogen (Σ N, mgNL <sup>-1</sup> )	0.303	0.360	0.051	0.017	0.164	-0.100	<b>0.533</b>	0.297	-0.232
Orthophosphates (PO <sub>4</sub> <sup>3-</sup> , mgPL <sup>-1</sup> )	<b>0.528</b>	0.279	-0.032	-0.010	-0.027	-0.099	0.424	0.421	-0.367
Total phosphorous (Σ P, mgPL <sup>-1</sup> )	0.095	-0.107	-0.188	-0.194	-0.181	-0.180	<b>0.534</b>	0.233	-0.180
<b>Landuse</b>									
% Near natural	-0.141	-0.283	-0.303	-0.170	-0.129	-0.122	-0.236	-0.153	0.101
% Intensive agriculture	0.187	-0.212	-0.074	-0.210	-0.287	-0.217	-0.056	-0.144	0.161
% Extensive agriculture	0.034	<b>0.567</b>	<b>0.457</b>	<b>0.489</b>	0.440	<b>0.453</b>	0.285	0.044	-0.038
% Total agriculture	0.107	0.144	0.237	0.102	0.013	0.083	0.163	0.019	0.031
% Urban	0.282	0.272	-0.052	-0.038	0.120	-0.114	0.353	<b>0.448</b>	-0.421
<b>EN 15843:2010 - 100 m scale</b>									
AVERAGE SCORE	-0.371	-0.039	0.254	0.231	0.072	0.244	-0.093	0.218	-0.199
Morphology	-0.423	-0.043	0.253	0.251	0.078	0.270	-0.039	0.142	-0.125
Flow	0.036	0.061	0.012	-0.061	0.061	-0.158	0.231	0.061	-0.036
Continuity	-0.029	-0.130	0.058	-0.029	-0.145	-0.029	0.289	-0.029	0.029
Channel	-0.220	-0.068	0.147	0.143	-0.006	0.153	-0.054	0.272	-0.230
Riparian zone	-0.337	-0.003	0.287	0.243	0.073	0.252	0.018	0.079	-0.067
Floodplain	-0.387	-0.118	0.106	0.122	-0.002	0.144	-0.009	0.106	-0.101
<b>EN 15843:2010 - 500 m scale</b>									
AVERAGE SCORE	-0.310	0.084	0.303	0.304	0.201	0.281	-0.130	0.141	-0.139
Morphology	-0.343	0.076	0.301	0.295	0.203	0.273	-0.096	0.109	-0.105
Flow	-0.150	0.128	0.237	0.172	0.191	0.090	0.098	-0.073	0.053
Continuity	-0.029	-0.130	0.058	-0.029	-0.145	-0.029	0.289	-0.029	0.029
Channel	-0.230	0.063	0.279	0.289	0.147	0.270	-0.150	0.201	-0.199
Riparian zone	-0.268	0.125	0.375	0.345	0.214	0.310	-0.050	0.094	-0.092
Floodplain	-0.380	-0.021	0.140	0.179	0.118	0.167	0.005	0.100	-0.092
<b>Habitat Modification Score</b>									
HMS Resectioned Bank Bed	-0.349	-0.087	0.072	0.095	0.042	0.113	-0.250	0.405	-0.398
HMS Reinforced Bank Bed	-0.258	-0.183	-0.018	-0.007	-0.120	0.032	-0.206	0.439	-0.422
HMS Realigned	-0.322	0.266	<b>0.492</b>	0.414	0.364	0.381	0.131	-0.186	0.204
HMS Realigned	-0.226	-0.010	0.199	0.247	0.007	0.267	-0.103	0.377	-0.363
<b>Habitat Quality Assessment</b>									
HQA flow type	0.230	0.194	-0.057	-0.050	0.108	-0.068	0.275	<b>-0.477</b>	<b>0.477</b>
HQA channel substrate	0.181	0.164	-0.005	0.059	0.046	0.062	0.057	-0.206	0.193
HQA channel features	-0.163	-0.361	-0.286	-0.236	-0.286	-0.169	-0.215	<b>-0.482</b>	<b>0.453</b>
HQA channel vegetation structure	0.228	0.325	0.293	0.291	0.280	0.269	0.152	-0.437	0.405
HQA bank vegetation structure	-0.093	<b>0.498</b>	0.203	0.250	<b>0.473</b>	0.205	0.362	<b>-0.449</b>	<b>0.451</b>
HQA channel vegetation	0.298	0.166	0.216	0.093	0.102	0.007	0.108	-0.333	0.371
HQA landuse	0.157	0.242	0.013	0.089	0.157	0.052	0.004	-0.084	0.048
HQA trees	0.188	0.082	-0.238	-0.192	-0.015	-0.200	0.377	-0.128	0.143
HQA special features	0.284	-0.107	-0.088	-0.083	-0.205	-0.029	-0.283	-0.077	0.058
<b>Riparian Quality Index</b>									
RQI Complexity subscore	0.171	0.344	0.029	0.075	0.236	0.042	<b>0.513</b>	-0.314	0.329
RQI Naturalness subscore	0.045	0.431	0.122	0.166	0.345	0.147	<b>0.570</b>	-0.282	0.305
RQI Continuity subscore	0.397	0.054	-0.189	-0.136	-0.092	-0.153	0.115	-0.335	0.318
Channel Substrate Index (CSI)	0.190	0.236	-0.063	-0.001	0.118	0.000	<b>0.488</b>	-0.050	0.084
Flow Regime Index (FRI)	0.333	<b>0.596</b>	0.368	0.371	0.374	0.254	0.273	-0.047	0.047
Channel Vegetation Index (CVI)	0.636	0.338	-0.066	-0.009	0.123	-0.112	-0.060	-0.167	0.141
Depth (average)	-0.049	0.297	0.129	0.247	0.289	0.196	-0.263	0.187	-0.249
Water velocity (average)	-0.250	-0.020	-0.235	-0.307	0.029	-0.350	<b>0.481</b>	0.232	-0.170
Water velocity (average)	0.247	0.277	0.198	0.198	0.120	0.189	0.423	<b>-0.471</b>	<b>0.460</b>



Stressors and benthic macroinvertebrate metrics with significant correlations from Tab. 4.3.2. are displayed as RDA in Fig. 4.3.2.

The RDA shows that explanatory variables (stressors) account for 100% of the variation. The eigenvalues of the first two axes are 0.435 and 0.267 (Fig. 4.3.2.).

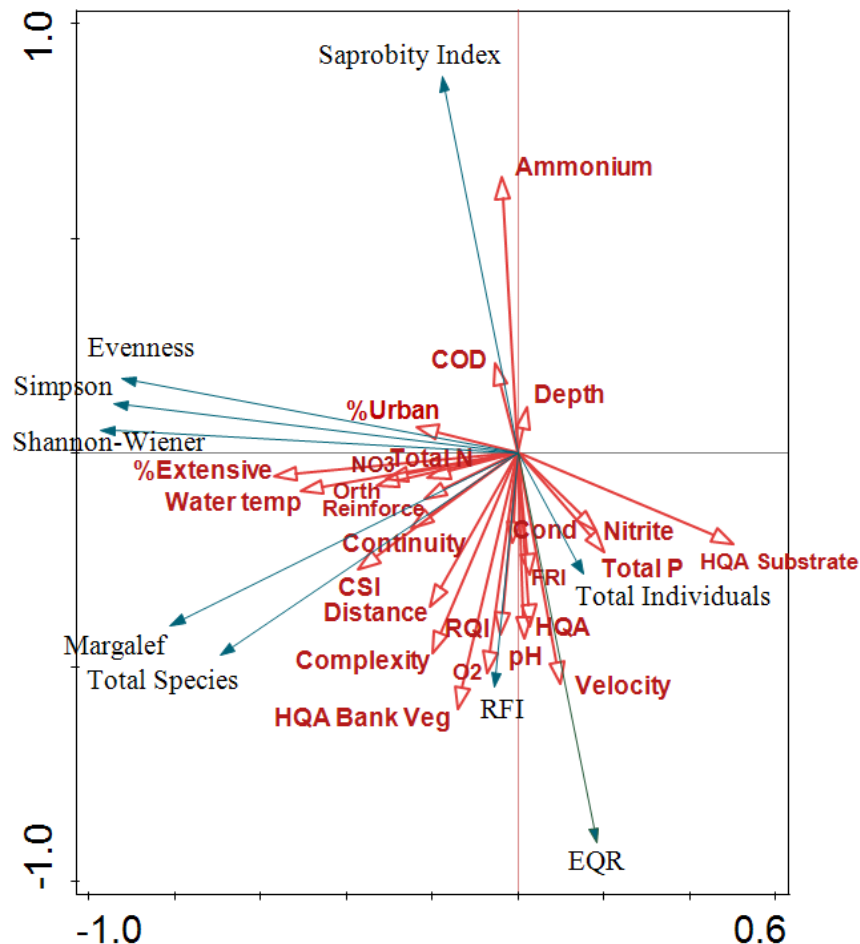


Figure 4.3.2. Redundancy analysis of significant relationships between benthic macroinvertebrate diversity metrics and stressors. Arrow lengths on the ordination show the relative importance of variables, and their direction relative to each other. Abbreviations: EQR = Ecological Quality Ratio, %Urban = urban landuse, %Extensive = extensive agriculture, RFI = River Fauna Index; HQA = Habitat Quality Assessment score, O2 = dissolved oxygen, RQI = Riparian Quality Index, HQA Bank Veg = Habitat Quality Assessment bank vegetation subscore, Complexity = Riparian Quality Index complexity subscore, CSI = Channel Substrate Index, Continuity = Riparian Quality Index continuity subscore, Reinforce = Habitat Modification reinforced Bank Bed subscore; Water temp = water temperature, Orth = orthophosphates, NO3 = nitrates, Total N = total nitrogen, COD = chemical oxygen demand, Cond = conductivity, FRI = Flow Regime Index, Total P = total phosphorous, HQA Substrate = Habitat Quality Assessment channel substrate subscore.

For functional and sensitivity metrics, the metric Average score per Taxon (ASPT) gives the greatest number of significant negative correlations with nutrient enrichment, and a strong negative correlation with urban landuse. The share of taxa preferring akal substrate metric gives the greatest number of significant negative correlations to RHS Habitat modification scores. The HQA bank vegetation structure subscore gives the overall greatest number of significant correlations with selected metrics (Tab. 4.3.3.).

Table 4.3.3. Results of Spearman's rank correlation coefficient (R) for the relationship between benthic macroinvertebrate metrics and stressors. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Part 2 – selected functional and sensitivity metrics.

	BMWP Score (Biological Monitoring Working Party)	ASPT (Average score per Taxon)	BBI (Belgian Biotic Index)	IBE AQEM (Altered Indice Biotico Estesio)	MTS (Mayfly Total Score)	r-dominance	(%) Type Pelal	(%) Type Psammal	(%) Type Akal	(%) Type Lithal	(%) Type Akal+Lithal+ Psammal
	r	r	r	r	r	r	r	r	r	r	r
<b>Water Quality</b>											
Water temperature (°C)	0.030	-0.372	0.069	0.045	0.367	0.278	-0.108	-0.242	-0.107	0.358	0.083
Dissolved oxygen (mgL <sup>-1</sup> )	0.387	0.344	<b>0.481</b>	0.268	0.175	<b>-0.446</b>	<b>-0.520</b>	-0.384	0.429	0.331	0.354
Oxygen saturation (%)	0.306	0.136	<b>0.472</b>	0.238	0.324	-0.221	-0.400	-0.356	0.269	0.315	0.227
Conductivity (µScm <sup>-1</sup> )	0.173	-0.118	0.127	0.394	0.153	-0.070	-0.208	-0.166	0.065	0.244	0.194
pH	0.150	0.024	0.312	0.108	0.221	-0.263	-0.353	-0.279	0.263	0.326	0.281
Chemical Oxygen Demand (COD <sub>Mn</sub> , mgO <sub>2</sub> L <sup>-1</sup> )	0.330	-0.153	-0.033	0.309	0.052	<b>0.664</b>	0.112	0.064	-0.354	0.040	-0.091
Biological oxygen demand (BOD <sub>5</sub> , mg O <sub>2</sub> L <sup>-1</sup> )	0.348	-0.149	-0.074	0.228	0.051	<b>0.636</b>	0.184	0.131	-0.418	-0.076	-0.244
Ammonium concentration (NH <sub>4</sub> <sup>+</sup> , mgNL <sup>-1</sup> )	-0.020	-0.351	-0.133	-0.159	-0.195	-0.035	0.390	-0.016	-0.384	-0.185	<b>-0.680</b>
Nitrites (NO <sub>2</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	-0.417	-0.169	0.182	-0.190	0.280	-0.069	-0.008	0.117	0.143	-0.082	0.060
Nitrates (NO <sub>3</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	0.131	<b>-0.491</b>	0.108	0.359	0.366	0.312	0.053	-0.118	-0.273	0.204	-0.110
Kjeldahl Nitrogen (mgNL <sup>-1</sup> )	0.075	-0.235	<b>-0.482</b>	-0.169	-0.357	0.217	0.362	0.230	-0.348	-0.230	-0.321
Organic Nitrogen (mgNL <sup>-1</sup> )	0.159	-0.100	-0.411	-0.080	-0.314	0.295	0.124	0.197	-0.178	-0.106	0.017
Total nitrogen (Σ N, mgNL <sup>-1</sup> )	0.016	<b>-0.490</b>	0.009	0.150	0.377	0.400	0.056	-0.015	-0.277	0.141	-0.075
Orthophosphates (PO <sub>4</sub> <sup>3-</sup> , mgPL <sup>-1</sup> )	-0.170	<b>-0.598</b>	-0.096	0.056	0.089	0.192	0.133	-0.164	-0.302	0.235	-0.192
Total phosphorous (Σ P, mgPL <sup>-1</sup> )	-0.401	-0.296	-0.055	-0.242	-0.024	0.119	0.023	-0.029	-0.193	0.069	-0.124
<b>Landuse</b>											
% Near natural	-0.162	0.169	-0.331	-0.280	<b>-0.478</b>	-0.165	0.094	0.018	0.122	-0.047	0.059
% Intensive agriculture	0.064	0.003	0.130	-0.079	0.150	-0.081	-0.278	0.001	0.130	-0.017	-0.018
% Extensive agriculture	0.100	-0.015	0.205	0.392	0.233	-0.072	0.012	-0.271	0.043	0.343	0.287
% Total agriculture	0.102	-0.034	0.354	0.177	0.443	0.014	-0.153	0.027	0.011	-0.019	-0.025
% Urban	-0.098	<b>-0.668</b>	-0.318	0.039	-0.078	0.392	0.215	-0.053	-0.379	0.195	-0.105
<b>EN 15843:2010 - 100 m scale</b>											
AVERAGE SCORE	-0.024	-0.041	-0.138	-0.136	0.173	0.149	0.148	-0.063	-0.373	-0.001	-0.241
Morphology	-0.057	0.045	-0.113	-0.177	0.228	0.100	0.083	-0.144	-0.273	0.091	-0.154
Flow	0.109	-0.231	-0.197	-0.209	0.171	0.328	0.012	0.109	-0.304	-0.036	-0.158
Continuity	-0.058	-0.058	-0.050	<b>-0.468</b>	-0.014	0.116	-0.058	0.000	-0.173	-0.058	-0.202
Channel	-0.080	-0.145	-0.158	-0.222	0.096	0.156	0.239	0.072	-0.434	-0.134	-0.396
Riparian zone	-0.029	-0.005	-0.074	-0.080	0.196	0.019	-0.021	-0.226	-0.202	0.196	-0.018
Floodplain	-0.147	0.084	-0.213	-0.263	0.128	0.114	0.035	-0.173	-0.244	0.143	-0.062
<b>EN 15843:2010 - 500 m scale</b>											
AVERAGE SCORE	0.069	-0.062	-0.076	-0.014	0.127	0.096	0.083	-0.069	-0.317	0.058	-0.102
Morphology	0.080	0.000	-0.052	-0.002	0.183	0.094	0.034	-0.132	-0.260	0.126	-0.043
Flow	0.192	-0.006	-0.051	-0.132	0.225	0.137	-0.082	-0.061	-0.149	0.073	-0.023
Continuity	-0.058	-0.058	-0.050	<b>-0.468</b>	-0.014	0.116	-0.058	0.000	-0.173	-0.058	-0.202
Channel	0.033	-0.119	-0.119	-0.104	0.043	0.071	0.187	0.010	-0.385	-0.057	-0.245
Riparian zone	0.154	-0.014	-0.011	0.079	0.227	0.052	-0.023	-0.239	-0.210	0.230	0.011
Floodplain	-0.069	0.043	-0.127	-0.105	0.179	0.084	0.028	-0.135	-0.199	0.163	0.024
<b>Habitat Modification Score</b>											
HMS Resectioned Bank Bed	-0.244	-0.284	-0.323	-0.091	-0.169	0.021	0.382	0.139	<b>-0.447</b>	-0.171	-0.287
HMS Reinforced Bank Bed	0.155	0.052	0.116	0.166	<b>0.462</b>	0.018	-0.302	-0.433	0.127	<b>0.483</b>	0.275
HMS Realigned	-0.182	-0.257	-0.183	-0.132	-0.034	-0.007	0.315	0.048	<b>-0.452</b>	-0.075	-0.254

Table 4.3.3. (Continued). Results of Spearman's rank correlation coefficient (R) for the relationship between benthic macroinvertebrate metrics and stressors. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Part 2 – selected functional and sensitivity metrics.

	BMWP Score (Biological Monitoring Working Party)	ASPT (Average score per Taxon)	BBi (Belgian Biotic Index)	IBE AQEM (Altered Index Biotico Esteso)	MTS (Mayfly Total Score)	r-dominance	(%) Type Pelal	(%) Type Psammal	(%) Type Akal	(%) Type Lithal	(%) Type Akal+Lithal+ Psammal
	r	r	r	r	r	r	r	r	r	r	r
<b>Habitat Quality Assessment</b>	0.112	0.194	0.415	0.163	0.090	-0.145	-0.405	-0.238	<b>0.573</b>	0.270	0.316
HQA flow type	0.062	0.058	0.222	0.190	-0.112	-0.246	-0.135	-0.358	0.311	0.309	0.103
HQA channel substrate	-0.098	<b>0.579</b>	0.259	-0.188	-0.072	-0.396	-0.233	0.133	0.442	-0.201	0.228
HQA channel features	0.307	0.390	0.426	0.247	0.085	-0.227	-0.398	<b>-0.498</b>	<b>0.498</b>	0.428	0.298
HQA bank vegetation structure	0.140	0.086	<b>0.482</b>	0.314	0.336	0.089	<b>-0.485</b>	-0.311	<b>0.505</b>	<b>0.511</b>	<b>0.621</b>
HQA channel vegetation	0.306	-0.077	0.300	0.347	0.242	-0.263	-0.389	-0.248	0.163	0.244	0.102
HQA landuse	0.202	0.167	0.173	0.156	0.140	0.084	-0.073	-0.100	0.253	0.139	0.117
HQA trees	-0.073	-0.006	0.180	-0.047	0.025	0.050	-0.038	0.098	0.253	-0.048	0.040
HQA special features	0.005	0.131	-0.052	-0.085	-0.196	-0.223	0.016	-0.052	0.138	-0.072	-0.197
<b>Riparian Quality Index</b>	-0.024	-0.005	0.406	0.198	0.226	0.021	-0.317	-0.189	<b>0.460</b>	0.290	0.341
RQI Complexity subscore	-0.041	-0.040	<b>0.455</b>	0.298	0.231	0.017	-0.285	-0.192	0.434	0.326	0.424
RQI Naturalness subscore	-0.095	0.026	0.124	-0.019	-0.069	-0.214	-0.308	-0.116	<b>0.458</b>	0.188	0.302
RQI Continuity subscore	-0.207	-0.227	0.155	0.082	-0.017	-0.021	-0.013	-0.074	0.188	0.128	0.073
Channel Substrate Index (CSI)	0.146	-0.038	0.163	0.289	0.366	0.069	-0.303	<b>-0.635</b>	0.069	<b>0.695</b>	0.281
Flow Regime Index (FRI)	0.077	-0.104	0.099	0.373	-0.129	-0.145	-0.310	-0.313	0.181	0.396	0.343
Channel Vegetation Index (CVI)	0.146	-0.032	-0.179	0.163	-0.046	0.177	0.132	-0.238	-0.041	0.291	0.160
Depth (average)	0.115	-0.193	-0.009	0.093	0.243	0.376	0.310	<b>0.489</b>	-0.227	-0.359	-0.092
Water velocity (average)	-0.191	0.035	0.407	0.100	0.052	-0.355	<b>-0.636</b>	<b>-0.686</b>	<b>0.472</b>	<b>0.698</b>	<b>0.493</b>

Stressors and benthic macroinvertebrate functional and sensitivity metrics with statistically significant correlations from Tab. 4.3.3. are displayed as RDA in Fig. 4.3.3.

The RDA shows that explanatory variables account for 100% of the variation. Total variation is 55.38. The eigenvalues of the first two axes are 0.586 and 0.236 (Fig. 4.3.3.).

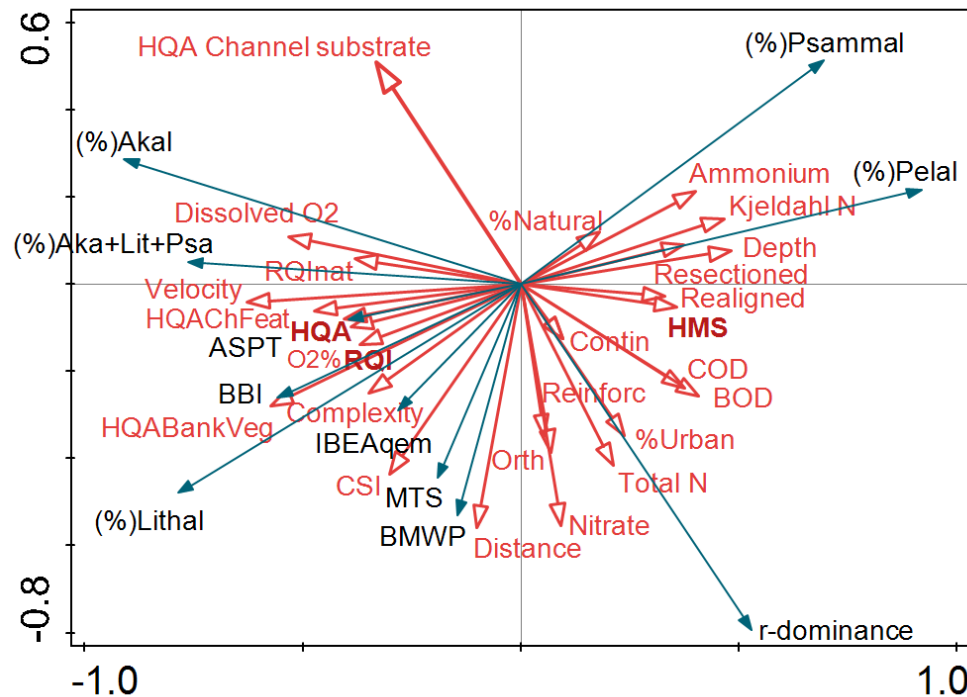


Figure 4.3.3. Redundancy analysis of significant relationships between benthic macroinvertebrate functional and sensitivity metrics, and stressors. Arrow lengths on the ordination show the relative importance of variables, and their direction relative to each other. Abbreviations: (%)Aka+Lit+Psa = share of taxa with a preference for akal, lithal, and sand, ASPT = Average Score per taxon, BBI = Belgian Biotic Index, IBEAqem = Altered Indice Biotico Estesio, MTS = Mayfly Total Score, BMWP = Biological Monitoring Working Party, %Urban = urban landuse, %Natural = natural land cover, HQA = Habitat Quality Assessment, RQI = Riparian Quality assessment, HMS = Habitat Modification Score, Resectioned = HMS Resectioned bank bed subscore, Realigned = HMS Realigned bank bed subscore, Reinforce = HMS Reinforced bank bed subscore, RQInat = Riparian Quality Index naturalness subscore, HQAChFeat = HQA channel features subscore, O2% = oxygen saturation, HQABankVeg = HQA bank vegetation subscore, Complexity = RQI complexity subscore, Orth = orthophosphates, CSI = Channel Substrate Index, Total N = total nitrogen, Contin = EN 15843:2010 continuity score, COD = chemical oxygen demand, BOD = biological oxygen demand.

For feeding groups, the strongest significant positive correlation is found between the predators and the Channel Substrate Index (CSI) ( $R = 0.706$ ,  $p < 0.05$ ). The strongest significant negative correlation is found between the shredder feeding group and share of urban landuse ( $R = -0.686$ ,  $p < 0.05$ ).

Hydromorphological modification scores both of EN 15843:2010 and RHS are significantly positively correlated with share of miners and share of predator feeding group (Tab. 4.3.4.).

Table 4.3.4. Results of Spearman's rank correlation coefficient (R) for the relationship between benthic macroinvertebrate metrics and stressors. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Part 3 - Feeding groups. Abbreviations: GRA = grazers/scrapers, MIN = miners, XYL = xylophagous taxa, SHR = shredders, DET = gatherers/collectors, AFIL = active filter feeders, PFIL = passive filter feeders, PRE = predators, PAR = parasites, OTH = other feeding types.

	GRA	MIN	XYL	SHR	DET	AFIL	PFIL	PRE	PAR	OTH
	r	r	r	r	r	r	r	r	r	r
<b>Water Quality</b>										
Water temperature (°C)	0.238	0.037	-0.348	-0.270	-0.059	0.014	0.216	0.063	0.119	0.272
Dissolved oxygen (mgL <sup>-1</sup> )	0.426	-0.069	-0.346	0.401	<b>-0.549</b>	-0.128	0.142	0.028	-0.120	<b>0.500</b>
Oxygen saturation (%)	0.359	0.001	-0.334	0.251	-0.425	-0.167	0.231	0.099	0.000	<b>0.583</b>
Conductivity (µScm <sup>-1</sup> )	0.213	-0.417	<b>-0.506</b>	0.043	-0.188	0.354	0.129	-0.121	0.080	-0.270
pH	0.309	-0.022	-0.229	0.108	-0.350	-0.211	0.143	-0.101	-0.060	<b>0.516</b>
Chemical Oxygen Demand (COD <sub>Mn</sub> , mgO <sub>2</sub> L <sup>-1</sup> )	0.097	0.283	<b>0.483</b>	-0.347	0.215	0.294	0.199	0.218	-0.060	-0.194
Biological oxygen demand (BOD <sub>5</sub> , mg O <sub>2</sub> L <sup>-1</sup> )	0.020	0.238	<b>0.472</b>	-0.369	0.285	0.308	0.167	0.201	-0.165	-0.278
Ammonium concentration (NH <sub>4</sub> <sup>+</sup> , mgNL <sup>-1</sup> )	-0.229	-0.271	-0.091	<b>-0.466</b>	0.386	0.119	0.212	0.018	-0.359	<b>-0.629</b>
Nitrites (NO <sub>2</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	-0.180	-0.302	-0.093	0.081	-0.059	-0.121	-0.095	-0.252	0.319	0.245
Nitrates (NO <sub>3</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	0.099	-0.238	-0.427	-0.346	0.122	0.298	0.312	-0.017	0.080	-0.211
Kjeldahl Nitrogen (mgNL <sup>-1</sup> )	-0.259	0.363	0.319	-0.418	0.389	-0.014	-0.017	-0.065	-0.199	-0.112
Organic Nitrogen (mgNL <sup>-1</sup> )	-0.105	<b>0.549</b>	0.365	-0.188	0.196	-0.028	-0.115	-0.124	-0.080	0.092
Total nitrogen (Σ N, mgNL <sup>-1</sup> )	0.026	-0.082	-0.331	-0.400	0.142	0.254	0.215	-0.152	0.160	-0.021
Orthophosphates (PO <sub>4</sub> <sup>3-</sup> , mgPL <sup>-1</sup> )	0.002	-0.198	-0.243	<b>-0.536</b>	0.162	0.189	0.312	-0.071	0.080	-0.204
Total phosphorous (Σ P, mgPL <sup>-1</sup> )	-0.040	-0.123	0.165	-0.338	0.093	0.142	0.014	-0.230	-0.080	-0.177
<b>Landuse</b>										
% Near natural	-0.114	0.416	0.368	0.062	0.031	-0.428	-0.064	0.034	-0.020	0.161
% Intensive agriculture	0.011	<b>-0.497</b>	-0.182	0.284	-0.223	0.434	-0.238	-0.224	-0.139	-0.389
% Extensive agriculture	0.333	0.192	-0.388	-0.265	-0.052	-0.248	0.394	0.262	0.219	<b>0.546</b>
% Total agriculture	0.076	<b>-0.538</b>	-0.322	0.132	-0.100	0.345	-0.082	-0.122	-0.080	-0.191
% Urban	-0.068	0.173	-0.068	<b>-0.686</b>	0.300	0.239	0.238	-0.066	0.060	-0.158
<b>EN 15843:2010 - 100 m scale</b>										
AVERAGE SCORE	0.032	<b>0.596</b>	-0.091	-0.187	0.182	-0.050	0.074	<b>0.616</b>	0.020	0.314
Morphology	0.133	<b>0.636</b>	-0.104	-0.160	0.106	-0.128	0.090	<b>0.627</b>	0.020	0.395
Flow	-0.036	0.152	-0.176	-0.182	0.134	0.061	-0.036	0.061	-0.096	0.095
Continuity	0.000	0.016	-0.140	-0.087	0.029	-0.116	0.000	0.000	-0.076	-0.016
Channel	-0.133	0.393	-0.009	-0.189	0.314	-0.121	-0.018	0.386	-0.101	0.130
Riparian zone	0.201	<b>0.609</b>	-0.193	-0.151	-0.015	-0.023	0.115	<b>0.634</b>	0.101	<b>0.470</b>
Floodplain	0.148	<b>0.684</b>	-0.082	-0.179	0.047	-0.119	0.100	<b>0.568</b>	0.080	0.402
<b>EN 15843:2010 - 500 m scale</b>										
AVERAGE SCORE	0.145	<b>0.572</b>	-0.064	-0.195	0.129	-0.097	0.096	<b>0.539</b>	0.040	0.363
Morphology	0.211	<b>0.579</b>	-0.089	-0.173	0.069	-0.069	0.091	<b>0.556</b>	0.020	0.404
Flow	0.196	0.270	-0.208	-0.128	-0.032	-0.109	0.139	0.279	-0.114	0.282
Continuity	0.000	0.016	-0.140	-0.087	0.029	-0.116	0.000	0.000	-0.076	-0.016
Channel	0.021	<b>0.476</b>	-0.047	-0.237	0.236	-0.206	0.104	<b>0.445</b>	-0.060	0.256
Riparian zone	0.305	<b>0.570</b>	-0.245	-0.201	-0.010	0.024	0.154	<b>0.602</b>	0.101	<b>0.453</b>
Floodplain	0.223	<b>0.592</b>	-0.010	-0.199	0.046	-0.075	0.034	<b>0.490</b>	0.060	0.417
<b>Habitat Modification Score</b>										
HMS Resectioned Bank Bed	-0.120	<b>0.586</b>	0.137	-0.395	0.374	0.068	-0.074	<b>0.506</b>	-0.060	0.166
HMS Reinforced Bank Bed	-0.262	0.382	0.232	-0.324	0.356	0.120	-0.125	<b>0.459</b>	-0.020	0.072
HMS Realigned	-0.062	<b>0.480</b>	-0.033	-0.377	0.329	-0.103	0.034	<b>0.456</b>	0.181	0.233
<b>Habitat Quality Assessment</b>										
HQA flow type	0.222	-0.420	0.123	0.383	-0.416	-0.227	0.158	-0.380	-0.100	-0.053
HQA channel substrate	0.156	0.039	0.162	0.108	-0.252	-0.262	0.347	0.193	0.187	0.181
HQA channel features	-0.005	-0.274	0.233	<b>0.674</b>	-0.327	-0.300	-0.312	-0.237	0.145	0.099
HQA channel vegetation	<b>0.490</b>	-0.159	-0.224	0.205	<b>-0.448</b>	-0.369	<b>0.457</b>	-0.137	-0.206	0.168
HQA bank vegetation structure	<b>0.514</b>	-0.011	0.081	0.246	-0.415	-0.233	0.189	-0.080	0.213	0.269
HQA channel vegetation	0.212	0.028	-0.267	0.234	-0.349	0.125	0.170	0.038	-0.020	0.317
HQA landuse	0.214	-0.369	-0.065	0.025	-0.127	0.022	0.167	-0.101	0.124	-0.242
HQA trees	-0.087	<b>-0.491</b>	0.218	0.092	-0.036	-0.149	-0.010	<b>-0.517</b>	-0.040	-0.246
HQA special features	-0.164	-0.262	-0.052	0.123	-0.023	-0.102	0.135	-0.182	-0.248	-0.365
<b>Riparian Quality Index</b>										
RQI Complexity subscore	0.245	-0.368	0.077	0.208	-0.308	-0.217	0.202	-0.308	0.099	0.006
RQI Naturalness subscore	0.269	-0.240	0.141	0.154	-0.275	-0.244	0.192	-0.209	0.159	0.174
RQI Continuity subscore	0.117	<b>-0.482</b>	-0.053	0.243	-0.305	-0.142	0.043	<b>-0.534</b>	-0.040	-0.261
RQI Substrate Index (CSI)	-0.052	-0.165	0.214	-0.063	-0.009	-0.305	0.212	-0.295	-0.021	0.006
Channel Substrate Index (CSI)	<b>0.594</b>	0.117	<b>-0.531</b>	-0.277	-0.321	-0.150	<b>0.706</b>	0.299	-0.060	0.317
Flow Regime Index (FRI)	0.279	-0.190	-0.176	-0.020	-0.329	-0.018	0.430	-0.264	0.060	-0.040
Channel Vegetation Index (CVI)	0.258	0.328	-0.086	-0.332	0.056	-0.113	0.318	0.341	0.100	0.067
Depth (average)	-0.331	-0.043	0.185	-0.162	0.311	0.413	-0.424	-0.157	0.338	0.189
Water velocity (average)	<b>0.572</b>	0.036	-0.085	0.137	<b>-0.642</b>	-0.371	<b>0.582</b>	0.009	0.020	0.315

Stressors and benthic macroinvertebrate feeding groups with statistically significant correlations from Tab. 4.3.4. are displayed as RDA in Fig. 4.3.4.

The RDA shows that explanatory variables account for 100% of the variation. Total variation is 57.56. The eigenvalues of the first two axes are 0.469 and 0.428 (Fig. 4.3.4.).

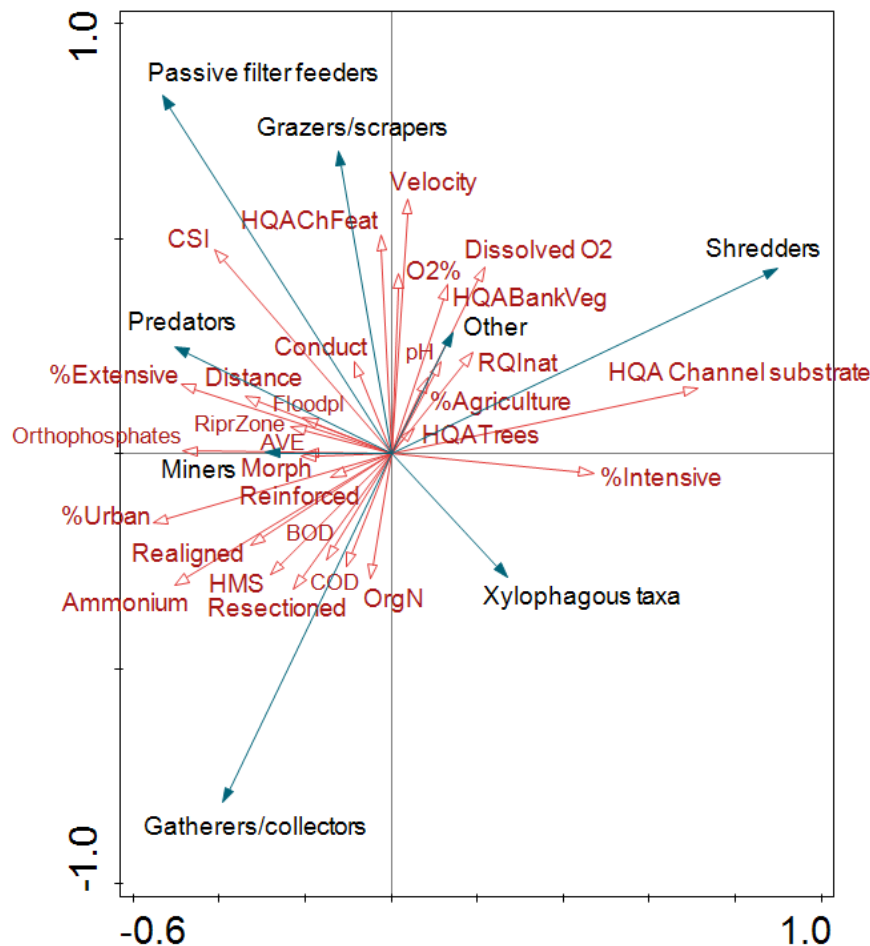


Figure 4.3.4. Redundancy analysis of significant relationships between benthic macroinvertebrate feeding groups and stressors. Arrow lengths on the ordination show the relative importance of variables, and their direction relative to each other. Significant correlations with EN 15843:2010 500 m scores are not shown due high level of correspondence with 100 m scores. Abbreviations: AVE = EN 15843:2010 100 m average score; RiprZone = EN 15843:2010 riparian zone modification, Morpho = EN 15843:2010 morphological modification, Floodpl = EN 15843:2010 modification of floodplain, HMS = Habitat Modification Score, Reinforced = HMS Reinforced bank bed subscore, Realigned = HMS Realigned bank bed subscore, Resectioned = HMS Resectioned bank bed subscore, %Urban = urban landuse, %Extensive = extensive agriculture, %Intensive = intensive agriculture, Conduct = conductivity, O2% = oxygen saturation, BOD = biological oxygen demand, COD = chemical oxygen demand, OrgN = organic nitrogen, CSI = Channel Substrate Index, HQAChFeat = Habitat Quality Assessment channel features subscore, HQABankVeg = Habitat Quality Assessment bank vegetation subscore, RQInat = Riparian Quality Index naturalness subscore.

For functional metrics zonation and current preference, the strongest significant positive correlation is found between % Type RP (rheophile taxa) and water velocity ( $R = 0.749$ ,  $p < 0.05$ ). The strongest significant negative correlation is between the Rhithron Type Index and % Urban landuse ( $R = -0.794$ ,  $p < 0.05$ ) (Table 4.3.5.). The Rheoindex responds to the greatest number of RHS modification and quality scores.

Table 4.3.5. Results of Spearman's rank correlation coefficient (R) for the relationship between benthic macroinvertebrate metrics and stressors. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Part 4 - zonation and current preference.

	(%) Hypocrenal	(%) Epirhithral	(%) Metarhithral	(%) Hyporhithral	(%) Epipotamal	(%) Metapotamal	(%) Littoral	(%) Type RP (rheophile taxa)	Rheoindex (Banning, with abundance)	Rhithron Type Index
	r	r	r	r	r	r	r	r	r	r
<b>Water Quality</b>										
Water temperature (°C)	-0.290	-0.286	-0.123	0.177	0.233	0.063	-0.171	0.154	-0.007	-0.395
Dissolved oxygen (mgL <sup>-1</sup> )	<b>0.556</b>	0.210	0.346	0.374	0.114	-0.424	-0.410	0.382	0.378	0.344
Oxygen saturation (%)	0.335	0.111	0.151	0.290	0.196	-0.333	-0.328	0.306	0.202	0.123
Conductivity (µS/cm)	0.002	0.077	0.254	0.363	0.116	-0.129	-0.302	0.236	0.223	-0.060
pH	0.226	-0.012	0.160	0.231	0.069	-0.246	-0.294	0.304	0.259	-0.049
Chemical Oxygen Demand (COD <sub>Mn</sub> , mgO <sub>2</sub> L <sup>-1</sup> )	-0.228	-0.362	-0.286	0.122	0.361	<b>0.495</b>	-0.055	-0.068	-0.071	-0.271
Biological oxygen demand (BOD <sub>5</sub> , mg O <sub>2</sub> L <sup>-1</sup> )	-0.243	-0.395	-0.371	0.038	0.277	<b>0.544</b>	0.033	-0.162	-0.152	-0.282
Ammonium concentration (NH <sub>4</sub> <sup>+</sup> , mgNL <sup>-1</sup> )	-0.431	<b>-0.506</b>	-0.304	-0.095	-0.104	<b>0.618</b>	0.183	-0.371	-0.369	-0.248
Nitrites (NO <sub>2</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	-0.104	-0.001	-0.007	-0.047	0.060	-0.118	-0.023	0.095	0.035	-0.356
Nitrates (NO <sub>3</sub> <sup>-</sup> , mgNL <sup>-1</sup> )	-0.431	-0.260	-0.099	0.283	0.282	0.229	-0.194	0.012	-0.096	<b>-0.512</b>
Kjeldahl Nitrogen (mgNL <sup>-1</sup> )	-0.300	-0.312	<b>-0.488</b>	<b>-0.464</b>	-0.191	0.402	0.338	-0.295	-0.297	-0.364
Organic Nitrogen (mgNL <sup>-1</sup> )	-0.072	-0.070	-0.317	-0.321	-0.110	0.145	0.200	-0.106	-0.102	-0.218
Total nitrogen (Σ N, mgNL <sup>-1</sup> )	<b>-0.476</b>	-0.342	-0.229	0.164	0.317	0.267	-0.172	0.054	-0.062	<b>-0.734</b>
Orthophosphates (PO <sub>4</sub> <sup>3-</sup> , mgPL <sup>-1</sup> )	<b>-0.577</b>	<b>-0.445</b>	-0.187	0.088	0.144	0.378	-0.076	0.023	-0.122	<b>-0.608</b>
Total phosphorous (Σ P, mgPL <sup>-1</sup> )	-0.369	-0.363	-0.069	0.113	0.119	0.344	-0.105	0.077	0.018	-0.442
<b>Landuse</b>										
% Near natural	0.145	0.179	-0.012	-0.397	-0.335	-0.104	0.282	-0.058	-0.042	0.302
% Intensive agriculture	0.211	0.128	0.222	0.244	-0.103	-0.117	-0.079	0.145	0.202	0.155
% Extensive agriculture	-0.160	-0.262	-0.012	0.208	0.434	0.027	-0.373	0.130	0.066	-0.295
% Total agriculture	0.020	-0.009	0.153	0.363	0.148	-0.062	-0.212	0.059	0.093	-0.091
% Urban	<b>-0.651</b>	<b>-0.604</b>	-0.330	0.014	0.241	<b>0.597</b>	-0.058	-0.063	-0.127	<b>-0.794</b>
<b>EN 15843:2010 - 100 m scale</b>										
AVERAGE SCORE	-0.175	-0.004	-0.272	-0.143	0.060	-0.014	0.178	-0.269	-0.374	0.193
Morphology	-0.115	0.000	-0.194	-0.087	0.091	-0.051	0.105	-0.182	-0.286	0.237
Flow	-0.206	-0.182	-0.279	-0.134	-0.085	0.061	0.158	-0.109	-0.134	-0.182
Continuity	-0.087	-0.260	-0.173	-0.029	-0.058	0.116	0.058	-0.029	-0.029	0.029
Channel	-0.272	0.033	-0.251	-0.235	-0.168	-0.004	0.344	-0.395	<b>-0.474</b>	0.163
Riparian zone	-0.068	-0.043	-0.164	-0.004	0.187	-0.067	-0.023	-0.063	-0.178	0.179
Floodplain	-0.107	-0.001	-0.186	-0.104	0.097	-0.063	0.077	-0.079	-0.195	0.209
<b>EN 15843:2010 - 500 m scale</b>										
AVERAGE SCORE	-0.120	-0.077	-0.252	-0.078	0.100	-0.017	0.058	-0.180	-0.266	0.139
Morphology	-0.081	-0.062	-0.201	-0.018	0.142	-0.045	0.007	-0.127	-0.219	0.159
Flow	0.019	-0.257	-0.251	-0.017	0.081	0.047	-0.049	0.016	0.008	-0.033
Continuity	-0.087	-0.260	-0.173	-0.029	-0.058	0.116	0.058	-0.029	-0.029	0.029
Channel	-0.185	-0.107	-0.310	-0.184	-0.052	0.027	0.185	-0.299	-0.364	0.116
Riparian zone	-0.074	-0.132	-0.200	0.064	0.271	-0.019	-0.115	-0.037	-0.157	0.098
Floodplain	-0.099	-0.072	-0.165	-0.041	0.140	-0.041	-0.006	-0.053	-0.151	0.105
<b>Habitat Modification Score</b>										
HMS Resectioned Bank Bed	-0.332	-0.075	-0.356	-0.361	-0.160	0.120	0.392	<b>-0.456</b>	<b>-0.520</b>	0.010
HMS Reinforced Bank Bed	0.147	-0.024	0.159	0.324	0.333	-0.087	-0.322	0.199	0.154	0.154
HMS Realigned	-0.363	-0.175	-0.363	-0.230	0.000	0.089	0.199	-0.356	<b>-0.452</b>	-0.014

Table 4.3.5. (Continued). Results of Spearman's rank correlation coefficient (R) for the relationship between benthic macroinvertebrate metrics and stressors. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Part 4 - zonation and current preference.

	(%) Hypocrenal	(%) Epirhithral	(%) Metarhithral	(%) Hyporhithral	(%) Epipotamal	(%) Metapotamal	(%) Littoral	(%) Type RP (rheophile taxa)	Rheoindex (Banning, with abundance)	Rhithron Type Index
	r	r	r	r	r	r	r	r	r	r
<b>Habitat Quality Assessment</b>	0.404	0.139	<b>0.503</b>	0.367	0.057	-0.105	-0.325	<b>0.454</b>	<b>0.520</b>	0.120
HQA flow type	0.203	0.079	0.203	0.067	0.064	-0.034	-0.100	0.221	0.150	0.253
HQA channel substrate	<b>0.628</b>	<b>0.614</b>	0.339	-0.064	-0.341	<b>-0.645</b>	0.086	0.158	0.218	<b>0.674</b>
HQA channel features	0.382	-0.031	0.403	<b>0.451</b>	0.266	-0.046	<b>-0.522</b>	<b>0.492</b>	<b>0.494</b>	0.135
HQA bank vegetation structure	0.300	-0.012	<b>0.490</b>	<b>0.597</b>	0.436	-0.084	<b>-0.573</b>	<b>0.537</b>	<b>0.588</b>	0.048
HQA channel vegetation	0.279	0.232	0.172	0.222	0.092	-0.266	-0.262	0.282	0.194	0.061
HQA landuse	0.083	-0.201	0.141	0.215	0.217	0.175	-0.238	0.245	0.301	-0.063
HQA trees	0.026	-0.081	0.174	0.085	-0.049	0.123	-0.055	0.145	0.224	-0.228
HQA special features	0.121	0.154	0.194	-0.053	-0.269	0.031	0.112	-0.016	0.066	0.190
<b>Riparian Quality Index</b>	0.170	-0.059	0.382	0.386	0.272	0.042	-0.411	<b>0.460</b>	<b>0.480</b>	-0.159
RQI Complexity subscore	0.126	-0.093	0.370	0.414	0.364	0.045	<b>-0.449</b>	0.416	0.436	-0.195
RQI Naturalness subscore	0.238	0.065	0.419	0.238	-0.090	-0.075	-0.259	0.412	<b>0.497</b>	-0.032
RQI Continuity subscore	-0.112	-0.120	0.158	0.085	0.066	0.188	-0.122	0.143	0.143	-0.328
Channel Substrate Index (CSI)	-0.074	-0.291	0.128	<b>0.460</b>	0.428	0.066	<b>-0.520</b>	0.442	0.308	-0.186
Flow Regime Index (FRI)	0.097	0.031	0.230	0.285	0.112	-0.089	-0.389	<b>0.470</b>	0.397	-0.173
Channel Vegetation Index (CVI)	-0.137	-0.296	-0.147	0.032	0.217	0.220	-0.097	0.014	-0.019	-0.084
Depth (average)	-0.287	-0.149	-0.424	-0.244	0.129	0.099	0.229	-0.304	-0.305	<b>-0.571</b>
Water velocity (average)	0.331	-0.069	<b>0.487</b>	<b>0.590</b>	0.364	-0.068	<b>-0.696</b>	<b>0.749</b>	<b>0.665</b>	0.102

Stressors and benthic macroinvertebrate metrics with significant correlations from Tab. 4.3.5. are displayed as RDA in Fig. 4.3.5.

The RDA shows that explanatory variables account for 100% of the variation. Total variation is 32.08. The eigenvalues of the first two axes are 0.657 and 0.238 (Fig. 4.3.5.).



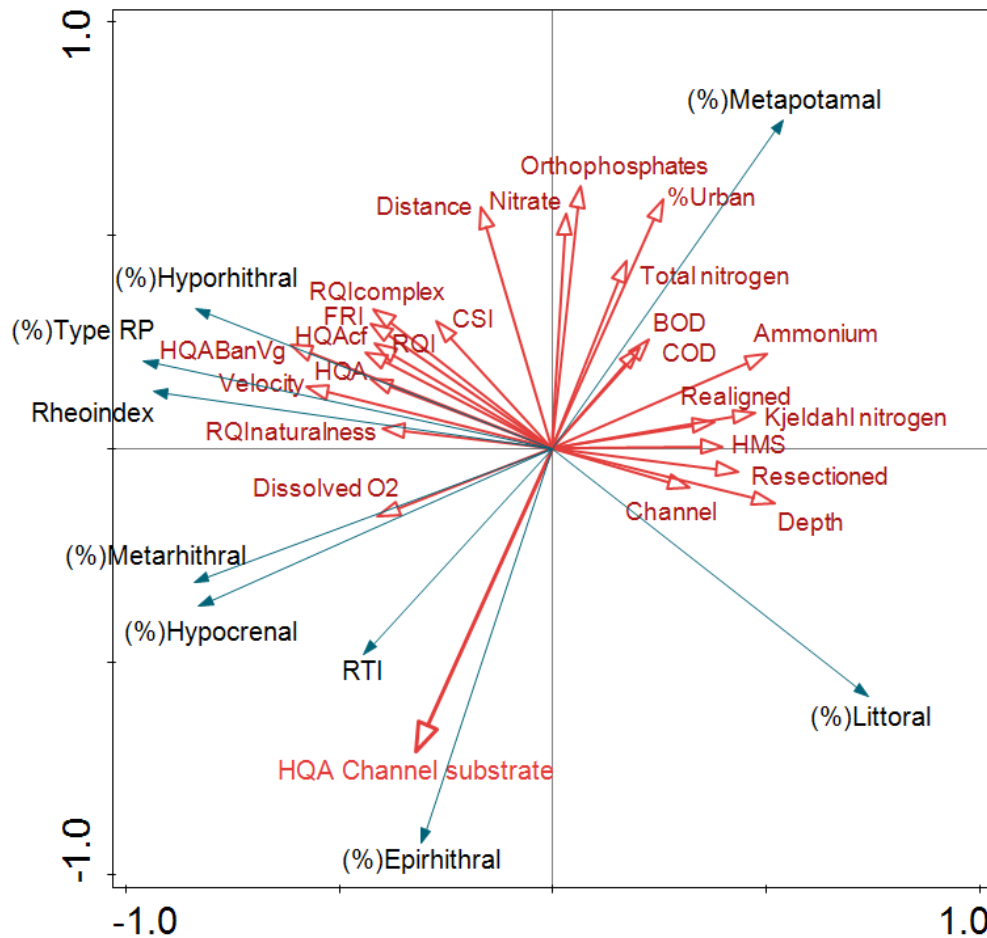


Figure 4.3.5. Redundancy analysis of significant relationships between benthic macroinvertebrate functional metrics zonation and current preference, and stressors. Arrow lengths on the ordination show the relative importance of variables, and their direction relative to each other. Abbreviations: Type RP = = rheophile taxa, RTI = Rhithron Type Index, Channel = EN 15843:2010 channel modification, HMS = Habitat Modification Score, Resectioned = HMS Resectioned bank bed subscore, Realigned = HMS Realigned bank bed subscore, BOD = biological oxygen demand, COD = chemical oxygen demand, % Urban = urban landuse, CSI = Channel Substrate Index, FRI = Flow Regime Index, RQI = Riparian Quality Index, RQIcomplex = Riparian Quality Index complexity subscore, RQInaturalness = Riparian Quality Index naturalness subscore, HQA = Habitat Quality Assessment, HQAch = Habitat Quality Assessment channel features subscore, HQABanVg = Habitat Quality Assessment bank vegetation subscore.

#### 4.4 Relationship between hydromorphological status and the benthic macroinvertebrate community longitudinally along the Bednja River

The Objective to examine the relationship between the hydromorphological status and the benthic macroinvertebrate community has partially been fulfilled by the previous chapter where hydromorphological scores together with other stressors were analysed with benthic macroinvertebrate metrics. This section will examine the taxonomic and species relationships with hydromorphological quality and modification.

The greatest number of significant correlations with hydromorphological modification (assessed by EN 15843:2010 and RHS) occurs with the taxonomic group Hydrachnidia (Tab. 4.4.1.).

Table 4.4.1. Results of Spearman's rank correlation coefficient (R) for the relationship between taxonomic group share (%) and hydromorphological scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Abbreviations: EPT = Ephemeroptera, Plecoptera Trichoptera, EPT/OL = Ephemeroptera, Plecoptera, Trichoptera / Oligochaeta, EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata, HMS = Habitat Modification Score, HQA = Habitat Quality Assessment, RQI = Riparian Quality Index, CSI = Channel Substrate Index, FRI = Flow Regime Index, CVI = Channel Vegetation Index.

	Coelenterata (%)	Turbellaria (%)	Gastropoda (%)	Bivalvia (%)	Oligochaeta (%)	Hirudinea (%)	Crustacea (%)	Ephemeroptera (%)	Odonata (%)	Plecoptera (%)	Heteroptera (%)	Megaloptera (%)	Trichoptera (%)	Coleoptera (%)	Diptera (%)	Hydrachnidia (%)	(%) EPT-Taxa	EPT (%) (abundance classes)	EPT/OL taxa	EPTCBO
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<b>EN 15843:2010 - 100 m scale</b>																				
AVERAGE SCORE	0.084	-0.257	0.081	-0.323	0.047	0.244	-0.214	0.004	0.033	-0.140	0.212	-0.050	0.322	0.319	0.175	<b>0.516</b>	0.118	0.210	0.283	0.007
Morphology	0.082	-0.222	0.155	-0.344	-0.017	0.183	-0.197	0.078	-0.018	-0.090	0.222	-0.155	0.370	0.376	0.115	<b>0.555</b>	0.180	0.242	0.255	0.000
Flow	0.013	-0.239	0.061	<b>-0.547</b>	-0.061	-0.012	-0.206	-0.085	-0.036	-0.314	0.085	0.122	-0.036	-0.036	0.304	0.328	-0.085	0.109	0.255	-0.110
Continuity	-0.285	-0.190	0.058	-0.390	-0.058	-0.174	-0.116	0.116	-0.203	-0.194	-0.116	0.227	0.058	0.116	0.087	0.376	0.087	0.231	0.347	-0.203
Channel	0.082	-0.379	-0.125	-0.425	0.193	0.222	-0.204	-0.071	-0.070	-0.206	0.063	0.137	0.175	0.317	0.141	0.438	0.014	0.073	0.187	-0.042
Riparian zone	0.089	-0.177	0.274	-0.257	-0.103	0.188	-0.196	0.092	-0.004	-0.097	0.280	-0.287	0.358	0.301	0.084	<b>0.541</b>	0.187	0.217	0.268	-0.024
Floodplain	0.130	-0.174	0.251	-0.282	-0.097	0.154	-0.223	0.043	-0.046	-0.145	0.198	-0.298	0.420	0.282	0.126	<b>0.577</b>	0.170	0.256	0.191	-0.105
<b>EN 15843:2010 - 500 m scale</b>																				
AVERAGE SCORE	0.130	-0.121	0.152	<b>-0.451</b>	0.014	0.226	-0.239	0.084	-0.029	-0.149	0.227	-0.125	0.369	0.323	0.099	<b>0.599</b>	0.180	0.180	0.144	0.029
Morphology	0.123	-0.097	0.196	<b>-0.450</b>	-0.043	0.184	-0.218	0.118	-0.028	-0.107	0.243	-0.186	0.362	0.331	0.106	<b>0.595</b>	0.205	0.227	0.182	0.056
Flow	-0.111	-0.014	0.237	<b>-0.553</b>	-0.143	-0.084	-0.174	0.160	-0.031	-0.087	0.191	0.032	0.201	0.101	0.112	0.409	0.160	0.283	0.280	-0.004
Continuity	-0.285	-0.190	0.058	-0.390	-0.058	-0.174	-0.116	0.116	-0.203	-0.194	-0.116	0.227	0.058	0.116	0.087	0.376	0.087	0.231	0.347	-0.203
Channel	0.097	-0.156	0.030	<b>-0.520</b>	0.150	0.222	-0.273	0.066	-0.095	-0.150	0.133	0.032	0.353	0.319	0.042	<b>0.564</b>	0.159	0.109	0.071	-0.022
Riparian zone	0.181	-0.055	0.272	-0.389	-0.114	0.202	-0.252	0.185	0.003	-0.069	0.279	-0.308	0.381	0.249	0.101	<b>0.560</b>	0.269	0.246	0.209	0.072
Floodplain	0.217	-0.126	0.227	-0.406	-0.069	0.144	-0.252	0.102	-0.054	-0.191	0.230	-0.379	0.355	0.290	0.087	<b>0.629</b>	0.177	0.207	0.091	-0.074
<b>HMS</b>																				
Resectioned Bank Bed	0.240	-0.238	-0.176	-0.105	0.333	<b>0.451</b>	-0.326	-0.237	0.012	-0.361	0.005	-0.057	0.153	0.133	0.074	0.382	-0.144	-0.110	-0.005	-0.266
Reinforced Bank Bed	0.024	0.053	0.300	-0.313	-0.333	0.065	-0.020	0.300	-0.028	0.218	0.335	-0.354	0.264	0.285	0.052	0.326	0.282	0.269	0.219	0.261
Realigned	0.285	-0.315	-0.055	-0.322	0.260	0.391	-0.418	0.000	-0.130	-0.322	0.117	-0.142	0.356	0.281	0.034	<b>0.614</b>	0.110	-0.041	-0.048	-0.196

Table 4.4.1. (Continued). Results of Spearman's rank correlation coefficient (R) for the relationship between taxonomic group share (%) and hydromorphological scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are **bolded**. Abbreviations: EPT = Ephemeroptera, Plecoptera Trichoptera, EPT/OL = Ephemeroptera, Plecoptera, Trichoptera / Oligochaeta, EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata, HMS = Habitat Modification Score, HQA = Habitat Quality Assessment, RQI = Riparian Quality Index, CSI = Channel Substrate Index, FRI = Flow Regime Index, CVI = Channel Vegetation Index.

	Coelenterata (%)	Turbellaria (%)	Gastropoda (%)	Bivalvia (%)	Oligochaeta (%)	Hirudinea (%)	Crustacea (%)	Ephemeroptera (%)	Odonata (%)	Plecoptera (%)	Heteroptera (%)	Megaloptera (%)	Trichoptera (%)	Coleoptera (%)	Diptera (%)	Hydrachnidia (%)	(%) EPT-Taxa	EPT (%) (abundance classes)	EPT/OL taxa	EPTCBO
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<b>HQA</b>	-0.256	0.242	0.027	0.219	-0.313	-0.279	0.410	0.209	0.026	0.329	-0.099	0.083	-0.131	-0.017	-0.199	<b>-0.519</b>	0.118	-0.056	-0.202	0.283
HQA flow type	0.074	0.227	0.112	0.338	-0.147	0.216	0.129	0.114	0.151	0.183	-0.095	-0.104	0.306	0.084	-0.250	-0.316	0.216	-0.216	-0.409	0.123
HQA channel substrate	<b>-0.684</b>	0.123	0.352	0.315	-0.071	<b>-0.593</b>	<b>0.667</b>	-0.169	-0.059	0.229	0.019	0.311	-0.173	0.375	<b>-0.445</b>	-0.210	-0.162	0.075	0.311	0.040
HQA channel features	-0.072	0.432	0.269	-0.093	-0.318	-0.320	0.190	<b>0.576</b>	-0.061	<b>0.608</b>	0.019	-0.242	0.268	0.061	-0.308	-0.195	<b>0.530</b>	0.167	-0.283	0.369
HQA bank vegetation structure	-0.113	0.218	0.169	0.011	<b>-0.480</b>	-0.082	0.243	0.356	-0.043	0.196	0.099	-0.097	0.130	0.262	0.073	-0.066	0.278	0.038	-0.222	0.412
HQA channel vegetation	0.026	-0.146	0.258	-0.060	-0.317	-0.035	0.204	0.009	0.203	0.038	0.432	-0.290	-0.167	-0.113	-0.180	-0.135	0.030	-0.024	0.200	0.232
HQA landuse	0.024	0.245	-0.122	0.171	-0.171	-0.069	0.045	0.245	0.027	0.244	-0.253	0.152	0.028	-0.176	0.091	<b>-0.453</b>	0.172	0.118	-0.107	0.208
HQA trees	-0.080	-0.013	-0.214	0.120	0.014	-0.194	0.122	0.061	0.036	0.006	-0.160	0.171	-0.227	-0.113	-0.021	-0.354	-0.048	-0.193	-0.302	0.008
HQA special features	-0.118	0.142	-0.245	0.355	0.041	-0.020	0.172	-0.055	-0.063	0.362	-0.322	0.284	-0.145	-0.239	-0.226	<b>-0.603</b>	-0.055	-0.098	-0.073	0.043
<b>RQI</b>	-0.025	0.038	-0.035	0.138	-0.269	-0.163	0.214	0.304	0.045	0.135	0.035	-0.112	0.008	0.005	-0.021	-0.329	0.195	-0.051	-0.331	0.243
Complexity	0.033	0.018	0.038	0.098	-0.210	-0.092	0.153	0.338	0.071	0.110	0.148	-0.220	0.084	0.168	-0.013	-0.129	0.231	-0.135	-0.441	0.245
Naturalness	-0.117	0.196	-0.087	0.245	-0.231	-0.235	0.253	0.088	-0.250	0.186	-0.253	0.007	-0.189	-0.264	-0.242	<b>-0.526</b>	-0.008	-0.091	-0.222	0.028
Continuity	0.226	-0.089	-0.180	0.086	0.059	0.096	-0.048	0.146	0.056	-0.039	0.030	-0.212	0.062	-0.028	-0.095	-0.143	0.103	-0.357	<b>-0.657</b>	-0.009
CSI	0.435	-0.095	0.085	<b>-0.489</b>	<b>-0.475</b>	0.141	-0.328	<b>0.574</b>	-0.181	0.216	0.205	<b>-0.507</b>	<b>0.556</b>	-0.104	0.168	0.002	<b>0.629</b>	0.357	-0.204	0.369
FRI	0.365	0.116	0.011	-0.009	-0.364	0.095	-0.042	0.156	-0.124	0.080	0.046	-0.400	0.190	-0.428	-0.087	-0.373	0.236	0.008	-0.425	0.214
CVI	0.315	<b>0.480</b>	-0.008	-0.081	-0.050	0.365	-0.323	0.205	-0.094	0.149	-0.201	-0.077	<b>0.528</b>	-0.062	0.141	0.036	0.305	0.062	-0.386	0.118
Depth (average)	0.079	-0.304	0.117	0.029	0.290	-0.027	-0.147	-0.361	<b>0.542</b>	<b>-0.495</b>	0.385	0.035	-0.439	-0.012	0.398	0.260	-0.414	-0.356	0.068	-0.203
Velocity (average)	0.043	-0.004	0.208	-0.028	<b>-0.606</b>	-0.038	0.083	<b>0.535</b>	-0.217	0.146	0.170	<b>-0.618</b>	0.430	0.030	-0.298	-0.020	<b>0.550</b>	0.205	-0.275	0.184

RDA of benthic macroinvertebrate taxonomic groups and EN 15843:2010 hydromorphological modification scores shows that hydromorphological modification scores are positioned relatively close to each other, with the majority of taxa groups positioned away from hydromorphological modification. Total variation is 112.36 with hydromorphological scores accounting for 61.7% of the variation. The eigenvalues of the first two axes are 0.278 and 0.133 (Fig. 4.1.1.).

RDA of benthic macroinvertebrate taxonomic groups and River Habitat Survey scores shows taxonomic group affiliation to both habitat modification scores and habitat quality scores. Total variation is 112.36 and RHS scores account for 83.8% of the variation. The eigenvalues of the first two axes are 0.316 and 0.280 (Fig. 4.4.2.).

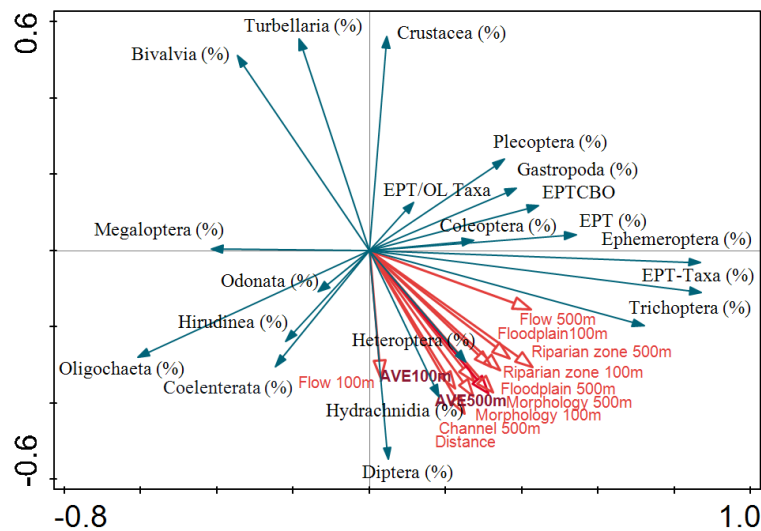


Figure 4.4.1. Redundancy analysis of EN 15843:2010 hydromorphological scores and benthic macroinvertebrate taxonomic group share. Arrow lengths on the ordination show the relative importance of variables, and their direction relative to each other. Abbreviations: EPT (%) = Ephemeroptera, Plecoptera, Trichoptera abundance classes, EPT-Taxa (%) = Ephemeroptera, Plecoptera, Trichoptera taxa, EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata, AVE100m/AVE500m = EN 15843:2010 average score for 100 m or 500 m reach.

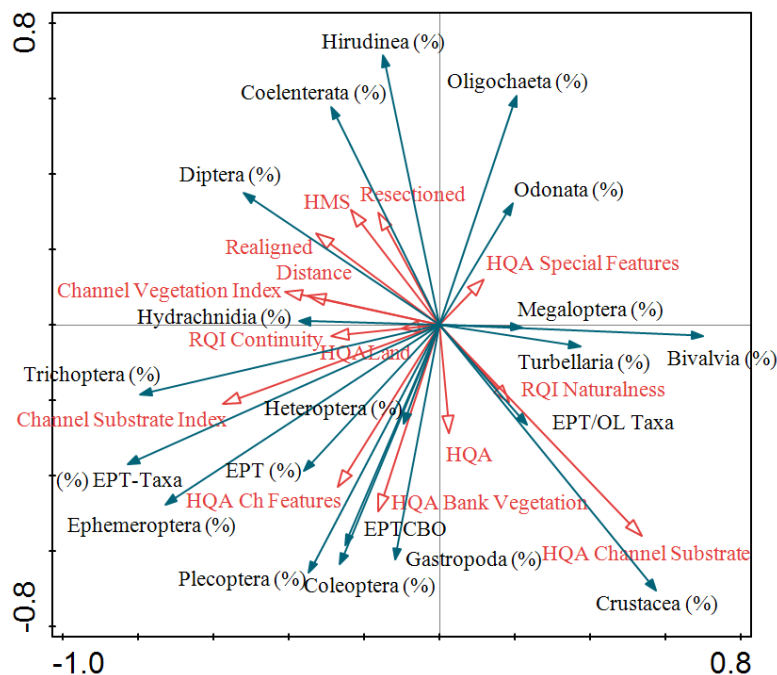


Figure 4.4.2. Redundancy analysis of River Habitat Survey scores and benthic macroinvertebrate taxonomic group share. Arrow lengths on the ordination show the relative importance of variables, and their direction relative to each other. Abbreviations: EPT (%) = Ephemeroptera, Plecoptera, Trichoptera abundance classes, EPT-Taxa (%) = Ephemeroptera, Plecoptera, Trichoptera taxa, EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata, HMS = Habitat Modification Score, Realigned = Habitat Modification Realigned bank bed subscore, Resectioned = Habitat Modification Resectioned bank bed subscore, RQI = Riparian Quality Index continuity subscore, HQA = Habitat Quality Assessment, HQALand = Habitat Quality Assessment landuse subscore, HQA Ch Features = Habitat Quality Assessment Channel features subscore.

### River Habitat Survey

River Habitat Survey scores were correlated with all 259 taxa. 108 taxa gave significant Spearman correlations with RHS scores. The highest number of significant correlations is found for substrate related scores: the HQA channel substrate subscore and the Chanel Substrate Index. The strongest significant correlation is found between subfamily Orthocladiinae (Diptera) and the Channel Substrate Index ( $R = 0.833$ ,  $p < 0.05$ ). Taxa giving significant correlations to RHS scores are given in Tab 4.4.2.

Table 4.4.2. Results of Spearman's rank correlation coefficient (R) for the relationship between Bednja River taxa abundance and hydromorphological scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Only taxa with at least one significant correlation to a hydromorphological score is given. Abbreviations: HMS = Habitat Modification Score, HQA = Habitat Quality Assessment, RQI = Riparian Quality Index, HI = Hydromorphological Indices, CSI = Channel Substrate Index, FRI = Flow Regime Index, CVI = Channel Vegetation Index.

	HMS					HQA							RQI			HI				
	HMS	Resectioned Bank Bed	Reinforced Bank Bed	Realigned	HQA	Flow type	Channel substrate	Channel features	Bank vegetation structure	Channel vegetation	Landuse	Trees	Special features	Riparian Quality Index	Complexity	Naturalness	Continuity	CSI	FRI	CVI
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<i>Baetis fuscatus</i>	-0.131	-0.220	0.337	-0.038	0.261	0.237	<b>-0.459</b>	<b>0.629</b>	0.355	0.158	0.109	0.169	-0.118	0.395	<b>0.465</b>	0.119	<b>0.448</b>	<b>0.671</b>	0.301	0.208
<i>Baetis buceratus</i>	-0.124	-0.064	0.038	0.014	0.162	0.217	<b>-0.482</b>	0.243	0.063	0.214	0.171	0.112	0.221	0.229	0.155	0.155	0.282	<b>0.525</b>	0.408	0.136
<i>Cloeon dipterum</i>	0.384	0.257	0.225	0.263	<b>-0.467</b>	<b>-0.493</b>	-0.199	-0.409	-0.399	0.192	-0.360	-0.286	-0.360	<b>-0.460</b>	<b>-0.461</b>	-0.409	-0.388	0.135	-0.216	-0.133
<i>Procloeon bifidum</i>	0.099	-0.028	0.101	-0.060	-0.215	<b>-0.563</b>	-0.209	-0.026	-0.244	-0.027	-0.199	-0.002	-0.421	-0.073	-0.072	-0.240	-0.074	0.079	-0.166	-0.176
<i>Caenis luctuosa</i>	0.076	-0.065	0.134	0.070	-0.071	-0.014	-0.194	0.232	0.078	-0.041	0.111	-0.016	-0.408	0.005	0.045	-0.195	-0.108	<b>0.454</b>	0.012	0.182
<i>Serratella ignita</i>	-0.244	-0.305	0.308	-0.075	0.226	0.157	-0.319	0.384	0.424	0.161	0.172	0.185	-0.226	<b>0.465</b>	<b>0.519</b>	0.087	0.341	<b>0.601</b>	0.128	0.070
<i>Electrogena ujhelyii</i>	-0.161	-0.204	0.170	-0.239	0.168	0.155	<b>0.514</b>	0.191	0.053	0.079	0.094	-0.217	0.346	-0.142	-0.272	0.074	-0.417	-0.088	-0.067	0.081
<i>Heptagenia longicauda</i>	-0.222	-0.069	-0.149	-0.072	0.278	0.296	0.183	0.051	<b>0.462</b>	-0.037	0.139	0.276	-0.371	<b>0.448</b>	<b>0.522</b>	0.160	0.305	0.253	0.323	0.084
<i>Ephemera danica</i>	-0.200	-0.275	0.005	-0.208	0.020	<b>-0.507</b>	0.338	0.061	0.024	-0.222	0.126	0.032	0.083	-0.042	-0.083	0.227	-0.281	-0.249	-0.227	-0.093
<i>Leuctra</i> sp.	-0.121	-0.252	0.348	-0.158	0.301	0.227	-0.066	<b>0.703</b>	0.304	-0.159	0.171	0.117	0.066	0.307	0.377	0.105	0.279	0.432	0.007	0.320
<i>Nemurella pictetii</i>	-0.118	-0.056	-0.149	-0.098	-0.067	-0.180	<b>0.549</b>	-0.046	-0.180	0.060	0.125	-0.258	0.216	-0.258	-0.372	0.078	<b>-0.539</b>	-0.213	0.146	-0.002
<i>Beraeodes minutus</i>	0.252	0.232	-0.015	-0.033	-0.084	0.006	-0.101	-0.308	0.081	<b>-0.534</b>	0.078	0.010	-0.052	-0.038	-0.008	-0.157	-0.025	-0.335	-0.349	0.259
<i>Goeridae</i> Gen. sp.	-0.099	-0.101	-0.271	-0.173	-0.050	-0.243	<b>0.488</b>	0.000	-0.198	-0.035	0.175	-0.155	0.221	-0.210	-0.331	0.087	<b>-0.447</b>	-0.272	0.183	-0.095
<i>Hydropsyche contubernalis contubernalis</i>	-0.253	-0.264	-0.005	-0.220	0.168	0.079	-0.341	0.087	0.439	-0.064	0.378	0.199	-0.199	0.428	0.417	0.210	0.207	<b>0.597</b>	<b>0.501</b>	0.354
<i>Hydropsyche incognita</i>	-0.073	-0.184	0.172	-0.135	0.269	0.162	-0.100	0.327	<b>0.529</b>	-0.274	0.210	0.107	-0.037	0.292	0.342	0.132	0.144	0.398	0.179	0.417
<i>Hydropsyche incognita /pellucidula</i>	<b>-0.446</b>	-0.406	0.088	-0.308	0.442	0.032	-0.121	0.338	0.359	0.214	0.119	0.426	-0.066	<b>0.520</b>	<b>0.467</b>	0.302	0.377	0.322	0.072	-0.319
<i>Hydropsyche pellucidula</i>	-0.342	-0.337	-0.128	-0.328	0.352	-0.026	0.046	0.205	<b>0.461</b>	-0.408	<b>0.452</b>	0.350	0.045	<b>0.470</b>	0.389	0.381	0.180	0.348	0.288	0.327
<i>Hydropsyche saxonica</i>	-0.249	-0.142	-0.298	-0.210	0.035	-0.151	<b>0.524</b>	-0.188	-0.150	0.077	0.138	-0.123	0.368	-0.164	-0.332	0.188	<b>-0.446</b>	-0.391	0.079	-0.201
<i>Hydropsyche fulvipes</i>	0.372	0.177	<b>0.469</b>	0.263	-0.138	0.165	0.067	0.389	0.082	0.054	-0.171	-0.407	-0.160	-0.290	-0.120	-0.315	-0.220	0.297	0.025	0.411
<i>Hydroptila</i> sp.	0.365	0.218	0.367	0.418	-0.175	0.148	<b>-0.468</b>	0.112	0.169	-0.179	-0.223	-0.136	-0.246	-0.017	0.143	-0.217	0.246	0.209	-0.179	0.359
<i>Adicella</i> sp.	<b>0.466</b>	0.410	<b>0.563</b>	0.263	-0.426	-0.278	<b>-0.470</b>	<b>-0.508</b>	-0.032	0.109	-0.189	-0.396	-0.360	-0.268	-0.214	-0.355	-0.288	0.396	-0.051	0.224
<i>Ceraclea</i> sp.	0.196	0.151	0.399	0.033	-0.156	-0.130	<b>-0.540</b>	-0.301	0.117	0.179	0.064	-0.143	-0.102	-0.058	-0.048	-0.114	-0.113	<b>0.449</b>	0.107	0.155
<i>Mystacides azurea</i>	0.077	-0.022	-0.017	0.121	-0.156	-0.331	0.037	0.286	-0.119	-0.095	-0.012	-0.074	<b>-0.452</b>	-0.123	-0.082	-0.120	-0.216	0.212	0.084	0.184
<i>Mystacides longicornis/nigra</i>	0.212	0.220	-0.354	0.216	-0.397	<b>-0.485</b>	0.122	-0.225	<b>-0.474</b>	-0.081	-0.306	-0.205	-0.396	-0.353	-0.338	-0.394	-0.350	-0.362	-0.147	-0.336
<i>Mystacides</i> sp.	0.201	0.206	-0.160	0.026	-0.250	-0.263	-0.269	-0.180	<b>-0.496</b>	-0.100	-0.071	0.006	-0.069	-0.216	-0.215	-0.319	-0.077	-0.242	-0.355	-0.244
<i>Oecetis lacustris</i>	<b>0.466</b>	0.410	<b>0.563</b>	0.263	-0.426	-0.278	<b>-0.470</b>	<b>-0.508</b>	-0.032	0.109	-0.189	-0.396	-0.360	-0.268	-0.214	-0.355	-0.288	0.396	-0.051	0.224
<i>Anabolia furcata</i>	-0.069	-0.137	0.082	-0.089	-0.038	<b>-0.494</b>	-0.261	-0.135	-0.070	0.175	-0.017	0.077	-0.039	0.049	-0.065	-0.051	-0.071	-0.121	-0.236	-0.346



Table 4.4.2. (Continued). Results of Spearman's rank correlation coefficient (R) for the relationship between Bednja River taxa abundance and hydromorphological scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Only taxa with at least one significant correlation to a hydromorphological score is given. Abbreviations: HMS = Habitat Modification Score, HQA = Habitat Quality Assessment, RQI = Riparian Quality Index, HI = Hydromorphological Indices, CSI = Channel Substrate Index, FRI = Flow Regime Index, CVI = Channel Vegetation Index.

	HMS			HQA							RQI			HI						
	HMS	Resectioned Bank Bed	Reinforced Bank Bed	Realigned	HQA	Flow type	Channel substrate	Channel features	Bank vegetation structure	Channel vegetation	Landuse	Trees	Special features	Riparian Quality Index	Complexity	Naturalness	Continuity	CSI	FRI	CVI
<i>Radix labiata</i>	-0.130	0.007	-0.237	-0.112	-0.173	-0.120	0.381	-0.253	-0.367	0.299	-0.104	-0.372	0.202	-0.356	<b>-0.467</b>	0.003	<b>-0.543</b>	-0.407	0.150	-0.269
<i>Physella acuta</i>	0.063	0.018	0.108	0.110	-0.174	-0.142	-0.334	-0.109	0.089	0.218	0.040	-0.091	<b>-0.452</b>	0.085	0.105	-0.115	-0.013	0.316	0.279	0.179
<i>Gyraulus crista</i>	0.087	0.015	0.204	0.000	-0.116	-0.271	<b>-0.465</b>	0.030	-0.015	0.248	-0.015	-0.058	-0.360	0.058	0.043	-0.087	0.015	0.392	0.290	0.145
<i>Hippeutis complanatus</i>	0.110	0.034	0.225	0.013	-0.137	-0.278	<b>-0.470</b>	0.003	-0.032	0.261	-0.032	-0.080	-0.360	0.033	0.020	-0.107	-0.002	0.396	0.280	0.141
<i>Nais pardalis</i>	0.110	0.034	0.225	0.013	-0.137	-0.278	<b>-0.470</b>	0.003	-0.032	0.261	-0.032	-0.080	-0.360	0.033	0.020	-0.107	-0.002	0.396	0.280	0.141
<i>Pristina longiseta</i>	0.259	0.085	0.415	0.110	-0.109	-0.063	<b>-0.447</b>	0.150	0.089	0.284	-0.163	-0.211	-0.294	-0.068	0.007	-0.235	-0.013	0.403	0.170	0.223
<i>Stylaria lacustris</i>	0.030	-0.022	0.263	-0.079	-0.024	-0.177	<b>-0.626</b>	-0.036	0.183	0.204	0.166	0.014	-0.225	0.145	0.134	0.011	0.040	<b>0.519</b>	0.308	0.194
<i>Propappus volki</i>	-0.192	-0.033	-0.343	-0.147	0.299	0.202	-0.068	0.071	0.369	-0.432	0.240	<b>0.477</b>	-0.118	<b>0.579</b>	<b>0.711</b>	0.269	<b>0.631</b>	-0.109	0.110	0.077
<i>Branchiura sowerbyi</i>	0.334	0.306	-0.131	0.213	0.010	0.407	-0.194	0.245	-0.056	-0.356	-0.022	0.057	0.075	-0.024	0.105	-0.085	0.361	-0.033	0.078	<b>0.447</b>
<i>Limnodrilus</i> sp.	<b>0.466</b>	0.273	<b>0.456</b>	0.393	-0.358	-0.050	-0.290	0.142	-0.238	0.272	-0.403	<b>-0.446</b>	-0.072	-0.439	-0.335	-0.331	-0.107	0.385	0.188	0.317
<i>Limnodrilus claparedeanus</i>	-0.090	-0.027	-0.203	0.182	0.085	0.190	-0.189	0.142	0.006	-0.035	-0.087	0.307	-0.083	0.200	0.287	0.101	<b>0.473</b>	0.138	0.002	-0.112
<i>Potamothrix hammoniensis</i>	-0.308	-0.255	<b>-0.454</b>	-0.327	0.130	-0.078	0.188	0.087	-0.332	-0.199	0.163	0.167	<b>0.533</b>	-0.085	-0.230	0.265	-0.088	<b>-0.455</b>	-0.139	-0.146
<i>Psammorectes barbatus</i>	-0.412	-0.329	-0.405	-0.368	0.413	0.103	<b>0.476</b>	0.242	0.209	-0.130	0.252	0.336	0.294	0.275	0.161	<b>0.588</b>	0.183	-0.068	<b>0.518</b>	0.152
<i>Lumbricidae</i> Gen. sp.	<b>-0.447</b>	-0.393	-0.405	-0.387	0.217	0.062	-0.047	0.339	-0.125	0.170	0.257	0.143	0.443	0.092	-0.060	0.405	0.076	0.060	<b>0.635</b>	0.114
<i>Lumbricidae</i> Gen. sp.	0.066	0.134	0.031	0.025	0.259	<b>0.575</b>	-0.244	0.115	0.372	-0.151	0.214	0.181	0.163	0.260	0.384	0.135	0.373	0.442	0.261	0.281
<i>Erpobdella octoculata</i>	0.213	0.211	-0.085	0.170	-0.015	0.424	-0.311	0.273	-0.145	-0.193	0.043	-0.044	0.247	-0.092	-0.019	-0.074	0.230	0.060	0.117	<b>0.496</b>
<i>Erpobdellidae</i> Gen. sp.	0.290	0.228	0.171	0.169	-0.079	0.195	<b>-0.774</b>	0.001	0.025	-0.178	0.063	0.022	0.067	0.052	0.087	-0.040	0.321	0.325	0.090	0.434
<i>Trocheta</i> sp.	-0.207	-0.197	0.015	-0.199	0.220	-0.004	<b>-0.452</b>	0.173	0.389	-0.046	0.361	0.235	-0.102	0.367	0.362	0.237	0.189	0.365	0.292	0.200
<i>Glossiphonia complanata</i>	0.242	0.256	0.009	0.331	-0.274	0.076	-0.088	-0.075	-0.349	0.317	<b>-0.453</b>	-0.384	0.201	-0.435	-0.373	-0.308	-0.124	-0.134	0.056	-0.114
<i>Glossiphoniinae</i> Gen. sp.	0.226	0.233	0.172	0.306	-0.242	0.008	<b>-0.713</b>	-0.023	-0.235	0.346	-0.177	-0.212	0.005	-0.129	-0.121	-0.152	0.115	0.424	0.310	0.093
<i>Helobdella stagnalis</i>	0.295	0.341	0.033	0.333	-0.270	0.011	<b>-0.501</b>	-0.125	-0.242	0.018	-0.097	-0.199	0.042	-0.175	-0.146	-0.148	0.057	0.292	0.242	0.271
<i>Piscicola</i> sp.	0.073	0.191	-0.298	0.331	-0.111	0.023	0.377	-0.376	0.097	0.055	-0.204	0.049	<b>-0.452</b>	-0.034	0.091	-0.122	-0.033	-0.223	-0.070	-0.281
<i>Aturax scaber scaber</i>	0.202	0.029	<b>0.526</b>	0.264	-0.014	0.181	-0.090	0.210	0.139	0.335	-0.361	-0.276	-0.015	-0.116	-0.058	-0.217	-0.030	0.116	-0.261	-0.145
<i>Atractides</i> sp.	-0.425	-0.431	-0.299	<b>-0.554</b>	<b>0.547</b>	0.266	0.252	0.189	0.390	<b>-0.527</b>	<b>0.543</b>	<b>0.536</b>	<b>0.504</b>	<b>0.449</b>	0.304	<b>0.450</b>	0.291	-0.061	0.024	0.340
<i>Hygrobatas calliger</i>	0.326	0.259	0.433	0.357	-0.100	0.143	-0.348	0.108	<b>0.451</b>	0.073	-0.050	-0.203	<b>-0.515</b>	0.111	0.359	-0.216	0.100	0.435	0.154	0.288
<i>Hygrobatas fluvialis</i>	0.373	0.306	-0.158	<b>0.523</b>	-0.304	-0.051	0.050	0.010	-0.233	-0.230	-0.345	-0.075	-0.304	-0.227	-0.073	-0.280	0.116	-0.205	-0.206	-0.002
<i>Lebertia</i> sp.	0.354	0.237	0.315	<b>0.448</b>	-0.182	0.131	-0.265	0.094	0.182	-0.080	-0.132	-0.151	-0.443	0.014	0.195	-0.329	0.092	0.146	-0.295	0.070
<i>Mideopsis orbicularis</i>	0.199	0.202	0.051	0.330	-0.352	-0.437	-0.153	<b>-0.458</b>	-0.153	0.175	-0.408	-0.078	-0.367	-0.186	-0.074	-0.207	-0.005	-0.101	-0.180	-0.315
<i>Sperchon compactilis</i>	0.202	0.029	<b>0.526</b>	0.264	-0.014	0.181	-0.090	0.210	0.139	0.335	-0.361	-0.276	-0.015	-0.116	-0.058	-0.217	-0.030	0.116	-0.261	-0.145
<i>Sperchon hibernicus</i>	-0.182	-0.313	0.060	-0.199	0.360	0.261	-0.334	<b>0.568</b>	0.389	0.172	0.202	0.197	0.083	0.291	0.336	0.160	0.282	0.301	0.292	0.149
<i>Hydrachnidia</i> Gen. sp.	0.135	0.001	<b>0.448</b>	0.056	-0.015	-0.161	-0.276	-0.099	0.259	-0.197	-0.185	-0.022	-0.050	0.001	0.104	-0.132	0.026	-0.219	<b>-0.719</b>	-0.076
<i>Dugesia</i> sp.	-0.102	-0.152	-0.043	-0.232	0.225	0.126	-0.089	0.391	0.207	-0.278	0.346	0.200	-0.051	0.220	0.218	0.131	0.139	0.038	0.096	<b>0.519</b>
<i>Hydra</i> sp.	0.266	0.217	0.044	0.270	-0.202	0.145	<b>-0.700</b>	-0.015	-0.044	0.013	0.068	-0.057	-0.103	0.021	0.089	-0.088	0.254	<b>0.476</b>	0.370	0.364

Canonical correlation analysis (CCA) of all taxa with the main River Habitat Survey scores (HMS, HQA, RQI, CVI, CSI and FRI) is given in Fig. 4.4.3. The CCA analysis of all taxa with the RHS subscores of HMS, HQA and RQI are given in Fig. 4.4.4.

For RHS main scores, total variation is 2.454 and the explanatory variables account for 32.4% of the variation. The eigenvalues of the first 2 axis are 0.272 and 0.239 respectively (Fig. 4.4.3.).

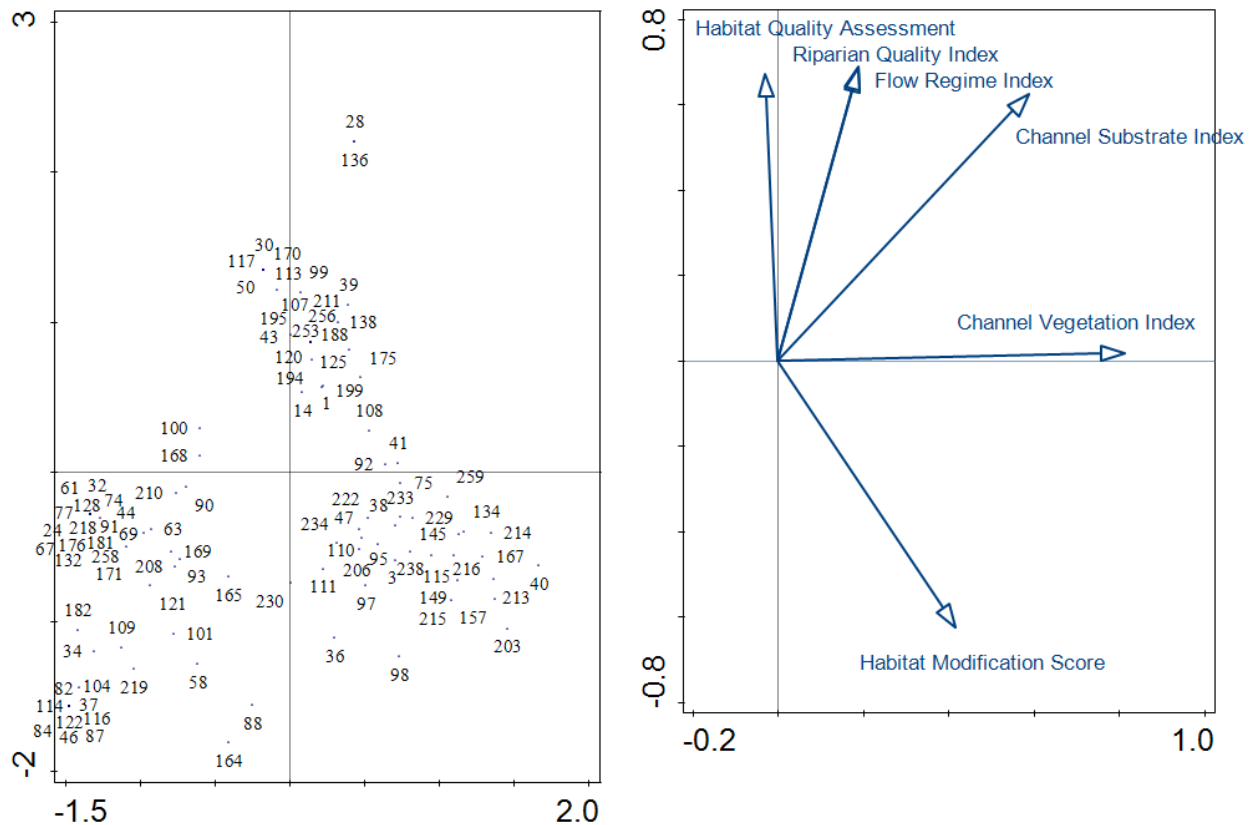


Figure 4.4.3. Canonical correspondence analysis showing variation in taxa composition explained by River Habitat Survey scores (RHS). The Subplot 1 is focused on taxa. The distance between the symbols (dots) approximates the dissimilarity of distribution of relative abundance of those taxa across the samples. The Subplot 2 shows RHS score arrows. Each arrow points in the direction of the steepest increase of RHS score value.

Index value ranges for taxa groups in Subplot 1 are: 1 – 23 (Ephemeroptera), 24 – 31 (Plecoptera), 32 – 78 (Trichoptera), 79 – 85 (Odonata), 86 – 88 (Megaloptera), Diptera (89 – 102), Chironomidae (103 – 110), Coleoptera (111 – 157), Heteroptera (158 – 166), Crustacea (167 – 169), Bivalvia (170 – 174), Gastropoda (175 – 189), Oligochaeta (190 – 214), Hirudinea (215 – 224), Hydrachnidia (225 – 255), Turbellaria (256 – 258), Hydra (259). Corresponding index label for each taxon is given in Annex I.

For RHS subscores, total variation is 2.591 and the explanatory variables account for 81.7% of the variation. The eigenvalues of the first 2 axis are 0.534 and 0.394 respectively (Fig. 4.4.4.).



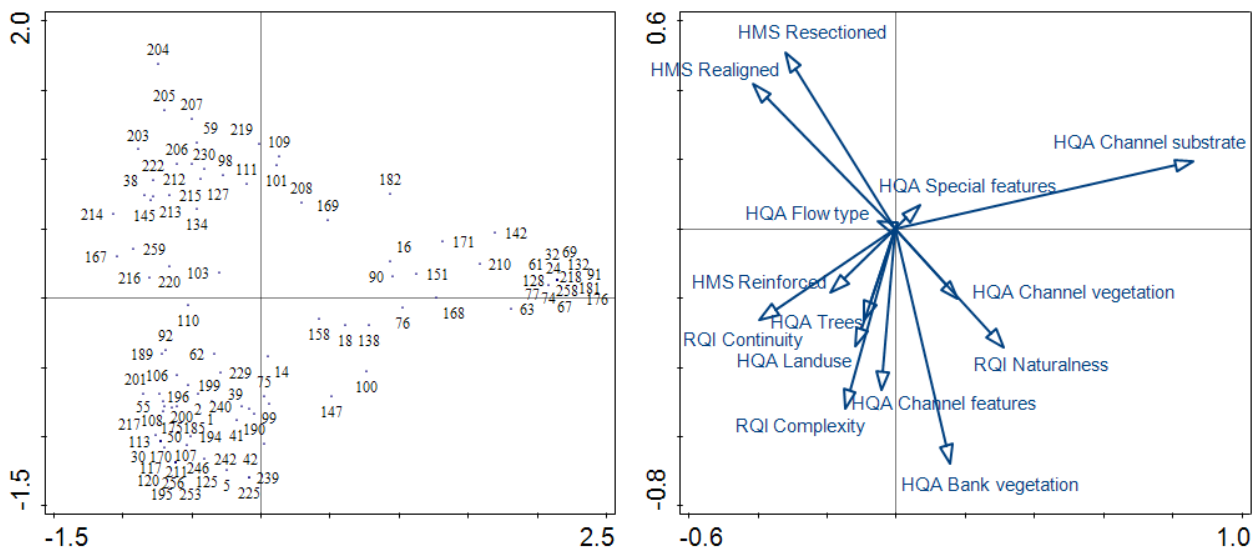


Figure 4.4.4. Canonical correspondence analysis showing variation in taxa composition explained by River Habitat Survey subscores (RHS). The Subplot 1 is focused on taxa. The distance between the symbols (dots) approximates the dissimilarity of distribution of relative abundance of those taxa across the samples. The Subplot 2 shows RHS subscore arrows. Each arrow points in the direction of the steepest increase of RHS subscore value.

Index value ranges for taxa groups in Subplot 1 are: 1 – 23 (Ephemeroptera), 24 – 31 (Plecoptera), 32 – 78 (Trichoptera), 79 – 85 (Odonata), 86 – 88 (Megaloptera), Diptera (89 – 102), Chironomidae (103 – 110), Coleoptera (111 – 157), Heteroptera (158 – 166), Crustacea (167 – 169), Bivalvia (170 – 174), Gastropoda (175 – 189), Oligochaeta (190 – 214), Hirudinea (215 – 224), Hydrachnidia (225 – 255), Turbellaria (256 – 258), Hydra (259). Corresponding index label for each taxon is given in Annex I.

#### EN 15843:2010

EN 15843:2010 hydromorphological modification grouped and average scores were correlated with all 259 taxa. 50 taxa gave significant Spearman correlations with EN 15843:2010 scores. The highest number of significant correlations is found for flow related modification. The strongest significant positive correlation is found between *Cloeon dipterum* (Linnaeus, 1761) (Ephemeroptera) and modification of flow for the 100 m reach ( $R = 0.792$ ,  $p < 0.05$ ). The strongest significant negative correlation is found between the family Ceratopogonidae (Diptera) and 500 m Channel modification ( $R = -0.655$ ,  $p < 0.05$ ). Ceratopogonidae also give the greatest number of significant correlations to all EN 15843:2010 scores except for continuity. Taxa giving significant correlations to EN 15843:2010 hydromorphological modification grouped and average scores are given in Tab 4.4.3.

Table 4.4.3. Results of Spearman's rank correlation coefficient (R) for the relationship between Bednja River taxa abundance and EN 15843:2010 grouped and average scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Only taxa with at least one significant correlation to a hydromorphological score is given.

	EN 15843:2010 - 100 m scale							EN 15843:2010 - 500 m scale						
	AVERAGE SCORE	Morphology	Flow	Continuity	Channel	Riparian zone	Floodplain	AVERAGE SCORE	Morphology	Flow	Continuity	Channel	Riparian zone	Floodplain
	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<i>Baetis lutheri</i>	0.299	0.368	0.350	<b>0.472</b>	0.320	0.316	0.327	0.174	0.211	0.334	<b>0.472</b>	0.179	0.254	0.234
<i>Cloeon dipterum</i>	0.385	0.327	<b>0.792</b>	0.416	<b>0.456</b>	0.316	0.285	0.398	0.385	<b>0.621</b>	0.416	<b>0.452</b>	0.346	0.301
<i>Ecdyonurus macani</i>	0.182	0.219	0.216	0.327	0.172	0.136	0.135	0.292	0.255	<b>0.523</b>	0.327	0.366	0.185	0.171
<i>Paraleptophlebia submarginata</i>	0.379	0.339	<b>0.546</b>	-0.076	0.343	0.324	0.302	0.379	0.379	0.428	-0.076	0.340	0.363	0.341
<i>Potamanthus luteus</i>	0.003	0.026	<b>0.482</b>	0.262	0.017	0.047	0.047	-0.015	0.032	0.333	0.262	-0.043	0.137	0.071
<i>Hydropsyche fulvipes</i>	0.282	0.355	-0.140	-0.111	0.087	0.393	0.346	0.422	<b>0.464</b>	0.247	-0.111	0.365	<b>0.485</b>	0.412
<i>Adicella</i> sp.	0.440	0.437	0.350	-0.111	0.289	<b>0.470</b>	0.438	0.357	0.412	0.247	-0.111	0.217	0.443	0.439
<i>Mystacides azurea</i>	-0.049	-0.032	0.196	0.303	-0.083	0.020	-0.042	0.214	0.227	<b>0.468</b>	0.303	0.257	0.255	0.193
<i>Oecetis lacustris</i>	0.440	0.437	0.350	-0.111	0.289	<b>0.470</b>	0.438	0.357	0.412	0.247	-0.111	0.217	0.443	0.439
<i>Anabolia furcata</i>	0.105	0.010	<b>0.554</b>	0.377	0.282	-0.106	-0.145	0.119	0.052	0.398	0.377	0.220	-0.033	-0.102
<i>Potamophylax rotundipennis</i>	0.415	0.361	0.415	0.228	0.409	0.282	0.213	<b>0.472</b>	0.413	<b>0.587</b>	0.228	<b>0.528</b>	0.335	0.229
<i>Cyrnus trimaculatus</i>	0.179	0.181	<b>0.449</b>	0.196	0.075	0.259	0.177	0.296	0.340	<b>0.528</b>	0.196	0.241	0.402	0.330
<i>Lype reducta</i>	0.140	0.100	<b>0.546</b>	<b>0.688</b>	0.283	0.101	0.080	0.160	0.140	0.428	<b>0.688</b>	0.280	0.101	0.060
<i>Sialis sordida</i>	-0.072	-0.116	0.327	<b>0.444</b>	0.044	-0.162	-0.132	-0.116	-0.145	0.228	<b>0.444</b>	0.015	-0.205	-0.219
<i>Ceratopogonidae</i> Gen. sp.	<b>-0.638</b>	<b>-0.624</b>	<b>-0.452</b>	-0.291	<b>-0.612</b>	<b>-0.582</b>	<b>-0.623</b>	<b>-0.618</b>	<b>-0.593</b>	<b>-0.450</b>	-0.291	<b>-0.655</b>	<b>-0.551</b>	<b>-0.571</b>
<i>Limoniidae</i> Gen. sp.	-0.411	-0.336	-0.246	-0.308	<b>-0.518</b>	-0.213	-0.250	-0.408	-0.318	-0.303	-0.308	<b>-0.570</b>	-0.195	-0.183
<i>Antocha</i> sp.	0.426	<b>0.509</b>	0.123	0.220	0.309	<b>0.515</b>	<b>0.593</b>	<b>0.527</b>	<b>0.560</b>	0.281	0.220	<b>0.465</b>	<b>0.558</b>	<b>0.642</b>
<i>Stratiomyidae</i> Gen. sp.	<b>-0.463</b>	<b>-0.463</b>	-0.140	-0.111	<b>-0.455</b>	-0.382	-0.395	-0.304	-0.290	-0.166	-0.111	-0.348	-0.205	-0.262
<i>Diamesinae</i> Gen. sp.	<b>-0.522</b>	<b>-0.522</b>	-0.140	-0.111	<b>-0.458</b>	<b>-0.470</b>	<b>-0.526</b>	-0.398	-0.385	-0.166	-0.111	-0.399	-0.286	-0.358
<i>Laccophilus</i> sp. Lv.	0.428	0.431	0.303	-0.111	0.267	<b>0.470</b>	0.438	0.337	0.398	0.208	-0.111	0.188	0.435	0.434
<i>Pomatinus substriatus</i> Ad.	<b>0.500</b>	<b>0.489</b>	0.140	0.250	<b>0.495</b>	<b>0.463</b>	0.373	0.348	0.369	0.109	0.250	0.327	0.407	0.229
<i>Hydraena riparia</i> Ad.	-0.188	-0.147	<b>-0.487</b>	-0.386	-0.196	-0.127	-0.134	-0.034	-0.031	-0.261	-0.386	-0.002	-0.069	-0.007
<i>Esolus parallelepipedus</i> Ad.	0.293	0.411	-0.176	-0.139	0.125	0.407	0.389	0.313	0.417	0.121	-0.139	0.230	<b>0.478</b>	0.432
<i>Esolus</i> sp. Ad.	0.343	0.352	0.369	0.172	0.321	0.348	0.346	0.440	0.378	<b>0.542</b>	0.172	<b>0.539</b>	0.373	0.366
<i>Limnius</i> sp. Ad.	0.174	0.174	0.327	<b>0.444</b>	0.308	0.191	0.117	0.261	0.203	0.311	<b>0.444</b>	0.319	0.117	0.175
<i>Oulimnius tuberculatus</i> Lv.	<b>0.456</b>	0.441	<b>0.451</b>	<b>0.493</b>	<b>0.585</b>	0.341	0.300	0.440	0.398	0.401	<b>0.493</b>	<b>0.511</b>	0.301	0.317
<i>Riolus</i> sp. Lv.	0.296	0.368	-0.140	-0.111	0.118	0.400	0.355	0.417	<b>0.461</b>	0.208	-0.111	0.360	<b>0.481</b>	0.403
<i>Mesoveliidae</i> Gen. sp.	0.379	0.339	<b>0.546</b>	-0.076	0.343	0.324	0.302	0.379	0.379	0.428	-0.076	0.340	0.363	0.341
<i>Pisidium</i> sp.	-0.399	-0.430	-0.417	-0.277	-0.394	-0.299	-0.404	<b>-0.467</b>	<b>-0.480</b>	<b>-0.516</b>	-0.277	<b>-0.496</b>	-0.386	<b>-0.468</b>
<i>Chaetogaster</i> sp.	0.379	0.339	<b>0.546</b>	-0.076	0.343	0.324	0.302	0.379	0.379	0.428	-0.076	0.340	0.363	0.341
<i>Nais behningi</i>	0.341	0.376	0.139	0.248	0.312	0.314	<b>0.457</b>	0.262	0.265	0.108	0.248	0.243	0.202	0.261
<i>Limnodrilus</i> sp.	0.377	0.413	0.087	-0.166	0.259	0.407	0.413	<b>0.462</b>	<b>0.470</b>	0.296	-0.166	<b>0.464</b>	<b>0.477</b>	0.436
<i>Potamothrix hammoniensis</i>	-0.331	-0.404	-0.160	0.015	-0.138	<b>-0.451</b>	<b>-0.467</b>	-0.415	<b>-0.450</b>	-0.311	0.015	-0.268	<b>-0.458</b>	<b>-0.561</b>
<i>Psammoryctides barbatus</i>	<b>-0.534</b>	<b>-0.511</b>	-0.329	-0.261	<b>-0.468</b>	<b>-0.530</b>	-0.442	-0.384	-0.408	-0.193	-0.261	-0.310	<b>-0.480</b>	-0.365
<i>Lumbricidae</i> Gen. sp.	<b>-0.484</b>	<b>-0.539</b>	-0.329	-0.261	<b>-0.550</b>	<b>-0.454</b>	<b>-0.465</b>	-0.402	-0.438	-0.193	-0.261	-0.370	-0.353	<b>-0.485</b>
<i>Hemicleipsis marginata</i>	0.379	0.339	<b>0.546</b>	-0.076	0.343	0.324	0.302	0.379	0.379	0.428	-0.076	0.340	0.363	0.341
<i>Aturus scaber scaber</i>	<b>0.463</b>	<b>0.492</b>	0.327	<b>0.444</b>	<b>0.484</b>	<b>0.470</b>	0.439	0.420	0.405	0.311	<b>0.444</b>	0.436	0.396	0.291
<i>Atractides</i> sp.	-0.377	-0.377	-0.176	-0.140	-0.296	<b>-0.519</b>	-0.356	<b>-0.487</b>	<b>-0.499</b>	-0.209	-0.140	-0.390	<b>-0.603</b>	<b>-0.514</b>
<i>Hygrobatas calliger</i>	0.176	0.242	-0.127	-0.166	0.015	0.354	0.241	0.340	0.388	0.034	-0.166	0.184	<b>0.462</b>	<b>0.445</b>
<i>Lebertia</i> sp.	0.343	0.422	0.074	0.234	0.299	0.430	0.418	0.387	0.416	0.106	0.234	0.314	<b>0.459</b>	<b>0.493</b>
<i>Mideopsis orbicularis</i>	0.161	0.116	<b>0.449</b>	0.232	0.245	0.163	0.071	0.206	0.164	0.287	0.232	0.256	0.139	0.134

Table 4.4.3. (Continued). Results of Spearman's rank correlation coefficient (R) for the relationship between Bednja River taxa abundance and EN 15843:2010 grouped and average scores. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Only taxa with at least one significant correlation to a hydromorphological score is given.

	EN 15843:2010 - 100 m scale							EN 15843:2010 - 500 m scale						
	AVERAGE SCORE	Morphology	Flow	Continuity	Channel	Riparian zone	Floodplain	AVERAGE SCORE	Morphology	Flow	Continuity	Channel	Riparian zone	Floodplain
	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<i>Mideopsis</i> sp.	0.140	0.100	<b>0.546</b>	<b>0.688</b>	0.283	0.101	0.080	0.160	0.140	0.428	<b>0.688</b>	0.280	0.101	0.060
<i>Nudomideopsis</i> cf. <i>Motasi</i>	0.379	0.339	<b>0.546</b>	-0.076	0.343	0.324	0.302	0.379	0.379	0.428	-0.076	0.340	0.363	0.341
<i>Sperchon compactilis</i>	<b>0.463</b>	<b>0.492</b>	0.327	<b>0.444</b>	<b>0.484</b>	<b>0.470</b>	0.439	0.420	0.405	0.311	<b>0.444</b>	0.436	0.396	0.291
<i>Sperchon insignis</i>	0.339	0.339	<b>0.546</b>	<b>0.688</b>	0.383	0.324	0.302	0.319	0.259	<b>0.542</b>	<b>0.688</b>	0.380	0.242	0.180
<i>Sperchon papillosus</i>	0.339	0.339	<b>0.546</b>	<b>0.688</b>	0.383	0.324	0.302	0.319	0.259	<b>0.542</b>	<b>0.688</b>	0.380	0.242	0.180
<i>Torrenticola amplexa</i>	0.379	0.339	<b>0.546</b>	-0.076	0.343	0.324	0.302	0.379	0.379	0.428	-0.076	0.340	0.363	0.341
<i>Torrenticola elliptica</i>	0.140	0.100	<b>0.546</b>	<b>0.688</b>	0.283	0.101	0.080	0.160	0.140	0.428	<b>0.688</b>	0.280	0.101	0.060
<i>Torrenticola hyporheica</i>	0.339	0.339	<b>0.546</b>	<b>0.688</b>	0.383	0.324	0.302	0.319	0.259	<b>0.542</b>	<b>0.688</b>	0.380	0.242	0.180
<i>Hydrachnidia</i> Gen. sp.	0.233	0.242	0.303	<b>0.469</b>	0.236	0.252	0.170	0.103	0.112	0.214	<b>0.469</b>	0.121	0.107	0.020

CCA analysis of all taxa with the EN 15843:2010 grouped and average score assessed for 100 m are given in Fig. 4.4.5. CCA analysis of all taxa with the EN 15843:2010 grouped and average score assessed for 500 m are given in Fig. 4.4.6.

For EN 15843:2010 grouped and average scores for hydromorphological modification of a 100 m long reach, total variation is 2.454 with hydromorphological scores accounting for 29.0% of the variation. The eigenvalues of the first 2 axis are 0.255 and 0.135 respectively (Fig. 4.4.5).

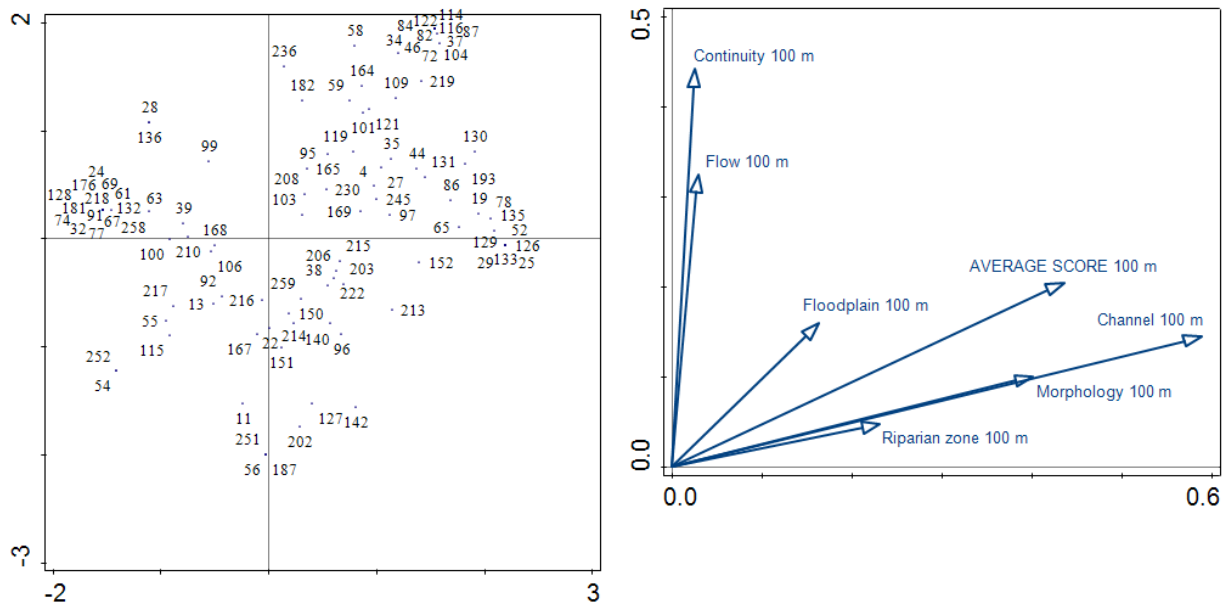


Figure 4.4.5. Canonical correspondence analysis showing variation in taxa explained by EN 15843:2010 grouped and average score for hydromorphological modification assessed on a 100 m long reach. The Subplot 1 is focused on taxa. The distance between the symbols (dots) approximates the dissimilarity of distribution of relative abundance of those taxa across the samples. The Subplot 2 shows EN 15843:2010 grouped and average score arrows. Each arrow points in the direction of the steepest increase of modification.

Index value ranges for taxa groups in Subplot 1 are: 1 – 23 (Ephemeroptera), 24 – 31 (Plecoptera), 32 – 78 (Trichoptera), 79 – 85 (Odonata), 86 – 88 (Megaloptera), Diptera (89 – 102), Chironomidae (103 – 110), Coleoptera (111 – 157), Heteroptera (158 – 166), Crustacea (167 – 169), Bivalvia (170 – 174), Gastropoda (175 – 189), Oligochaeta (190 – 214), Hirudinea (215 – 224), Hydrachnidia (225 – 255), Turbellaria (256 – 258), Hydra (259). Corresponding index label for each taxon is given in Annex I.

For EN 15843:2010 grouped and average scores for hydromorphological modification of a 500 m long reach, total variation is 2.454 with hydromorphological scores accounting for 29.7% of the variation. The eigenvalues of the first 2 axis are 0.270 and 0.168 respectively (Fig. 4.4.6).

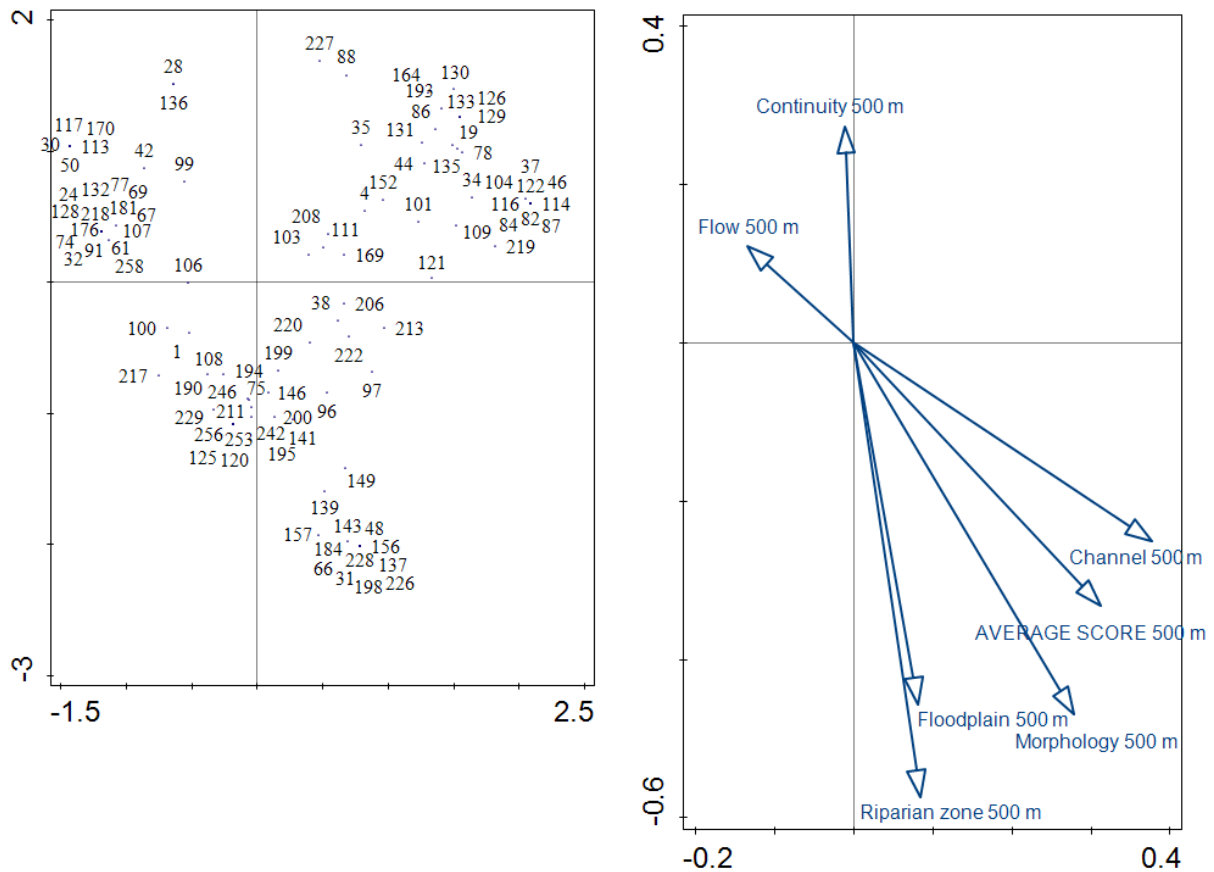


Figure 4.4.6. Canonical correspondence analysis showing variation in taxa explained by EN 15843:2010 grouped and average score for hydromorphological modification assessed on a 500 m long reach. The Subplot 1 is focused on taxa. The distance between the symbols (dots) approximates the dissimilarity of distribution of relative abundance of those taxa across the samples. The Subplot 2 shows EN 15843:2010 grouped and average score arrows. Each arrow points in the direction of the steepest increase of modification.

Index value ranges for taxa groups in Subplot 1 are: 1 – 23 (Ephemeroptera), 24 – 31 (Plecoptera), 32 – 78 (Trichoptera), 79 – 85 (Odonata), 86 – 88 (Megaloptera), Diptera (89 – 102), Chironomidae (103 – 110), Coleoptera (111 – 157), Heteroptera (158 – 166), Crustacea (167 – 169), Bivalvia (170 – 174), Gastropoda (175 – 189), Oligochaeta (190 – 214), Hirudinea (215 – 224), Hydrachnidia (225 – 255), Turbellaria (256 – 258), Hydra (259). Corresponding index label for each taxon is given in Annex I.

EN 15843:2010 hydromorphological modification individual scores were correlated with all 259 taxa. 101 taxa gave significant Spearman correlations with EN 15843:2010 100 m individual scores. The highest number of significant taxa correlations is found features 2a, 3a, and 3b corresponding to substrate, management of aquatic vegetation and woody debris. The strongest significant positive correlation is found between *Aturus scaber scaber* Kramer, 1875 (Hydrachnidia) and feature 2a - Extent of artificial material ( $R = 0.793, p < 0.05$ ). The strongest significant negative correlation is found between the family Ceratopogonidae (Diptera) and feature 10a - Degree of lateral connectivity of river and floodplain ( $R = -0.774, p < 0.05$ ). Taxa giving significant correlations to EN 15843:2010 100 m individual scores are given in Tab 4.4.4.

Table 4.4.4. Results of Spearman's rank correlation coefficient (R) for the relationship between Bednja River taxa abundance and EN 15843:2010 scores for individual hydromorphological features on a 100 m long reach. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Only taxa with at least one statistically significant correlation to a hydromorphological score is given. Hydromorphological feature code given in Fig. 4.4.7.

	EN 15843:2010 - 100 m scale – individual features													
	1a	1b	2a	2b	3a	3b	4	5b	6	7	8	9	10a	10b
	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<i>Baetis lutheri</i>	0.214	0.287	0.350	0.122	-0.111	0.225	0.021	0.350	<b>0.472</b>	0.416	0.190	0.362	0.151	0.354
<i>Cloeon dipterum</i>	0.214	0.287	0.350	<b>0.454</b>	<b>0.472</b>	0.225	0.430	<b>0.792</b>	0.416	0.173	0.361	0.218	0.210	0.118
<i>Heptagenia longicauda</i>	-0.036	-0.114	-0.269	<b>-0.471</b>	-0.214	-0.319	-0.393	-0.269	-0.214	-0.123	-0.027	0.020	-0.149	-0.192
<i>Paraleptophlebia submarginata</i>	0.147	0.198	<b>0.546</b>	0.313	<b>0.688</b>	0.399	0.296	<b>0.546</b>	-0.076	0.376	0.249	0.249	0.256	0.320
<i>Potamanthus luteus</i>	-0.134	-0.119	0.262	0.031	0.180	0.082	-0.165	<b>0.482</b>	0.262	0.123	0.023	0.183	-0.113	0.020
<i>Perla marginata/pallida</i>	0.147	0.198	<b>0.546</b>	0.313	-0.076	<b>0.513</b>	0.296	-0.096	-0.076	0.376	0.249	0.249	0.256	0.320
<i>Beraeodes</i> sp.	-0.192	-0.092	-0.140	0.122	<b>0.472</b>	0.312	0.021	-0.140	-0.111	-0.238	-0.039	-0.025	-0.371	-0.265
<i>Goera pilosa</i>	-0.237	-0.046	0.192	0.183	0.228	<b>0.467</b>	0.185	-0.209	-0.166	-0.020	0.016	0.188	-0.236	-0.083
<i>Silo</i> sp.	0.214	0.029	-0.140	<b>0.454</b>	<b>0.472</b>	0.312	0.226	-0.140	-0.111	-0.238	-0.039	0.090	-0.371	-0.265
<i>Hydropsyche bulbifera</i>	0.274	0.228	0.242	0.075	-0.237	0.134	0.276	-0.071	0.034	0.188	0.146	-0.041	<b>0.635</b>	0.346
<i>Hydropsyche saxonica</i>	-0.054	-0.190	-0.176	0.307	0.325	0.173	0.018	-0.176	-0.139	-0.300	-0.173	0.032	<b>-0.467</b>	-0.333
<i>Hydropsyche fulvipes</i>	0.214	0.002	0.303	0.087	-0.111	0.267	0.204	-0.140	-0.111	<b>0.456</b>	0.093	0.140	0.371	<b>0.464</b>
<i>Hydropsyche tenuis</i>	0.147	0.198	-0.096	0.313	<b>0.688</b>	<b>0.513</b>	0.296	-0.096	-0.076	-0.164	0.249	0.249	-0.256	-0.183
<i>Hydroptila</i> sp.	0.239	0.268	0.342	-0.073	-0.349	0.112	0.287	0.098	0.218	0.390	0.282	0.063	<b>0.593</b>	0.365
<i>Hydroptila sparsa</i>	0.147	0.198	<b>0.546</b>	0.313	-0.076	<b>0.513</b>	0.296	-0.096	-0.076	0.376	0.249	0.249	0.256	0.320
<i>Adicella</i> sp.	0.214	0.287	0.350	0.122	<b>0.472</b>	0.225	0.226	0.350	-0.111	<b>0.545</b>	0.361	0.362	0.371	<b>0.464</b>
<i>Oecetis lacustris</i>	0.214	0.287	0.350	0.122	<b>0.472</b>	0.225	0.226	0.350	-0.111	<b>0.545</b>	0.361	0.362	0.371	<b>0.464</b>
<i>Anabolia furcata</i>	-0.077	-0.069	0.343	<b>0.572</b>	0.377	0.328	0.292	<b>0.554</b>	0.377	0.062	-0.075	-0.014	-0.203	0.090
<i>Potamophylax rotundipennis</i>	0.366	0.092	0.415	<b>0.550</b>	<b>0.475</b>	<b>0.489</b>	0.420	0.415	0.228	0.341	0.106	0.247	0.127	0.295
<i>Odontocerum albicorne</i>	0.147	0.198	<b>0.546</b>	0.313	-0.076	<b>0.513</b>	0.296	-0.096	-0.076	0.376	0.249	0.249	0.256	0.320
<i>Cyrnus trimaculatus</i>	0.037	-0.022	0.105	0.067	0.232	-0.020	0.110	<b>0.449</b>	0.196	0.306	0.107	0.039	0.208	0.250
<i>Lype reducta</i>	0.147	0.198	-0.096	0.313	-0.076	-0.114	0.296	<b>0.546</b>	<b>0.688</b>	-0.164	0.249	0.042	0.021	-0.183
<i>Lype</i> sp.	0.214	0.029	-0.140	<b>0.454</b>	-0.111	-0.165	0.226	-0.140	-0.111	-0.238	-0.039	0.090	0.019	0.118
<i>Notidobia ciliaris</i>	0.269	0.111	0.176	<b>0.570</b>	0.325	<b>0.516</b>	0.342	-0.176	-0.139	-0.004	0.066	0.191	-0.187	-0.058
<i>Orthetrum albistylum</i>	0.147	0.198	-0.096	0.313	<b>0.688</b>	<b>0.513</b>	0.296	-0.096	-0.076	-0.164	0.249	0.249	-0.256	-0.183
<i>Coenagrionidae</i> Gen. sp.	0.147	0.198	-0.096	0.313	<b>0.688</b>	<b>0.513</b>	0.296	-0.096	-0.076	-0.164	0.249	0.249	-0.256	-0.183
<i>Sialis fuliginosa</i>	0.214	0.002	-0.140	<b>0.454</b>	-0.111	-0.165	0.204	0.303	0.416	-0.238	-0.081	-0.082	-0.180	-0.265
<i>Sialis lutaria</i>	0.147	0.198	-0.096	0.313	<b>0.688</b>	<b>0.513</b>	0.296	-0.096	-0.076	-0.164	0.249	0.249	-0.256	-0.183
<i>Sialis sordida</i>	-0.143	0.016	-0.140	0.105	-0.111	-0.166	0.215	0.327	<b>0.444</b>	-0.239	-0.060	-0.075	-0.170	-0.265
<i>Ceratopogonidae</i> Gen. sp.	-0.436	<b>-0.611</b>	<b>-0.525</b>	-0.329	-0.218	-0.415	<b>-0.631</b>	<b>-0.452</b>	-0.291	-0.438	<b>-0.519</b>	-0.327	<b>-0.774</b>	<b>-0.505</b>
<i>Empididae</i> Gen. sp.	-0.337	-0.190	0.012	<b>-0.466</b>	-0.305	-0.157	-0.332	-0.244	-0.218	-0.021	-0.101	-0.320	0.077	-0.154
<i>Limoniidae</i> Gen. sp.	-0.432	<b>-0.473</b>	-0.160	-0.424	-0.205	-0.306	<b>-0.630</b>	-0.246	-0.308	0.003	-0.241	-0.126	-0.388	-0.073
<i>Antocha</i> sp.	0.343	0.236	0.345	0.148	-0.294	0.137	0.290	0.123	0.220	0.378	0.415	0.326	<b>0.620</b>	<b>0.546</b>
<i>Stratiomyidae</i> Gen. sp.	<b>-0.517</b>	<b>-0.511</b>	-0.140	-0.245	-0.111	-0.166	-0.430	-0.140	-0.111	-0.239	-0.377	-0.257	-0.372	-0.265
<i>Tipulidae</i> Gen. sp.	-0.272	-0.288	-0.243	-0.182	0.192	0.029	-0.149	-0.243	-0.192	-0.236	-0.178	-0.240	<b>-0.504</b>	-0.310
<i>Chironomus</i> sp.	0.147	0.198	-0.096	0.313	<b>0.688</b>	<b>0.513</b>	0.296	-0.096	-0.076	-0.164	0.249	0.249	-0.256	-0.183
<i>Diamesinae</i> Gen. sp.	<b>-0.523</b>	<b>-0.510</b>	-0.140	-0.244	-0.111	-0.165	-0.430	-0.140	-0.111	-0.238	<b>-0.482</b>	<b>-0.452</b>	-0.371	-0.265
<i>Prodiamesa olivacea</i>	0.074	0.171	<b>-0.462</b>	-0.109	0.203	-0.176	-0.090	-0.195	-0.159	<b>-0.454</b>	0.077	-0.036	-0.264	-0.344



99 taxa gave significant Spearman correlations with EN 15843:2010 500 m individual scores. The highest number of significant taxa correlations is found with feature 3b - Extent of woody debris, followed by 2a - Extent of artificial material. The strongest significant positive correlation is found between *Ecdyonurus macani* Thomas & Sowa, 1970 (Ephemeroptera) and feature 5a - Impacts of artificial in-channel structures within the reach ( $R = 0.793$ ,  $p < 0.05$ ). The strongest significant negative correlation is found between the family Ceratopogonidae (Diptera) and feature 10a - Degree of lateral connectivity of river and floodplain ( $R = -0.709$ ,  $p < 0.05$ ). Taxa giving significant correlations to EN 15843:2010 500 m individual scores are given in Tab 4.4.5.

Table 4.4.5. Results of Spearman's rank correlation coefficient (R) for the relationship between Bednja River taxa abundance and EN 15843:2010 scores for individual hydromorphological features on a 500 m long reach. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Only taxa with at least one statistically significant correlation to a hydromorphological score is given. Hydromorphological feature code given in Fig. 4.4.8.

	EN 15843:2010 - 500 m scale – individual features														
	1a	1b	2a	2b	3a	3b	4	5a	5b	6	7	8	9	10a	10b
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<i>Baetis fuscatus</i>	-0.067	-0.135	0.409	-0.239	-0.342	0.101	-0.286	0.405	-0.085	0.029	<b>0.479</b>	-0.006	-0.008	0.098	0.370
<i>Baetis lutheri</i>	0.013	0.147	0.229	0.066	-0.111	0.169	0.021	<b>0.472</b>	0.350	<b>0.472</b>	0.314	0.147	0.365	0.005	0.222
<i>Cloeon dipterum</i>	0.262	0.336	0.288	<b>0.509</b>	<b>0.500</b>	0.169	0.430	-0.111	<b>0.792</b>	0.416	0.185	0.336	0.163	0.268	0.148
<i>Ecdyonurus macani</i>	0.110	0.154	0.383	<b>0.465</b>	-0.140	0.336	0.362	<b>0.793</b>	0.216	0.327	0.302	0.103	0.281	0.064	0.200
<i>Heptagenia longicauda</i>	-0.060	-0.191	-0.180	<b>-0.516</b>	-0.214	-0.367	-0.393	-0.214	-0.269	-0.214	-0.058	-0.067	0.027	-0.085	-0.091
<i>Paraleptophlebia submarginata</i>	0.181	0.231	<b>0.497</b>	0.439	<b>0.725</b>	0.341	0.296	-0.076	<b>0.546</b>	-0.076	0.405	0.231	0.251	0.294	0.372
<i>Potamanthus luteus</i>	-0.205	-0.143	0.310	0.028	0.202	-0.015	-0.165	0.000	<b>0.482</b>	0.262	0.223	0.033	0.069	-0.123	0.134
<i>Perla marginata/pallida</i>	0.181	-0.042	0.419	0.254	-0.076	<b>0.472</b>	0.000	-0.076	-0.096	-0.076	0.338	0.231	0.251	0.063	0.262
<i>Hydropsyche angustipennis</i>	-0.044	-0.013	-0.262	-0.234	0.046	-0.214	-0.074	-0.285	-0.359	-0.285	-0.321	-0.222	<b>-0.452</b>	-0.012	-0.232
<i>Hydropsyche bulbifera</i>	0.372	0.389	0.328	0.224	-0.237	0.327	0.302	<b>0.475</b>	-0.071	0.034	0.191	0.288	0.026	<b>0.549</b>	0.292
<i>Hydropsyche pellucidula</i>	-0.315	<b>-0.449</b>	-0.068	-0.127	-0.165	-0.284	-0.320	-0.165	-0.208	-0.165	-0.009	-0.330	-0.042	-0.294	-0.035
<i>Hydropsyche saxonica</i>	-0.197	-0.184	-0.239	0.223	0.302	0.128	0.018	-0.139	-0.176	-0.139	-0.329	-0.148	0.102	<b>-0.460</b>	-0.359
<i>Hydropsyche fulvipes</i>	0.262	0.147	<b>0.548</b>	0.368	-0.111	<b>0.585</b>	0.226	<b>0.472</b>	-0.140	-0.111	<b>0.542</b>	0.336	0.365	0.268	<b>0.464</b>
<i>Hydropsyche tenuis</i>	0.181	0.231	-0.131	0.254	<b>0.649</b>	<b>0.472</b>	0.296	-0.076	-0.096	-0.076	-0.180	0.231	0.251	-0.252	-0.197
<i>Hydroptila sp.</i>	0.426	0.263	0.401	-0.033	-0.341	0.167	0.220	0.335	0.098	0.218	0.397	0.314	-0.019	<b>0.483</b>	0.337
<i>Hydroptila sparsa</i>	0.181	-0.042	0.419	0.254	-0.076	<b>0.472</b>	0.000	-0.076	-0.096	-0.076	0.338	0.231	0.251	0.063	0.262
<i>Adicella sp.</i>	0.262	0.147	0.288	0.207	<b>0.500</b>	0.169	0.226	-0.111	0.350	-0.111	<b>0.495</b>	0.336	0.365	0.427	<b>0.464</b>
<i>Oecetis lacustris</i>	0.262	0.147	0.288	0.207	<b>0.500</b>	0.169	0.226	-0.111	0.350	-0.111	<b>0.495</b>	0.336	0.365	0.427	<b>0.464</b>
<i>Anabolia fuscata</i>	-0.104	0.017	0.346	<b>0.500</b>	0.386	0.202	0.292	0.126	<b>0.554</b>	0.377	0.133	-0.059	0.028	-0.257	0.165
<i>Halesus digitatus/tesselatus</i>	0.181	0.231	0.340	0.254	-0.076	0.341	0.296	<b>0.688</b>	-0.096	-0.076	0.405	0.231	0.251	0.294	0.372
<i>Potamophylax rotundipennis</i>	0.234	0.312	<b>0.494</b>	<b>0.718</b>	<b>0.477</b>	<b>0.618</b>	<b>0.559</b>	<b>0.589</b>	0.415	0.228	0.351	0.261	<b>0.450</b>	0.062	0.239
<i>Odontocerum albicorne</i>	0.181	-0.042	0.419	0.254	-0.076	<b>0.472</b>	0.000	-0.076	-0.096	-0.076	0.338	0.231	0.251	0.063	0.262
<i>Cyrnus trimaculatus</i>	0.143	0.113	0.365	0.276	0.254	0.117	0.242	0.125	<b>0.449</b>	0.196	0.443	0.302	0.155	0.292	0.388
<i>Lype reducta</i>	0.181	0.231	-0.131	0.254	-0.076	-0.131	0.296	-0.076	<b>0.546</b>	<b>0.688</b>	-0.180	0.231	-0.042	0.063	-0.197
<i>Psychomyia pusilla</i>	0.109	-0.116	0.438	-0.068	-0.271	0.193	-0.176	0.293	0.074	0.161	<b>0.519</b>	0.256	0.266	0.052	0.328
<i>Notidobia ciliaris</i>	0.088	-0.023	0.062	<b>0.463</b>	0.302	<b>0.458</b>	0.180	-0.139	-0.176	-0.139	-0.045	0.070	0.262	-0.288	-0.108
<i>Orhetrum albistylum</i>	0.181	0.231	-0.131	0.254	<b>0.649</b>	<b>0.472</b>	0.296	-0.076	-0.096	-0.076	-0.180	0.231	0.251	-0.252	-0.197
<i>Coenagrionidae</i> Gen. sp.	0.181	0.231	-0.131	0.254	<b>0.649</b>	<b>0.472</b>	0.296	-0.076	-0.096	-0.076	-0.180	0.231	0.251	-0.252	-0.197
<i>Sialis lutaria</i>	0.181	0.231	-0.131	0.254	<b>0.649</b>	<b>0.472</b>	0.296	-0.076	-0.096	-0.076	-0.180	0.231	0.251	-0.252	-0.197
<i>Sialis sordida</i>	-0.115	0.015	-0.190	0.050	-0.111	-0.191	0.215	-0.111	0.327	<b>0.444</b>	-0.262	-0.122	-0.274	-0.137	-0.286
<i>Athericidae</i> Gen. sp.	0.171	0.229	0.290	0.066	-0.286	0.271	0.000	<b>0.461</b>	0.174	0.350	0.358	0.141	0.270	0.134	0.186





Table 4.4.5. (Continued). Results of Spearman's rank correlation coefficient (R) for the relationship between Bednja River taxa abundance and EN 15843:2010 scores for individual hydromorphological features on a 500 m long reach. Marked correlations are significant at  $p < 0.05$ . Significant correlations are bolded. Only taxa with at least one statistically significant correlation to a hydromorphological score is given. Hydromorphological feature code given in Fig. 4.4.8.

	EN 15843:2010 - 500 m scale – individual features														
	1a	1b	2a	2b	3a	3b	4	5a	5b	6	7	8	9	10a	10b
	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r
<i>Lebertia</i> sp.	0.427	0.215	0.300	0.057	-0.390	0.066	0.148	0.234	0.074	0.234	0.341	<b>0.458</b>	0.179	0.424	0.438
<i>Mideopsis orbicularis</i>	0.329	0.310	-0.029	0.073	0.216	-0.114	0.186	-0.214	<b>0.449</b>	0.232	-0.025	0.186	-0.151	0.232	-0.117
<i>Mideopsis</i> sp.	0.181	0.231	-0.131	0.254	-0.076	-0.131	0.296	-0.076	<b>0.546</b>	<b>0.688</b>	-0.180	0.231	-0.042	0.063	-0.197
<i>Nudomideopsis</i> cf. <i>Motasi</i>	0.181	0.231	<b>0.497</b>	0.439	<b>0.725</b>	0.341	0.296	-0.076	<b>0.546</b>	-0.076	0.405	0.231	0.251	0.294	0.372
<i>Sperchon compactilis</i>	0.263	0.138	<b>0.608</b>	0.369	-0.111	<b>0.591</b>	0.215	<b>0.444</b>	0.327	<b>0.444</b>	0.442	0.336	0.365	0.092	0.302
<i>Sperchon insignis</i>	0.181	0.231	0.419	0.254	-0.076	0.341	0.296	<b>0.688</b>	<b>0.546</b>	<b>0.688</b>	0.270	0.231	0.251	0.063	0.153
<i>Sperchon papillosus</i>	0.181	0.231	0.419	0.254	-0.076	0.341	0.296	<b>0.688</b>	<b>0.546</b>	<b>0.688</b>	0.270	0.231	0.251	0.063	0.153
<i>Sperchonopsis verrucosa</i>	0.176	0.075	<b>0.541</b>	<b>0.550</b>	-0.165	<b>0.535</b>	0.289	<b>0.580</b>	0.104	0.207	0.423	0.188	0.370	0.061	0.295
<i>Sperchon</i> sp.	0.081	-0.018	<b>0.498</b>	0.184	-0.190	0.235	-0.022	0.209	0.096	0.209	0.259	-0.001	-0.022	-0.067	0.142
<i>Torrenticola amplexa</i>	0.181	0.231	<b>0.497</b>	0.439	<b>0.725</b>	0.341	0.296	-0.076	<b>0.546</b>	-0.076	0.405	0.231	0.251	0.294	0.372
<i>Torrenticola elliptica</i>	0.181	0.231	-0.131	0.254	-0.076	-0.131	0.296	-0.076	<b>0.546</b>	<b>0.688</b>	-0.180	0.231	-0.042	0.063	-0.197
<i>Torrenticola hyporheica</i>	0.181	0.231	0.419	0.254	-0.076	0.341	0.296	<b>0.688</b>	<b>0.546</b>	<b>0.688</b>	0.270	0.231	0.251	0.063	0.153
<i>Hydrachnidia</i> Gen. sp.	0.066	-0.049	0.122	0.067	-0.216	0.111	0.091	0.126	0.303	<b>0.469</b>	0.325	0.062	0.045	-0.045	0.166

CCA analysis of all taxa with the EN 15843:2010 individual scores assessed for 100 m are given in Fig. 4.4.7. CCA analysis of all taxa with the EN 15843:2010 individual scores assessed for 500 m are given in Fig. 4.4.8.

For EN 15843:2010 individual scores for hydromorphological modification of a 100 m long reach, total variation is 2.454 with hydromorphological scores accounting for 71.7% of the variation. The eigenvalues of the first 2 axis are 0.380 and 0.325 respectively (Fig. 4.4.7).

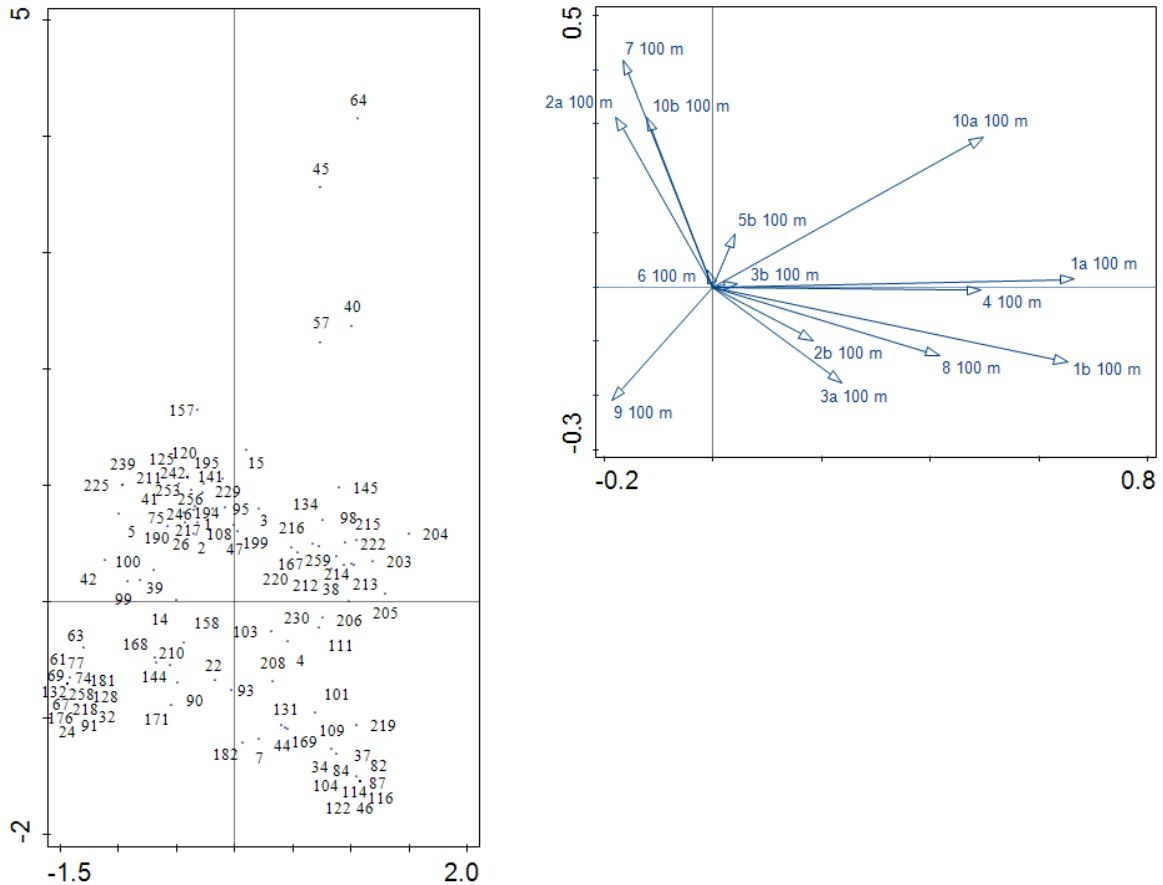


Figure 4.4.7. Canonical correspondence analysis showing variation in taxa explained by EN 15843:2010 individual scores of hydromorphological features assessed on a 100 m long reach. The Subplot 1 is focused on taxa. The distance between the symbols (dots) approximates the dissimilarity of distribution of relative abundance of those taxa across the samples. The Subplot 2 shows EN 15843:2010 individual hydromorphological feature arrows. Each arrow points in the direction of the steepest increase of modification.

Index value ranges for taxa groups in Subplot 1 are: 1 – 23 (Ephemeroptera), 24 – 31 (Plecoptera), 32 – 78 (Trichoptera), 79 – 85 (Odonata), 86 – 88 (Megaloptera), Diptera (89 – 102), Chironomidae (103 – 110), Coleoptera (111 – 157), Heteroptera (158 – 166), Crustacea (167 – 169), Bivalvia (170 – 174), Gastropoda (175 – 189), Oligochaeta (190 – 214), Hirudinea (215 – 224), Hydrachnidia (225 – 255), Turbellaria (256 – 258), Hydra (259). Corresponding index label for each taxon is given in Annex I.

Hydromorphological features in Subplot 2: 1a = Planform; 1b = Channel section (long-section and cross-section); 2a = Extent of artificial material; 2b = "Natural" substrate mix or character altered; 3a = Aquatic vegetation management; 3b = Extent of woody debris if expected; 4 = Erosion/deposition character; 5b = Effects of catchment-wide modifications to natural flow character; 6 = Longitudinal continuity as affected by artificial structures; 7 = Bank structure and modifications; 8 = Vegetation type/structure on banks and adjacent land; 9 = Adjacent landuse and associated features; 10a = Degree of lateral connectivity of river and floodplain; 10b = Degree of lateral movement of river channel.

For EN 15843:2010 individual scores for hydromorphological modification of a 500 m long reach, total variation is 2.454 with hydromorphological scores accounting for 82.7% of the variation. The eigenvalues of the first 2 axis are 0.488 and 0.398 respectively (Fig. 4.4.8).

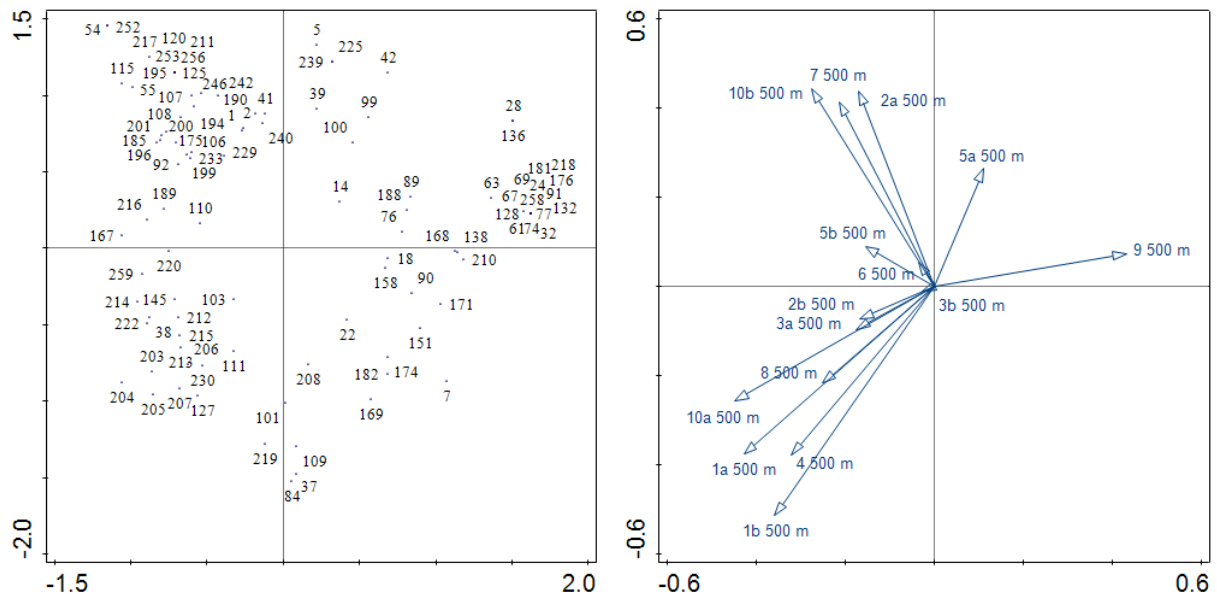


Figure 4.4.8. Canonical correspondence analysis showing variation in taxa explained by EN 15843:2010 individual scores of hydromorphological features assessed on a 500 m long reach. The Subplot 1 is focused on taxa. The distance between the symbols (dots) approximates the dissimilarity of distribution of relative abundance of those taxa across the samples. The Subplot 2 shows EN 15843:2010 individual hydromorphological feature arrows. Each arrow points in the direction of the steepest increase of modification.

Index value ranges for taxa groups in Subplot 1 are: 1 – 23 (Ephemeroptera), 24 – 31 (Plecoptera), 32 – 78 (Trichoptera), 79 – 85 (Odonata), 86 – 88 (Megaloptera), Diptera (89 – 102), Chironomidae (103 – 110), Coleoptera (111 – 157), Heteroptera (158 – 166), Crustacea (167 – 169), Bivalvia (170 – 174), Gastropoda (175 – 189), Oligochaeta (190 – 214), Hirudinea (215 – 224), Hydrachnidia (225 – 255), Turbellaria (256 – 258), Hydra (259). Corresponding index label for each taxon is given in Annex I.

Hydromorphological features in Subplot 2: 1a = Planform; 1b = Channel section (long-section and cross-section); 2a = Extent of artificial material; 2b = "Natural" substrate mix or character altered; 3a = Aquatic vegetation management; 3b = Extent of woody debris if expected; 4 = Erosion/deposition character; 5a = Impacts of artificial in-channel structures within the reach; 5b = Effects of catchment-wide modifications to natural flow character; 6 = Longitudinal continuity as affected by artificial structures; 7 = Bank structure and modifications; 8 = Vegetation type/structure on banks and adjacent land; 9 = Adjacent landuse and associated features; 10a = Degree of lateral connectivity of river and floodplain; 10b = Degree of lateral movement of river channel.

#### 4.5 Response gradient of the benthic macroinvertebrate community towards anthropogenic stressors and hydromorphological alterations

The response gradient of the benthic macroinvertebrate community is given as the Ecological Quality Ratio (EQR) gradient towards anthropogenic stressors. The response gradient of the Ecological Quality Ratio (EQR) towards nutrients is given in Fig. 4.5.1.

The steepest gradient is created between EQR and ammonium concentration (Fig. 4.5.1.).

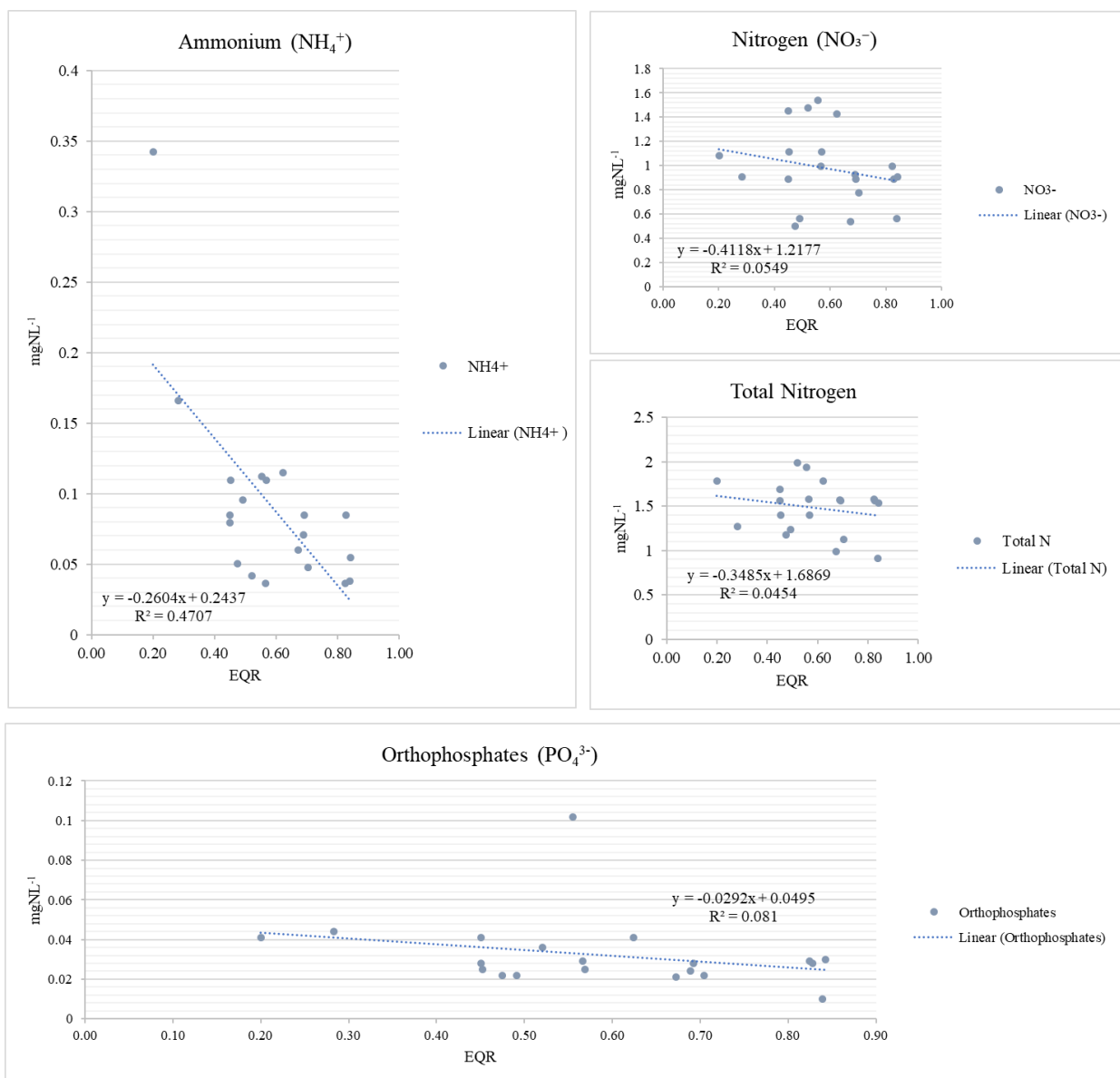


Figure 4.5.1. Response gradient of the Ecological Quality Ratio (EQR) to nutrients: ammonium, nitrates, total nitrogen and orthophosphates.

The response gradient of the Ecological Quality Ratio (EQR) towards landuse is given in Fig. 4.5.2.

The steepest gradient is created for EQR and share of urban landuse (Fig. 4.5.2.).

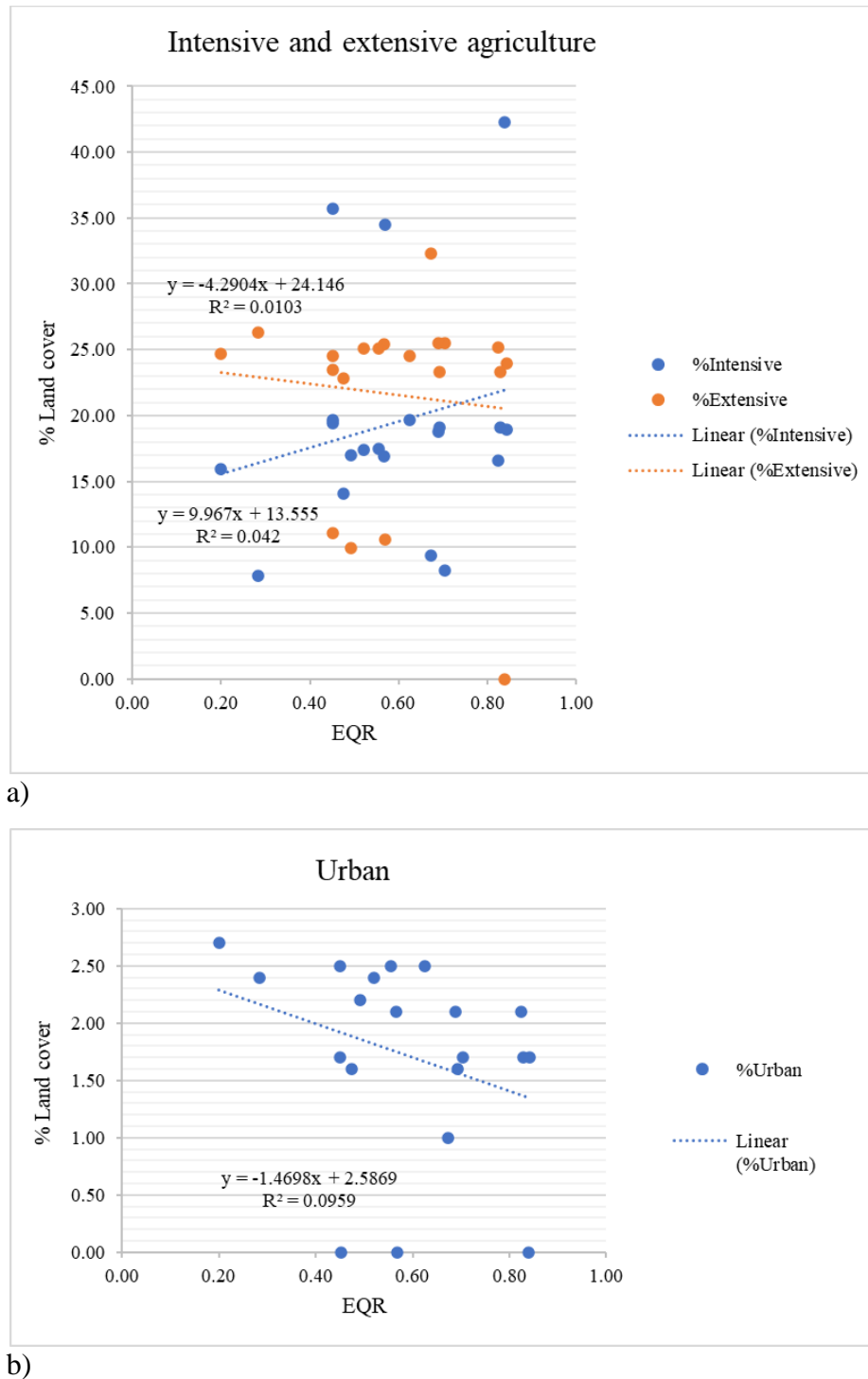


Figure 4.5.2. Response gradient of the Ecological Quality Ratio (EQR) towards: a) intensive and extensive agriculture landuse; b) urban landuse.

The response gradient of the Ecological Quality Ratio (EQR) towards hydromorphological modification EN 15843:2010 is given in Fig. 4.5.3.

The steepest gradient is created for EQR and EN 15843:2010 100 m average score for hydromorphological modification (Fig. 4.5.3.).

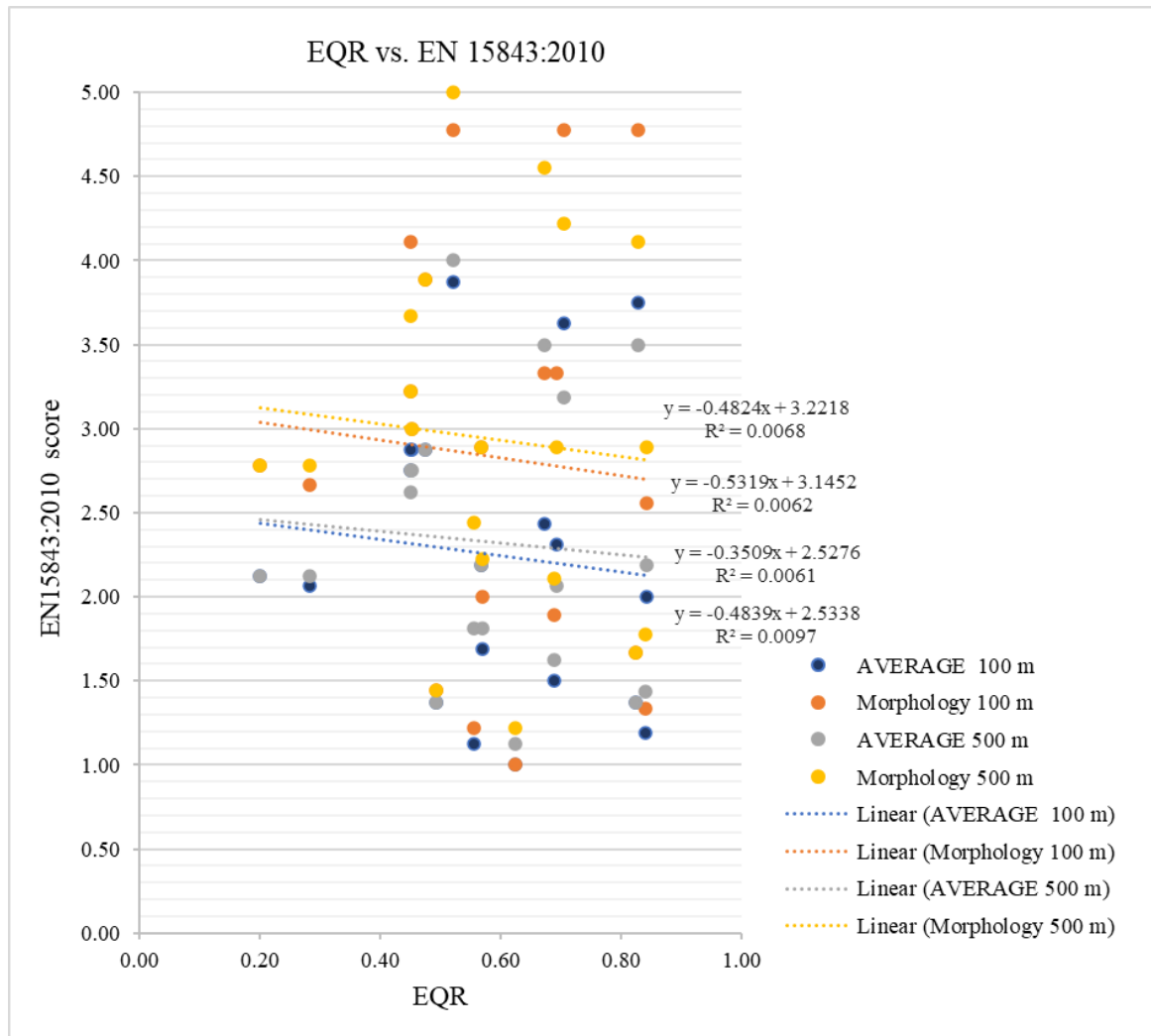
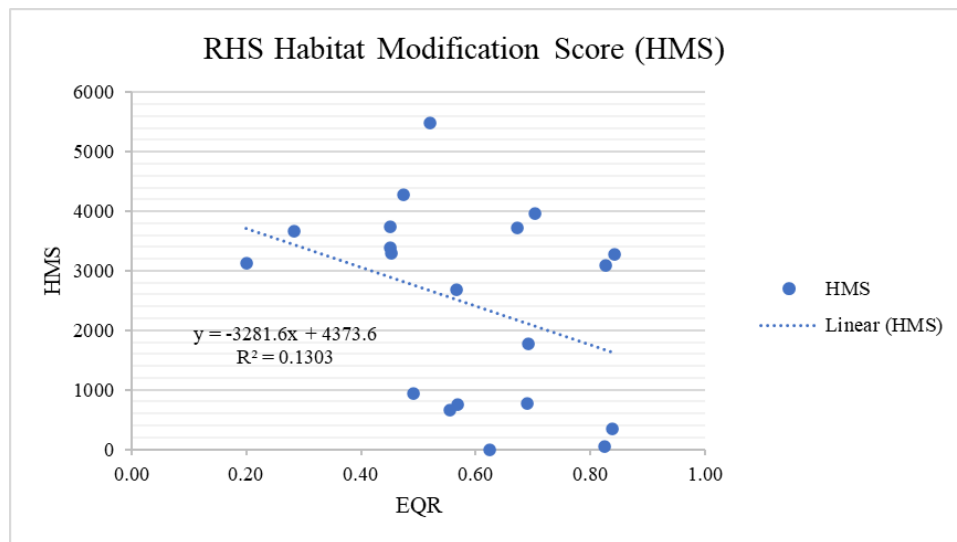


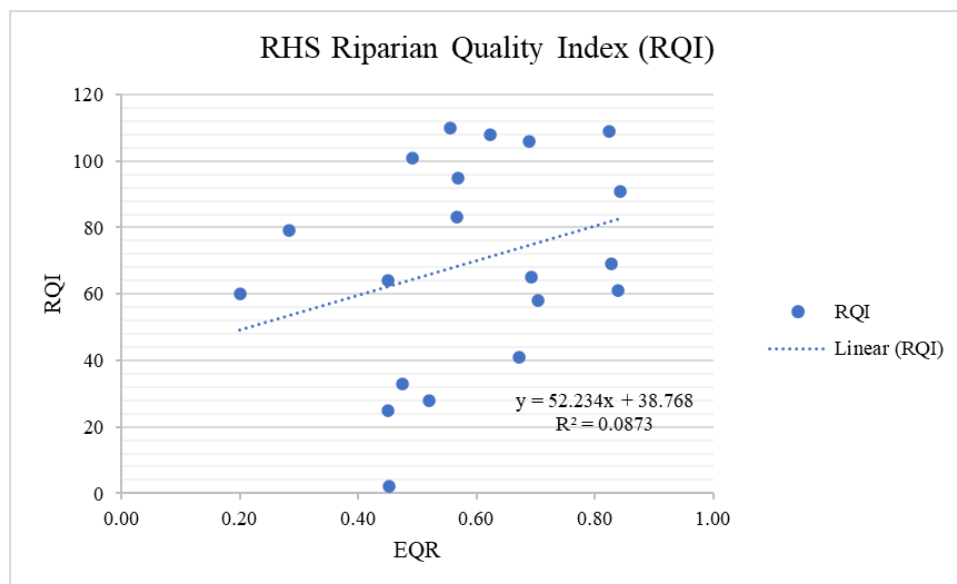
Figure 4.5.3. Response gradient of the Ecological Quality Ratio (EQR) towards hydromorphological modification EN 15843:2010 given for the average score and score for morphological modification on a 100 m and 500 m long reach.

The Response gradient of the Ecological Quality Ratio (EQR) towards River Habitat Survey scores: the Habitat Modification Score (HMS), Riparian Quality Index (RQI), and Habitat Quality Assessment (HQA) score and subscores is given in Fig. 4.5.4. and 4.4.5.

The steepest gradient is created for EQR and the HQA Bank vegetation structure subscore (Fig. 4.5.5.).



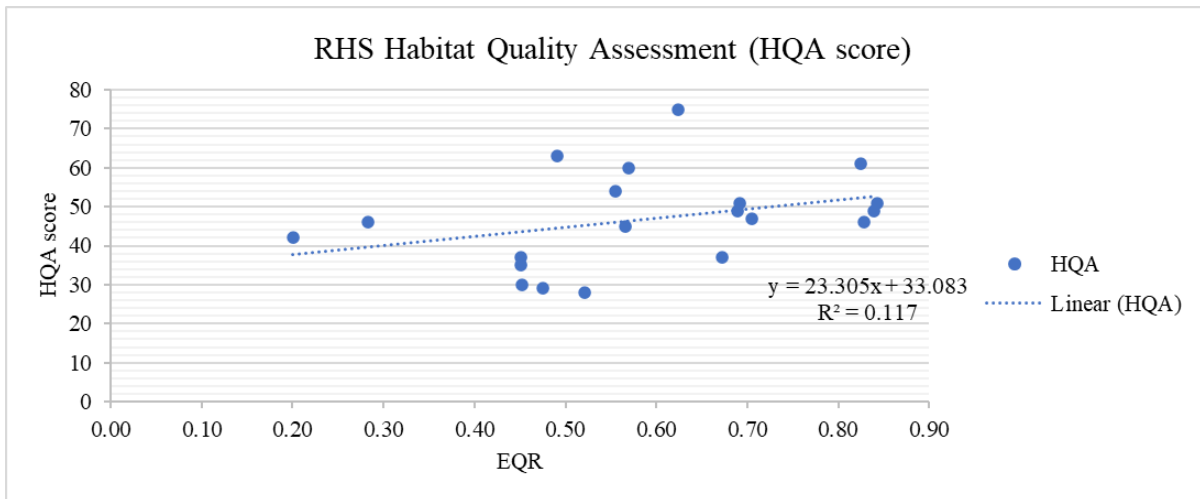
a)



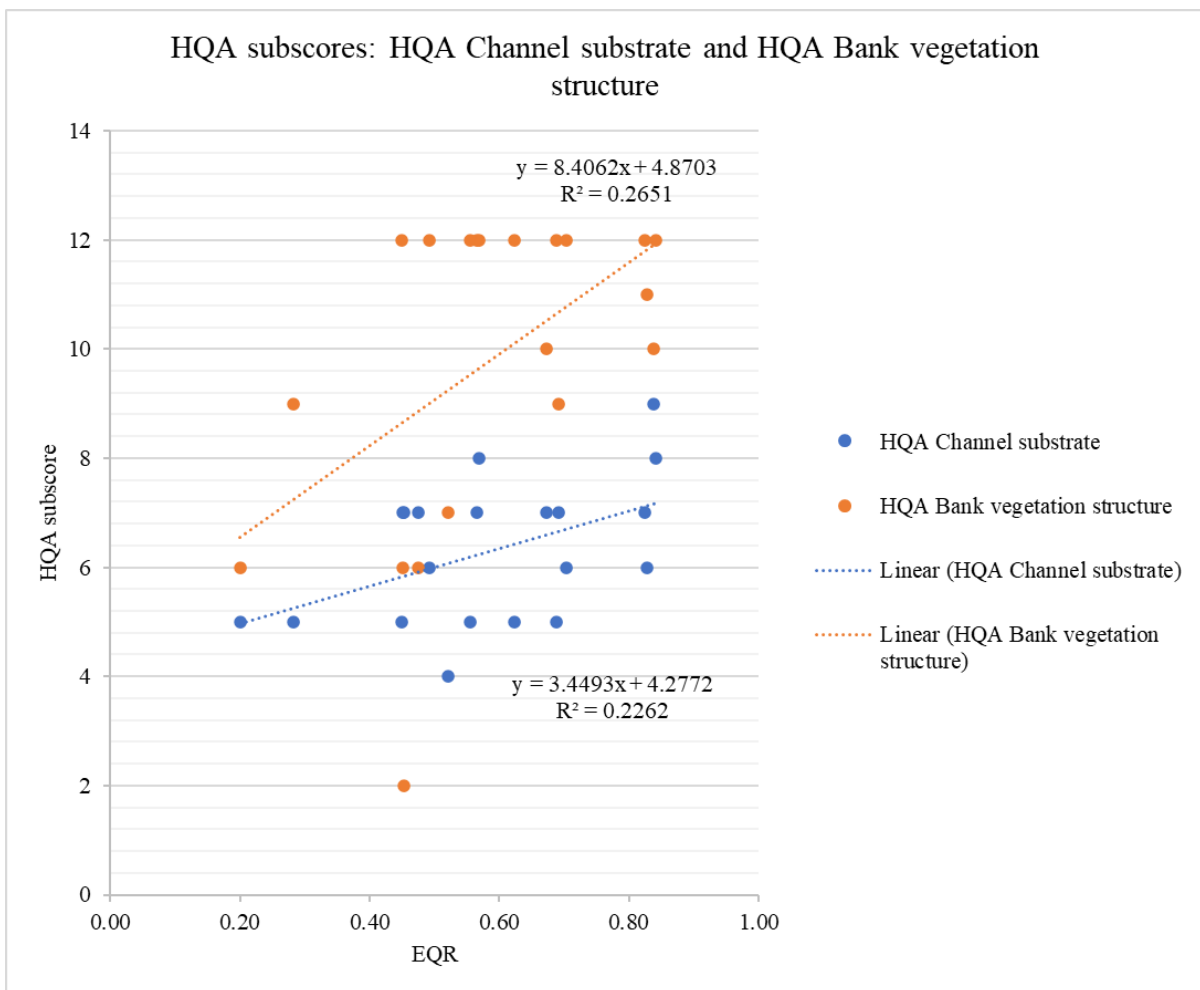
b)

Figure 4.5.4. Response gradient of the Ecological Quality Ratio (EQR) towards: a) Habitat Modification Score (HMS); b) Riparian Quality Index (RQI). A higher HMS score represents higher level of modification, a higher RQI score represents better habitat quality.





a)



b)

Figure 4.5.5. Response gradient of the Ecological Quality Ratio (EQR) towards: a) Habitat Quality Assessment score (HQA); b) River Habitat Survey subscores HQA Bank vegetation structure and HQA Channel substrate. A higher HQA score represents better habitat quality.

## 5. DISCUSSION

### *Environmental variables and stressors*

To meet the objectives of this research and identify the impacts of different stressors and natural factors on the benthic macroinvertebrate assemblages, several environmental variables and stressors were selected and measured. The characteristics of abiotic and environmental parameters, and their relationships with each other in the Bednja River are discussed below.

### Microhabitats

The most frequently and abundantly occurring substrate was medium-sized and coarse gravel (akal and microlithal). This result is not in line with the substrates defined for the corresponding Bednja River types. Rivers belonging to type HR-R\_4A (study sites 10 – 20) are expected to have sand and silt as the dominant substrate, while study sites 1 – 9 belonging to type HR-R\_1 are expected to primarily be dominated by gravel (Mihaljević et al., 2020). Sand or “psammal” microhabitats were present at several sites but overall amounted to only 14.5% of sampled microhabitats. Silt, which can be a result of natural and anthropogenic activities (Wood and Armitage, 1999), was observed at some study site, but was not further quantified. According to Mihaljević et al. (2020) the initial biological typology of rivers in Croatia (i.e. Mihaljević et al., 2011) was mainly based on expert opinion. It is possible that this discrepancy in substrate is a result of expert judgement. Regulated river sections and intensified land-use contribute to deposition of fine sediments in the riverbed (Graf et al., 2016). Therefore, the fine substrate covering the river bed and the larger grained substrate underneath could lead to the misinterpretation of silt being the dominant substrate based on observation. Regarding the measured depth and water velocity, the Bednja River possesses sites with high velocity and depth diversity, but also sites with very low water velocities which are a result of channelization or weirs which have modified the flow (Poff et al., 1997).

### Water quality

Measured water quality parameters, especially BOD in summer, indicate that the main problem for the Bednja River is discharge of untreated wastewater. In summer, several of the parameters reached their maximum concentration due to low water level (e.g. Vero et al., 2019) and the highest water temperature values was recorded for all 20 study sites. This is not a surprise since

water temperature is strongly influenced by air temperature (e.g. Durfee et al., 2021) and exposure to solar radiation (e.g. Wondzell et al., 2019).

The Bednja River receives nutrient input longitudinally along the entire course with some of the upstream study sites already achieving highest values for certain parameters. For example, the upstream reach of the Bednja River is under impact of higher levels of ammonium, and these elevated values are also in line with results of the National Surveillance monitoring data from Hrvatske vode for the upper course. The source of ammonium in rivers is untreated wastewater (European Environment Agency, 2018). Ammonium indicates fresh pollution and as the nitrogen cycle proceeds, it is converted to nitrite, and ultimately nitrate, which is then transported downstream (Xia et al., 2018).

#### Hydromorphological modification

##### *Difference in scores for hydromorphological modification between assessment systems*

In Europe, there are several methods for assessing river hydromorphology (see review by Belletti et al. (2014)). In this study, two separate assessment systems for hydromorphological modification were applied to the same river reach at 20 study sites corresponding to where benthic macroinvertebrates were collected. The two assessment systems gave differing results for extent of hydromorphological modification when applied to the same river reach. There are no available studies and literature exploring differences in EN 15843:2010 and River Habitat Survey (RHS) subscores to discuss these findings, but the results are interpreted as follows:

The RHS Habitat Modification Score (HMS) gave worse scores, indicating greater habitat modification for all study sites as compared to modification classes (average score) calculated using EN 15843:2010. The difference can be ascribed to two factors: different number of encompassed hydromorphological input features between the two systems and different scoring weight for features, primarily channel resectioning in RHS. Firstly, the EN 15843:2010 standard is based on assessing the “departure from naturalness” through quantitative and qualitative estimation of only 16 hydromorphological features deriving an average final score (DIN, 2010). The RHS is an additive system where multiple natural and artificial features are recorded, including the presence of features of special interest which represent valuable microhabitats for benthic macroinvertebrates. These features add up to a final, cumulative score (Raven et al., 1998a, 1998b). Secondly, unlike EN 15843:2010, the RHS weighs heavily any present resectioning even when there is no reinforcement with rip-rap and only a small reach

length is affected. With EN 15843:2010, over 75% of the assessed reach length must have an altered planform (morphology) or banks modified with hard artificial material to receive the worst score 5. Among the 16 assessed features, the EN 15843:2010 also scores flow through three subscores and the longitudinal continuity as a single score (four out of the 16 scores). If there are no significant alterations to these features, this can contribute to improving the average hydromorphological score even if the morphology of the river is heavily altered. A good example where this is evident is study site 2 which represents an over-deepened, straight channel with grass banks. The study site received the worst (class 5) RHS scores for HMS, HQA and RQI while only moderate (class 3) average score according to EN 15843:2010. For this study site to be classified as severely modified (class 5) according to EN 15843:2010 it would further need to have concrete banks and riverbed, disconnected longitudinal continuity, embankments and have greatly altered discharge and daily flow (e.g. hydropeaking) (DIN, 2010). No study site received the worst score (class 5) according to EN 15843:2010. With RHS, 13 study sites fall under the worst Habitat Modification Class (HMC) (Class 5) due to high Habitat Modification Scores (HMS). The biggest contributor to high Habitat Modification Scores (HMS) was the subscore for resectioning of bank and bed. Erba et al. (2006) also report that the subscore for bed and bank resectioning is most responsible for high HMS in their study encompassing a much larger number of sites. According to Szoszkiewicz et al. (2006), bank resectioning is the most common modification found in European rivers.

Despite RHS being more stringent, the two assessment systems have highly correlated subscores. The RHS HMS Realigned channel subscore is almost perfectly correlated with the EN 15843:2010 morphological scores, especially the score for feature 1a - modification of channel planform. The HMS score only encompasses physical alterations as a results of engineering works (and not hydrological or habitat quality features) (Raven et al., 1998b), so its correlation with morphological features of EN 15843:2010 is assumed. Furthermore, correlations with RHS were generally higher when EN 15843:2010 was applied to a 500 m long reach (same as RHS) as opposed to just 100 m. This shows that for two assessment systems to be comparable, the same length of reach should be assessed. This can be explained by existence of upstream disturbances which RHS records, but the 100 m EN 15843:2010 (shorter) assessment misses. A good example of this is study site 6 where there is quite extensive morphological modification further upstream but not as much at the sampling site.

### Sub-catchment landuse

Near natural areas are the most represented land cover on the Bednja catchment due to large areas of forests on the hills and mountains within the boundaries of the catchment (CLC category 311 Broad-leaved forest make up 45% of the total landuse within the catchment). This was expected as different vegetation types are immediately connected to altitude (Townsend et al., 2003). Furthermore, the floodplains of rivers have a long history of being cultivated (Blann et al., 2009) so agriculture being the dominant landuse on the floodplain is common. Urbanized and artificial land cover surfaces are usually much smaller compared to other landuse types (Allan, 2004; Herringshaw et al., 2011) which is also the case with the Bednja River catchment.

### *Correlations between natural factors, landuse, water quality, and hydromorphology*

On the Bednja catchment, several relationships on the longitudinal gradient have been observed. Near natural land cover (which is associated with higher altitude) decreases downstream, while the share of agriculture and urban landuse increases with distance from source. This situation is not a surprise since suitable locations for agricultural or urban development on a river catchment are influenced by natural landscape features such as terrain (Allan, 2004). Pursuantly, agricultural activities are primarily situated in the floodplains, while the main towns are distributed longitudinally along the Bednja River itself (HAOP, 2012), contributing to the longitudinal increase in total share.

Water temperature significantly correlated with distance from source, and this is in line with the generally accepted concept that rivers warm as their water moves downstream from source to mouth (Ward, 1985; Bogan, 2003). However, studies have since demonstrated that the temperature regime of rivers is a complex issue and not all rivers exhibit this downstream trend (e.g. Fullerton et al., 2015). Although ultimately the lower course of the Bednja River is warmer, some upstream unshaded sites (e.g. study site 2) recorded warmer water temperatures than their subsequent downstream site, especially in summer. This longitudinal variation in water temperature can be accounted for by riparian vegetation which contributes to local cooling of shaded sections (e.g. Broadmeadow, 2011) but also lateral contributions from tributaries (e.g. Mejia et al., 2020). Apart from distance from source as a factor, the RDA ordination also places % of extensive agriculture and water temperature close. Quinn et al. (1997) found that pasture streams had higher average temperatures than native streams because

of lower riparian shade. In this study, although water temperature had a positive Spearman's correlation with increased share of extensive agricultural land, and negative with riparian vegetation scores, these correlations were not statistically significant. The concentration of some nutrients, especially total nitrogen, also increased towards the downstream sites. This is an expected trend especially for catchments draining agricultural and urban land (e.g. Edwards et al., 2000; Jarvie et al., 2018).

Hydromorphological modification scores of both RHS and EN 15843:2010 assessment systems do not follow a longitudinal gradient as reported by some studies (e.g. Tavzes et al., 2006; Gündüz & Şimşek, 2021). This shows that hydromorphological degradation is present to different extents along the entire Bednja River, including most upstream sites. Some RHS habitat quality subscores however did give significant longitudinal correlations. The negative correlation of HQA channel substrate subscore with distance from source indicates there is a downstream decrease in diversity of available substrates (heterogeneity) while the positive longitudinal correlation with the Channel Substrate Index (CSI) means there is a gradual increase in average substrate size. Some downstream sites such as study site 18 could have larger substrate as a result of tributaries contributing to the substrate. However, this result also shows that finer substrate at upstream study sites is a result of hydromorphological degradation. A higher Riparian Complexity subscore indicates the presence of greater number of vegetation types (i.e. on the bank of the river there are trees, shrubs, grasses, and bryophytes as opposed to just shrubs and grass) (Environment Agency, 2003). The Riparian Complexity subscore increases with river size. It is possible that the reason lies in management practices i.e. smaller streams are easier to manage so riparian vegetation is more frequently impacted while larger downstream reaches are left to succession.

Results of correlations between landuse, water quality and hydromorphology show that landuse influences water quality parameters more than it does hydromorphological features. The results also show that urban areas are strongly correlated with increased orthophosphates and total nitrogen, while agricultural surfaces in general are connected to increased nitrate concentrations. These findings are in line with existing knowledge on influence of different landuse on nutrient input (e.g. Strayer et al., 2003; Paul & Meyer, 2001).

Only a few hydromorphological features/subscores and not the main hydromorphological scores were correlated to water quality variables. Similar findings were reported by Erba et al. (2006) which, alike their results, shows that the Bednja River morphology is relatively

independent from water quality i.e. the two stressors are not correlated. The strongest identified relationship was found between the HQA subscore which measures instream channel vegetation quality, and oxygen saturation. This is not a surprise since submerged angiosperms can increase oxygen levels in a river (e.g. Caraco et al., 2006). Hydromorphological scores are also poorly correlated with landuse. Although there are studies which have shown that hydromorphological status is significantly related to sub-catchment landuse (e.g. Kail et al., 2009), findings of this study are more in line with results of Buffagni et al. (2009) who also didn't find relationships between landuse at the sub-catchment scale and hydromorphological features. This is probably due to the fact that the Bednja floodplains have been used for agriculture since the Middle Ages (Petrić, 2010) and sections of the Bednja River are subject to continuous regulations regardless of landuse (Hrvatske Vode, 2015b).

On the other hand, presence of technolithal and absence of xylal microhabitats has shown to be a strong predictor of hydromorphological modification scores, especially EN 15843:2010 scores at both 100 m and 500 m assessed reach scale. Share of xylal is negatively correlated to modification of morphology, channel, and the riparian zone. Xylal represents an important microhabitat for benthic macroinvertebrates (Benke & Wallace, 2003) and it is often removed during river management. Therefore, more natural reaches have better riparian vegetation and more available xylal microhabitats, which is also shown through the positive correlation of share of xylal microhabitats with the RHS Habitat Quality Assessment (HQA) subscore for trees. Technolithal in the Bednja River is usually placed as rip rap as after resectioning/channelization to prevent erosion (personal observation). When the morphology of a river channel is altered by resectioning, the diversity of both in-stream and bank habitats are adversely impacted because features such as riffles or tree cover are lost (Raven et al., 1998b). Pursuantly, sites with technolithal are already under impact of multiple other hydromorphological modifications, so it is not unusual that these sites are associated with higher level of modification.

#### *Composition and structure of benthic macroinvertebrates in the Bednja River*

In accordance with the first research objective, the composition and structure of benthic macroinvertebrates in the Bednja River was investigated. Chironomidae larvae are the most abundant group constituting 32.6% of all sampled benthic macroinvertebrate individuals. At 11 study sites their abundance exceeded 30% in the total share of all benthic macroinvertebrates

out of which at 7 study sites their abundance exceeded 50%. This result is in line with existing knowledge on their dominance in freshwater ecosystems (Rosenberg, 1993; Armitage et al., 1995b). Similar results are reported by Rios & Bailey (2006) where Chironomidae larvae made up 47% of all sampled organisms based on 33 sites, and Herringshaw et al. (2011) where Chironomidae larvae constituted 48% of all recorded invertebrates from 20 study sites, and Leitner (2021b) where Chironomidae larvae dominated samples from both reference and impacted sites.

High variation in macroinvertebrate abundance and diversity between study sites can be explained by several factors. Sampling effort can be excluded because the same method was applied at all sites. Study site 18 with the highest macroinvertebrate abundance also possesses the highest total species and relatively high values for all diversity indices, despite water quality issues (highest nitrate concentrations in summer, and orthophosphate and total phosphorous greatly exceeding limit values in spring). This site possesses high habitat heterogeneity resulting from several fallen over trees which have created variations in flow and depth. The contribution of woody debris to creating morphological complexity has been demonstrated by Buffington et al. (2002). Anlanger et al. (2022) studied the effects of large wood placement in a gravel-bed river 8 months after installation for restoration purposes and found an increase in both morphological (by 821%) and flow diversity (by 127%), while macroinvertebrate diversity increased by 35%. Furthermore, it has long been established that a greater variety of available microhabitats will allow a greater number of species to find suitable habitats (Junk et al., 1989).

The second highest abundance is found at study site 1 just below the rheohelocene type source of the Bednja River. Unlike study site 18, the high abundance at study site 1 is accompanied by the lowest recorded values for all tested diversity indices and is dominated by Amphipoda. Springs are known to be areas of high abundance of non-insect taxa and low macroinvertebrate diversity in general (e.g. Baraquin & Death, 2004). At study site 1, taxa lacking flying adults dominated (Gammaridae, Oligochaeta, Gastropoda). Unlike insects, these taxa cannot be affected by thermal constancy i.e. lack of crucial thermal stimuli for various life-cycle phenomena (Ward, 1976). Another reason could be lack of drift possibilities from upstream areas to provide recolonization and redistribution of benthic macroinvertebrates (William & Hynes, 1976). Furthermore, the high abundance of *Gammarus fossarum* could also be due to the species good current resistance and upstream migration abilities. By moving upstream into fishfree headwaters, *G. fossarum* avoids predation (Meijering, 1980).



Study sites 8 and 9 are typical example of sites under influence of organic pollution (e.g. Shieh et al., 1999) - high abundance and domination of gatherers/collectors (89% at study site 9), while the abundance of just one subsample of sand at study site 8 reaches 102,480 individuals m<sup>2</sup> and has a 99% share of gatherers/collectors due to Oligochaeta. Both sites are located downstream of the towns Lepoglava and Ivanec which at the time of sampling had no waste water treatment (Hrvatske Vode, 2015a).

The overall high species diversity in the Bednja River could partially be a result of isolation effort. Since this study was performed for the purpose of research, and not monitoring, it was decided to isolate 100% of a given microhabitat sample (as opposed to subsampling until 500 organisms are found as recommended by AQEM (2002)). This resulted in some individual finds of small taxa which would have otherwise been missed.

Interestingly, despite significant hydromorphological modification affecting several study sites, it is the Saprobity Index responsible for the poorer, deciding score of the final EQR, and not the module for general degradation. This shows that there is still sufficient habitat diversity to 'neutralize' the degradation. Generally, smaller streams are more adversely affected by organic pollution which causes greater degradation of the ecological status, while with large rivers the hydromorphology is mostly influences the status (e.g. Urbanič et al., 2020). Furthermore, Vilenica et al. (2022) suggest the high mayfly species richness in the Bednja River could be in relation to numerous tributaries, and variety of available microhabitats. A possible reason why the Saprobity Index was able to give a gradient from high to poor class, while General Degradation places almost all sites in the highest status is because the Saprobic Index works best when identification is done to species level, as opposed to genus or family level (Sanding & Hering, 2004), which is the case with this study.

#### *Similarity and dissimilarity between the study sites*

Pursuant to Bray-Curtis similarity index between the study sites, highest level of similarity between benthic macroinvertebrate communities primarily occurs between study sites based on their vicinity (the most similar sites are from 0.4 to 8.6 km apart). This is not unexpected as benthic invertebrates are known for their high dispersal abilities which can be active and passive

both in water and over land (Ptatscheck et al., 2020) so several taxa are shared between those sites despite some habitat differences.

There are exceptions however where proximity of sites did not result in assemblage similarity because differences in habitat conditions are too great. Study sites 10 and 11 (600 m apart) and study sites 15 and 16 (800 m apart) are characterised by the upstream sites (10, 15) being slower flowing with finer sediment and the downstream sites (11, 16) reaching high water velocities and with larger substrate size. It has long been established that flow velocity and sediment size are among the most important variables in explaining benthic macroinvertebrate community variations (Hynes, 1970). In both cases, the downstream of the two sites have very high abundances of Simuliidae (black flies, Diptera), Ephemeroptera (mayflies) taxa and *Hydropsyche* (caddisflies, Trichoptera) taxa. Subsequently, these sites also have the highest share of passive filter feeders from all the other sites on the Bednja River. Although passive downstream dispersal by water currents (drift) is generally the most common dispersal method for benthic macroinvertebrates (e.g. Bruno et al., 2012) it is obvious that these taxa did not drift from the upstream site where they were scarce or missing. All three insect groups have flying adults that can migrate overland and cover larger distances in search of suitable sites for oviposition (Finn & Poff, 2008).

Contrastingly, the upstream slow flowing sites (10 and 15) have among the highest abundances of taxa belonging to the Chironomini tribe. These sites offer favourable conditions for Chironomini as they generally like stagnant and slow flowing water (Moller Pilot, 2009). Chironomini are also responsible for having the highest contribution to similarity between all the studied sites on the Bednja River in the SIMPER analysis. Chironomini inhabit a wide range of habitats, depending on species, but in general are mostly bottom dwellers, preferring fine substrates. Some species even build tubes or live in plants as miners, while their feeding preference ranges from carnivores to phytodetritophagous species. Identification of Chironomini was in most cases not performed to species level so it is not possible to go into detail, but the wide range of suitable habitats in the Bednja River facilitates their presence at all study sites. Furthermore, species belonging to *Polypedilum* which were identified are known to inhabit running water, so this further supports their presence in the studied lotic system (Moller Pilot, 2009).

Another observation regarding grouping of study sites based on Bray-Curtis similarity index is grouping of several upstream study sites with study sites belonging to the lower course, and no distinct grouping of the study sites based on typology (small mid-altitude category study sites 1 - 9 are located above 200 m a.s.l., and medium lowland category study sites 10 - 20 are below 200 m a.s.l.). The fact that the Bednja River doesn't follow the River Continuum Concept (RCC) proposed by Vannote et al. (1980) is also evident from the domination of gatherer/collectors in the upper course sites (when it should be shredders and grazers/scrapers), and the taxa longitudinal zonation preference analysis showing several upstream sites are dominated by potamal and litoral preferring taxa. These conditions are probably the result of a combination of sediment size and water velocity which are influenced by hydromorphological modification and landuse, and which have led to the phenomenon of 'potamalization' of the faunal structure (Moog & Chovanec, 2000). For example, study site 8 and 9 still belong to the upper reach of the Bednja River but have grouped with the mid and downstream sites. Both sites are dominated by finer sediment, which has a higher capability to trap the fine particulate organic matter (FPOM) than larger substrate (Parker, 1989). Gatherers / collectors feed from sedimented FPOM so it is no wonder this feeding group dominates at these sites, especially with also the nutrient enrichment being present (e.g. Sakai et al., 2021). For the same reason, study sites 4 and 5 have grouped with study site 10, which is more 30 km downstream and has a 300 km<sup>2</sup> larger sub-catchment area. Study sites 4, 5 and 10 apart from also having fine, sandy substrate, are distinguished from other sites by having very slow water velocity, which is the reason they have grouped slightly separately from the remaining downstream sites.

Several upstream study sites also have degraded riparian zones, which are the source of leaf litter in rivers (Gregory et al., 1991) so consequently there is a deficit of food source for the shredders. However no significant correlations were found between hydromorphological indices scoring riparian vegetation quality and share of shredders. This is probably because several downstream study sites possessed much higher scores for riparian vegetation, but these lowland reaches naturally aren't expected to be dominated by shredders.

As previously stated, there is also no distinct grouping of assemblages between the two Bednja River types (HR-R\_1 and HR-R\_4A). For both types, the highest contributing taxa to similarity within each type are Chironomini tribe, followed by *Limnodrilus hoffmeisteri* Claparede, 1862 and *Gammarus fossarum*, both of which are widely distributed across Europe (Timm, 2013; Vonk, 2013), and it is not surprising they were abundantly found at all the study sites.

Interestingly, the non-native Oligochaet *Branchiura sowerbyi* Beddard, 1982 is the highest contributor to dissimilarity between the two types, respectively the upper and lower Bednja River reach. This species has previously been confirmed in the old river channel of the Drava River at hydropower plant Dubrava (Mihaljević et al., 2007), but its highest abundance in the most upstream reaches of the Bednja River and absence from the downstream study sites suggest they did not arrive from the Drava. The species seem to be proliferating in artificial and heavily modified water bodies of the Pannonian Lowland Ecoregion (ER 11) (Illies, 1978) in Croatia (Vučković et al., 2020). Regarding non-native species in the Bednja River, another important record is the one of the highly invasive zebra mussel *Dreissena polymorpha* (Pallas, 1771) at the most downstream study site 20. Although this species started colonizing the Drava in the 1980's (Mišetić et al., 1991) this first record of its presence in the Bednja River is alarming for two reasons. At the moment it seems that the species has not yet moved higher upstream past study site 20 which is near the river mouth. At the time of sampling, study site 19 at Mali Bukovec had just been freshly reinforced with rip rap, and this is the most downstream presence of technolithal on the Bednja River. Rip rap is a specially favoured habitat by the zebra mussel (Jude & DeBoe, 1996) so there is a possibility it can facilitate its spread to more upstream areas of the Bednja River. Secondly, the zebra mussels are known to pose a threat to native mussels through fouling (e.g. Pilotto et al., 2016; Ožgo et al., 2020) especially the thick shelled river mussel *Unio crassus* (Philipsson, 1778) which has also been recorded at 8 study sites on the Bednja River. Moreover, *U. crassus* is listed on the IUCN Red List as Endangered (Lopes-Lima et al., 2014) and in Annex II of the European Habitats Directive (European Commission, 1992).

#### *Composition and structure of benthic macroinvertebrates on sampled microhabitats*

The sampled technolithal blocks achieved high values for diversity metrics. While this came as a surprise at first, upon examining the characteristics of technolithal as a microhabitat for benthic macroinvertebrates, several explanations can be deduced. Technolithal blocks are irregularly shaped with a relatively large available surface area for colonization, and interstitial space of different sizes between the blocks. One rock can offer different microhabitat conditions - the underside, protected from water velocity, and the outer exposed surface which in the Bednja River was covered in periphyton (personal observation). Although not widely available in literature, there are records of artificially introduced technolithal (referred to as rip rap)

supporting higher invertebrate abundance and diversity in rivers (e.g. Wolf et al., 1972) but also in coastal zones (e.g. Seitz et al., 2019).

Technolithal most probably has this effect because the depressions and grooves on the stone surface offer habitat complexity, and these features are less evident on surfaces of smaller stones (Douglas & Lake, 1994). In the Bednja River however, it is observed that technolithal community assemblages differ from other lithal substrate assemblages. While *Gammarus fossarum*, followed by Chironomini, contribute most to similarity between lithal microhabitats (macrolithal, mesolithal, microlithal), in the Bednja River, Orthocladiinae contribute most to similarity between the sampled technolithal substrate which belongs to the same size category as lithal. Interestingly, Orthocladiinae don't even make it to the top 5% similarity contribution on natural substrate while contribution of Chironomini on technolithal are preceded in abundance by several other taxa. This phenomenon probably has to do with food availability. The larger lithal substrates are found at more natural sites which at the same time are characterised by having higher level of shading (e.g. study site 1, 6, 16) and technolithal is present on reaches where riparian trees have been removed so there is more primary production (Gregory et al., 1991). Unlike the larvae of Chironomini, many Orthocladiinae species are scrapers which feed mainly on algae and other periphyton. Some Orthocladiinae are also deposit-collectors, and the periphyton layer on technolithal probably also contains detritus, bacteria, fungi and protozoans. The presence of Orthocladiinae on technolithal can further be explained by their ability to inhabit a wide range of microhabitat conditions, as they can be they can be tolerant to less optimal conditions if other factors such as food availability are optimal (Moller Pillot, 2014).

Lithal is a favoured microhabitat by *G. fossarum* and its affiliation for this substrate is often confirmed in studies (e.g. Mauchart et al., 2014). Amphipoda in general can have densities exceeding 10,000 individuals m<sup>2</sup> (Pennak, 1989) and this was also the case with *G. fossarum* at several of the lithal substrates. Apart from lithal, *G. fossarum* also has preference for smaller sized substrates (Schmedtje & Colling, 1996) and has no dominant stream zone affiliation (Pöckl et al., 2017) all which has also been demonstrated in this study. Although Chironomidae as a group had the highest share within the total benthic macroinvertebrate community, *G. fossarum* was the most abundant and frequently found species in the Bednja River (36,588 individuals identified making up 18.9% of all taxa).

*Effects of natural factors and anthropogenic stressors on the benthic macroinvertebrate community*

Biological response to human stressors is conditioned by natural gradients which influence natural variability (Vannote et al., 1980). In line with the second objective of this study: to identify the effects of natural factors and anthropogenic stressors on the benthic macroinvertebrate community, the following relationships are observed and discussed:

Diversity and functional feeding metrics do not display a longitudinal gradient on the Bednja River and this has finding already been discussed above. Some taxa groups and individual taxa on the other hand show a longitudinal increase or decrease in abundance which is in relation to the temperature gradient (e.g. Vilenica et al., 2022). For example, Diptera are also usually more abundant downstream (Williams & Hogg, 1988). A relationship that was expected but not found with the longitudinal gradient was a shift in dominance of *G. fossarum* upstream to dominance of *Gammarus roeselii* (Gervais, 1835) downstream as reported by Mauchart et al. (2017). In the Bednja River, *G. fossarum* co-exists with *G. roeselii*, the latter having a wider range of oxygen tolerance (Meijering, 1991). In studies on the coexistence between the two species Mauchart et al. (2014) found that *G. roeselii* occurred at sites with degraded riparian vegetation i.e. site with higher anthropogenic impact. It is possible this shift in dominance between *G. fossarum*, and *G. roeselii* doesn't take place along the Bednja River due to degradations present even in the most upstream study sites. Another possible explanation could lay in the great cryptic diversity of *G. fossarum* in the Bednja River which could influence their tolerance to non-optimal conditions (Wattier et al., 2020).

Stream zonation preference of the communities show some consistency along the longitudinal gradient in accordance with benthic macroinvertebrate zonation preferences as elaborated by Moog (2002). Pursuantly, the increase in taxa preferring the epipotamal zone (barbel region) with distance from source is expected. An example of a taxon inhabiting the Bednja River with a high preference for the epipotamal zone is the gastropod *Holandriana holandrii* (C. Pfeiffer, 1828) (Reischütz et al., 2017) which is found in highest abundance at study site 15. The Rhithron Type Index (RTI) positively correlated with altitude, showing that taxa with rhithral zone preferences are more associated with the upper reaches, also in line with (Moog, 2002). This relationship of RTI with altitude explains the strong negative correlation of the same index

with urban landuse, which, as previously discussed, on the Bednja catchment increases its total share with distance from source.

The results further show that even though the share of urban land cover does not significantly correlate with ammonium concentrations, both stressors significantly correlate with the Saprobity Index (SI). Between urban areas and ammonium, it is the ammonium which had a stronger correlation with SI. This result could suggest that urban areas do not have to impose such a negative impact on benthic macroinvertebrates (e.g. Herringshaw et al., 2011) if the source of ammonium, i.e. the wastewaters are treated. This result also stresses the urgency to increase wastewater treatment efforts on the Bednja River catchment and to prevent point source discharge from individual households into the river.

Some authors argue that physical habitat degradation is the dominant stressor in the hierarchy of stressors affecting benthic macroinvertebrates, even more than nutrients (e.g. Gieswein et al., 2017). In the Bednja River, benthic macroinvertebrate metrics responded better to water quality and landuse parameters than with hydromorphological variables. Both total individuals (N) and total species (S) increased with nutrient enrichment. This could be due to the fact that nutrients display a longitudinal increase in concentration, but the water temperature also follows this gradient in the Bednja River (Vidaković Maoduš et al., 2022). The importance of water temperature as a factor in species distribution and richness has long been established (Ward, 1985).

Four diversity metrics (Total species, Simpson, Shannon-Wiener, and Evenness) all have significant correlations with extensive landuse. It is possible that this phenomenon is also related to the downstream temperature gradient as extensive landuse and water temperature have already shown high mutual relationship. Extensive landuse on the Bednja River catchment is represented by pastures and agricultural land with significant areas of natural vegetation. Maybe the positive impact of extensive agriculture should be viewed from the aspect of being a suitable terrestrial habitat for maturation, foraging and mating of adult insects (e.g. Wildermuth, 2012). Hykel et al. (2016) demonstrated that vegetation structure on extensively used land could be especially important for the conservation management of the threatened dragonfly *Sympetrum depressiusculum* (Selys, 1841).

The Average Score Per Taxon metric (ASPT) gave the greatest number of significant negative correlations with nutrient enrichment and urban landuse, justifying the metrics' inclusion into the Croatian national method for ecological status assessment (Mihaljević et al., 2020). The ASPT metric was also the overall best correlated metric to landuse and physico-chemistry parameters in Austrian and German mountain and lowland streams (Dahm et al. 2013). ASPT also strongly responded to organic pollution in a lowland tropical river system (Eriksen et al., 2022).

*Relationship between hydromorphological status and the benthic macroinvertebrate community longitudinally along the Bednja River*

Diversity metrics, but also benthic macroinvertebrate metrics in general, did not correlate well with hydromorphological degradation in this study. Similar findings have also been reported by Friberg et al. (2009) whose results are based on a much larger dataset from over 1000 study sites. One of their arguments was that macroinvertebrates indicators are not sensitive enough to detect hydromorphological stress because they are mainly developed to detect nutrient enrichment or general ecological quality changes. They further argued that RHS does not directly assess the hydrological dynamics of the site. However, their explanation cannot be applied to this study because RHS was applied on the Bednja River to calculate a special Flow Regime Index (FRI) which Friberg et al. (2009) did not consider. Furthermore, hydromorphological modification scores of the EN 15843:2010 were even worse at correlating with macroinvertebrate metrics, and this assessment system incorporates flow conditions among others.

One of the possible explanations which can be applied to the Bednja River is the high diversity associated with artificial bank enforcement (technolithal) which seems to be a favoured habitat for benthic macroinvertebrates but is at the same time responsible for hydromorphological degradation (discussed previously).

Lack of significant correlations between diversity metrics and metrics in general with hydromorphological pressures initiated the inclusion of the River Fauna Index (RFI) into the General Degradation module for assessment of Croatian rivers (Mihaljević et al., 2020). Even though it has been established for the Bednja River that hydromorphological degradation does



not follow a longitudinal gradient, the significant longitudinal increase in RFI could be misleading in suggesting there is a downstream improvement of hydromorphological status after all. RFI is a metric based on indicator responses to hydromorphological degradation. The reason for this downstream increase lies in naturally higher reference values of RFI for larger rivers, i.e. the downstream Bednja reach (type HR-R\_4a) has a reference RFI value of 0.52, while the upstream Bednja reach (type HR-R\_1) has a reference RFI value of 0.054 (Mihaljević et al., 2020).

The best responding functional metrics to River Habitat Survey modification and habitat quality scores were flow related and substrate related metrics ((%) Type RP (rheophile), Rheoindex, and % Type Akal). The decrease of rheophile taxa and the Rheoindex with increased modification shows that hydromorphological modification has altered the natural flow regime. Although rivers are primarily modified (channelized) for the faster conveyance of water during floods, as a consequence, the base flows often decline the remaining time (Poff et al., 1997). Furthermore, several of the Bednja sites with the highest level of hydromorphological modification are also characterized by low flow velocities due to downstream impoundments by weirs. All of these factors contribute to the ‘potamalization’ of the benthic macroinvertebrate community (Moog & Chovanec, 2000).

The share of taxa with a preference for akal is the substrate metric with the greatest number of significant correlations with hydromorphological modification and quality scores. This metric is expected to decrease as a response to morphological degradation (Hering et al., 2004b). Moreover, this result showing the positive association of akal preferring taxa with less modified sites (i.e. higher habitat quality) is additional confirmation that akal is the natural, type-specific substrate in the Bednja River, and not silt / sand as defined by the typology, and discussed above.

Significant positive correlation of littoral taxa with altitude indicates morphological degradation of the upper course (Hering et al., 2004b), despite the same index giving significant correlations with any of the hydromorphological modification scores. An increase in share of potamal or littoral preferring taxa in the benthic macroinvertebrate community as well as an increase in detritus feeders could be a result of fine sediment deposition in the river-bed (e.g. Leitner et al., 2021b). As previously mentioned, siltation at the study sites was observed, but as a stressor it was not further quantified.

Since studies have already demonstrated that metrics such as ASPT do not correlate with morphological and riparian modification (e.g. Zelnik & Muc, 2021), similar results were expected in this study. As discussed previously, most of the tested sensitivity metrics are simply better at detecting organic pollution. However, because of findings by Erba et al. (2006), it was expected to find significant correlations between EPT taxa and morphological modification scores, especially the degradation of river banks. Leitner et al. (2021a) also found negative correlations of EPT taxa metrics with channelization in large European rivers. EPT taxa are generally pollution-intolerant with relatively high habitat requirements (Rosenberg and Resh, 1993; Graf et al., 2016). In the Bednja River, EPT metrics and some individual Ephemeroptera and Trichoptera species were contradictorily positively correlated with higher modification. This result is probably related to substrate particle size. Some of the sites with the highest level of modification, and high EPT abundance, are also characterized by a large share of lithal substrate (e.g. sites 6, 7, and 11). Therefore, this finding is in accordance with knowledge of EPT taxa preference for such substrate (e.g. Graf et al., 2016). Furthermore, it is also possible to find high EPT taxa at degraded sites with finer homogenous substrate if xylal microhabitats are present, but as Leitner et al. (2021b) point out, the higher taxa number does not mean the taxa composition is type-specific.

The strongest taxa correlations with hydromorphological modification are found for *Cloeon dipterum* and *Ecdyonurus macani* (Ephemeroptera) and modification of the flow. Flow modification at the study sites is either reduced velocity due to downstream impoundment by weir, or increased velocity downstream of the weir. *C. dipterum* is known to prefer lentic habitats and has a high ecological tolerance to disturbance, and this finding at impounded sites has already been described by Vilenica et al. (2022). *Ecdyonurus macani* on the other hand has a very high rhithral preference (Leitner & Lorenz, 2020) so acceleration of flow velocity agrees with this affinity. Another taxon related result which stands out is the significant negative correlation of Ceratopogonidae larvae (Diptera) to the greatest number of hydromorphological modification scores. Literature provides only scarce connections between Ceratopogonidae and hydromorphological modification. For example, Ceratopogonidae together with 394 taxa, qualified as indicators of hydromorphological quality of Estonian surface waters for an index developed by Timm et al. (2011). Their hydromorphological index (MESH – Macroinvertebrates in Estonia: Score of Hydromorphology) which considers velocity and waterbody bottom type, grouped Ceratopogonidae with taxa with the affinity for slow-flowing water and a stony bottom. For Croatian rivers, Ceratopogonidae as a family receive a very low

hydromorphological indicative weight ( $HM_i$ ) and a low River fauna ( $Rf_i$ ) value (Mihaljević et al., 2020). Since Ceratopogonidae larvae develop in a very wide range of aquatic and semi-aquatic environments, and different genera can simultaneously inhabit the same habitat (Foxi et al., 2020) it would be interesting to further investigate these results on a lower taxonomic level.

The greatest number of significant taxa and taxa groups correlations was with the HQA channel substrate score which is an indicator of substrate heterogeneity (i.e. the diversity and abundance of available substrates at the assessed reach) (Raven et al., 1998b). Crustacea (represented dominantly with *Gammarus fossarum*) had a strong significant correlation with the HQA channel substrate score which is in accordance with the general affiliation of Amphipoda to more structurally complex habitats which offer shelter opportunities (e.g. Kley et al., 2009). Furthermore, sites with a higher HQA channel substrate score also have lithal present which is the substrate of choice for *G. fossarum* (Mauchart et al., 2017). Because *G. fossarum* are shredders (Moog, 2002) and as such have an important role in leaf litter decomposition (Tank et al., 2010), significant correlations were expected between riparian vegetation scores and Amphipoda but were not established. HQA channel substrate score was the best correlating score for also Friberg et al. (2009).

In order to interpret the results of significant correlations between hydromorphological modification and share of the feeding group miners and predators, first the representatives of those feeding groups in the Bednja river had to be identified. After observing that a few specimens of Hydroptilidae (Trichoptera) on technolithal are the result of the positive correlation of miners with hydromorphological modification, these findings are not as important. Hydroptilidae are known piercers that feed by puncturing algal cells (Huryn, 2009) and have probably been attracted to the technolithal for the periphyton, the same reason as Orthocladinae (discussed above). A more interesting result is the positive correlation of predators with hydromorphological modification scores and the Channel Substrate Index (CSI), which is once again in relation to the presence of technolithal. This study revealed a high affiliation of water mite assemblages (Hydrachnidia) towards technolithal where they are probably drawn by their most favoured food – Chironomidae (Pozojević et al., 2019).

The UK River Habitat Survey which is an advanced method as compared to other available assessment systems, has already been applied in several European countries due to numerous scores and details on physical habitat it can provide (e.g. Szoszkiewicz et al., 2006; Costa &

Vieira, 2021). The RHS HQA cannot fully be applied to Croatian rivers because in order to assign a HQA class (1 - 5) to a study site, comparison with a large database of several hundred assessed sites of similar typology is required (Raven et al., 1998b). For this reason, it is recommended to increase application of RHS in Croatia especially because the HQA channel substrate score has shown to be one of the few hydromorphological scores correlating with benthic macroinvertebrates. A further reason is the possibilities HQA can offer in monitoring river restoration success (e.g. Zieliński & Suchowolec, 2013).

In applying the EN 15843:2010 on two different reach length, it is not clear which assessment lengths is more suitable. On one hand, in degraded rivers it is difficult to find uniform reaches so assessing 500 m can encompass some upstream disturbances. However, this might not result in better correlation with benthic macroinvertebrates because they can be governed by much smaller scale and local factors. Pedersen & Friberg (2007) found that two visually similar adjacent riffles had significant differences in benthic macroinvertebrate composition in a lowland stream.

*Response gradient of the benthic macroinvertebrate community towards anthropogenic stressors and hydromorphological alterations*

A response gradient was able to be created between the Ecological Quality Ratio (EQR) and stressors from the three tested stressor groups: hydromorphology, nutrients and landuse. EQR gave a linear negative response towards all stressors showing a degradation relationship even for stressors not significantly correlating to EQR.

The steepest response gradient however was achieved between EQR and parameters which gave a statistically significant Spearman's Correlation Coefficient: ammonium concentration, HQA, the HQA bank vegetation structure subscore and the HQA channel substrate subscore.

The EQR response gradients suggest that improvement in condition of the three identified parameters (lowering the ammonium concentration, increasing the HQA bank vegetation subscore by fostering the development of more complex bank vegetation, and increasing the HQA channel substrate subscore by increasing size heterogeneity) could contribute to an improvement of the WFD Ecological Status by offering more suitable habitat conditions which can supporting a higher taxonomic composition and abundance of benthic macroinvertebrates.

## 6. CONCLUSION

The Bednja River is degraded river with a history of human interventions to the floodplain and morphology. Despite human influence there are still sites supporting high benthic macroinvertebrate taxonomic diversity and sampled microhabitat played a large role in benthic macroinvertebrate metrics.

Regarding the composition and structure of benthic macroinvertebrates in the Bednja River the following conclusions are brought up:

- Chironomidae (Diptera) larvae are the most abundant taxa group.
- The amphipod *Gammarus fossarum* Koch in Panzer, 1836 is the most abundant and widespread species.
- Collector gatherers are the overall dominant feeding group.
- Benthic macroinvertebrate abundance and diversity greatly varies longitudinally along the Bednja River between the study sites and microhabitats.
- The Tribus Chironomini (Diptera), oligochaete *Limnodrilus hoffmeisteri* Claparede 1862, amphipod *Gammarus fossarum* and Tribus Tanytarsini (Diptera) contribute most to similarity between all study sites.
- There is no distinct grouping of communities according to river type.
- The non-native oligochaete *Branchiura sowerbyi* Beddard, 1892 is the highest contributor to dissimilarity between the two Bednja river types.

In regard to the influence of natural factors and anthropogenic stressors on the benthic macroinvertebrate community, the following is concluded:

- Akal (fine to medium-sized gravel with a grain size >0.2 cm to 2 cm) is the dominant substrate in the Bednja River.
- There is a difference in assemblages between microhabitats based on substrate type and grain size. *Gammarus fossarum* contributes most to similarity between the xylal (wood) samples and the lithal substrates (macrolithal, mesolithal, microlithal). The Tribus Chironomini contributes most to similarity between the smaller grained substrates (sand, akal, argyllal).
- Assemblages of natural lithal substrates differ from artificial technolithal substrate and Orthocladiinae (Diptera) contribute most to similarity between the technolithal samples.

- Total nitrogen has the strongest correlation with the longitudinal gradient.
- Share of urban landcover increases with distance from source on the Bednja catchment.
- Urban landcover has a stronger correlation with nutrient concentrations than agricultural landuse.
- Extensive agriculture has a positive influence on benthic macroinvertebrate diversity.
- The Saprobity module is the lower, decisive score of the Ecological Quality Ratio for all sites not achieving good status showing that organic pollution is a bigger problem on the Bednja River than hydromorphological degradation.
- Ammonium concentration have a stronger correlation with the Saprobity index than share of urban landcover showing urban areas do not have to impose such a negative effect on rivers if their wastewater is treated.
- Untreated wastewater from the town Ivanec (study site 9) is responsible for the poorest ecological status on the Bednja River and this issue needs to be addressed urgently.
- The metric Average Score per Taxon (ASPT) gives the greatest number of significant negative correlations with nutrient enrichment and urban landcover.

Regarding the relationship between hydromorphological status and the benthic macroinvertebrate community longitudinally along the Bednja River.

- Hydromorphological modification is present along the entire course of the river and does not follow a longitudinal gradient.
- The RHS Channel Substrate Index indicates an increase average substrate size along the longitudinal gradient.
- The presence of technolithal and absence of xylal microhabitats is a strong predictor of hydromorphological modification.
- The scores derived through EN 15843:2010 and River Habitat Survey are highly correlated.
- Benthic macroinvertebrate diversity metrics are not a good indicator of hydromorphological modification.
- The best performing benthic macroinvertebrate metrics to detect hydromorphological modification are (%) Type Akal, Rheoindex, and (%) Type RP.
- The HQA bank vegetation structure subscore gives the greatest number of significant correlations with all tested metrics.
- Hydromorphological modification is associated with increased miners and predators feeding group.

- The increase in Hydrachnidia taxonomic group with increased hydromorphological modification is because of their association with technolithal as a habitat.
- The River Habitat Survey scores explain more variation in benthic macroinvertebrates assemblages than the EN 15843:2010 scores.
- The subscores of both assessment systems (River Habitat Survey and EN 15843:2010) explain more variation in taxa than the main scores.
- The River Habitat Survey HQA channel substrate subscore and the Channel Substrate Index have the biggest influence on benthic macroinvertebrate assemblages.
- The EN 15843:2010 subscores corresponding to substrate, woody debris and aquatic vegetation management give the highest number of significant correlations with taxa.

The response gradient shows that ecological status can be improved by lowering ammonium levels, improving bank vegetation structure (measured by the HQA bank vegetation structure subscore) and increasing substrate size heterogeneity (measured by the HQA channel substrate subscore).

This study represents the first systematic analysis of benthic macroinvertebrate fauna of the Bednja River. The results contribute to understanding of structuring of the benthic macroinvertebrate community as a response to natural and anthropogenically altered environmental conditions. The results show that there are connections between hydromorphological features and the benthic macroinvertebrate assemblages, and there is a need to further investigate these relationships and test the results on a larger number of study sites.

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**8. ANNEXES**

- ANNEX I Benthic macroinvertebrate taxa list for the Bednja River
- ANNEX II Abiotic characteristics of sampled microhabitats (water depth and flow velocity) at study sites on the Bednja River
- ANNEX III Hydromorphological modification of study sites according to EN 15843:2010: individual scores for 16 assessed features
- ANNEX IV Nutrient concentrations and physicochemical water properties measures at the study sites in spring, summer and autumn

**ANNEX I** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>CLASS: INSECTA</b>																						
<b>ORDER: EPHEMEROPTERA</b>																						
Total individuals: 20,188																						
BAETIDAE	<i>Baetis</i> sp. Juv.		6	207	150	15	57	99	433	410	217		323	43	28	126	52	157	25	870	257	428
	<i>Baetis fuscatus</i> (Linnaeus, 1761)	1		1		1	6	615	336	127	140	4	782	249	167	95	102	498	7	3486	246	1255
	<i>Baetis buceratus</i> Eaton, 1870	2		111	33	1		13	20	107	96		719	1	3			48	18	968	86	246
	<i>Baetis rhodani</i> (Pictet, 1843)	3	242	89	17		2	200	265	256	15		66	1	7	1		23		82		
	<i>Baetis vernus</i> Curtis, 1834	4		120	174		29	33	51	85	7		27		7	6	17	53				69
	<i>Baetis liebenauae</i> Keffermüller, 1974	5											39					4		28		
	<i>Baetis lutheri</i> Müller-Liebenau, 1967	6											70	9								
	<i>Centropilum luteolum</i> (Müller, 1776)	7			13	9									31							1
	<i>Cloeon dipterum</i> (Linnaeus, 1761)	8										2								8		
	<i>Procloeon</i> sp.	9															2					
	<i>Procloeon bifidum</i> (Bengtsson, 1912)	10					6					10		1		3		1	33	7		
	<i>Procloeon pennulatum</i> (Eaton, 1870)	11			1											6					4	
CAENIDAE	<i>Caenis luctuosa</i> (Burmeister, 1839)	12					2	4	3	1		283	1	8			4	1	1	30	30	48
	<i>Caenis</i> sp. Juv.																3	1		3	4	15
	<i>Caenis pseudorivulorum</i> Keffermüller, 1960	13													1	6	19		5		6	17
EPHEMERELLIDAE	<i>Serratella ignita</i> (Poda, 1761)	14		13	27		5	47	111	68	6	45	218	147	32	186	230	481	76	229	62	133
HEPTAGENIIDAE	Heptageniidae juv.						11	1	2						19	13	9	10				5
	<i>Ecdyonurus macani</i> Thomas & Sowa, 1970				2			2						2								
	<i>Electrogena ujhelyii</i> (Sowa, 1981)	15	82	20	107	1		3	2				2	3				1			1	
	<i>Heptagenia flava</i> Rostock, 1878	16					2	12					2		2	5	2	6	9	4		6
	<i>Heptagenia longicauda</i> (Stephens, 1835)	17												1	2		4	4		4	2	
LEPTOPHLEBIIDAE	Leptophlebiidae Gen. sp. Juv.			26	73												1		4			
	<i>Habrophlebia lauta</i> Eaton, 1884	19	1	31	249	4		6	4	3		1					7				1	1
	<i>Paraleptophlebia submarginata</i> (Stephens, 1835)	20																	8			
EPHEMERIDAE	<i>Ephemera danica</i> Müller, 1764	21	1		2			1				1				10						
	<i>Ephemera lineata</i> Eaton, 1870	22													3	2	2	3		1		3
POTAMANTHIDAE	<i>Potamanthus luteus</i> (Linnaeus, 1767)	23										1	1	8			1	1	10	4		2
<b>ORDER: PLECOPTERA</b>																						
Total individuals: 57																						
PERLODIDAE	<i>Isoperla tripartita tripartita</i> Illies, 1954	24	3																			
LEUCTRIDAE	<i>Leuctra braueri</i> Kempny, 1898	25			1																	
	<i>Leuctra</i> sp. Stephens, 1835	26			1			4	11	2			1	3		4		8		3		
NEMOURIDAE	<i>Nemurella pictetii</i> Klapálek, 1900	27	1	2	4			1														
	<i>Nemoura</i> sp. Pictet, 1841	28																	1			
	<i>Amphinemura</i> sp. Ris, 1902	29			2																	
	<i>Nemoura cinerea cinerea</i> (Retzius, 1783)	30																				2
PERLIDAE	<i>Perla marginata/pallida</i>	31							3													

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>CLASS: INSECTA</b>																						
<b>ORDER: TRICHOPTERA</b>																						
Total individuals: 9,398																						
BERAEIDAE	Beraeidae Gen. sp. Lv.	1																				
	Beraeidae Gen. sp. pupa ♀	1																				
	<i>Beraea dira</i> Mclachlan, 1875	32	2																			
	<i>Beraeodes minutus</i> (Linnaeus, 1761)	33				17	1													1		
	<i>Beraeodes</i> sp.	34		18																	2	
GOERIDAE	Goeridae Gen. sp.	35	7	1	27		14	5													1	
	<i>Goera pilosa</i> (Fabricius, 1775)	36		1		1			4													
	<i>Goera</i> sp.	37	1																			
	<i>Silo</i> sp.	37		17	1																	
HYDROPSYCHIDAE	<i>Hydropsyche</i> sp.	6	166	57	21	19	424	175	999	454	1	913	68	52	15	66	742	1	831	200	202	
	<i>Hydropsyche angustipennis angustipennis</i> (Curtis, 1834)	38		64	30	8	4			110	29								117	22	36	
	<i>Hydropsyche contubernalis contubernalis</i> Mclachlan, 1865	39														24	90	1	98	54	29	
	<i>Hydropsyche bulbifera</i> Mclachlan, 1878	40				3	44	5	15	1		1									2	
	<i>Hydropsyche incognita</i> Pitsch, 1993	41							6								5		8	11		
	<i>Hydropsyche incognita/pellucidula</i>	42			10												46		16	5	84	
	<i>Hydropsyche pellucidula</i> (Curtis, 1834)	43										156	1	1			1		3	1		
	<i>Hydropsyche saxonica</i> Mclachlan, 1884	44	3	6	13																	
	<i>Hydropsyche fulvipes</i> (Curtis, 1834)	45						29	5													
	<i>Hydropsyche tenuis</i> Navás, 1932	46		6																		
HYDROPTILIDAE	Hydroptilidae Gen. sp. Lv.				7	3	4												3	5	1	
	<i>Hydroptila</i> sp. Lv.				3	1	6	33	5	16	3	13	1	2	3	9	2	2	3	3		
	<i>Hydroptila</i> sp. pupa	47			1					6	1	9			1	1		1	4	6		
	<i>Hydroptila sparsa</i> Curtis, 1834	48						2														
	<i>Ithytrichia</i> sp.	49										7			3	13	4	5	2	3		
LEPIDOSTOMATIDAE	<i>Lepidostoma</i> sp.	50																			1	
LEPTOCERIDAE	Leptoceridae Gen. sp.				4		1									5		3	1			
	<i>Adicella</i> sp.	51															21		2			
	<i>Athripsodes bilineatus bilineatus</i> (Linnaeus, 1758)	52			16																	
	<i>Athripsodes</i> sp.	53			1						12		1			7	1					
	<i>Ceraclea dissimilis</i> (Stephens, 1836)	54																		3		
	<i>Ceraclea</i> sp.	55																3		5	1	
	<i>Leptocerus interruptus</i> (Fabricius, 1775)	56													1							
	<i>Mystacides azurea</i> (Linnaeus, 1761)	57									1								1			
	<i>Mystacides longicornis/nigra</i>	58		1			4					2							1			
	<i>Mystacides</i> sp.	59		2		1	23			5		7		2	3			2	1		3	



**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																																					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20																		
<b>ORDER: TRICHOPTERA</b>																																								
Total individuals: 9,398																																								
		60																																						
	<i>Oecetis lacustris</i> (Pictet, 1834)																																							
	<i>Oecetis</i> sp.																																							
LIMNephilidae	<i>Allogamus uncatu</i> s (Brauer, 1857)	61	5																		1	1																		
	<i>Anabolia furcata</i> Brauer, 1857	62		1	2	1	1												1	8	1	7	2	1																
	Chaetopterygini/Stenophylacini Gen. sp.	63	28																		5																			
	<i>Halesus digitatus/tesselatus</i>	64																		2																				
	<i>Potamophylax rotundipennis</i> (Brauer, 1857)	65		1	63												4	6																						
ODONTOCERIDAE	<i>Odontocerum albicorne</i> (Scopoli, 1763)	66																		2																				
PHRYGANEIDAE	<i>Oligostomis reticulata</i> (Linnaeus, 1761)	67	1																																					
POLYCENTROPODIDAE	Polycentropodidae Gen. sp.	68	9																																					
	<i>Cyrnus trimaculatus</i> (Curtis, 1834)	68																		4	36																			
	<i>Plectrocnemia conspersa conspersa</i> (Curtis, 1834)	69	16																																					
PSYCHOMYIIDAE	<i>Lype phaeopa</i> (Stephens, 1836)	70																		34																				
	<i>Lype reducta</i> (Hagen, 1868)	71																		16																				
	<i>Lype</i> sp.	72		2												7																								
	<i>Tinodes</i> sp.	73																		2	4																			
	<i>Tinodes</i> sp. pupa ♀	74	1																																					
	<i>Tinodes rostocki</i> Mclachlan, 1878	74	2																																					
	<i>Psychomyia pusilla</i> (Fabricius, 1781)	75																		145	701	3	13	90	60	10	13	272	221	4	198	28	9							
RHYACOPHILIDAE	<i>Rhyacophila</i> sp. Lv.	76	4	3												1	3																							
	<i>Rhyacophila</i> sp. pupa	76	1																																					
	<i>Rhyacophila hirticornis</i> Mclachlan, 1879 ♂ pupa	77	1																																					
SERICOSTOMATIDAE	<i>Notidobia ciliaris</i> (Linnaeus, 1761)	78		3	17												1																							
<b>ORDER: ODONATA</b>																																								
Total individuals: 341																																								
CALOPTERYGIDAE	<i>Calopteryx virgo</i> (Linnaeus, 1758)	79																		2	3	2	1	2	5	1				1				2						
GOMPHIDAE	<i>Gomphus vulgatissimus</i> (Linnaeus, 1758)	80																		1	3	1												2	6	6	1	1		
	<i>Onychogomphus forcipatus forcipatus</i>	81	1	2	1	1												3	1	1	3	6	9	4	1	6														
	Gomphidae Gen. sp. Juv.	81																		1												2	22	2	4	1	11	3	31	
LIBELLULIDAE	<i>Orithetrum albistylum</i> (Selys, 1848)	82	1																																					
LIBELLULIDAE/ CORDULIIDAE	Libellulidae/corduliidae juv.	83																		1																				
COENAGRIONIDAE	Coenagrionidae Gen. sp.	84	97																																					
PLATYCNEMIDIDAE	<i>Platycnemis pennipes</i> (Pallas, 1771)	85																		33												3	19	1						
[UOrd:Zygoptera]	Zygoptera Gen. sp. Juv. non det.																														1	27	4							

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>ORDER: MEGALOPTERA</b>																						
Total individuals: 8																						
SIALIDAE	<i>Sialis fuliginosa</i> Pictet, 1836	86			3							1										
	<i>Sialis lutaria</i> (Linnaeus, 1758)	87		1																		
	<i>Sialis sordida</i> Klingstedt, 1932	88				1						1										
	<i>Sialis</i> sp. Klingstedt, 1932 juv.				1																	
<b>ORDER: DIPTERA 1</b>																						
Total individuals: 11,792																						
ATHERICIDAE	Athericidae Gen. sp.	89						1				1	20	24	4	2		4				
	<i>Atherix ibis</i> (Fabricius, 1789)							3	5		4	16										
CERATOPOGONIDAE	Ceratopogonidae Gen. sp.	90	99	6	45	4	2	4	2	3		4		18	7	23	27	8		6	1	8
DIXIDAE	<i>Dixa nebulosa</i> Meigen, 1830	91	2																			
	<i>Dixa</i> sp.	91	12																			
EMPIDIDAE	Empididae Gen. sp.	92				1			1	125	25	1	2		5	13	27	15	3	114	173	172
	<i>Chelifera</i> sp.		4							1										1		
	<i>Hemerodromia</i> sp.		4			2	1		36											1		
	<i>Wiedemannia</i> sp.								1													
EPHYDRIDAE	Ephydriidae Gen. sp.	93	1	3													1			1		
LIMONIIDAE	Limoniidae Gen. sp.	94	6		1		1	1	2			1		10	6	36	110	16	2	5	2	191
	<i>Antocha</i> sp. Osten-Sacken, 1860	95					29	23	44	9	1	3	22	1	1	1	5	10	1	16	5	
	<i>Scleroprocta</i> sp. Edwards, 1938								1													
	<i>Linnophora</i> sp.	96							2	1	1					2						
PEDICIIDAE	<i>Dicranota</i> sp. Zetterstedt, 1838	97	5	29	29	2	3	8	94	22	1		1		1	7				1		1
PSYCHODIDAE	Psychodidae Gen. sp.	98		1		1		1		1												
SIMULIIDAE	Simuliidae Gen. sp.	99		85	26	8		41	388	117	10	6	1560	34	15	14	5	4221	104	1221	27	1596
	<i>Simulium ornatum</i> -Gr.	99		269	46	1			6	2												
	<i>Simulium aureum</i> -Gr.	99		29																		
	<i>Simulium trifasciatum</i> Curtis, 1839	99		1																		
	<i>Simulium (Eusimulium) angustipes</i> Edwards, 1915	99				1																
	<i>Simulium (Wilhemia) lineatum</i> (Meigen, 1804)	99						6	1				5	1						1		
	<i>Simulium (Boophthora) erythrocephalum</i> (De Geer, 1776)	99											1									
STRATIOMYIDAE	Stratiomyidae Gen. sp.	100	1																		1	
TABANIDAE	Tabanidae Gen. sp.	101	7	36			3		1	8	3							1				3
TIPULIDAE	Tipulidae Gen. sp.	102		1		1										1	1				1	
<b>ORDER: DIPTERA 2</b>																						
Total individuals: 64,503																						
CHIRONOMIDAE	CHIRONOMINAE-Tribus Chironomini	103	257			1671	1350	194	257	687	1455	3763	75	365	445	1350	2729	588	3582	3363	668	1866
	<i>Chironomus</i> sp.	104		3																		

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>ORDER: DIPTERA 2</b>																						
Total individuals: 64,503																						
	<i>Chironomus thummi</i> -Gr.	105									6	68			4	38		19	60			
	<i>Cryptochironomus</i> sp.			15	2																	
	<i>Microtendipes</i> sp.			1249	2204																	
	<i>Paratendipes albinus</i> (Meigen, 1818)			6																		
	<i>Polypedilum pedestre</i> (Meigen, 1830)			828	307																	
	<i>Polypedilum scalaenum</i> (Schrank, 1803)			1																		
	CHIRONOMINAE-Tribus Tanytarsini	106	501			64	92	44	117	519	122	960	13	36	48	35	46	7	1431	2588	1695	4073
	<i>Micropsectra</i> sp.			3																		
	<i>Rheotanytarsus</i> sp.			2	18						75											
	<i>Tanytarsus</i> sp.			1	189																	
	Diamesinae Gen. sp.	107																			3	6
	Orthoclaadiinae Gen. sp.	108	8	5	28	2	9	40	2046	1245	415	17	572	16	11	6	16	107	1908	7787	2507	2446
	<i>Prodiamesa olivacea</i> (Meigen, 1818)	109	5	1383	3	21	64	2	1	15	93	43		120	41	92	44	32	13	18		3
	Tanytopodinae Gen. sp.	110	24	44	40	24	6	17	40	161	42	55	27	23	4	37	58	7	73	89	281	92
	<i>Procladius choreus</i> (Meigen, 1804)			12																		
<b>ORDER: COLEOPTERA</b>																						
Total individuals: 2,424																						
GYRINIDAE	<i>Gyrinus</i> sp. Lv.	111	3			1	1				4	3	2			2				3		
	<i>Orectochilus villosus</i> (Müller, 1776) Ad.	112					1															
DYTISCIDAE	<i>Bidessus delicatulus</i> (Schaum, 1844) Ad.	113																				1
	<i>Ilybius aenescens</i> Thomson, 1870	114		3																		
	<i>Laccophilus</i> sp. Lv.	115																	3		5	
	<i>Platambus maculatus</i> (Linnaeus, 1758) Ad.	116		1																		
	<i>Hygrotus</i> sp. Lv.	117																				5
DRYOPIDAE	<i>Dryops</i> sp. Lv.	118															1					1
	<i>Pomatinus substriatus</i> (Müller, 1806)	119		1					1					1	1							
HYDROPHILIDAE	<i>Hydrophilus</i> sp. Lv.	120																			1	
	<i>Laccobius</i> sp. Lv.	121			2																1	
	<i>Laccobius</i> sp. Ad. ♀	122			1																	
	<i>Anacaena lutescens</i> (Stephens, 1829)	123				1																
	Hydrophilidae Lv. Juv.	124	1			1						1									1	
HELOPHORIDAE	<i>Helophorus</i> sp. ♀	125																			1	
HYDRAENIDAE	<i>Hydraena belgica</i> D'orchymont, 1930	126			12																	
	<i>Hydraena cf. Croatia</i> Kuwert, 1888	127									1						1					
	<i>Hydraena excisa</i> Kiesenwetter, 1849	128	6																			
	<i>Hydraena gracilis</i> Germar, 1824 Ad.	129			1																	
	<i>Hydraena intermedia</i> Rosenhauer, 1847 Ad.	130			2		1															
	<i>Hydraena melas</i> Dalla Torre, 1877 Ad.	131	1	2	8		2									1						

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																																																		
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20																															
<b>ORDER: COLEOPTERA</b>																																																					
Total individuals: 2,424																																																					
ELMIDAE	<i>Hydraena nigrita</i> Germar, 1824 Ad.	132	6																																																		
	<i>Hydraena pulchella</i> Germar, 1824 Ad.	133		3																																																	
	<i>Hydraena riparia</i> Kugelann, 1794 Ad.	134	1	1	2		1	11	7	40	3			4	4			8		4	1																																
	<i>Hydraena</i> sp. Ad. ♀	135		1	18																																																
	<i>Hydraena</i> sp. Lv.	136																				1																															
	<i>Elmis rioloides</i> Kuwert, 1890 Ad.	137																				1																															
	<i>Elmis obscura</i> (Müller, 1806)	138																				1	1	2																													
	<i>Elmis maugetii</i> Latreille, 1798 Ad.	139	2																				1																														
	<i>Elmis</i> sp. Ad. ♀	140																				1	1																														
	<i>Elmis</i> sp. Lv.	141	1	1	8	1		10	57	1			8	1	2	2	2	2	1	2		2																															
	<i>Esolus angustatus</i> (Müller, 1821) Ad.	142																				2																															
	<i>Esolus parallelepipedus</i> (Müller, 1806) Ad.	143																				4	151																														
	<i>Esolus pygmaeus</i> (Müller, 1806) Ad.	144																				4	5	1		1	12	1	31	69	136	82	2	18	11																		
	<i>Esolus</i> sp. Ad. ♀	145																				1		4	1		1																										
	<i>Esolus</i> sp. Ad. Juv.	146																				4				8	69	7	1		2	31	12	19	48	19		10	1														
	<i>Elmis</i> sp. Lv.	147																								1				1	1																						
	<i>Limnius volckmari</i> (Panzer, 1793) Ad.	148																												1	1																						
	<i>Limnius</i> sp. Ad. ♀	149		3	22	1																																															
	<i>Limnius</i> sp. Lv.	150																				14	187	19	1		1	3	3		1	2																					
	<i>Macronychus quadrituberculatus</i> Müller, 1806 Ad.	151																											1																								
<i>Macronychus quadrituberculatus</i> Müller, 1807 Lv.	152																											3	1		1																						
<i>Oulimnius tuberculatus</i> (Müller, 1806) Ad.	153		13	298	1	13	12	19	11	5	2	16	14	48	46	23	7	1	8																																		
<i>Oulimnius tuberculatus</i> (Müller, 1806) Lv.	154		10	49	2	15	6	6	3	3	58	110	46	23	34	75	1	11	1																																		
<i>Oulimnius</i> sp. Lv.	155		2		2																																																
<i>Potamophilus acuminatus</i> (Fabricius, 1792) Lv.	156																											1	3	1	1		1	1																			
<i>Riolus subviolaceus</i> (Müller, 1817) Ad.	157																							5																													
<i>Riolus</i> sp. Lv.	157																						2	19																													
<b>ORDER: HEMIPTERA</b>																																																					
Total individuals: 342																																																					
APHELOCHEIRIDAE	<i>Aphelocheirus aestivalis</i> (Fabricius, 1794)	158																				1	1			1	1	4	3	1		2		5																			
	<i>Aphelocheirus</i> sp. Juv.	158																				2	7	7	4																												
CORIXIDAE	Corixidae Gen. sp.	159																															21	1	2																		
	<i>Micronecta</i> sp.	160																								2		2	1	4																							
GERRIDAE	Gerridae Gen. sp.	161																																4																			
HYDROMETRIDAE	Hydrometridae Gen. sp. Juv.	162																															7	2																			
MESOVELIIDAE	Mesoveliidae Gen. sp.	163																														1																					
NEPIDAE	<i>Nepa</i> sp.	164		1		1																																															
VELIIDAE	Veliidae Gen. sp.	165	4	24	2			5	2			5																																									
	Heteroptera Gen. sp. Juv non det.	166	1																														5		2																		

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
<b>CLASS: MALACOSTRACA</b>																							
<b>ORDER: ISOPODA</b>																							
Total individuals: 338																							
ASELLIDAE	<i>Asellus aquaticus aquaticus</i> (Linnaeus, 1758)	167				1				153	11	1						30	19	102	21		
<b>ORDER: AMPHIPODA</b>																							
Total individuals: 43,442																							
GAMMARIDAE	<i>Gammarus fossarum</i> Koch in Panzer, 1836	168	15019	393	1069	218	87	331	1211	108	10	20	1230	2872	5737	1509	664	4036	325	1356	8	385	
	<i>Gammarus roeselii</i> Fabricius, 1775	169		1823	691	251	179	83	25	8	20	195	196	1672	849	628	129	8	12	21	1	63	
<b>CLASS: BIVALVIA</b>																							
<b>ORDER: MYIDA</b>																							
Total individuals: 3																							
DREISSENIDAE	<i>Dreissena polymorpha</i> (Pallas, 1771)	170																				3	
<b>ORDER: SPHAERIIDA</b>																							
Total individuals: 1,085																							
SPHAERIIDAE	<i>Pisidium</i> sp. Pfeiffer, 1821	171	585	235		4				1	21	14		3	41	48	40	35	21		2	1	4
	<i>Sphaerium rivicola</i> (Lamarck, 1818)	172				20									2	1	2	1					4
<b>ORDER: UNIONIDA</b>																							
Total individuals: 20																							
UNIONIDAE	<i>Anodonta anatina</i> (Linnaeus, 1758)	173				5																	
	<i>Unio crassus</i> ssp.	174			1	1	1	1	1					6		1	3						
<b>CLASS: GASTROPODA</b>																							
<b>ORDER: NEOTAENIOGLOSSA</b>																							
Total individuals: 1,647																							
BITHYNIIDAE	<i>Bithynia tentaculata</i> (Linnaeus, 1758)	175																	8	7		3	
HYDROBIIDAE	<i>Bythinella opaca opaca</i> (M. Von Gallenstein, 1848)	176	487																				
	<i>Sadleriana fluminensis</i> (Küster, 1852)	177																24					
MELANOPSIDAE	<i>Esperiana (Microcolpia) daudebartii acicularis</i> (Ferussac, 1823)	178												1			24	9					
	<i>Holandriana holandrii</i> (C. Pfeiffer, 1828)	179					2	81	198	1		9	73	105	50	81	352	114	9				9
<b>ORDER: PULMONATA</b>																							
Total individuals: 63																							
ACROLOXIDAE	<i>Acroloxus lacustris</i> (Linnaeus, 1758)	180				3				2			1								1		1
LYMNAEIDAE	<i>Galba truncatula</i> (O.F. Müller, 1774)	181	1																				
	<i>Radix labiata</i> (Rossmässler, 1835)	182	2	4																			
PHYSIDAE	<i>Physella acuta</i> (Draparnaud, 1805)	183															1		22	2			
PLANORBIDAE	<i>Ancylus fluviatilis</i> O.F. Müller, 1774	184							10														
	<i>Gyraulus crista</i> (Linnaeus, 1758)	185																		1	1		
	<i>Hippeutis complanatus</i> (Linnaeus, 1758)	186																		8	2		
	<i>Segmentina nitida</i> Fleming, 1818	187															1						

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>ORDER: NERITOPSINA</b>																						
Total individuals: 304																						
NERITIDAE	<i>Theodoxus danubialis danubialis</i> (C. Pfeiffer, 1828)	188					49					31	10	19	15	44	123	13				
<b>ORDER: ECTOBRACHIA</b>																						
Total individuals: 6																						
VALVATIDAE	<i>Valvata piscinalis piscinalis</i> (O.F. Müller, 1774)	189									2									4		
<b>CLASS: CLITELLATA (Oligochaeta)</b>																						
<b>ORDER: HAPLOTAXIDA</b>																						
Total individuals: 35,290																						
ENCHYTRAEIDAE	Enchytraeidae Gen. sp.	190	17						3			3	1								64	
NAIDIDAE	<i>Chaetogaster</i> sp. Von Baer, 1827	191																	18			
	<i>Nais behningi</i> Michaelsen, 1923	192				1		14				3					3					
	<i>Nais breitscheri</i> Michaelsen, 1899	193			80	38	3		4	2												
	<i>Nais communis</i> Piguët, 1906	194			27		7	4								4			4		286	
	<i>Nais elinguis</i> Müller, 1773	195																			75	
	<i>Nais pardalis</i> Piguët, 1906	196																	111		95	
	<i>Nais stolci</i> Hrabec, 1979	197										11				7	3					
	<i>Nais simplex</i> Piguët, 1906	198						5														
	<i>Nais</i> sp. Müller, 1773	199			53			6		32					3	2	2	77		274		
	<i>Pristina longiseta</i> Ehrenberg, 1828	200							38										123		84	
	<i>Stylaria lacustris</i> (Linnaeus, 1767)	201																114		62	13	
PROPAPPIDAE	<i>Propappus volki</i> Michaelsen, 1916	202			1	1				184					510	1111	1	10		22	13	
TUBIFICIDAE	<i>Branchiura sowerbyi</i> Beddard, 1892	203			353	124	110	152	1586	140				4						42		
	<i>Limnodrilus</i> sp. Claparède, 1862	204					6	6		140									5			
	<i>Limnodrilus claparedeanus</i> Ratzel, 1868	205						15	247	1906	814	8	78	66	52	122	37		40	7	13	
	<i>Limnodrilus hoffmeisteri</i> Claparède, 1862	206	51	1543	472	405	320	144	229	6307	4997	1224	106	947	371	448	850	204	740	1194	417	
	<i>Limnodrilus udekemianus</i> Claparède, 1862	207								210					38	62				6	29	
	<i>Potamothenrix hammoniensis</i> (Michaelsen, 1901)	208	769	718	303	65	3		32	306	241	509		137		280		20		47	13	
	<i>Potamothenrix</i> sp. Vějdovský & Mrázek, 1902	209									3						61			21	1	
	<i>Psammorectides barbatus</i> (Grube, 1891)	210	456		47	22		26			32				101			31		11		
	<i>Tubifex ignotus</i> (Stolc, 1886)	211																		19		
<b>ORDER: LUMBRICULIDA</b>																						
Total individuals: 1,339																						
LUMBRICIDAE	Lumbricidae Gen. sp.	212	36	44		2		16			350						7		20		127	
	<i>Eiseniella tetraedra</i> (Savigny, 1826)	213			65				66	235												
LUMBRICULIDAE	Lumbriculidae Gen. sp.	214							10					4			1			28	73	
	Lumbriculidae Gen. sp. Juv.	214								245											10	

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>CLASS: CLITELLATA (Hirudinea)</b>																						
<b>ORDER: ARHYNCHOBELLIDA</b>																						
Total individuals: 205																						
ERPOBDELLIDAE	<i>Erpobdella octoculata</i> (Linnaeus, 1758)	215		1		3		1	2	8	3								1			
	<i>Erpobdella</i> sp. Juv.				15	1		7	6													
	Erpobdellidae Gen. sp. Juv.	216								30	23		1					14	20	45	4	
	<i>Trocheta</i> sp.	217																	7	8	3	
HAEMOPIIDAE	<i>Haemopsis sanguisuga</i> (Linnaeus, 1758)	218	2																			
<b>ORDER: RHYNCHOBELLIDA</b>																						
Total individuals: 155																						
GLOSSIPHONIIDAE	<i>Glossiphonia complanata</i> (Linnaeus, 1758)	219		14				1		2												
(Glossiphoniinae)	<i>Glossiphonia</i> sp. Juv.			4				1														
	Glossiphoniinae Gen. sp. Juv.	220							4	9		1						6	7	1	1	
	<i>Hemiclepsis marginata</i> (O.F. Müller, 1774)	221																1				
GLOSSIPHONIIDAE	<i>Helobdella stagnalis</i> (Linnaeus, 1758)	222		9	1			1	22	29								5	16	3		
(Haementeriinae)																						
PISCICOLIDAE	<i>Piscicola geometra</i> (Linnaeus, 1761)	223										1		1		2			1			
	<i>Piscicola</i> sp.	224										1		3		4						
	Hirudinea Gen. sp. Juv								1										2			
<b>CLASS: ARACHNIDA</b>																						
<b>ORDER: TROMBIDIFORMES (Hydrachnidia)</b>																						
Total individuals: 452																						
ATURIDAE	<i>Atrurus scaber</i> Kramer, 1875	225						1				1										
HYDRYPHANTIDAE	<i>Protzia</i> sp. Piersig, 1896	226						1														
HYGROBATIDAE	<i>Atractides</i> sp. Koch, 1837	227			1	1											1					
	<i>Atractides loricatus</i>	228							2													
	<i>Hygrobatas calliger</i> Piersig, 1896	229					1	7	12	1		1	3	1	4		1	10	11	3		
	<i>Hygrobatas fluviatilis</i> (Ström, 1768)	230		1	2		20	6	5	5	27	24	2	1	4	3	16		2			
	<i>Hygrobatas longiporus</i> Thor, 1898	231					2														2	
	<i>Hygrobatas trigonicus</i> Koenike, 1895	232										3					5					
	<i>Hygrobatas</i> sp. Koch, 1837 nymph	233						2		1										1	1	
LEBERTIIDAE	<i>Lebertia</i> sp. Neumann, 1880	234		1	1	10	4	14	9	1	4	13	4	3	4	14		1	5	5	4	
MIDEOPSISIDAE	<i>Mideopsis orbicularis</i> (Müller, 1776)	235								1	41			1	4	3		7				
	<i>Mideopsis</i> sp. Neuman, 1880 nymph	236									1											
NUDOMIDEOPSISIDAE	<i>Nudomideopsis cf. Motasi</i>	237																	1			
SPERCHONTIDAE	<i>Sperchon clupeiifer</i> Piersig, 1896	238						1	1			1										
	<i>Sperchon compactilis</i> Koenike, 1911	239						1				1										
	<i>Sperchon denticulatus</i> -Gr.	240										1		1					1	1	1	
	<i>Sperchon hispidus</i>	241						6													1	
	<i>Sperchon hibernicus</i> Halbert, 1944	242						2											3		1	

**ANNEX I (Continued).** Benthic macroinvertebrate taxa list for the Bednja River. Number of identified individuals per study site is given. Corresponding taxa index number from Canonical correlation analysis (CCA) plot is given in a separate column.

		Number in CCA plot	Study site																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
<b>ORDER: TROMBIDIFORMES (Hydrachnidia)</b>																							
Total individuals: 452																							
TORRENTICOLIDAE	<i>Sperchon insignis</i>	243																				1	
	<i>Sperchon papillosus</i>	244																				3	
	<i>Sperchonopsis verrucosa</i> (Protz, 1896)	245			3			1		3												1	
	<i>Sperchon</i> sp. Kramer, 1877 nymph	246			1					2		1										3	
	<i>Torrenticola amplexa</i> (Koenike, 1908)	247																		8		27	
	<i>Torrenticola elliptica</i>	248										1											
	<i>Torrenticola hyporheica</i>	249											1										
	<i>Torrenticola ischnophallus</i>	250																5					1
	<i>Torrenticola laskai</i>	251														1							
	<i>Torrenticola ungeri</i>	252																				1	
NEOACARIDAE	<i>Torrenticola</i> sp. Piersig, 1896 nymph	253																		1			
	<i>Neoacarus hibernicus</i> Halbert, 1944	254															2					1	
	Hydrachnidia Gen. sp. Juv. Non. det.	255				1				1		1			2						1		
<b>CLASS: TURBELLARIA</b>																							
<b>ORDER: TRICLADIDA</b>																							
DENDROCOELIDAE	<i>Dendrocoelum</i> sp.	256																				1	
DUGESIIDAE	<i>Dugesia</i> sp.	257				10			5													2	
PLANARIIDAE	<i>Polycelis</i> sp.	258	45							1													
<b>CLASS: HYDROZOA</b>																							
<b>ORDER: ANTHOATHECATA</b>																							
HYDRIDAE	<i>Hydra</i> sp.	259					1			2		64	16				4		49	23	3	11	



**ANNEX II** Abiotic characteristics of sampled microhabitats (water depth and flow velocity) at study sites on the Bednja River.

Study site	Substrate	Number of subsamples	Substrate share%	Sampling depth (cm)			Velocity (ms <sup>-1</sup> )		
				Min	Max	Average	Min	Max	Average
1	Mesolithal	7	35%	2	10	<b>4.29</b>	0.01	0.70	<b>0.324</b>
	Psammal	5	25%	4	14	<b>8.60</b>	0.01	0.17	<b>0.064</b>
	Microlithal	3	15%	2	8	<b>5.67</b>	0.05	0.37	<b>0.177</b>
	Macrolithal	2	10%	2	4	<b>3.00</b>	0.06	0.07	<b>0.065</b>
	Argyllal	2	10%	7	10	<b>8.50</b>	0.03	0.06	<b>0.045</b>
	Akal	1	5%	13	13	<b>13.00</b>	0.02	0.02	<b>0.020</b>
2	Psammal	8	40%	16	29	<b>21.25</b>	0.01	0.11	<b>0.063</b>
	Macrophytes	8	40%	18	33	<b>24.25</b>	0.01	0.35	<b>0.081</b>
	Akal	4	20%	16	44	<b>27.00</b>	0.01	0.11	<b>0.083</b>
3	Argyllal	6	30%	5	12	<b>7.17</b>	0.04	0.12	<b>0.062</b>
	Microlithal	6	30%	14	26	<b>18.50</b>	0.01	0.11	<b>0.058</b>
	Mesolithal	4	20%	12	20	<b>14.00</b>	0.01	0.09	<b>0.048</b>
	Akal	2	10%	8	9	<b>8.50</b>	0.10	0.30	<b>0.200</b>
	C POM	1	5%	5	5	<b>5.00</b>	0.22	0.22	<b>0.220</b>
	Psammal	1	5%	7	7	<b>7.00</b>	0.01	0.01	<b>0.010</b>
4	Psammal	10	50%	20	53	<b>32.00</b>	0.02	0.22	<b>0.060</b>
	Xylal	9	45%	15	30	<b>22.22</b>	0.00	0.25	<b>0.064</b>
	Microlithal	1	5%	16	16	<b>16.00</b>	0.14	0.14	<b>0.140</b>
5	Psammal	9	45%	11	41	<b>31.67</b>	0.01	0.12	<b>0.039</b>
	Argyllal	8	40%	11	33	<b>22.00</b>	0.05	0.14	<b>0.105</b>
	Xylal	3	15%	10	10	<b>10.00</b>	0.12	0.25	<b>0.207</b>
6	Mesolithal	15	75%	6	35	<b>18.33</b>	0.01	0.70	<b>0.313</b>
	Macrolithal	2	10%	10	20	<b>15.00</b>	0.47	0.60	<b>0.535</b>
	Microlithal	2	10%	15	16	<b>15.50</b>	0.03	0.23	<b>0.130</b>
	Akal	1	5%	8	8	<b>8.00</b>	0.20	0.20	<b>0.200</b>
7	Microlithal	11	55%	5	28	<b>19.36</b>	0.04	0.57	<b>0.220</b>
	Technolithal	6	30%	12	20	<b>16.50</b>	0.00	0.41	<b>0.153</b>
	Akal	2	10%	11	23	<b>17.00</b>	0.07	0.15	<b>0.110</b>
	Macrophytes	1	5%	12	12	<b>12.00</b>	0.06	0.06	<b>0.060</b>
8	Akal	16	80%	1	43	<b>17.31</b>	0.00	0.57	<b>0.205</b>
	Xylal	3	15%	10	15	<b>12.67</b>	0.01	0.08	<b>0.047</b>
	Psammal	1	5%	5	5	<b>5.00</b>	0.00	0.00	<b>0.000</b>
9	Akal	16	80%	4	38	<b>19.81</b>	0.00	0.50	<b>0.281</b>
	Xylal	2	10%	10	20	<b>15.00</b>	0.02	0.20	<b>0.110</b>
	Psammal	2	10%	20	30	<b>25.00</b>	0.01	0.50	<b>0.010</b>
10	Akal	19	95%	80	110	<b>92.11</b>	0.00	0.01	<b>0.006</b>
	Xylal	1	5%	5	10	<b>7.50</b>	0.01	0.01	<b>0.010</b>
11	Technolithal	11	55%	4	35	<b>15.18</b>	0.20	1.29	<b>0.638</b>
	Macrophytes	5	25%	4	12	<b>7.00</b>	0.06	0.45	<b>0.280</b>
	Microlithal	4	20%	20	25	<b>22.50</b>	0.24	1.24	<b>0.558</b>
12	Akal	11	55%	4	90	<b>53.55</b>	0.01	0.25	<b>0.085</b>
	Psammal	4	20%	27	90	<b>65.50</b>	0.01	0.01	<b>0.010</b>
	Microlithal	3	15%	2	36	<b>17.00</b>	0.03	0.41	<b>0.207</b>
	Xylal	2	10%	10	15	<b>12.50</b>	0.03	0.70	<b>0.365</b>
13	Akal	10	50%	18	55	<b>36.30</b>	0.08	3.10	<b>1.012</b>
	Psammal	4	20%	10	50	<b>33.75</b>	0.01	0.08	<b>0.045</b>
	Xylal	4	20%	5	10	<b>7.50</b>	0.10	3.20	<b>1.173</b>
	Microlithal	2	10%	27	30	<b>28.50</b>	0.20	0.21	<b>0.205</b>
14	Akal	11	55%	10	65	<b>42.27</b>	0.02	0.23	<b>0.129</b>
	Psammal	8	40%	8	70	<b>40.50</b>	0.01	0.08	<b>0.038</b>
	Xylal	1	5%	15	15	<b>15.00</b>	0.30	0.30	<b>0.30</b>
15	Akal	17	85%	11	80	<b>51.12</b>	0.00	0.31	<b>0.186</b>
	Psammal	2	10%	30	34	<b>32.00</b>	0.01	0.04	<b>0.025</b>
	Xylal	1	5%	10	15	<b>12.50</b>	0.35	0.35	<b>0.350</b>
16	Mesolithal	10	50%	6	50	<b>21.80</b>	0.40	0.83	<b>0.648</b>
	Xylal	4	20%	5	10	<b>7.00</b>	0.07	0.37	<b>0.193</b>
	Microlithal	2	10%	12	22	<b>17.00</b>	0.19	0.57	<b>0.380</b>
	Akal	3	15%	28	62	<b>40.00</b>	0.25	0.38	<b>0.330</b>
	Psammal	1	5%	10	10	<b>10.00</b>	0.01	0.01	<b>0.010</b>

**ANNEX II (Continued).** Abiotic characteristics of sampled microhabitats (water depth and flow velocity) at study sites on the Bednja River.

Study site	Substrate	Number of subsamples	Substrate share%	Sampling depth (cm)			Velocity (ms <sup>-1</sup> )		
				Min	Max	Average	Min	Max	Average
<b>17</b>	Technolithal	19	95%	20	60	<b>41.63</b>	0.00	0.02	<b>0.008</b>
	Macrophytes	1	5%	42	42	<b>42.00</b>	0.01	0.01	<b>0.010</b>
<b>18</b>	Microlithal	6	30%	6	50	<b>27.17</b>	0.06	0.86	<b>0.370</b>
	Mesolithal	6	30%	10	25	<b>17.83</b>	0.02	0.40	<b>0.213</b>
	Xylal	4	20%	20	35	<b>24.75</b>	0.02	0.43	<b>0.153</b>
	Akal	4	20%	15	50	<b>31.00</b>	0.05	0.52	<b>0.270</b>
<b>19</b>	Technolithal	10	50%	20	30	<b>24.00</b>	0.01	0.12	<b>0.039</b>
	Microlithal	10	50%	20	42	<b>28.80</b>	0.24	0.53	<b>0.344</b>
<b>20</b>	Microlithal	14	70%	10	47	<b>28.36</b>	0.00	1.19	<b>0.534</b>
	Psammal	3	15%	9	25	<b>16.33</b>	0.01	0.08	<b>0.033</b>
	Xylal	2	10%	20	20	<b>20.00</b>	0.07	0.09	<b>0.080</b>
	Akal	1	5%	12	12	<b>12.00</b>	0.65	0.65	<b>0.650</b>

**ANNEX III** Hydromorphological modification of study sites according to EN 15843:2010: individual scores for 16 assessed features.

Results for 16 individual scores for hydromorphological modification of study sites according to EN 15843:2010 assessed on a 100 m long reach. Scored hydromorphological features are as follows: 1a = Planform; 1b = Channel section (long-section and cross-section); 2a = Extent of artificial material; 2b = "Natural" substrate mix or character altered; 3a = Aquatic vegetation management; 3b = Extent of woody debris if expected; 4 = Erosion/deposition character; 5a = Impacts of artificial in-channel structures within the reach; 5b = Effects of catchment-wide modifications to natural flow character; 5c = Effects of daily flow alteration (e.g. hydropeaking); 6 = Longitudinal continuity as affected by artificial structures; 7 = Bank structure and modifications; 8 = Vegetation type/structure on banks and adjacent land; 9 = Adjacent landuse and associated features; 10a = Degree of lateral connectivity of river and floodplain; 10b = Degree of lateral movement of river channel

		Study site																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Hydromorphological feature score (100 m reach)	1a	1	5	5	2	5	5	5	5	5	5	5	5	4	5	2	5	3	5	1	
	1b	1	5	3	3	5	3	5	5	5	5	5	5	5	3	4	1	5	1	5	1
	2a	1	1	1	1	1	1	5	1	1	1	5	1	1	1	1	1	5	1	1	1
	2b	1	3	3	1	3	1	3	1	1	3	3	1	1	1	1	1	3	1	1	1
	3a	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1
	3b	1	5	1	1	1	1	5	1	1	1	3	1	1	1	1	1	3	1	1	1
	4	1	5	3	3	5	3	5	3	3	5	5	1	3	1	3	1	5	1	3	1
	5a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5b	1	1	1	1	1	1	1	1	1	3	3	1	1	1	1	1	3	1	1	1
	5c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1	1	1
	7	1	1	1	1	1	4	5	1	1	1	5	2	1	2	1	1	5	1	5	1
	8	2	5	1	1	5	3	5	4	4	5	5	4	4	2	5	2	5	1	5	1
	9	3	5	2	2	5	3	5	1	2	4	5	5	1	1	4	5	5	1	5	1
	10a	1	1	1	1	5	5	5	5	5	4	5	3	4	1	4	1	5	1	5	1
	10b	1	1	1	1	5	5	5	1	1	1	5	4	1	2	1	1	5	1	5	1

Results for 16 individual scores for hydromorphological modification of study sites according to EN 15843:2010 assessed on a 500 m long reach. Scored hydromorphological features are as follows: 1a = Planform; 1b = Channel section (long-section and cross-section); 2a = Extent of artificial material; 2b = "Natural" substrate mix or character altered; 3a = Aquatic vegetation management; 3b = Extent of woody debris if expected; 4 = Erosion/deposition character; 5a = Impacts of artificial in-channel structures within the reach; 5b = Effects of catchment-wide modifications to natural flow character; 5c = Effects of daily flow alteration (e.g. hydropeaking); 6 = Longitudinal continuity as affected by artificial structures; 7 = Bank structure and modifications; 8 = Vegetation type/structure on banks and adjacent land; 9 = Adjacent landuse and associated features; 10a = Degree of lateral connectivity of river and floodplain; 10b = Degree of lateral movement of river channel

		Study site																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Hydromorphological feature score (500 m reach)	1a	2	5	4	2	5	5	5	5	5	5	5	4	5	4	5	2	5	4	5	1
	1b	2	5	3	3	5	5	4	5	5	5	5	4	5	3	4	2	5	3	4	1
	2a	1	1	1	1	1	3	4	1	1	1	4	1	1	1	1	1	5	2	1	1
	2b	1	3	3	1	3	3	3	1	1	3	3	1	1	1	1	1	5	1	1	1
	3a	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1
	3b	1	5	1	1	1	3	5	1	1	1	3	1	1	1	1	1	3	1	1	1
	4	1	5	3	3	5	5	3	3	3	5	5	1	3	1	3	1	5	1	3	1
	5a	1	1	1	1	1	3	1	1	1	1	3	1	1	1	1	1	1	1	1	1
	5b	1	1	1	1	1	1	1	1	1	3	3	1	1	1	1	1	3	1	1	1
	5c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1	1	1
	7	1	1	1	1	1	5	4	1	1	1	3	2	1	2	1	1	5	2	3	1
	8	3	5	2	1	5	5	5	4	4	5	5	4	4	2	5	2	5	3	5	2
	9	4	5	4	2	5	5	5	2	2	4	5	5	4	3	4	4	5	3	5	2
	10a	1	1	1	1	5	5	4	5	5	4	4	2	4	1	4	1	5	1	5	1
	10b	1	1	1	1	5	5	4	1	1	1	3	3	1	2	1	1	5	3	4	1

## ANNEX IV Nutrient concentrations and physicochemical water properties measures at the study sites in spring, summer and autumn.

### Results of physicochemical water properties measured in March (spring) 2015.

	Water temp. °C	Dissolved O <sub>2</sub> mgL <sup>-1</sup>	Oxygen saturation %	Conductivity µScm <sup>-1</sup>	pH	KMnO <sub>4</sub> mgO <sub>2</sub> L <sup>-1</sup>	BOD <sub>5</sub> mgO <sub>2</sub> L <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> mgNL <sup>-1</sup>	NO <sub>2</sub> <sup>-</sup> mgNL <sup>-1</sup>	NO <sub>3</sub> <sup>-</sup> mgNL <sup>-1</sup>	Kjeldahl N mgNL <sup>-1</sup>	ORG. N mgNL <sup>-1</sup>	Σ N mgNL <sup>-1</sup>	PO <sub>4</sub> <sup>3-</sup> mgPL <sup>-1</sup>	Σ P mgPL <sup>-1</sup>	Alkalinity HCL ml
1	4.5	11.5	88.7	556	8.22	1.0	<0.6	0.0077	0.0016	0.5156	0.046	0.038	0.56	0.007	0.035	5.5
2	5.6	12.5	99.3	562	8.17	2.7	1.7	0.1763	0.0057	0.4841	0.200	0.024	0.69	0.019	0.037	5.9
3	9.0	12.0	97.3	552	8.13	2.7	1.7	0.1763	0.0057	0.4841	0.200	0.024	0.69	0.019	0.037	5.8
4	6.9	12.0	98.5	-	8.16	3.6	1.8	0.0218	0.0170	0.3523	0.060	0.038	0.41	0.005	0.010	4.5
5	7.8	11.5	96.6	501	8.02	5.0	3.2	0.0450	0.0490	0.3447	0.264	0.219	0.66	0.011	0.084	4.0
6	8.3	13.6	115.7	510	8.36	2.3	1.4	0.0433	0.0072	0.4101	0.160	0.120	0.53	0.009	0.020	4.1
7	9.1	12.8	111.1	502	8.37	2.4	1.2	0.0639	0.0045	0.6271	0.141	0.077	0.77	0.026	0.062	4.3
8	9.6	11.1	97.5	509	8.32	2.4	1.3	0.2043	0.0099	0.6778	0.268	0.064	0.96	0.025	0.082	3.9
9	8.0	10.6	89.5	508	8.35	1.9	0.9	0.3160	0.7468	0.7468	0.950	0.630	1.71	0.029	0.115	4.5
10	9.6	12.9	114.2	495	8.48	2.0	1.1	0.1572	0.8422	0.0146	0.503	0.350	1.36	0.018	0.096	4.7
11	9.7	13.0	114.5	495	8.48	2.0	1.1	0.1572	0.8422	0.0146	0.503	0.350	1.36	0.018	0.096	4.7
12	9.7	13.0	114.5	495	8.48	2.0	1.1	0.1572	0.8422	0.0146	0.503	0.350	1.36	0.018	0.096	4.7
13	9.5	11.7	102.5	506	8.62	3.3	1.5	0.1459	0.9384	0.0709	0.477	0.330	1.49	0.013	0.078	4.8
14	10.2	11.2	99.8	517	8.28	2.1	1	0.1100	0.9342	0.0187	0.340	0.230	1.35	0.011	0.072	4.8
15	9.5	10.8	94.7	548	8.15	1.7	<0.6	0.0777	0.9309	0.1440	0.310	0.240	1.25	0.009	0.060	5.1
16	9.5	10.8	94.7	548	8.15	1.7	<0.6	0.0777	0.9309	0.1440	0.310	0.240	1.25	0.009	0.060	5.1
17	9.4	11.2	97.9	563	8.06	3.2	1.4	0.0408	0.0134	1.0859	0.076	0.035	1.13	0.011	0.024	5.1
18	9.3	11.1	96.8	564	8.08	4.0	1.8	0.1729	0.0142	1.1260	0.300	0.127	1.27	0.168	0.248	5.3
19	8.8	11.9	102.5	574	8.02	2.8	1.3	0.0821	0.0159	1.1038	0.154	0.072	1.19	0.022	0.085	5.5
20	7.5	10.9	90.9	575	8.17	2.8	1.5	0.1286	0.0155	1.0020	0.163	0.034	1.05	0.020	0.085	5.4

### Results of physicochemical water properties measured during benthic macroinvertebrate sampling in end of June (summer) 2015.

	Water temp. °C	Dissolved O <sub>2</sub> mgL <sup>-1</sup>	Oxygen saturation %	Conductivity µScm <sup>-1</sup>	pH	KMnO <sub>4</sub> mgO <sub>2</sub> L <sup>-1</sup>	BOD <sub>5</sub> mgO <sub>2</sub> L <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> mgNL <sup>-1</sup>	NO <sub>2</sub> <sup>-</sup> mgNL <sup>-1</sup>	NO <sub>3</sub> <sup>-</sup> mgNL <sup>-1</sup>	Kjeldahl N mgNL <sup>-1</sup>	ORG. N mgNL <sup>-1</sup>	Σ N mgNL <sup>-1</sup>	PO <sub>4</sub> <sup>3-</sup> mgPL <sup>-1</sup>	Σ P mgPL <sup>-1</sup>
1	14.7	8.9	88.1	620	8.10	3.6	2.4	0.0659	0.0046	0.7991	0.600	0.530	1.40	0.015	0.127
2	24.8	8.7	106.0	608	8.03	3.7	2.7	0.1288	0.0688	0.8725	0.160	0.031	1.10	0.022	0.130
3	22.5	6.7	78.1	609	8.03	3.7	2.7	0.1288	0.0688	0.8725	0.160	0.031	1.10	0.022	0.130
4	22.5	6.0	69.9	419	7.90	6.6	4.6	0.0949	0.0172	0.4377	0.860	0.764	1.32	0.011	0.081
5	21.5	6.3	72.0	448	8.03	9.6	7.7	0.0469	0.0194	0.5134	1.200	1.150	1.73	0.023	0.296
6	22.5	7.9	92.1	513	8.24	5.0	3.5	0.0812	0.0283	0.6257	0.820	0.770	1.47	0.025	0.170
7	23.1	8.2	96.7	516	8.21	3.9	2.7	0.0405	0.0197	0.7902	0.370	0.330	1.18	0.024	0.136
8	22.3	7.2	83.5	529	8.01	3.7	2.4	0.0560	0.0385	0.8770	0.300	0.240	1.22	0.033	0.100
9	23.5	7.7	91.4	528	8.02	3.8	2.8	0.2612	0.0778	1.0931	0.622	0.360	1.79	0.037	0.161
10	25.7	7.6	92.6	512	8.39	3.6	2.2	0.0626	0.0609	1.2196	0.166	0.100	1.45	0.035	0.144
11	24.8	7.6	92.6	510	8.31	3.6	2.2	0.0626	0.0609	1.2196	0.166	0.100	1.45	0.035	0.144
12	23.6	7.6	92.6	511	8.28	3.6	2.2	0.0626	0.0609	1.2196	0.166	0.100	1.45	0.035	0.144
13	23.2	7.6	89.8	520	8.31	3.4	2.0	<0.008	0.0423	1.2822	0.080	0.072	1.40	0.035	0.152
14	20.4	7.4	82.7	531	8.08	3.4	1.9	0.0586	0.0436	1.3246	0.200	0.140	1.57	0.035	0.146
15	23.5	7.6	90.3	566	8.21	4.2	3.0	<0.008	0.0390	1.4872	0.140	0.132	1.67	0.036	0.142
16	23.5	7.6	90.3	566	8.21	4.2	3.0	<0.008	0.0390	1.4872	0.140	0.132	1.67	0.036	0.142
17	25.7	7.4	91.7	570	8.48	5.8	4.6	0.0068	0.0466	1.8374	1.000	0.930	2.88	0.051	0.141
18	25.5	7.7	95.1	574	8.41	5.9	4.4	0.1170	0.0496	1.8915	0.540	0.473	2.48	0.063	0.186
19	24.5	7.0	84.8	580	8.27	5.4	3.6	0.0444	0.0627	1.7868	0.220	0.176	2.07	0.046	0.198
20	22.5	8.0	93.2	580	8.24	5.3	4.2	0.1033	0.0710	1.8096	0.467	0.364	2.43	0.052	0.156

## Results of physicochemical water properties measured in October (autumn) 2015.

	Water temp. °C	Dissolved O <sub>2</sub> mgL <sup>-1</sup>	Oxygen saturation %	KMnO <sub>4</sub> mgO <sub>2</sub> L <sup>-1</sup>	BOD <sub>5</sub> mgO <sub>2</sub> L <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> mgNL <sup>-1</sup>	NO <sub>2</sub> <sup>-</sup> mgNL <sup>-1</sup>	NO <sub>3</sub> <sup>-</sup> mgNL <sup>-1</sup>	Kjeldahl N mgNL <sup>-1</sup>	ORG. N mgNL <sup>-1</sup>	Σ N mgNL <sup>-1</sup>	PO <sub>4</sub> 3 <sup>-</sup> mgPL <sup>-1</sup>	Σ P mgPL <sup>-1</sup>
1	14.0	8.1	78.9	3.4	1.5	0.040	0.0497	0.3716	0.380	0.340	0.76	0.009	0.020
2	12.5	7.1	66.9	3.9	2.2	0.024	0.0092	1.9892	0.420	0.396	2.40	0.035	0.070
3	12.5	7.1	66.9	3.9	2.2	0.024	0.0092	1.9892	0.420	0.396	2.40	0.035	0.070
4	13.5	4.5	43.4	7.8	4.7	0.170	0.0585	0.8966	1.210	1.040	2.00	0.051	0.097
5	13.0	6.3	60.0	6.2	3.6	0.060	0.0128	0.6308	0.550	0.490	1.14	0.032	0.065
6	14.0	8.0	78.0	4.2	2.6	0.055	0.0095	0.5827	0.440	0.390	0.98	0.029	0.059
7	14.0	8.4	81.9	3.0	1.2	0.038	0.0147	0.9006	0.560	0.522	1.44	0.015	0.028
8	14.5	7.2	70.9	3.0	1.3	0.238	0.0798	1.1716	0.620	0.382	1.63	0.074	0.109
9	14.5	8.0	78.8	3.0	1.4	0.450	0.0933	1.4010	0.800	0.350	1.84	0.056	0.113
10	15.0	8.5	84.7	3.1	1.7	0.034	0.0544	1.4227	0.420	0.386	1.86	0.032	0.062
11	15.0	8.5	84.7	3.1	1.7	0.034	0.0544	1.4227	0.420	0.386	1.86	0.032	0.062
12	15.0	8.5	84.7	3.1	1.7	0.034	0.0544	1.4227	0.420	0.386	1.86	0.032	0.062
13	15.0	9.4	93.7	3.5	1.8	0.014	0.0286	1.3611	0.360	0.346	1.74	0.041	0.780
14	14.5	8.6	84.7	3.0	1.6	0.044	0.0456	1.4326	0.350	0.306	1.78	0.027	0.068
15	15.0	8.7	86.7	3.4	1.8	0.028	0.0400	1.3511	0.460	0.432	1.82	0.041	0.079
16	15.0	8.7	86.7	3.4	1.8	0.028	0.0400	1.3511	0.460	0.432	1.82	0.041	0.079
17	16.0	8.8	89.6	3.7	2.3	0.078	0.0362	1.5126	0.500	0.422	1.97	0.045	0.088
18	16.0	8.4	85.5	3.7	2.1	0.046	0.0460	1.5988	0.460	0.414	2.06	0.074	0.145
19	15.5	6.6	66.5	3.5	1.8	0.112	0.0653	1.4742	0.380	0.268	1.81	0.055	0.112
20	15.5	8.6	86.7	3.7	2.0	0.113	0.0519	1.4618	0.450	0.337	1.85	0.051	0.069

## 9. CURRICULUM VITAE

Iva Vidaković Maoduš was born in Zagreb on the 15<sup>th</sup> of January 1985. For her elementary and middle school education she attended the American British Academy, International School in Muscat, Sultanate of Oman. She completed her high school education under the International Baccalaureate Programme at the XV. Gymnasium in Zagreb. In 2009, she graduated from the Faculty of Science, University of Zagreb with the title Master of Educational Biology. Her graduation thesis titled “Assessment of Surface Running Water Quality in the Pannonian Ecoregion Based on the Macrozoobenthos Community” was supervised by Prof. Zlatko Mihaljević, PhD. In her final year of studies, she interned on the EU CARDS Twinning Project "Capacity Building and Development of Guidelines for the Implementation of the Water Framework Directive“ in Croatia.

She has been employed in Elektroprojekt d.d. in Zagreb since 2011, where she participated in the development of over 50 projects in the field of environmental and nature protection, and water management. Her project references also include the development of river basin management plans, strategic environmental assessment, protected area management plans, and hydromorphological monitoring of rivers. She gained her experience on projects located in Croatia, but also in Bosnia and Herzegovina and North Macedonia.

She has been an active member of the Croatian Water Pollution Control Society since 2010 where she also held the position of Vice-President from 2013 to 2016. She has organized, moderated and presented at numerous conferences, symposiums, and workshops. Since 2016, she has been the representative of Croatia in the European Technical and Scientific Committee of the European Water Association.

So far, she has published, as the lead author and co-author, two scientific papers cited in the WoS database, co-authored two papers in the Scopus database, and three professional papers.