

# Effects of physical-chemical parameters on the EPT assemblages in surface waters in Pannonian Ecoregion

Lidija Kladarić<sup>1</sup>, Renata Ćuk<sup>1</sup>, Ivor Dukić<sup>2</sup> & Andrea Marinković Ruždjak<sup>1</sup>

<sup>1</sup>Hrvatske vode, Central Water Management Laboratory, Ulica Grada Vukovara 220, HR-10000 Zagreb, Croatia ([lvukovic@voda.hr](mailto:lvukovic@voda.hr), [rcuk@voda.hr](mailto:rcuk@voda.hr), [amarinkovic@voda.hr](mailto:amarinkovic@voda.hr)),  
<sup>2</sup>student, Faculty of science in Split, Ruđera Boškovića 33, HR-21000 Split, Croatia ([idukic@pmfst.hr](mailto:idukic@pmfst.hr))

## 1. Introduction

In recent years, much legislation has been developed in order to assess the ecological integrity of freshwaters worldwide (e.g. Water Framework Directive in Europe) (Poikane et al., 2016). Among the many biological quality elements used in biomonitoring, macroinvertebrates are one of the most commonly used, fulfilling many of the criteria characterizing the ideal biomonitoring tool (Hickey & Clements, 1998).

In general, three orders of aquatic insects: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), known together as EPT assemblage, are sensitive to the pollution and degradation of stream ecosystems (Hickey & Clements, 1998). For this reason, EPT metrics calculated from a macroinvertebrate taxa list are commonly used in Europe and worldwide to assess stream ecosystem health (Girgin et al., 2010).

Nitrogen and phosphorus are essentials found in aquatic ecosystems, however, in excess amounts, they can cause significant water problems (Beketov 2004). Nitrates and phosphates enter the water systems through freshwater runoff from wastewater treatment plants, fertilized lawns and agriculture lands, faulty septic tanks, and industrial waste discharge. The increase in phosphorus concentration in the stream or river water has a negative effect on macroinvertebrate family and EPT diversity (Weijters, 2009).

In this work, we investigated the relationship between EPT metrics (the number of Ephemeroptera (E), Plecoptera (P), Trichoptera (T) taxa, total number of EPT taxa (EPT), ratio of EPT taxa (EPT%)), physical-chemical parameters (water temperature, pH, conductivity, TSS, alkalinity, hardness) and nitrogen and phosphorus compounds as well (ammonia, nitrite, nitrate, total nitrogen, inorganic and organic nitrogen, orthophosphate and total phosphorus) in the riverine ecosystems.

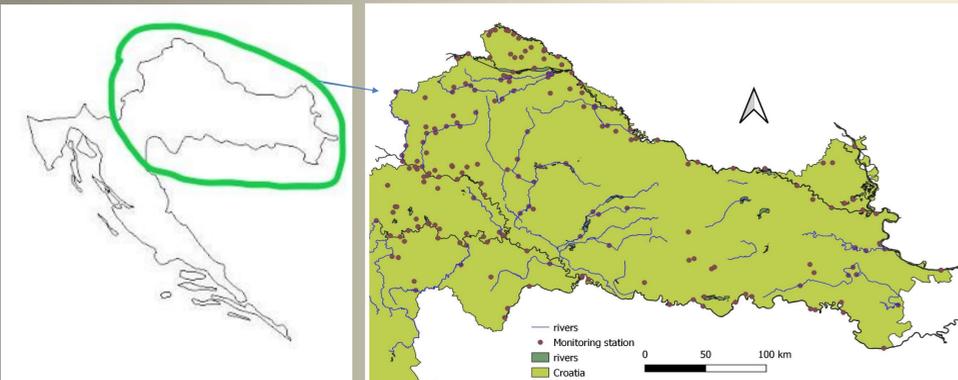


Figure 1. Map of Croatia with the sampling sites.



Figure 2. Some of the studied localities.

## 2. Materials and Methods

Data used in this study originate from the national monitoring programme of surface water quality within a period of 8 years (2010-2017), including 332 samples collected at 239 sites within the Pannonian lowland Ecoregion of Croatia (ER11) (Illies, 1978) (Figure 1., 2.).

Quantitative collection of benthic macroinvertebrates assemblages was done by a kick-sampling technique using hand net (25 x 25 cm, net mesh size 500 µm) by multihabitat sampling procedure (AQEM, 2002). Macroinvertebrates and associated organic material were immediately placed in plastic bottles and preserved in 80% ethanol (Figure 3.).

The concentrations of ammonia, nitrite, nitrate, inorganic nitrogen, organic nitrogen, orthophosphate and total phosphorus in water were measured according to ISO norms.

All variables, except pH, were log(x+1) transformed prior to analysis. A box-and-whisker plot was used to summarize and visualize the data. The relationship between variables was examined using Spearman rank correlation coefficients.



Figure 3. Some EPT taxa from Croatian freshwater macroinvertebrate fauna

## 3. Results and discussion

In 286 samples at least one member of EPT taxa was recorded. The most biodiverse was the Toplica River upstream of Daruvar sampled in 2012 with 28 recorded taxa: 7 Ephemeroptera, 6 Plecoptera and 15 of Plecoptera. In 46 samples no EPT specimens were recorded.

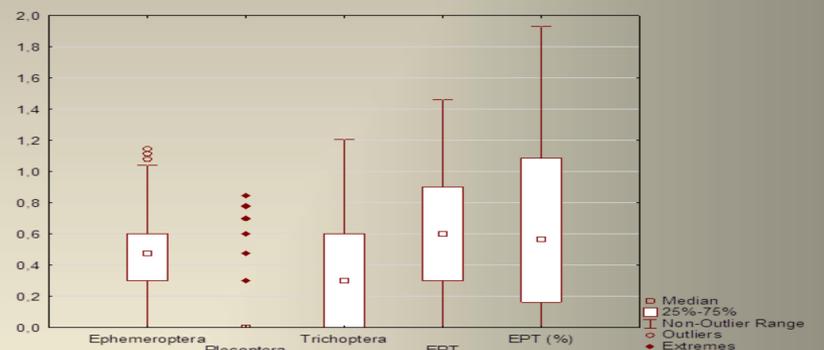


Figure 4. Box-and-whisker plots of the number of taxa of Ephemeroptera, Plecoptera, Trichoptera, EPT and EPT (%)

Table 1. Spearman rank correlation coefficients between the number of species of Ephemeroptera, Plecoptera, Trichoptera, EPT, and EPT (%), and physical-chemical and nutrients parameters

Variables		Plecoptera	Trichoptera	EPT	EPT (%)	
Physical-chemical	Water temperature (°C)	-0,110*	-0,262**	-0,185**	-0,175**	-0,102
	pH	0,060	0,176**	0,084	0,086	0,025
	Conductivity (µS/cm)	-0,154**	-0,210**	-0,180**	-0,189**	0,069
	Total suspended solids (mg/l)	-0,160**	-0,125*	-0,146**	-0,187**	-0,166**
	Alkalinity (mgCaCO <sub>3</sub> /l)	-0,030	-0,099	-0,157**	-0,105	0,115*
	Hardness (mgCaCO <sub>3</sub> /l)	0,050	-0,143*	-0,105	-0,035	0,263**
Nutrients	Ammonia (mgN/l)	-0,199**	-0,261**	-0,232**	-0,253**	-0,067
	Nitrite (mgN/l)	-0,183**	-0,275**	-0,140*	-0,186**	-0,010
	Nitrate (mgN/l)	-0,109*	-0,093	0,056	-0,034	-0,107
	Total nitrogen (mgN/l)	-0,293**	-0,212**	-0,173**	-0,270**	-0,193**
	Anorganic nitrogen (mgN/l)	-0,252**	-0,206**	-0,079	-0,199**	-0,194**
	Organic nitrogen (mgN/l)	-0,220**	-0,125*	-0,243**	-0,263**	-0,068
	Orthophosphate (mgP/l)	-0,245**	-0,309**	-0,230**	-0,288**	-0,168**
	Total phosphorus (mgP/l)	-0,194**	-0,191**	-0,181**	-0,220**	-0,133*

\*\* Statistically significant at p<0,01  
 \* Statistically significant at p<0,05

As it is shown in Figure 4. best metrics that describe effects of physical-chemical parameters on the EPT assemblages are EPT and EPT%.

Negative correlations have been found between EPT metrics, physical-chemical parameters and nitrogen and phosphorus compounds (Table 1), that is, increase in nitrogen and phosphorus compounds concentrations have been found to decrease the abundance and richness of EPT taxa.

The highest negative correlation was obtained between between E, P, T, EPT, %EPT and orthophosphates (-0,245, -0,309, -0,230, -0,288, -0,168 respectively). Also total nitrogen significantly correlated with E and %EPT (-0,293, -0,193 respectively). Nitrates did not affect EPT biodiversity nor share (Table 1.).

Furthermore, increase in electric conductivity, total suspended solids and water temperature is negatively correlated with EPT metrics, as it is found in previous studies (e.g. Stojanova *et al.*, 2014; Haidekker & Hering, 2008).

Although algal assemblage shows better response to nutrient enrichment (McCormick, 2019), EPT provides a significant response as well (e.g. Camargo, 2004) and some of EPT metrics could probably be used in water quality assessment regarding nutrient enrichment. However, more studies are needed.

Since the research was carried out on all types of rivers together, we assume that this is why correlations are weak. If the research were carried out on each river type separately, we assume that the correlations would be stronger for certain river types.

## 4. Conclusion

Higher values of EPT metrics indicate better water quality, and their presence show a biological community of higher integrity. In our research best metrics that described effects of physical-chemical parameters on the EPT assemblages were EPT and EPT%.

## 5. Literature

- AQEM (2002) *Manual for the application of the AQEM method. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive.* Version 1.0, 1-89.
- Beketov M. A. (2004) *Different sensitivity of mayflies (Insecta, Ephemeroptera) to ammonia, nitrite and nitrate: linkage between experimental and observed data.* Hydrobiologia 528: 209-216.
- Burton G.A.Jr (2002) *Sediment quality criteria in use around the world.* Limnology 3: 65-75.
- Camargo J.A., Alonso A., Puente M. (2004) *Multimetric assessment of nutrient enrichment in impounded rivers based on benthic macroinvertebrates.* Environmental Monitoring and Assessment 96: 233-249.
- Haidekker A. & Hering D. (2008) *Relationship between benthic insects (Ephemeroptera, Plecoptera, Coleoptera, Trichoptera) and temperature in small and medium-sized streams in Germany: A multivariate study.* Aquatic Ecology 42:463-481
- Illies J. (1978) *Limnofauna Europaetae.* Stuttgart: Gustav Fischer Verlag, 532 p.
- ISO 17294-2:2016 *Water quality -- Application of inductively coupled plasma mass spectrometry (ICP-MS)*
- McCormick A.R., Phillips J.S., Ives A. R. (2019) *Responses of benthic algae to nutrient enrichment in a shallow lake: Linking community production, biomass, and composition.* Freshwater Biology. 64(10): 1833-1847
- Poikane S., Johnson R.K., Sandin L., Schartau A.K., Solimini A.G., Urbanič G., Arbačiauskas K., Aroviita J., Gabriels W., Miler O., Pusch M.T., Timm H., Böhmer J. (2016) *Benthic macroinvertebrates in lake ecological assessment: A review of methods, intercalibration and practical recommendations.* Science of the Total Environment 543(A): 123-134.
- Regulation on water quality standards (OG 96/19)
- Stojanova T., Vidinova Y., Yaneva L., Tyufekchieva V., Parvanon D., Traykov I., Bogojev V. (2014) *Ephemeroptera, Plecoptera and Trichoptera as Indicators for Ecological Quality of the Luda Reka River, Southwest Bulgaria.* Acta Zoologica Bulgarica 66(2) 255-260
- Weijters, M. J., J. H. Janse, R. Alemaide & J. T. A. Verhoeven (2009) *Quantifying the effect of catchment land use and water nutrient concentrations on freshwater river and stream biodiversity.* Aquatic Conservation: Marine and Freshwater Ecosystems 19: 104-112.
- WFD 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy