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Ephemeroptera, Plecoptera and Trichoptera as Indicators for Ecological Quality of the Luda Reka River, Southwest Bulgaria

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Abstract: The Luda Reka River is a left tributary of the Struma River, one of the big rivers in Bulgaria, and is impacted by an abandoned uranium mine. The study presents the results of a research on the species composition of orders Ephemeroptera, Plecoptera and Trichoptera (EPT) in the Luda Reka River and its relation to river quality. The research was performed at six sites along the river in the period 2009–2011. During the study, a total of 19 mayfly, 26 stonefly and 15 caddisfly taxa were found. Mayflies, stoneflies and caddisflies were recorded at all studied sites. Four stonefly species are reported for the first time for the fauna of the Pirin Mountains. The ecological quality of the river was assessed by the EPT index.

Key words: Ephemeroptera, Plecoptera, Trichoptera, uranium mine, Struma River valley

Introduction

Aquatic insects are important elements in the ecological dynamics of river ecosystems and have been widely used as biological indicators of water quality (Hilsenhoff 1977, Uzunov, Kovachev 1987, Yaneva *et al*. 2001, Vidinova *et al*. 2007, Moskova *et al*. 2009). Among them, Ephemeroptera, Plecoptera and Trichoptera (EPT) are considered as important taxonomic groups in river ecosystems (Righi-Cavallaro *et al*. 2010). They live mainly in clean and well oxygenated waters and are good indicators of water quality (Karr 1991, Rosenberg, Resh 1993). The composition and distribution of the three orders is determined by their physiological tolerance to a wide array of environmental variables. The most important of them, which determine the EPT community structure, are considered to be the altitude and dissolved oxygen content (Hyness 1976, Dudgeon 1984, Pastuchova 2006).

The Struma River is one of the big Bulgarian rivers and is well studied with regard to the macrozoobenthic invertebrates and saprobiological state (Kovachev, Uzunov 1977, Kovachev *et al*. 1979, Natchev 1983, Islam *et al*. 1986, Uzunov, Kovachev 1987, Soufi *et al*. 2001, Vidinova *et al*. 2006), but some of its small tributaries have not yet been studied.

The Luda Reka River is a left tributary of the Struma River, flowing through the north-western slopes of the Pirin Mountains. The predominant substrate of the river bed is sand and gravel. The river almost completely dries up during the driest period of the year. An abandoned uranium mine is located

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in the upper reaches of the river and affects locally the ecological status of the river (Stoyanova *et al*. 2010). In the lower reaches of the river, the deposition of mine eroded materials caused the reduction of potential habitats.

The aim of the present work is to analyse the taxonomic diversity of the orders Ephemeroptera, Plecoptera and Trichoptera in the Luda Reka River and to assess the ecological quality of the river based on the representatives of these orders.

Material and Methods

The macrozoobenthic samples were collected according to ISO 7828/1985 from six sites, seasonally, in spring, summer and autumn of 2009 and 2010, and in spring and autumn of 2011 (Fig. 1). In the summer of 2009, the river almost completely dried up and samples were collected only from sites 5 and 6.

All samples were fixed in 4% formaldehyde and kept in 70% alcohol after laboratory sorting by systematic groups.

The physical and chemical variables of the river were measured *in situ*. Temperature and dissolved oxygen were measured by a WTW 3310 oxymeter, the hydrogen ion activity (pH) and the conductivity (EC) – by a HI 98129. The altitude of the sites was obtained by GPS Garmin-Etrex/Vista. The ecological status of the river was assessed by the EPT index (Lenat 1988). The relationship between the physical and chemical variables and the EPT index was evaluated with the use of Pearson's correlation coefficient (r). The statistical analysis was performed using Statistica 7.0.

Table 1. Altitude, average values and standard deviations of water physical and chemical variables in the Luda Reka River in 2009-2011. T – water temperature; O_2 – dissolved oxygen and oxygen saturation; EC – conductivity

Site	Altitude (m)	T (°C)	O_2 (mg/l)	O_2 (%)	pH	EC (µS/cm)
1	954	10.2 (±1.8)	9.6 (±0.6)	90.7 (±3.9)	7.1 (±0.6)	138 (±49)
2	672	10.7 (±2.7)	9.3 (±0.5)	88 (±4.8)	7.8 (±0.5)	803 (±275)
3	672	10.8 (±2.8)	9.5 (±0.7)	90.3 (±7.2)	7.5 (±0.6)	378 (±76)
4	638	10.7 (±3.6)	9.7 (±1)	90.6 (±7.4)	7.6 (±0.8)	253 (±48)
5	638	11.6 (±3.4)	9.5 (±1)	91 (±8)	7.6 (±0.6)	380 (±140)
6	561	12.6 (±4)	9.1 (±0.9)	89 (±7.1)	7.7 (±0.6)	388 (±56)

Results and Discussion

The river water was well oxygenated (9.1–9.7 mg/l, 88–91%) with average values of temperature varying from 10.2°C to 12.6°C (Table 1). According to Stoyanova *et al*. (2010) the village of Rakitna influenced the ecological quality of the Luda Reka River. The synergetic effect both of temperature and the influence of Rakitna village probably determines the decreased oxygen concentrations at Site 6.

The average values of pH varied from 7.1 to 7.8, while those of the conductivity (EC) increased downstream. An exception of this tendency was observed at Site 2, where the high EC is due to the inflow of the drainage water from the stulm. Site 4 is characterised by low EC.

A total of 19 Ephemeroptera, 26 Plecoptera and 15 Trichoptera taxa were found in the Luda Reka

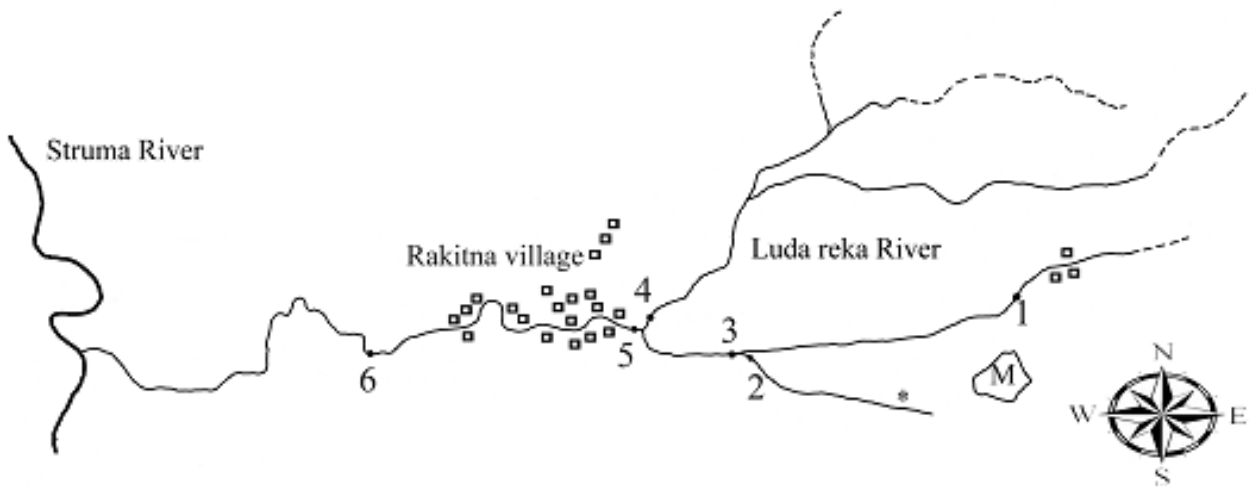

Fig. 1. Scheme of the sampling sites along the Luda Reka River. The mine is marked with M and the stulm with *

River during the three years of research (Table 2). In 2009, 44 EPT taxa were found, while in 2010 and 2011, the corresponding numbers were 42 and 39, respectively.

Mayflies, stoneflies and caddisflies were recorded at all sampling sites. The highest numbers of species of orders Ephemeroptera and Plecoptera were found in spring and in autumn during higher water levels and better oxygen conditions.

In the order Ephemeroptera, the family Heptageniidae was presented with the greatest number of species (12), but the most numerous species were *Baetis rhodani* and *Rhithrogena* sp.

The genera *Leuctra* and *Protonemura* were presented with the highest number of species among the Plecoptera (respectively 5 and 4 species) as the most numerous of them were *L. fusca fusca*, *P. montana,* and *P. praecox praecox*.

Four stonefly species are reported for the first time in the Pirin Mountains: *L. prima, P. auberti*, *Nemoura flexuosa,* and *N. marginata*. The species *P. auberti* and *N. flexuosa* were found at Sites 1, 3 and 4, respectively, during the spring and autumn of 2010. *L. prima* was found at Site 3 during the spring of 2009 and 2010, and *N. marginata* – at Sites 1 and 3 during the spring of 2010.

The most numerous species of the order Trichoptera were *Hydropsyche bulbifera, H. incognita,* and *Polycentropus* sp.

Some of the taxa found in the Luda Reka River, as *Baetis muticus*, *B. rhodani*, *Ephemera danica* and *Serratella ignita*, were also reported in the Struma River from 1999–2000 by Vidinova *et al*. (2006).

According to Bogoev *et al*. (2010) and Stoyanova *et al*. (2011, 2012), the surface erosion of the spoil banks of the abandoned uranium mine led to washout of radioactive materials and heavy metals into the Luda Reka River. This process was more intensive during the rainy 2010 and resulted in higher contents of heavy metals in the sediments of the Luda Reka River than those in 2009. According to Chadwick *et al*. (1986), Clements *et al*. (1992) and Garcia-Criado *et al*. (2002), the tolerance of benthic invertebrates to heavy metals decreases from caddisflies to stoneflies and mayflies. Some representatives of Baetidae (genus *Baetis*), Nemouridae (*Nemoura cinerea cinerea*) and *Leuctra* species, the genus *Hydropsyche* and family Rhyacophilidae are considered to be tolerant to heavy metals (Armitage 1980, Roline 1988, Garcia-Criado *et al*. 2002, Solà *et al*. 2004, Xiaodong Qu *et al*. 2010). The species *N. cinerea cinerea* was found only at Site 2, located 900 m downstream of the stulm. Comparing the EPT taxa upstream and downstream of the mine, we found that downstream of the mine there were no species of the family Lepidostomatidae, while others, such as *H. incognita*, *H. bulbifera*, *Rhyacophila tristis*, *Philopotamus montanus*, *P. montana*, *P. intricata intricata, L. inermis,* and *Ecdyonurus* gr. *helveticus* were characterised by a low number.

The ecological status of the Luda Reka River was well illustrated by the values of the EPT index (Fig. 2). The index values varied from 2 (Site 6 during the autumn of 2011) to 19 (Site 3 during the autumn of 2009).

In all three years of investigation, the good water quality at Site 1 resulted in high values of the EPT index (10–18), while the lower EPT values at Site 2 (Fig. 2) corresponded to the high values of EC and the toxic compounds in the seepage waters from the stulm. At Site 2, higher values of the EPT index, 13 and 16, respectively, were established only in the spring of both 2010 and 2011 (Fig. 2), probably as a result of the higher water discharge. Sites 3, 4 and 5 were characterised with high values of the EPT index (10-19) throughout the whole studied period, excepting Site 5 in the spring and summer of 2009.

Stoyanova *et al*. (2010, 2011, 2012) reported that the reduction of potential habitats, the low water discharge, the slight organic pollution from Rakitna village and the accumulation of heavy metals in water and sediments led to the deterioration of the water quality in the lower reaches of the Luda Reka River. All these variations in the environmental conditions probably resulted in low values of the EPT index (2–8) at Site 6.

According to Thiébaut *et al*. (2006), the taxonomic variety of the EPT larvae is strictly related to the physical and chemical parameters of water. The statistical analysis shows that the increased values of EC limits the occurrence of the EPT larvae ($r = -0.30$, $n = 39$, $\alpha < 0.05$).

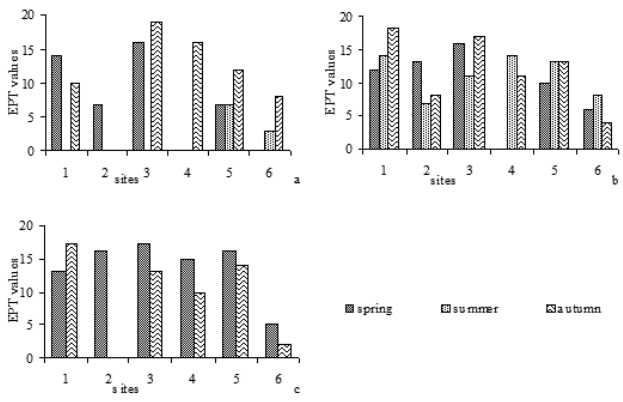

Fig. 2. EPT values at different stations along the Luda Reka River during 2009 (a), 2010 (b) and 2011 (c)

Table 2. Mayfly, stonefly and caddisfly taxa found in the Luda Reka River during 2009, 2010 and 2011

Taxon	2009	2010	2011
Ephemeroptera			
Baetidae			
Baetis alpinus (Pictet, 1845)	*	*	*
Baetis rhodani (Pictet, 1843)	*	*	*
Baetis muticus (Linné, 1758)	*		*
Heptageniidae			
Ecdyonurus epeorides Demoulin, 1955	*	*	*
Ecdyonurus sp. gr. *helveticus*	*	*	*
Ecdyonurus picteti (Meyer. – Dür, 1864)			*
Ecdyonurus sp.		*	*
Epeorus assimilis (Pictet, 1865)	*	*	*
Rhithrogena savoiensis Alba Tercedor & Sowa, 1987		*	
Rhithrogena sp. gr. *diaphana* (*savoiensis*)			*
Rhithrogena sp. gr. *hybrida*	*	*	
Rhithrogena sp. gr. *semicolorata*	*	*	*
Rhithrogena sp. gr. *sowai*			*
Rhithrogena sp.	*	*	*
Electrogena lateralis (Curtis, 1834)	*	*	*
Ephemerellidae			
Serratella ignita (Poda, 1761)		*	*
Leptophlebiidae			
Habroleptoides confusa Sartori & Jacob, 1986	*	*	*
Paraleptophlebia sp.	*		
Ephemeridae			
Ephemera danica Müller, 1764	*	*	*
Plecoptera			
Taeniopterygidae			
Taeniopteryx schoenemundi (Mertens,1923)			*
Brachyptera risi (Morton, 1896)	*		
Brachyptera seticornis (Klapálek, 1902)	*	*	*
Brachyptera sp.			*
Leuctridae			
Leuctra fusca fusca (Linnaeus, 1758)	*	*	*
Leuctra inermis (Kempny, 1899)	*	*	*
Leuctra prima (Kempny, 1899)	*	*	
Leuctra pseudosignifera (Aubert, 1954)	*	*	
Leuctra hippopus (Kempny, 1899)		*	
Leuctra sp.	*	*	*

Taxon	2009	2010	2011
Nemouridae			
Protonemura auberti (Illies, 1954)		*	
Protonemura intricata intricata (Ris, 1902)	*		*
Protonemura montana (Kimmins, 1941)	*	*	*
Protonemura praecox praecox (Morton, 1894)	*		
Protonemura sp.	*	*	
Nemoura cinerea cinerea (Retzius, 1783)	*	*	
Nemoura flexuosa (Aubert, 1949)		*	
Nemoura marginata (Pictet, 1836)		*	
Nemoura sp.	*	*	*
Amphinemura sp.			*
Perlodidae			
Isoperla grammatica (Poda, 1761)	*		*
Isoperla buresi (Raušer, 1962)		*	*
Isoperla sp.	*	*	*
Chloroperlidae			
Chloroperla sp.	*		*
Siphonoperla sp.		*	
Perlidae			
Perla marginata (Panzer, 1799)	*	*	*
Trichoptera			
Rhyacophilidae			
Rhyacophyla nubila (Zetterstedt, 1840)	*	*	*
Rhyacophyla tristis (Pictet, 1834)	*	*	*
Philopotamidae			
Philopotamus montanus (Donovan, 1813)	*	*	*
Polycentropidae			
Polycentropus cfm irrotatus (Curtis, 1835)	*	*	
Polycentropus sp.	*	*	
Hydropsychidae			
Hydropsyche bulbifera (McLachlan, 1878)	*	*	*
Hydropsyche incognita (Pitsch, 1993)	*	*	*
Sericostomatidae			
Sericostoma sp.	*	*	*
Limnephilidae			
Drusinae gen. sp.	*		
Chaeteropterigini gen sp.	*		
Chaeteropteryx sp.		*	*

Taxon	2009	2010	2011
Leptoceridae			
Glyphotaelius pellucidus (Retzius, 1783)	*		
Lepidostomatidae			
Lepidostoma hirtum (Fabricius, 1775)	*		

Taxon	2009	2010	2011
Psychomyidae			
Tinodes sp.		*	
Phryganeidae			
Phryganeidae gen. sp.			*

Conclusions

Mayflies, stoneflies and caddisflies were recorded at all sampling sites along the Luda Reka River. The changes in the EPT index downstream of the stulm were due to the impact of the abandoned mine. However, the variations in the water discharge and the reduction of potential habitats along the river had a stronger effect on the EPT than the abandoned mine.

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