



## Research paper

# Post-mining calcareous seepages as surrogate habitats for aquatic macroinvertebrate biota of vanishing calcareous spring fens



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## ABSTRACT

Many studies have investigated the vegetation and terrestrial fauna of post-mining spoil heaps, but little is known about aquatic macroinvertebrates in these areas, particularly at spring-fed habitats. We studied nine seepages (i.e. spring-fed habitats) located at two neighbouring spoil heaps in the Sokolov brown-coal basin (Czech Republic), characterised by basic water pH, calcium carbonate precipitation, high heavy metal and sulphate concentrations. Along with these nine artificial calcareous seepages, we also sampled macroinvertebrates at 15 natural and well-preserved calcareous spring fens to compare species richness and assemblage similarity between these two systems. We also categorised recorded species as spring specialists or generalists (i.e. matrix-derived species) to compare their importance for the similarity in diversity of these two habitat types. Our study revealed that post-mining calcareous seepages harboured unusually taxa-rich macroinvertebrate assemblages (158 taxa), despite their harsh conditions related to extreme water chemistry. Diptera, with 85 taxa, were the most diverse group, reaching taxon richness comparable with dipteran assemblages in natural calcareous spring fens. According to Sørensen pair-wise dissimilarity, dipteran assemblages of post-mining and natural sites were more similar in the composition of spring specialists than that of generalists, showing a strict relationship between specialist assemblages and specific environmental conditions of post-mining and natural sites. In addition, dipterans inhabiting post-mining seepages were demonstrated not to be limited by their extreme water chemistry, but they seemed to be associated with specific substratum properties, creating a mosaic of various microhabitats. We can therefore conclude that calcareous post-mining seepages have great potential to offer valuable analogues of natural habitats and may create biodiversity refuges for a high number of aquatic invertebrates, including spring habitat specialists and nationally threatened species. Thus, post-mining seepages seem to have similar or even higher conservation potential to more complex and larger post-mining freshwater habitats, such as post-mining streams and mine subsidence pools.

## 1. Introduction

Calcareous spring fens are defined as groundwater-fed nutrient-limited wetlands with calcium carbonate precipitation (Hájek et al., 2006). They are among the most species-rich habitats of the temperate zone, which harbour a high number of rare and highly specialised aquatic invertebrates (Horsák et al., 2015; Omelková et al., 2013; Schenková et al., 2016; Wassen et al., 2005; Zhai et al., 2015). For this reason, they are considered as biodiversity hotspots, significantly contributing to freshwater species richness. These isolated and island-like habitats are threatened by multiple impacts, such as water abstraction and conversion to agricultural fields, eutrophication, and the effect of climate change on the hydrologic cycle (Hartmann et al., 2014; van Diggelen et al., 2006). In temperate zones of Europe and North America, the destruction and degradation of calcareous spring fens is

very intense (Grootjans et al., 2006).

To our knowledge, the only habitats in Europe that can be considered as surrogates of calcareous fens are artificial calcareous seepages in the post-mining landscape of the Sokolov brown-coal basin. Specific bedrock chemistry of spoil heaps in this area supports the occurrence of seepages with basic pH and calcium carbonate (tufa) precipitation, but with the characteristics of high heavy metal and sulphate concentrations, in contrast to natural calcareous spring fens (Table 1, Appendix C). There is a growing body of evidence that spoil heaps with specific abiotic conditions and low levels of nutrients may offer valuable compensatory habitats for many terrestrial arthropods and plants that are rare in human-affected landscapes (Hendrychová et al., 2012; Hodeček et al., 2015; Tropek et al., 2012). Likewise, post-mining freshwater habitats, such as drainage ditches and mine subsidence pools, can provide refuges for endangered dragonfly species (Dolný and

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**Table 1**

Descriptive statistics (minimum, median, and maximum value) of environmental variables measured in post-mining seepages and natural spring fens. P-values of Mann–Whitney *U* test indicate significant differences between variable values measured in post-mining and natural sites. Adjusted  $R^2$  of significantly fitted variables in NMDS is given. Significance levels: \*\*\**P* < 0.001; \*\**P* < 0.01; \**P* < 0.05; n.s., not significant. CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; Inorg, proportion of inorganic substrate; Tufa crusts, proportion of tufa crusts.

	Post-mining sites			Natural sites			U test	Fitted variables
	MIN	MED	MAX	MIN	MED	MAX	p-value	(Adj. $R^2$ )
pH	6.76	7.78	8.49	6.7	7.8	8.5	n.s.	0.285*
Conductivity ( $\mu\text{S}/\text{cm}$ )	4760	5042	6790	214	450	633	< 0.001	0.918***
O <sub>2</sub> (mg/L)	0.01	4.57	12.03	0.1	8.1	10.4	< 0.050	0.399*
Temperature (°C)	10.7	14.9	22.1	8	12	20	< 0.050	0.365**
Discharge (l/s)	0	0.16	40	0.05	0.4	3	n.s.	0.765***
SO <sub>4</sub> (mg/L)	3130	3650	4150	8.8	26.5	67.7	< 0.001	0.918***
Ca (mg/L)	256	384	615	24.13	47.2	112	< 0.001	0.855***
Mg (mg/L)	230	250	337	1.14	7.47	20.1	< 0.001	0.855***
Na (mg/L)	548	914	1120	0.16	1.42	3.8	< 0.001	0.879***
K (mg/L)	11.9	17.86	24.8	0.15	0.53	2.7	< 0.001	0.879***
Fe (mg/L)	0.14	5.41	49.7	< 0.01	0.54	13.6	< 0.010	0.687***
Al (mg/L)	0	1	9.91	0.04	0.19	0.68	< 0.001	0.740***
Mn (mg/L)	0.07	1.96	19.6	< 0.01	0.17	1.82	< 0.010	0.310*
NH <sub>4</sub> (mg/L)	0.07	0.14	0.85	0.03	0.29	0.55	n.s.	n.s.
NO <sub>3</sub> (mg/L)	0.06	0.22	2.04	0	1.34	13.58	n.s.	0.281*
PO <sub>4</sub> (mg/L)	0.01	0.01	0.05	0	0.01	0.11	n.s.	0.373*
CPOM (%)	11.1	21.6	35.5	1.6	14.9	61.6	n.s.	n.s.
FPOM (%)	12.5	55.7	70	0.2	17.2	58	< 0.001	0.641***
Inorg (%)	5.2	17.1	59.2	0	68.4	96.1	< 0.001	0.648***
Tufa crusts (%)	0	7.5	100	0	0	40	< 0.050	0.295*
Size (m <sup>2</sup> )	75	225	2100	100	1127	4271	< 0.010	n.s.

Harabiš, 2012; Harabiš et al., 2013; Harabiš and Dolný, 2015; Tichanek and Tropek, 2016), freshwater molluscs (Lewin et al., 2015), and amphibians (Kolář et al., 2017; Vojar et al., 2016). Apart from a few studies dealing with adults of aquatic insects (Chvojka, 2011; Ježek, 2008), aquatic fauna of post-mining calcareous seepages remain virtually unexplored. Furthermore, there is no comprehensive study evaluating the species richness and the variation in composition of aquatic invertebrates in any type of freshwater post-mining habitats. Because of several environmental similarities with natural calcareous spring fens, artificial post-mining seepages have the potential to provide valuable alternatives of these rare and highly endangered habitats.

Within macroinvertebrates, Diptera are known to be the most diverse group of aquatic spring insects (Cantonati et al., 2006; Ferrington, 1998; Horsák et al., 2015). They are able to inhabit a wide range of aquatic and semiaquatic habitats and are represented by ubiquitous, highly specialised and rare taxa associated with specific environmental conditions in spring habitats (Omelková et al., 2013; Wagner et al., 1998). Diptera are thus the most suitable taxa for determining whether post-mining spring-fed seepages can provide biodiversity refuges in anthropogenically modified landscapes.

The main objectives of this study are 1) to describe the composition of aquatic macroinvertebrate assemblages colonising post-mining calcareous seepages in the Sokolov brown-coal basin, 2) to compare diversity and tolerance patterns of Diptera taxa in post-mining seepages and natural calcareous spring fens, and 3) to examine the potential of post-mining seepages to offer secondary habitats for species of highly threatened calcareous spring fens.

## 2. Methods

### 2.1. Study area and sites

#### 2.1.1. Post-mining sites

The studied post-mining calcareous seepages (referred to as “post-

mining sites” in the following text) were located in the Sokolov brown-coal basin in western Czech Republic (Fig. 1), where natural calcareous spring fens are completely absent in the landscape (Chytrý et al., 2010). This region has been largely re-sculpted by opencast brown-coal mining since the 19th century, becoming particularly intensive from the 1950s, and resulting in one of the most intensive opencast mining areas in Europe (Harabiš et al., 2013). The majority of the spoil material in the Sokolov area is formed by an alkaline tertiary clay material of so-called cypris formation, rich in fossil micro-organic material (Frouz et al., 2006). Cypris formation consists mainly of claystone with an essential presence of kaolinite (Rojík, 2004), pyrite, and calcium and magnesium carbonates (Pešek et al., 2010). The specific bedrock chemistry is reflected in the chemical composition of groundwater, which supports calcium carbonate (tufa) precipitation and causes high sulphate, calcium, magnesium and iron concentrations. Water pH is alkaline due to the neutralisation capacity of carbonates (Table 1). The studied sites have been originating at spoil heaps since the beginning of the 1990s. Some seepages originated in non-reclaimed parts of heaps left to spontaneous succession, and some seepages have originated spontaneously at technically reclaimed parts of the heaps. All sites were subsequently left to spontaneous succession for more than 20 years, and they therefore have very similar environmental characteristics, irrespective of the origin of sites (Appendix C).

Small, treeless seepages located at two neighbouring spoil heaps with similar water chemistry were sampled: seven sites were situated at the Velká podkrušnohorská heap (1957 ha) and two sites at the Smolnická heap (616 ha). These sites were located at an elevation of 460–496 m a.s.l., and the seepage area varied between 75 and 2100 m<sup>2</sup> (Fig. 1). All sites were fed by groundwater; two of them formed a head of small brooks draining the spoil heap. An intense precipitation of calcium carbonate (tufa) formed strong crusts as well as small tufa grains. Besides tufa precipitation, the substrate consisted mainly of fine and coarse particulate organic matter (FPOM, CPOM) and precipitates of iron and manganese oxides forming orange and black muddy

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