


# Rotifer Diversity in the Acidic Pyrite Mine Pit Lakes in the Sudety Mountains (Poland)

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**Abstract** The diversity of rotifers was studied in three artificial water bodies (Azure Lake, Yellow Lake, and Purple Lake), which were once pyrite mines. The physicochemical parameters and the zooplankton composition of the water were determined. Azure Lake had a pH of 3.4–6.9, conductivity values of 165–194  $\mu\text{S cm}^{-1}$ , and low concentrations of sulphate, calcium, magnesium, copper, and iron, while the other lakes had a pH of 2.6–2.9, a conductivity of 1636–3400  $\mu\text{S cm}^{-1}$ , and high concentrations of sulphate (up to 2863  $\text{mg dm}^{-3}$ ), Cu (up to 2650  $\mu\text{g dm}^{-3}$ ), and Fe (up to 178.3  $\text{mg dm}^{-3}$ ). The rotifer community in the lakes comprised 27 taxa (15 in Azure Lake, 13 in Purple Lake and 14 in Yellow Lake). We also found two species that are rarely observed in Poland (*Aspelta cincinator* and *Elosa spinifera*), and three species commonly found in acidic water (*E. worallii*, *Cephalodella delicata*, and *C. hoodi*). The types of rotifers in Azure Lake differed from those in the other two lakes. The Shannon–Weaver biodiversity index ( $H'$ ) was the highest in Purple Lake ( $H' = 1.255$ ) and lowest in Azure Lake ( $H' = 0.455$ ). The effect of some of the physicochemical parameters on rotifer diversity is discussed.

**Keywords** Shannon–Weaver index · Mining excavations · Water chemistry · pH

## Introduction

The chemistry of water that fills former mining excavations can vary greatly depending on the type of mine (e.g. pyrite, sulphur, coal, or metal ores), which creates specific conditions, such as high conductivity or low pH (Frömmichen et al. 2004; Blodau 2006; Lund and McCullough 2009; Jersabek et al. 2011; Geller et al. 2012, 2013). There have been relatively few studies on the organisms living in various post-mining water bodies, as these studies are difficult to conduct and require specialized techniques (Woelfl and Whitton 2000). However, researchers have begun to describe the organisms that live in these environments (Derham 2004; Geller et al. 2012, 2013; Ciszewski et al. 2013; Wołowski et al. 2013; Sienkiewicz and Gasiorowski 2015). There is a relatively large number of reports on the subject of the species diversity and physiology of cyanobacteria and algae (DeNicola 2000; Gross 2000; Lessmann et al. 2000; Steinberg et al. 2012; Wołowski et al. 2008, 2013; Lessman and Nixdorf 2013), protists and fungi (Geller et al. 2013; Wendt-Potthoff 2013), but fewer publications on the diversity of small animals including rotifers and cladocerans (Horyath and Hummon 1980; Deneke 2000; DeNicola 2000; Belyaeva and Deneke 2013; Ferrari et al. 2015).

The limited available information was not sufficient to define the role of planktonic animals in the food web of acidic mine lakes, which differ from that of more conventional lakes (Weinthoff et al. 2013). Rotifera are one of the most important groups present in mining lakes (Horyath and Hummon 1980; Deneke 2000; DeNicola 2000; Wollmann et al. 2000). Previous studies have shown that acidic pit lakes are dominated by only a few species, including *Cephalodella hoodi*, *Rotaria rotatoria*, *Elosa worallii*, *C. gibba*, and *Brachionus sericus* (Deneke 2000). The aim of this study was to investigate rotifer diversity and determine the effects of

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water properties on the rotifer community in three acidic pit lakes, created from abandoned pyrite opencast mines, in the Sudety Mountains in southern Poland. We hypothesized that the low pH and high metal concentrations would result in a low diversity of rotifers.

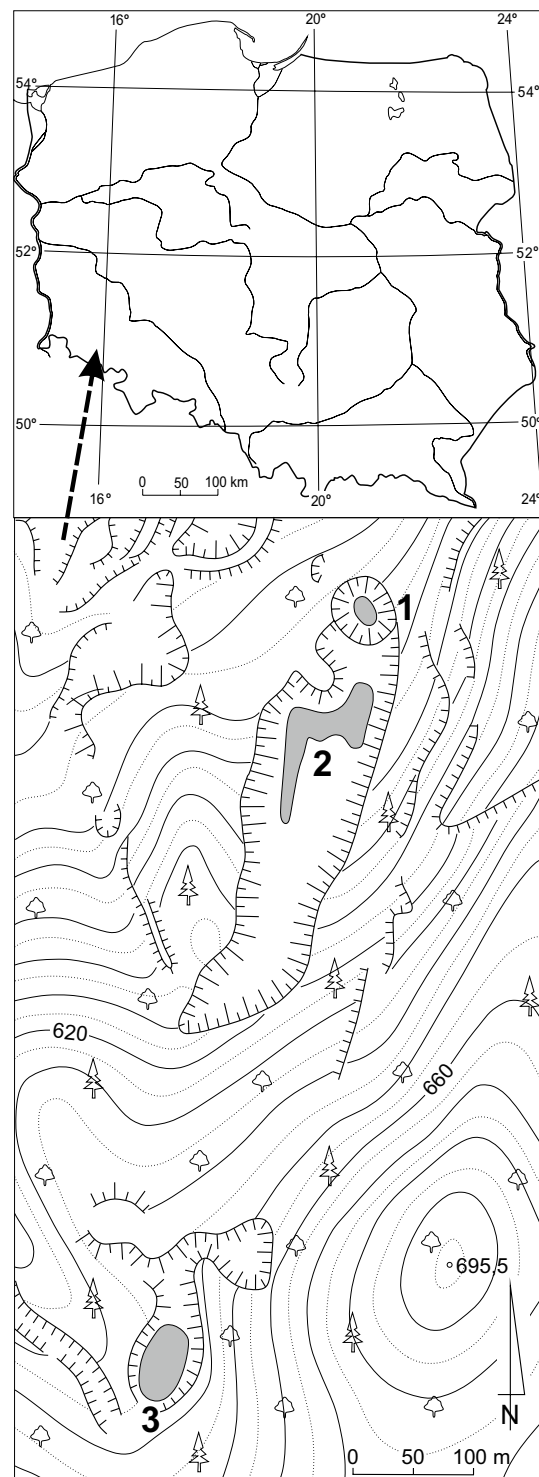
## Materials and Methods

### Study Area

“Colorful lakelets” is the name given to four artificial ponds that formed in an abandoned pyrite mine in the Rudawy Janowickie range of the Sudety Mountains in southwest Poland. These locations were mined for pyrite from 1785 to 1925. The color of the lake water depends on the chemical composition of the banks and beds, which are high in iron and copper compounds (Uzarowicz and Skiba 2011; Uzarowicz et al. 2008, 2011). The lakelets consist of: Azure Lake (also known as blue or emerald, 635 m above sea level, 50°49′22,4″N 15°58′18,7″E); Purple Lake (560 m above sea level, 50°49′41,6″N 15°58′26,1″E); Yellow Lake (555 m above sea level, 50°49′43,1″N 15°58′26,5″E); and Green Lake (730 m above sea level, 50°49′18,2″N 15°58′0,1″E). Depending on rainfall, Green Lake can temporarily disappear, which was the case during our study. The Azure Lake is fed by a small woodland stream, but the two remaining water bodies are supplied only by precipitation. Azure Lake is the largest, with a depth of approximately 11 m, length of 150 m, width of 30–40 m, and a surface area of 1770 m<sup>2</sup>. Purple Lake has an irregular shape, and is approximately 4 m deep in the central part; the length of the pit is 430 m, the width is about 10 m, and the surface area is 1290 m<sup>2</sup>. Yellow Lake is round in shape and is the smallest of the four lakes. It has a diameter of about 30 m, a surface area of 280 m<sup>2</sup>, and a maximum depth of about 6 m (Fig. 1) (Uzarowicz and Skiba 2011; Uzarowicz et al. 2008, 2011).

### Water and Rotifer Sampling

Samples for physicochemical and biological parameter analyses were taken from the deepest sampling points of the Azure, Yellow, and Purple Lakes in Spring (May–June) and Autumn (August–September) of 2011 and 2012. Samples were also collected at two other depths in each lakelet: Azure Lake, surface water and 5 m; Yellow Lake, surface water and 3 m; and Purple Lake, surface water and 2 m. Year 2011 was wet, but 2012 was dry, during which the water level of Purple and Yellow lakes fell by over a meter. Some physicochemical water properties [pH, temperature, electrical conductivity (EC), and dissolved oxygen (DO)] were measured in situ using a multi-parameter probe (6600V2; YSI Inc., Yellow Springs, OH, USA). Samples for chemical analysis



**Fig. 1** Map of the sampling sites in the “Colorful Lakelets” formed in an abandoned pyrite mine in the Rudawy Janowickie range of the Sudety Mountains (south–west Poland): 1 Yellow Lake, 2 Purple Lake, 3 Azure Lake

were collected from the same points and layers as biological samples, and immediately transported to the laboratory.

Anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ , and  $\text{NO}_3^-$ ) and cations ( $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$ ) were analyzed using ion chromatography (ICS-1000 IC25 ion chromatograph; Dionex, Sunnyvale, CA, USA). Water analyzed for metals (total concentrations of Cd, Pb, Cu, Zn, Mn, Fe, Ni, and Cr) were first acidified to pH 2 with ultrapure  $\text{HNO}_3$ , then analyzed using atomic absorption spectrophotometry (AA20; Varian, Mulgrave, Victoria, Australia).

Samples collected from the lakes were used to assess rotifer composition. For the quality and quantity analyses, the rotifers were concentrated from 30 L of water with a 30  $\mu\text{m}$  plankton net, then fixed with 4% formalin. These subsamples were analyzed under a light microscope (Eclipse 50i; Nikon, Tokyo, Japan) at 100–400 $\times$  magnification. The composition and density of rotifers was determined by counting, using 0.5 mL Kolkwitz chambers. The rotifer density in 1 L of water was counted and the mean value was calculated from five counts. Taxonomic zooplankton analyses were conducted using the identification keys described by Nogrady et al. (1995); Segers (1995); Norgady and Segers (2002), Ejsmont-Karabin et al. (2004).

The Shannon–Weaver and evenness indexes of species diversity were calculated using MVSP software. To determine the relationship between the physicochemical and

biological parameters, the Pearson correlation coefficient was calculated. The differences between lakes for the physicochemical and biological parameters were analyzed using the Mann–Whitney test with Statistica 12 software (StatSoft Inc., Tulsa, OK, USA).

## Results

### Water Chemistry

The temperature of the water during spring and late summer of 2011 and 2012 ranged from 9 to 15  $^\circ\text{C}$  (Table 1). The lakes can be divided into two groups: strongly acidic water characterized by a pH of 2.6–2.9, as observed in the Purple and Yellow Lakes; and acidic or neutral water, with a pH of 3.4–6.8, as observed in Azure Lake (Table 1). The water of Azure and Yellow Lakes were well oxygenated, while Purple Lake usually was weakly oxygenated (Table 1). The differences in DO content of the lakes were significant (Table 2).

The lakes also differed in their salt concentration, as expressed by their EC. The highest EC was in Purple Lake (up to 3400  $\mu\text{S cm}^{-1}$ ), while the lowest was determined in Azure Lake (up to 194  $\mu\text{S cm}^{-1}$ ). In each lake, the dominant

**Table 1** The values of physicochemical parameters and heavy metal concentrations in the water of ‘Colourful lakelets’

Parameter	Unit	Azure L.		Purple L.		Yellow L.	
		Median	Range	Median	Range	Median	Range
Depth	m		7.5–8.1		2.3–3.0		2.0–4.5
Temperature	$^\circ\text{C}$		9.5–13.4		11.9–15.0		11.4–13.3
Conductivity	$\mu\text{S cm}^{-1}$	171	165–194	2580	2040–3400	1685	1636–1789
pH		4.9	3.4–6.9	2.7	2.6–2.9	2.7	2.6–2.8
Dissolved oxygen	$\text{mg dm}^{-3}$	9.3	8.5–10.0	4.9	0.0–8.2	13.2	10.5–13.7
$\text{Cl}^-$	$\text{mg dm}^{-3}$	2.3	2.3–4.1	3.9	3.0–7.5	4.5	3.0–5.2
$\text{HCO}_3^-$	$\text{mg dm}^{-3}$	8.4	0.7–9.2	36.2	1.4–61.4	29.6	1.0–37.1
$\text{SO}_4^{2-}$	$\text{mg dm}^{-3}$	74.7	61.1–84.8	1340.6	1118–2863	710.4	629.3–871.5
$\text{NO}_3^-$	$\text{mg dm}^{-3}$	0.41	0.24–1.21	0.25	0.06–0.56	0.58	0.04–0.91
$\text{PO}_4^{3-}$	$\text{mg dm}^{-3}$	0.02	0.00–0.03	0.22	0.01–0.76	0.38	0.00–0.71
$\text{Na}^+$	$\text{mg dm}^{-3}$	3.5	2.4–4.5	5.8	5.0–7.0	4.8	4.7–5.8
$\text{K}^+$	$\text{mg dm}^{-3}$	0.4	0.3–0.9	2.0	0.4–3.1	1.0	0.8–2.3
$\text{Ca}^{2+}$	$\text{mg dm}^{-3}$	17.2	15.6–19.8	214.6	198.9–306.7	149.7	116.9–172.5
$\text{Mg}^{2+}$	$\text{mg dm}^{-3}$	7.2	6.6–8.1	135.2	114.5–201.8	52.0	40.2–60.0
$\text{NH}_4^+$	$\text{mg dm}^{-3}$	0.04	0.01–0.08	0.23	0.06–0.34	0.17	0.14–0.39
Cd	$\mu\text{g dm}^{-3}$	0.4	0.3–1.2	3.7	3.0–6.4	1.2	0.7–1.7
Pb	$\mu\text{g dm}^{-3}$	0.5	ND–1.2	2.1	1.3–5.6	1.0	0.9–1.3
Cu	$\mu\text{g dm}^{-3}$	8.9	4.9–12.1	1027.5	326–2650	240	205–257
Zn	$\mu\text{g dm}^{-3}$	32.5	12–43	267.5	75–503	67.5	53–108
Cr	$\mu\text{g dm}^{-3}$	0.5	0.1–1.1	72.9	17.4–140.0	29.3	20.8–135
Ni	$\mu\text{g dm}^{-3}$	10.2	6.4–22.0	196.7	71–370	50.0	39.9–86.0
Mn	$\text{mg dm}^{-3}$	0.14	0.12–0.16	1.7	0.36–3.5	0.4	0.33–0.47
Fe	$\text{mg dm}^{-3}$	0.11	0.07–0.56	84.7	28.8–178.3	29.5	26.1–35.9

ND not detected

**Table 2** Significance differences in the values of physicochemical parameters, heavy metal concentrations of the water and the density of chosen rotifers taxons between studied lakes (Mann–Whitney test) (Z statistic value; p level of significance; ns not significant)

Physicochemical and biological parameters	Lakes					
	Azure-Purple		Azure-Yellow		Yellow-Purple	
	Z	p	Z	p	Z	p
pH	3.18	0.0015	2.85	0.0043	–	ns
Dissolved oxygen	3.18	0.0015	–2.85	0.0043	2.76	0.0058
Conductivity	–3.18	0.0015	–2.85	0.0043	2.76	0.0058
Cl <sup>–</sup>	–2.72	0.0065	–2.56	0.0104	–	ns
SO <sub>4</sub> <sup>–</sup>	–3.18	0.0015	–2.85	0.0043	2.76	0.0058
HCO <sub>3</sub> <sup>–</sup>	–	ns	–	ns	–	ns
Na <sup>+</sup>	–3.18	0.0015	–2.85	0.0043	2.11	0.035
K <sup>+</sup>	–2.72	0.0065	–2.56	0.0104	–	ns
Ca <sup>2+</sup>	–3.18	0.0015	–2.85	0.0043	2.76	0.0058
Mg <sup>2+</sup>	–3.18	0.0015	–2.85	0.0043	2.76	0.0058
NO <sub>3</sub> <sup>–</sup>	–	ns	–	ns	–	ns
PO <sub>4</sub> <sup>3–</sup>	–2.49	0.0128	–1.98	0.0481	–	ns
Cd	–3.31	0.0009	–2.29	0.0219	2.63	0.0085
Pb	–3.31	0.0009	–2.12	0.0338	2.38	0.0174
Cu	–3.31	0.0009	–2.63	0.0085	2.63	0.0085
Zn	–3.31	0.0009	–2.63	0.0085	2.29	0.0219
Mn	–3.31	0.0009	–2.63	0.0085	2.29	0.0219
Fe	–3.31	0.0009	–2.63	0.0085	2.12	0.0338
Cr	–3.31	0.0009	–2.85	0.0043	–	ns
Ni	–3.31	0.0009	–2.63	0.0085	2.46	0.0138
<i>Bdelloidea</i> —density	2.20	0.028	–	ns	–	ns
<i>Cephalodella hoodi</i> —density	–	ns	–2.77	0.006	–	ns
<i>Elosa worallii</i> —density	–	ns	–2.28	0.023	–	ns

anion was SO<sub>4</sub><sup>2–</sup>. The dominant cations were Ca<sup>2+</sup> in Azure and Yellow Lakes and Ca<sup>2+</sup> and Mg<sup>2+</sup> in Purple Lake (Table 1). The lake water was characterized by a low amount of nutrients (NO<sub>3</sub><sup>–</sup>, NH<sub>4</sub><sup>+</sup>, and PO<sub>4</sub><sup>3–</sup>), though higher concentrations of NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3–</sup> ions were measured periodically in Purple and Yellow Lakes. The lake waters differed in the concentrations of the major anions, cations, and nutrients, with the exception of HCO<sub>3</sub><sup>–</sup> and NO<sub>3</sub><sup>–</sup> (Table 2). The water of Purple and Yellow Lakes had significantly higher EC values and concentrations of Cl<sup>–</sup>, SO<sub>4</sub><sup>2–</sup>, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, NH<sub>4</sub><sup>+</sup>, and PO<sub>4</sub><sup>3–</sup> ions, compared to Azure Lake. Additionally, the Purple Lake water had significantly higher EC values and SO<sub>4</sub><sup>2–</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> content compared to Yellow Lake (Table 2). The concentrations of the major ions and nutrients was not significantly different between the upper and bottom layers in Yellow and Azure Lakes. However, the SO<sub>4</sub><sup>2–</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> content in Purple Lake was approximately 1.5 times greater at a depth of 5 m than at the surface (Table 1).

Concentrations of metals in the studied lakes varied widely (Table 1). The Azure Lake water had lower concentrations of Cd, Pb, Zn, Mn and Ni (2–5-times lower), Cu (27 times lower), Cr (58 times lower), and Fe (268 times lower)

than Yellow Lake water, and lower concentrations of Cd, Pb, and Zn (4–9-times lower), Mn and Ni (12–19 times lower), Cu and Cr (146–162 times lower), and Fe (770 times lower) than Purple Lake water. The differences in metal concentrations between the lakes (with the exception of Cr in Yellow and Purple lakes) were statistically significant (Table 2).

**Rotifers**

The density of *Bdelloidea* in Azure Lake was significantly higher than in Purple Lake, while *C. hoodi* and *E. worallii* were higher in Yellow Lake than in Azure Lake. The differences in rotifer density indicates that *Bdelloidea* prefer water with a higher pH. Conversely, *C. hoodi* and *E. worallii* prefer water with a strongly acidic pH. However, the low total density of rotifers in Purple Lake confirmed that the physicochemical water conditions were unsuitable (Tables 2, 3).

The zooplankton community of the three lakes consisted of 26 rotifer taxa; however, only four taxa were present in all of the studied lakes: *Bdelloidea*, *Kellicottia longispina*, *Keratella cochlearis* and *K. quadrata*. The greatest number of total taxa (15) was found in Azure Lake, with 13 found in

**Table 3** Taxonomical characteristics and density (ind L<sup>-1</sup>) of rotifers in studied lakes (x—species were found in quality samples)

Taxa	Azure L.		Purple L.		Yellow L.	
	2011	2012	2011	2012	2011	2012
<b>Bdelloidea</b>						
<i>Rotaria neptunia</i> (Müll.)	x					
<i>Rotaria rotatoria</i> (Müll.)	x	3			x	
Bdelloidea n.d	20	14	3		3	9
<b>Monogononta</b>						
<i>Asplanchna priodonta</i> Gosse		2				
<i>Aspelta cincinator</i> (Gosse)			1			
<i>Brachionus angularis</i> Gosse		2				1
<i>Brachionus diversicornis</i> (Daday)				2		
<i>Brachionus rubens</i> Ehrb	6		2			
<i>Brachionus urceolaris</i> (Müll.)		28				
<i>Cephalodella auriculata</i> (Müll.)					886	
<i>Cephalodella catellina</i> (Müll.)					4	
<i>Cephalodella delicata</i> Wulfert			7			
<i>Cephalodella gibba</i> Ehrb					1	
<i>Cephalodella hoodi</i> (Gosse)			3	10	99	48
<i>Cephalodella ventripes</i> (Dix.-Nutt.)			9		1	
<i>Colurella colurus</i> (Ehrb.)						
<i>Elosa worallii</i> Lord			52		149	103
<i>Gastropus stylifer</i> Imh					1	
<i>Kellicottia longispina</i> Kell	1	688	x	16	x	20
<i>Keratella cochlearis</i> (Gosse)	57		1	4	x	2
<i>Keratella tecta</i> (Gosse)	3	3	1			
<i>Keratella quadrata</i> (Müll.)	5	24	x	3	20	1
<i>Lecane levistyla</i> (Olof.)	1					
<i>Lecane lunaris</i> (Ehrb.)	4		1			
<i>Lecane stichaea</i> (Harr.)	x	4				
<i>Polyarthra dolichoptera</i> Idel					2	
<i>Testudinella patina</i> (Herm.)	5					
<i>Trichocerca iernis</i> (Gosse)			x			
<i>Trichocerca rattus</i> (Müll.)	x					
Total density of year	102	768	80	35	1166	184
Total number of taxa	13	9	13	5	13	7
Total number of taxa in whole seasons	15		13		14	

Purple Lake, and 14 in Yellow Lake. The numbers of rotifer taxa were higher in the first year than the second (Table 3).

Species composition and dominant taxa were different between lakes and study years. *K. longispina* were dominant in Azure Lake, with a high number of Bdelloidea n.d., *Brachionus urceolaris*, *K. cochlearis*, and *Keratella quadrata*. In Purple Lake, *E. worallii* were the dominant species, and *K. longispina*, *C. hoodi*, and *Cephalodella ventripes* were also numerous. *Cephalodella auriculata*, *E. worallii*, and *C. hoodi* dominated Yellow Lake, while *K. longispina* and *K. quadrata* were also present in high numbers. The densities of the remaining species were less than ten individuals per L, with sometimes only a few individuals per L (Table 3). Among the species found in

the studied lakelets, we observed some rarely reported in Poland: *Aspelta cincinator* and *C. delicata*, as well as *C. auriculata*, *C. delicata*, *C. hoodi*, and *E. worallii*, which favour acidic water.

The highest Shannon (H') diversity rates (up to < 1) and evenness of distribution rates (up to < 0.5) occurred in Purple Lake in both years and Yellow Lake during the second year of investigation (Table 4). There were statistically significant correlations observed between the density of particular species and the physicochemical parameters of the water (Table 5a–c). In the Azure Lake, positive correlations were observed between specific species with the concentration of nutrients. *Lecane stichaea* was positively associated with PO<sub>4</sub><sup>3-</sup> and

**Table 4** Species richness, dominance, and indexes of diversity in studied mine lakes

Lake	Azure		Purple		Yellow	
	2011	2012	2011	2012	2011	2012
Dominant taxa	<i>Keratella cochlearis</i> 56%	<i>Kellicottia longispina</i> 89%	<i>Elosa worallii</i> 65%	<i>Kellicottia longispina</i> 45.7%	<i>Cephalodella auriculata</i> 75.9%	<i>Elosa worallii</i> 56%
H'	0.455	0.584	1.241	1.255	0.815	1.210
J	0.198	0.281	0.539	0.780	0.354	0.622

NO<sub>3</sub><sup>-</sup> concentrations, *K. quadrata* with NO<sub>3</sub><sup>-</sup>, and *K. longispina* with PO<sub>4</sub><sup>3-</sup>. A negative correlation was found between *Brachionus urceolaris* and NO<sub>3</sub><sup>-</sup> concentration. *K. cochlearis* and *Keratella tecta* showed negative correlations with pH, while *Branchionus rubens* and *Lecane lunaris* were negatively correlated with the dissolved oxygen and hydrocarbonate content. The total density of rotifers and *K. tecta* was negatively correlated with Ca<sup>2+</sup> content. While *K. cochlearis* and *K. quadrata* were positively correlated with conductivity, *B. urceolaris* showed a negative association. *L. stichaea* was positively correlated with sulphate concentrations. Among the rotifer species, positive correlations were found between Bdelloidea and *K. tecta*, *B. rubens* and *L. lunaris*, and between *L. stichaea*, *K. longispina* and *K. quadrata* (Table 5a).

In Purple Lake, *E. worallii* showed a negative correlation with SO<sub>4</sub><sup>2-</sup> and a positive association with K<sup>+</sup>. Bdelloidea were negatively correlated with the DO and bicarbonate content, but were positively associated with NH<sub>4</sub><sup>+</sup>. Positive relationships among rotifers living in strongly acidic conditions were found for *E. worallii* with *K. longispina*, *A. cincinnator*, *C. ventripes*, and *K. tecta*, as well as between *C. delicata* and Bdelloidea (Table 5B).

In Yellow Lake, *K. quadrata* was positively correlated with PO<sub>4</sub><sup>3-</sup> and Ca<sup>2+</sup> concentrations, while *K. longispina* was positively correlated with SO<sub>4</sub><sup>2-</sup>, but negatively correlated with K<sup>+</sup>. Total rotifer density was positively correlated with the Ca<sup>2+</sup> content (Table 5C).

Some rotifers were positively correlated with particular metals. In Azure Lake, *K. longispina* and *L. stichaea* were positively associated with Cr, *K. tecta* with Pb, *B. rubens* and *L. lunaris* with Ni, and *B. urceolaris* with Mn. In Yellow Lake, *K. quadrata* was positively associated with Zn and Fe. A positive relationship was found between total rotifer density and Cr in Azure Lake, while there was a positive correlation between the total rotifer density and the Cd and Zn content in Yellow Lake (Table 5A, C). In the highly contaminated Purple Lake, *E. worallii* was negatively correlated with Ni. Lead, which was present in small amounts in Purple Lake, was positively correlated with total rotifer density (Table 5B).

## Discussion and Conclusion

The chemistry of the water in these “colorful lakelets” was affected by their geochemical background and by the water supplying the lakes. In ecosystems like these, the biological communities are usually small, with low species diversity. There are typically no fish, and the role of top predator is taken over by invertebrates (Wollmann et al. 2000). While the composition of the zooplankton community is controlled by pH and related to water chemistry variables, the zooplankton biomass may be regulated by food availability (Wollmann et al. 2000; Moser and Weisse 2011). Based on their water chemistry, the studied lakes represent two clearly different groups. Azure Lake, situated higher than the other lakes and fed by a small rainwater stream and water from the degraded land surface, had water characterized by an acidic to neutral pH (3.4–6.9), and low EC and concentrations of major ions, nutrients, and metals such as Fe, Cu, Cr, Cd, and Ni. Conversely, the water of the Purple and Yellow lakes originates from rainwater that runs down the steep walls of the pyrite excavations, as well as from acidic water from neighboring adits, giving the water a strongly acidic pH (2.6–2.9) and significantly higher concentrations of the above parameters (with the exception of HCO<sub>3</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>). The Azure and Yellow lakes have a sulphate-calcium water type, whereas the Purple Lake has sulphate-calcium-magnesium water. Similar results for water composition have been reported in the pit lakes of the Iberian Pyrite Belt, with characteristics ranging from circumneutral water with relatively low metal concentrations to extremely acidic and metal(oid)-rich water (España et al. 2008). Another study from a pyrite mine pond in Portugal found that the water had a low pH and high concentrations of some metals (Turnau et al. 2009).

The chemical composition of water from the “colorful lakelets” creates varied environmental conditions for the organisms that live there. The best physicochemical conditions for biota appeared to occur in Azure Lake, which had an acidic to neutral pH and a relatively low concentration of metals. The strongly acidic pH of the Purple and Yellow Lakes favors the occurrence of metals as free metal ions, which are more bioavailable and therefore, have a higher

**Table 5** Significant correlations between values of physico-chemical and biological parameters (Spearman's rank order,  $p < 0.05$ ) in the “Colourful lakelets” (A—Azure L.; B—Purple L.; C—Yellow L.)

Parameters	<i>Brachionus rubens</i>	<i>Brachionus urceolaris</i>	<i>Kellicottia longispina</i>	<i>Keratella cochlearis</i>	<i>Keratella tecta</i>	<i>Keratella quadrata</i>	<i>Lecane lunaris</i>	<i>Lecane stichaea</i>	Total density of rotifers
<b>(A)</b>									
Pb					0.73				
Mn		0.76							
Cr			0.76					0.73	0.81
Ni	0.76						0.76		
Dissolved oxygen	-0.76					0.93	-0.77	0.77	
pH				-0.87	-0.76				
Conductivity		-0.78		0.71		0.71			
SO <sub>4</sub> <sup>2-</sup>								0.76	
HCO <sub>3</sub> <sup>-</sup>	-0.76						-0.76		
Ca <sup>2+</sup>					-0.77				-0.71
NO <sub>3</sub> <sup>-</sup>		-0.85				0.91		0.76	
PO <sub>4</sub> <sup>3-</sup>			0.87					0.76	
Bdelloidea					0.82				
<i>Brachionus rubens</i>							0.99		
<i>Kellicottia longispina</i>								0.88	0.79
<i>Keratella quadrata</i>								0.81	0.75
<i>Lecane stichaea</i>									0.76
Parameters	<i>Aspelta cincinnati</i>	<i>Bdelloidea</i>	<i>Cephalodella delicata</i>	<i>Cephalodella ventripes</i>	<i>Elosa worallii</i>	<i>Keratella tecta</i>	Total density of rotifers		
<b>(B)</b>									
Pb									0.82
Ni						-0.80			
Dissolved oxygen		-0.76							
SO <sub>4</sub> <sup>2-</sup>						-0.80			
HCO <sub>3</sub> <sup>-</sup>		-0.76							
K <sup>+</sup>						0.76			
NH <sub>4</sub> <sup>+</sup>		0.80							
<i>Cephalodella delicata</i>		0.76							
<i>Cephalodella gibba</i>									
<i>Elosa worallii</i>	0.76				0.76		0.76		
<i>Kellicottia longispina</i>									
Parameters	<i>Kellicottia longispina</i>			<i>Keratella quadrata</i>			Total density of rotifers		
<b>(C)</b>									
Cd									0.90
Zn						0.89			0.90
Fe						0.89			
SO <sub>4</sub> <sup>2-</sup>		0.89							
K <sup>+</sup>		-0.89							
Ca <sup>2+</sup>						0.89			0.90
PO <sub>4</sub> <sup>3-</sup>						0.89			

toxicity to organisms (Kushner 1993; Ciszewski et al. 2013). Complexes of metals with strong ligands, especially sulphates, found in high concentrations in the Yellow and Purple lakes, could potentially decrease this metal toxicity. The highest metal concentrations (with the exception of Cr) were found in the deeper water of Purple Lake, probably due to poor oxygenation (usually  $< 2 \text{ mg dm}^{-3}$ ) and the low pH, which favors the release of metals from an easily reductive phase (Calmano et al. 2005; Szarek-Gwiazda 2013) in the sediment to the overlying water. The positive correlations found between specific metals and the total density, or particular species, of rotifers in the Azure and Yellow lake water suggests that these metals were not toxic to these rotifers. The Cu and Fe concentrations in the Purple and Yellow lakes, and Zn in Purple Lake, were within the range determined to be lethal metal concentrations for freshwater rotifers. For example, the LC50 values for Cu, determined to be toxic to *Lecane hamata*, *L. luna*, and *L. quadridentata* in laboratory experiments, were in the range of 0.06–0.33  $\text{mg dm}^{-3}$  (Pérez-Legaspi and Rico-Martínez 2001), the Zn LC50 value was reported to be 0.12  $\text{mg dm}^{-3}$  for *L. quadridentata* (Guzmán et al. 2010), and the Fe LC50 for *Daphnia magna* was 23.78  $\text{mg dm}^{-3}$  (Burba 1999). Therefore, it is expected that the Cu, Zn, and Fe levels would be toxic to some rotifers living in the Purple and Yellow lakes. Furthermore, the negative correlation observed between Ni and *E. worallii* in the highly contaminated Purple Lake may indicate its negative effect on this organism.

In Azure Lake, the rotifers identified were clearly different from those in the Purple and Yellow lakes. In Azure Lake, which has a higher pH and lower conductivity, the dominant species (*K. longispina* and *K. cochlearis*) are species that are common and widespread. There were no acidophilic rotifer species in this lake, and the total number of species was higher than in the other studied lakes. In general, we observed a relatively low number of rotifer taxa (27 in total) in the three water bodies. This has also been observed in other water ecosystems with low pH, such as the identification of only one species in the most acidic Australian lake (Moser and Weisse 2011) and in an acidic meromictic lake in the Czech Republic (Hrdinka et al. 2013), three species in Chicken Creek lake (pH 2.4–2.9; Derham 2004), four taxa in an acidic uranium mine pit lake in Brazil (Ferrari et al. 2015), nine taxa in three acidic lakes in Lusatia Germany (pH 2–4; Wollmann et al. 2000), and 23 species in the Blue Waters, Stockton, and WO5B lakes (pH 3.9–4.4; Derham 2004). Substantially fewer rotifer species were found in an acidic quarry lake by Horyath and Hummon (1980). Most species observed in the lakes evaluated in our study are common and widely distributed, however, few of these species have been described as typically found at a low pH. The most abundant taxa observed in the studied lakes, including *C. auriculata*, *C. hoodi*, *E. worallii*, and *K. longispina*,

have also been observed in pit lakes with a pH  $< 3$  (Nixdorf et al. 1998; Deneke 2000). Some of these species demonstrate specific strategies in order to function well in these ecosystems, and occupy different “vertical niche habitats” (Weinhoff 2004). According to some authors, a low pH is the most important factor limiting the diversity of water organisms. In such ecosystems, the structure of the food web is less complex (Wollmann et al. 2000). This hypothesis has been confirmed by Deneke (2000), who found a positive correlation between the number of zooplankton species and an average pH of 2.3–3.9 in 21 acidic pit lakes in the Lusatian region (Germany). Another study conducted in three lakes (Blue Waters, Stockton, and WO5B), all with a pH between 3.8–5, showed a low H' index from 1.7 to 2.5 (Derham 2004). Our results also support this hypothesis. The Shannon–Weaver diversity index for the lakes included in this study was relatively low, ranging from 1.210 in Yellow Lake to 1.255 in Purple Lake, both of which have a low pH and high concentrations of some metals (especially Cu and Fe), which adversely affected rotifer diversity.

Our results indicate that physicochemical parameters other than pH can potentially influence the diversity of some rotifer species, as demonstrated by positive or negative correlation with phosphate, nitrate, DO, bicarbonate, EC, sulphate, and calcium in Azure Lake, ammonium, potassium, sulphate, DO, and bicarbonate in Purple Lake, and phosphate, sulphate, potassium, and calcium in Yellow Lake. A similar observation was reported in coal mine water by Radwan and Paleolog (1983). Apart from pH, the most important factors appeared to be chloride, sulphate, calcium, and DO, all of which affected the occurrence and quantitative structure of rotifer assemblages. High concentrations of sulphate, Fe, Ca, Mg, Mn, Zn, Cu, and Cd were shown to influence zooplankton species composition in other acidic pit lakes (Moser and Weisse 2011; Ferrari et al. 2015).

In conclusion, while the physicochemical parameters of the water showed considerable variability between the studied lakes, the pH and high concentration of some metals (especially Cu and Fe) had the greatest effect on the composition of rotifer communities. The highest species diversity of rotifers was observed in the Yellow and Purple lakes, where the dominant species were acidophilic species.

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