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ORIGINAL PAPER

The role of environmental factors in the composition of anuran species in several ponds under the influence of coal mining in southern Brazil

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Abstract Environmental pollution caused by the disposal of chemicals or toxins into the environment represent a great threat to amphibians. In this study, it was evaluated which environmental factors of ponds under the influence of coal-mining influence the composition of anuran species. For this purpose, anuran samples were performed in ponds in coal extraction areas in southern Brazil. Physical and chemical characteristics of water, sediment and structural characteristics of the ponds were determined. The content of copper in the sediments of the ponds was the main variable to explain the composition of pond species. Besides copper, it is evident the relevance of number of vegetation strata at pond border and pond area on the composition of anurans. It is shown that the composition of species in ponds under the influence of mineral coal mining activity may be a result of historical perturbations, remaining only the most tolerant towards colonization. From the results obtained in this work it can be suggested that there is a synergistic interaction between environmental factors and the coal mining effects on the anurans fauna of impacted ponds. Thus, efforts should be directed to elucidate the effects of pollutants exposure levels on the species, the understanding of sub-lethal and lethal effects of each pollutant on each species, as well as how local populations can be maintained through recruiting rates of larvae and anurans of the impacted ponds.

Keywords Amphibians decline · Copper effects · Heavy metals · Threats to conservation

Introduction

Environmental changes caused by anthropic actions are considered huge threats to world biodiversity (Ceballos et al. 2015). Based on fossil records and indexes of species extinction, theoretical models have pointed anthropogenic rates as high as one thousand greater than natural rates (de Vos et al. 2014), with losses in the range of 11,000–58,000 species per year (Dirzo et al. 2014). These biodiversity changes may generate dramatic impacts on the stability and functioning of ecosystems (Wang and Loreau 2016) and hence the knowledge of contributing factors by population declines and even extinction comprises

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one of the greatest ecological challenges (Verdade et al. 2011).

Among vertebrates, amphibians have been the most affected, with probably one-third of all species in the world suffering some extinction degree (Catenazzi 2015). The decline in amphibians and other aquatic vertebrates has been attributed to the presence of heavy metals as a consequence of progressive aquatic ecosystems contamination due to intense agricultural activity and mining (Ficken and Byrne 2013; Hopkins and Rowe 2010). Environmental pollution caused by accidental or deliberate disposal of chemical products or toxins (e.g., heavy metals) is considered a greatest threat to amphibians, with around 20% of all species in the world under such influence (Chanson et al. 2008).

Anurans amphibians are particularly susceptive to impacts caused by heavy metals due to their high permeable skin, allowing fast absorption of metallic ions (Loumbourdis et al. 2007). Though the effects of pollutants on the amphibians are not completely known (Collins and Storfer 2003), some toxicological studies confirm that even low concentrations of heavy metals may be lethal to larvae and adult amphibians (Mahaney 1994; Loumbourdis et al. 1999; Loman and Lardner 2006; Loumbourdis et al. 2007; Barth and Wilson 2010). Besides, some reports suggest that sub-lethal metals concentrations can lead to several deleterious effects on the amphibians, like reduced growth rates, sexual reversion and behavioral changes (Chen et al. 2009; Hayes et al. 2010; Chai et al. 2014; Edge et al. 2014; Babini et al. 2015). The susceptibility of amphibians to lethal and sub-lethal exposure to metals effects may vary depending on the species due to physiological characteristics, habitat demands and reproductive standards (Snodgrass et al. 2004). Thus, it is expected that heavy metals may have an important role in structural and compositional changes of the species in communities of amphibians.

Brazil has the greatest diversity of amphibians of the world, with 1080 species (Segalla et al. 2016), 3.8% (41 species) of them endangered threatened (Ministério Meio Ambiente 2014). Most of endangered species is located in Atlantic forest, which is considered a world biodiversity hotspot (Myers et al. 2000; Mittermeier et al. 2011). The number of endangered amphibians species in the Brazilian Atlantic forest is probably underestimated, as the agropastoral activities, urban/industrial occupation and mining activity have caused a severe reduction in plant cover, down to less than 10% of its original value (Ribeiro et al. 2009). The fast expansion of mining and agricultural activities over the last decades caused severe metal contamination of several aquatic ecosystems of this biome (Schiesari et al. 2007; Zocche et al. 2014). In South Brazil, since the end of XIX century, the extraction and processing of mineral coal led to many sources of acid draining, hence releasing great amount of metals. Residues produced from coal extraction activity are in fact complex mixtures that contain a variety of chemical elements such as hydrocarbons and metals that can cause negative impacts on the ecosystem, especially in the final aquatic receiver environment of such residues (Ferreira et al. 2007; Geremias et al. 2012; Lanctôt et al. 2016).

To the best of our knowledge there are few studies in the Brazilian context regarding the relationship of environmental pollution and amphibians reduction (Verdade et al. 2011), mainly due to the lack of data on the concentration of pollutants in nature, their spatial and temporal variation in natural environments, concentrations for which they may be biologically active and the how long the effects of pollutants may persist in certain species (Schiesari et al. 2007). In particular, no reports were found concerning contamination of heavy metals from coal mining on the amphibians communities in South Brazil. In this sense, this work investigates the environmental characteristics of ponds under the influence of coal mining, describing the anurans species, towards elucidating whether coal mining residues affect the characteristics of water and sediment of pond and which extent it may reflect on the anurans species composition around the pond.

Materials and methods

Study area

The present study was performed in several coal extraction areas located in South Brazil, namely in the cities of Criciúma (28°40′40″S; 49°22′12″W), Siderópolis (28°35′52″S; 49°25′26″W) and Treviso (28°30′57″S; 49°27′28″W). Eight ponds (P1, P2, P3, P4, P5, P6, P7 and P8) distant from each other at least 0.7 km and no more than 23 km were chosen near coal wastes, as shown in Fig. 1.

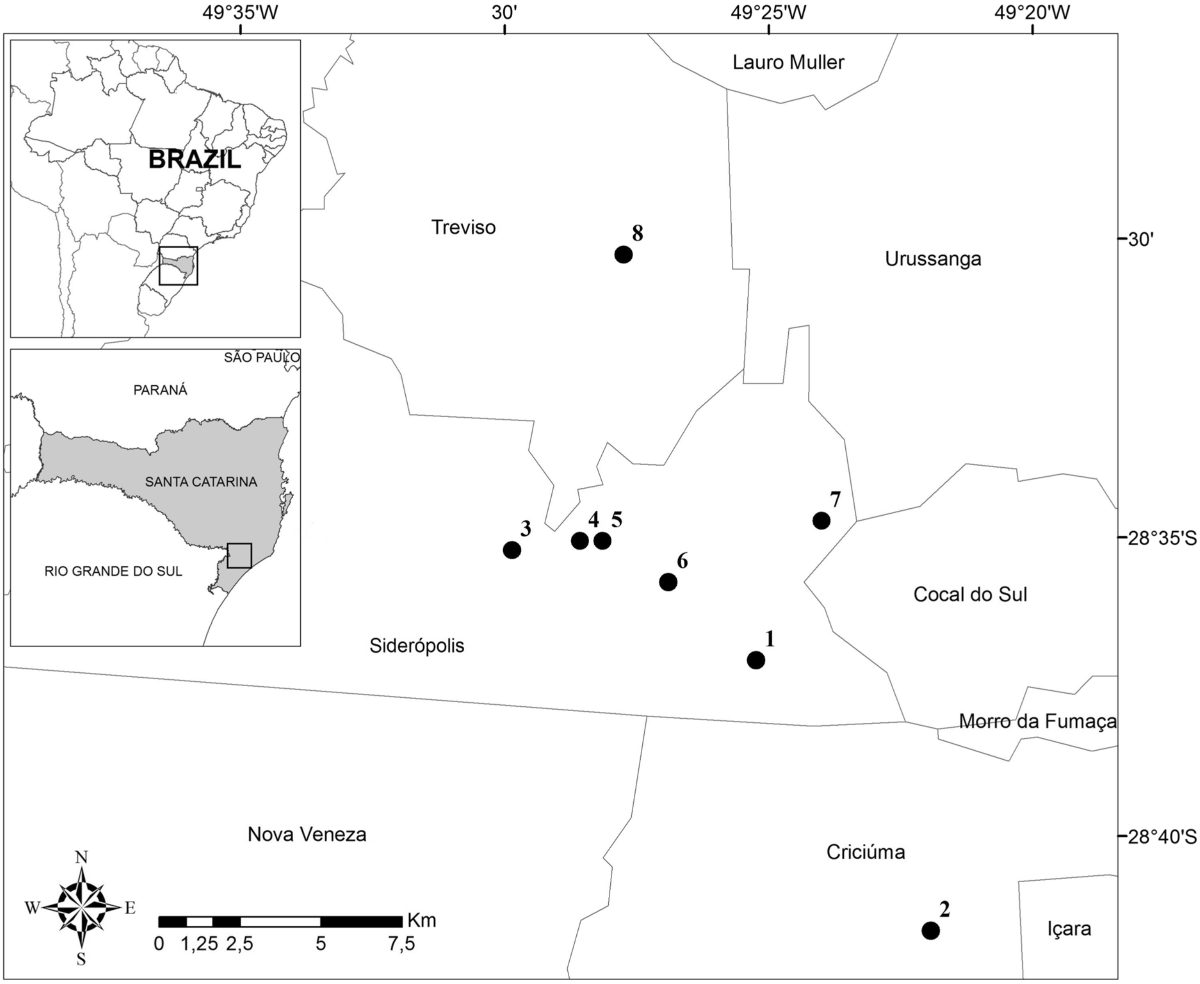

Fig. 1 Location of ponds sampled in the present study, in municipalities of Criciúma, Siderópolis and Treviso, Santa Catarina state, South Brazil

These ponds are inserted in the Coal Region Cities Association, belonging to Atlantic Forest domain. Currently, due to agropastoral activities, urban and industrial occupation and mineral coal extraction, there is only fragments of Submontane Dense Ombrophylous Forest at different succession stages, surrounded by cattle pasture, waste places of coal extraction and forestry areas of *Eucalyptus* sp. (IBGE—Instituto Brasileiro de Geografia e Estatística 2012).

The study region suffers from mineral coal exploration since the end of XIX century, with the highest production reached in 1940 decade due to governmental politics directed to the use of coal for steel industry. Today, a much smaller amount of mineral coal is locally produced, now directed to electric power generation. Wastes from mineral coal processing are laid out on the ground surface, which together with unsealed underground openings contribute to acid mine drainages (AMD). A vast territory in this region consists in sterile lands, which resembles to lunar landscape hence exposing to weathering hazardous materials, allowing diffusion of contamination sources (Filho et al. 2014).

Water and sediment sample collect

In order to determine the place of collection of water and sediment samples, the pond perimeter was divided in intervals of 2 m and at each month three intervals were drawn for each pond, in the same day that anurans were sampled. Water samples were carried out using 250 PET flasks, acidified (HNO_3, pH < 2) at the collection moment and stored in the fridge (−18 °C). Sediment samples were collected in the same intervals of water sampling, using stainless steel Petersen dredge (180 × 200 mm), stored in plastic bags and kept in a freezer prior to processing. Such process was performed by sieving (62 μm) the sediment samples, drying in oven at 80 °C for 24 h, milling, digestion and read of heavy metals.

Water and sediment analyses

Physico-chemical variables measured in water were: pH, electrical conductivity, turbidity, temperature, dissolved oxygen (DO), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). Dissolved oxygen, pH and temperature were measured in the field while the other variables were quantified at the laboratory following Public and Association—APHA (1998). For the analysis of heavy metals content, water samples was filtered using cellulose nitrate filters of 0.45 mm (Sartorius®), acidified with HNO_3 and analyzed in the atomic absorption spectrophotometer (AANALYST 800 AAS—Perkin Elmer precisely®), air-acetylene method and/or graphite oven. Analytical curves were built using *AA Test Mix Standard* Perkin Elmer and all analyses were performed in triplicate.

The content of the following metals were determined in the sediment ponds: Cd, Cr, Cu, Fe, Mn and Zn. Analysis of metals in the sediments were carried out according to USEPA (1996). Briefly, three sediment samples (0.5 g) were weighed and homogenized and transferred to flasks for digestion. Afterwards, 5 mL of nitric acid and 5 mL of ultrapure water was added, heating in reflux to 95 ± 5 °C for 15 min. After sample cooling, it was added 5 mL of HNO_3 leaving reacting for 30 min. Then, samples were heated again for 2 h (95 ± 5 °C) and after cooling at room temperature, 2 mL of ultra-pure water and 3 mL of H_2O_2 (30%) were added and wait reacting at natural boiling. Aliquots of 1 mL of H_2O_2 30% (no more than 10 mL) were added up to boiling was not observed. Afterwards, samples were heated for 4 h at 140 ± 5 °C and after cooling the whole content was diluted in 50 mL ultrapure water, filtered (0.45 μm cellulose nitrate filter), adding 10 mL of hydrochloric acid (HCl) and again heated (95 ± 5 °C) for 15 min. After digestion completion, spectrophotometer readings were performed in the atomic absorption.

Structural characteristics of ponds

For ponds characterization, the following structural characteristic parameters were taken into account: (i) pond area; (ii) minimum distance between pond and coal waste disposal; (iii) number of vegetation types at pond border: herbaceous; arboreal and shrub, where 1 = only one type; 2 = two types, 3 = three types; (iv) plant cover at water surface: 0–24; 25–49; >50%; (v) pond border profile: flat, downhill and ravine, where 1 = only one profile; 2 = two profiles;

Table 1 Spatial and structural characteristics of eight ponds in areas with influence of coal extraction in southern Santa Catarina, Brazil, from October 2012 to February 2013

Ponds	Area (m^2)	D (m)	MP	TM	VSM	PVC
P1	2558.0	0	fl, dm	DSW, DSV, HSV	HEV, BUV	2
P2	591.9	2000	dm, ra	DSW, DSV, HSV, HSW	HEV, BUV, ARV	1
P3	1428.9	2300	dm, ra	DSW, DSV, HSV	HEV, BUV, ARV	1
P4	685.0	230	dm, ra	DSW, DSV, HSV	HEV, BUV	2
P5	3468.9	0	dm, ra	DSW, DSV, HSV, HSW	HEV, BUV	1
P6	1438.5	130	fl, dm	DSW, DSV	HEV	1
P7	1348.7	0	fl, dm, ra	DSW, DSV, HSV	HEV, BUV, ARV	1
P8	1553.2	15	fl, dm, ra	DSV, HSV	HEV, BUV, ARV	1

D minimum distance from the ponds to areas of coal waste disposal, *MP* margin profile, *TM* types of margin, *VSM* vegetation strata in the margin, *PVC* percentage of vegetation cover on the water surface. Profile of margin: *fl* flat, *dm* downhill margin, *ra* ravine. Types margins: *DSW* dry soil without vegetation, *DSV* dry soil with vegetation, *HSW* humid soil without vegetation, *HSV* humid soil with vegetation. Vegetation: *HEV* herbaceous, *BUV* bushy, *ARV* arboreal. Water cover: 1 = 0–24%; 2 = 25–49%

3 = three border profiles (Table 1), adapted from Silva et al. (2011). In addition, atmospheric temperature and relative humidity were recorded at the beginning and at the end of each sampling.

Anurans samples

The collection of biological samples was performed from October 2012 to February 2013, period of major reproductive activity of anurans species in South Brazil (Colombo et al. 2008; Both et al. 2009; Bastiani and Lucas 2013). Samples were taken monthly from ponds, with three consecutive nights per month. For species registering of adult anurans, it was adopted hearing and visual observation in reproductive sites (Heyer et al. 1994), from 19 to 00 h. Three ponds were randomly sampled per night, which means that each pond was approached in approximately 1 h and half five times, totalizing 7 h and half for each pond. The abundance of anurans in each pond was estimated from the number of males in vocalization activity and for this purpose, ponds perimeter plus 5 m distance was approached slowly.

Towards taxonomic identification and maintenance of witness material, ten specimens for each species were collected in each sampled pond, being afterwards deposited at Amphibians Collection of Universidade Comunitária da Região de Chapecó (License Number SISBIO No. 35547-1), which is in accordance with Darrel Frost (2016).

Data analysis

To avoid population overestimation, due to recounting of species of different samples in the same site, the maximum abundance of male in active vocalization registered in the sampling period (Conte and Rossa-Feres 2006). The analysis of variance (ANOVA) was performed in order to check possible variations in anurans richness and abundance in each pond. When difference was detected, the Tukey posteriori HSD (Zar 1999) test was applied to identify which pairs differed. The same statistical test was also used to detect differences among variables of pond water and sediment.

To determine the variation of anurans species in the different sampled ponds, a biological matrix was built using the Bray–Curtis index from abundance data. Such matrix was employed to rank the composition variation of anuran species of the ponds through non-metrical multidimensional scaling (NMDS). The relationship between biological and environmental dissimilarity matrices was evaluated using BioEnv analysis (Clarke and Ainsworth 1993). Such analysis makes use of the Mantel test to correlate the biological dissimilarity matrix and a second matrix of environmental variables. Thus, in the BioEnv analysis an environmental dissimilarity matrix is constructed based on different number and combinations of environmental variables. The biological dissimilarity matrix is first correlated to an environmental matrix obtained from a single variable. Then, the analysis uses of a second environmental matrix, but now obtained from two variables, where the highest correlation is indicated. This procedure is repeated with three, four or even more environmental variables up to the total amount of variables have been considered. The combination that leads to the best correlation is selected, in the present work the Spearman coefficient was used to compute the correlation between matrices. The dissimilarity environmental matrix was calculated using Euclidian distances of environmental variables standardized by their standard deviations. The environmental variables used in the BioEnv analysis were: pond area (A), minimum distance between pond and coal waste disposal (D), margin profile (MP), number of vegetation strata in the margin (VSM), percentage of vegetation cover on the water surface (PVC), pH, electrical conductivity, turbidity, temperature, dissolved oxygen (DO), metals in water (As, Cd, Cr, Cu, Fe, Mn and Zn) and in the sediment (Cd, Cr, Cu, Fe, Mn and Zn), atmospheric temperature (ta) and relative air humidity (Rh).

Results

The values of physical and chemical water variables are presented in Table 2. As can be seen from this table, the water pH, dissolved oxygen, electrical conductivity and turbidity differed among ponds: pH: $F_{(7.32)} = 48.82$; $P < 0.0001$; OD: $F_{(7.32)} = 12.09$; $P < 0.0001$; electrical conductivity: $F_{(7.32)} = 13.80$; $P < 0.0001$; turbidity: $F_{(7.32)} = 2.84$; $P = 0.0201$). Nevertheless, no difference was found regarding temperature ($F_{(7.32)} = 1.58$; $P = 0.1774$) and the concentration of heavy metals in water. In the

Table 2 Mean values and their standard deviation of physical and chemical variables obtained from the sample sites of ponds analyzed in areas with influence of coal extraction in southern Santa Catarina, Brazil

Variable	P1	P2	P3	P4	P5	P6	P7	P8
Water								
pH	3.84 ± 0.11	6.29 ± 0.48	3.95 ± 0.45	5.83 ± 0.42	5.89 ± 0.22	6.75 ± 0.21	6.38 ± 0.26	6.24 ± 0.61
DO (mg/L)	3.91 ± 0.45	3.75 ± 0.46	6.33 ± 0.29	3.67 ± 0.005	3.82 ± 0.29	4.19 ± 0.78	4.98 ± 0.83	4.39 ± 0.59
T (°C)	26.94 ± 0.99	27.28 ± 1.63	28.02 ± 1.45	26.3 ± 2.31	27.16 ± 1.28	28.18 ± 1.59	29 ± 0.86	27.28 ± 1.56
EC (µS/cm)	80.2 ± 14.97	196.4 ± 143.67	24.4 ± 8.83	39.06 ± 8.98	51.22 ± 24.18	51.42 ± 8.32	30.02 ± 3.55	30.72 ± 7.46
Turbidity (NTU)	2.1 ± 2.16	28.41 ± 30.41	3.42 ± 2.5	3.77 ± 2.41	5.68 ± 5.73	14.21 ± 10.3	3.5 ± 2.79	3.88 ± 1.93
As (mg/L)	0.26 ± 0.27	1.99 ± 2.74	0.16 ± 0.17	0.36 ± 0.71	0.56 ± 0.95	0.34 ± 0.27	0.17 ± 0.27	0.09 ± 0.11
Cd (mg/L)	2.69 ± 3.69	5.43 ± 9.07	0.82 ± 0.9	0.95 ± 1.2	1.01 ± 1	0.56 ± 0.63	0.63 ± 0.56	0.88 ± 0.79
Cr (mg/L)	3.56 ± 7	1.08 ± 0.79	0.3 ± 0.25	0.44 ± 0.44	0.27 ± 0.19	0.36 ± 0.41	1.19 ± 1.65	0.1 ± 0.13
Cu (mg/L)	3.69 ± 3.56	9.07 ± 1.08	0.9 ± 0.3	1.2 ± 0.44	1 ± 0.27	0.63 ± 0.36	0.56 ± 1.19	0.79 ± 0.1
Fe (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND
Mn (mg/L)	0.86 ± 0.79	0.73 ± 1.13	0.31 ± 0.32	0.15 ± 0.14	0.46 ± 0.66	0.24 ± 0.23	0.14 ± 0.14	0.08 ± 0.11
Sediments								
Cd (mg/kg)	1.11 ± 2.48	1.44 ± 3.21	1.34 ± 3	1.85 ± 4.14	1.87 ± 4.18	3.01 ± 6.73	3.44 ± 7.68	3.55 ± 7.93
Cr (mg/kg)	23.77 ± 7.38	19.93 ± 12.23	26.59 ± 6.22	26.68 ± 8.66	27.41 ± 8.7	23.67 ± 9.43	27.54 ± 11.24	23.45 ± 10.21
Cu (mg/kg)	22.27 ± 7.6	19.84 ± 14.1	126.87 ± 40.55	41.17 ± 18.82	33.07 ± 10.87	40.65 ± 30.54	31.45 ± 3.7	37.56 ± 30.57
Fe (mg/kg)	66,962 ± 21,826.13	49,024 ± 29,785.41	209,483 ± 84,113.48	88,049 ± 47,382.18	89,305 ± 363,961	85,326 ± 37,731.62	112,108 ± 96,011.35	54,761 ± 34,994.54
Mn (mg/kg)	677.93 ± 286.99	252.4 ± 214.34	1028.47 ± 85.67	305.04 ± 420.14	209.7 ± 196.65	328.38 ± 279.63	481.37 ± 151.18	387.35 ± 375.33
Zn (mg/kg)	31.65 ± 18.29	37.12 ± 24.01	67.67 ± 10.52	41.78 ± 31.48	32.02 ± 28.19	18.1 ± 7.73	19.45 ± 6.09	13.25 ± 9.35

sediment, copper concentration was different among ponds ($F_{(7.32)} = 4.47$; $P < 0.01$), but for cadmium ($F_{(7.32)} = 0.17$; $P > 0.05$), chrome ($F_{(7.32)} = 0.80$; $P > 0.05$), iron ($F_{(7.32)} = 1.40$; $P > 0.05$), manganese ($F_{(7.32)} = 2.01$; $P > 0.05$) and zinc ($F_{(7.32)} = 2.18$; $P > 0.05$) no significant differences were detected.

Seventeen anuran species were registered, distributed into six families (Table 3). There was difference in anurans richness among ponds, being 16 the highest and 1 the smallest registered ($F_{(7.32)} = 18.231$; $P < 0.0001$). Ponds 1, 2, 5, 7 and 8 showed the greatest richness compared to ponds 3 and 6, whereas pond 4 did not present difference from the others (Fig. 1). Male abundance under vocalization activity differed significantly among ponds ($F_{(7.32)} = 9.68$; $P < 0.01$), with the highest found of 190 individuals registered pond 5. Abundance of anurans in pond 5 was higher than those found in ponds 3, 4, 6, 7 and 8, but did not differ from ponds 1 and 2. Ponds 3 and 6 presented the smallest abundance of anurans with regard to ponds 1, 2, 7 and 8, as shown in Fig. 2.

The NMDS was efficient to reduce the dimension of experimental data (stress 0.07) (Fig. 3). Ponds 3, 4 and 6 received the highest values in the first classification axis, while ponds 3 and 6 showed the lowest richness and abundance registered for all ponds.

The best correlation was reached for the dissimilarity matrix that employed two environmental variables: number of vegetation strata and copper in the sediment ($r = 0.6065$, see Table 4). Another model comprising the number of vegetation strata, copper in the sediment and also pond area produced a similar correlation, $r = 0.5977$, while the model using only the number of vegetation strata led to a poor correlation, $r = 0.4750$, thus indicating that copper in the sediment was relevant (Table 4). Ponds with the highest NMDS scores were those which showed reduced composition of species, i.e., pond with high copper concentration (pond 3) and pond with reduced number of vegetation strata (pond 6) presented low abundance and richness.

Discussion

In this investigation, water of ponds under the influence of coal mining activity presented differences regarding pH, dissolved oxygen, electrical conductivity and turbidity. On the other hand, pond sediment showed effect only for copper and the content of copper in the pond sediments was the main variable to explain the composition of anurans species in the ponds. Besides copper, it is evident the relevance of number of vegetation strata at pond border and pond area on the composition of anurans.

Some anuran species may be more pollutant tolerant than others (Snodgrass et al. 2008), but no ecotoxicological studies are available for most of the species registered in this study. Alone, the presence/absence of the species in the ponds does not confirm this tolerance to the pollutants. Indeed, all registered species have reproductive mode associated with water and the tadpole development is aquatic (Haddad et al. 2013). Even the most abundant species (*Scinax tymbamirim*) recorded in 75% of the studied ponds, presented few or none individuals in the ponds with the highest levels of copper.

Copper is a chemical element recognized for bioaccumulation at different levels in the biota (Vinot and Pihan 2005; Peltier et al. 2009; Soto et al. 2011), including amphibians (Unrine et al. 2007; Taiwo et al. 2014). Zocche et al. (2014), investigating the amount of metals in amphibian *Hypsiboas faber* tissue, collected in mineral coal areas, showed that copper was the second most abundant metal in the amphibian tissue. Here, copper affected negatively the composition of anurans, as the highest concentrations of such metal was found in ponds with the lowest abundances. Indeed, copper was also pointed as one of the main factors affecting the anurans community in areas under agriculture influence in Australia (Ficken and Byrne 2013).

Several negative and sub-lethal effects in amphibians were associated to copper exposure (Lance et al. 2013; Wang et al. 2016). For example, the toad larvae of *Bufo gargarizans* exposed to different concentration of copper showed clear increased mortality, inhibition of brooding development and delay in the metamorphosis process (Wang et al. 2016). Lance et al. (2013) evaluated the chronical effect of copper on the larvae and the toad embryos of *Anaxyrus terrestris* and concluded that spawns exposed to copper showed reduced survival. In the same way, the toad species *Lithobates sphanocephalus* presented low number of survival larvae when exposed to copper (Lance et al. 2012). Sub lethal effects of copper may vary and be species-specific, with different impact on the recruiting rates and lead to a decrease in the local

Table 3 List of species and number of individuals of species found in eight ponds in areas with influence of coal extraction in southern Santa Catarina, Brazil, from October 2012 to February 2013

Taxon	P1	P2	P3	P4	P5	P6	P7	P8
Bufonidae								
Rhinella abei (Baldissera-Jr, Caramaschi & Haddad, 2004)	0	0	0	0	1	0	0	0
Hylidae								
Dendropsophus minutus (Peters, 1872)	1	0	0	0	104	0	2	1
Dendropsophus sanborni (Schmidt, 1944)	0	0	0	21	0	0	0	4
Boana bischoffi (Boulenger, 1887)	0	8	0	0	0	0	2	2
Boana faber (Wied-Neuwied, 1821)	1	0	0	0	32	0	0	1
Phyllomedusa distincta A. Lutz in B. Lutz, 1950	0	2	0	0	0	0	7	0
Sphaenorhynchus caramaschii Toledo, Garcia, Lingnau & Haddad, 2007	2	1	0	0	1	0	0	2
Scinax fuscovarius (A. Lutz, 1925)	0	3	0	0	5	0	5	0
Scinax granulatus (Peters, 1871)	0	0	0	0	0	0	0	0
Scinax perereca Pombal, Haddad & Kasahara, 1995	0	0	0	0	0	0	3	0
Scinax tymbamirim Nunes, Kwet, and Pombal, 2012	48	3	3	27	32	0	0	19
Leptodactylidae								
Physalaemus cuvieri Fitzinger, 1826	0	0	0	2	9	0	11	5
Physalaemus nanus (Boulenger, 1888)	1	3	0	0	1	0	1	0
Leptodactylus gracilis (Duméril & Bibron, 1841)	0	6	0	0	0	0	0	0
Leptodactylus latrans (Steffen, 1815)	1	1	1	0	5	0	0	2
Microhylidae								
Elachistocleis bicolor (Valenciennes in Guérin-Menéville, 1838)	3	2	0	0	0	0	0	0
Odontophrynidae								
Proceratophrys boiei (Wied-Neuwied, 1825)	0	1	0	0	0	0	0	3

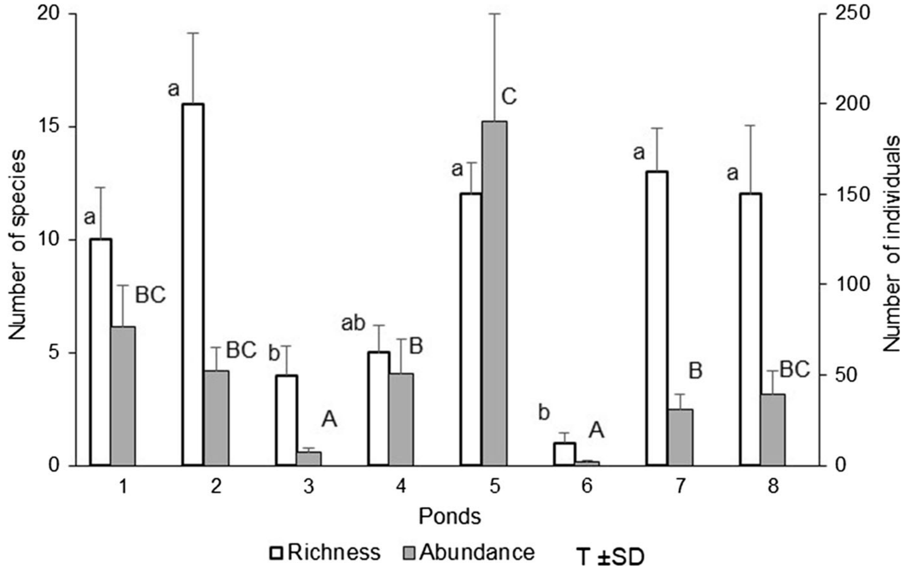

Fig. 2 Richness and highest abundance of anurans in eight ponds under the influence of mineral coal mining activity in south Santa Catarina state, from October 2012 to February 2013. Richness and abundance of each pond, with *different letters* in columns, differ from each other according to posteriori Tukey HSD test

population. In this sense, more toxicological studies are needed to better understand and detect possible lethal and sub-lethal effect patterns caused by heavy metals, including copper, in the different development stages of anurans exposed to these contaminants, as is the case of mineral coal mining areas in Brazil.

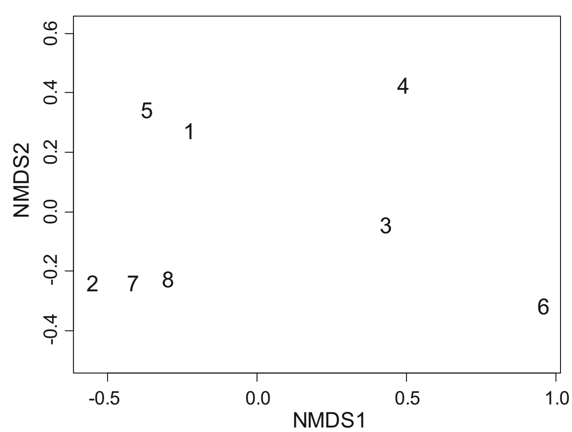

Fig. 3 Non-metrical multidimensional scaling of anurans registered in eight ponds under the influence of mineral coal mining activity in south Santa Catarina state, from October 2012 to February 2013. *Numbers* refer to the sites shown in Fig. 1. Stress = 0.07

Furthermore, this work demonstrated that the composition of anurans species was affected by the number of vegetation strata and pond size. The relationship between community structure and species tolerance and the biotic/abiotic factors suggests that the niche can overlap in the multidimensional space, limiting the species coexistence inside a community (Ricklefs and Schluter 1995). Relationship between structural characteristics of the environment and its use by anurans has been a matter of intense investigation over decades (Campos and Vaz-Silva 2010; De Fonseca and Jocqué 1982; Prado and Pombal 2005), thus strengthening the hypothesis that species composition depends on the vegetation structure (Peltzer et al. 2006; Vasconcelos et al. 2009) and on the size of reproduction sites (e.g., Iop et al. 2012). Thus, the influence of vegetation and pond area on the composition of species may be related to the availability of sources such as shelter, reproductive sites and food availability. Larger ponds with a greater number of micro-inhabitants will possibly offer better conditions to be colonized (da Silva et al. 2012; Iop et al. 2012) thus showing a greater abundance. On the other hand, other factors like competition and predation can also exert influence on the community structure (Kraus 2015; Anderson and Semlitsch 2016). Yet, there is no consensus on how synergistic effects occur between

Table 4 Matrix of dissimilarity using the environmental variables determined in the ponds analysed in areas with influence of coal extraction in southern Santa Catarina, Brazil

Model size	Model	Correlation
1	VSM	0.4750
2	VSM + Cus	0.6065
3	A + VSM + Cus	0.5977
4	A + VSM + Few + Cus	0.5818
5	A + VSM + Few + Fes + Cus	0.5473
6	A + VSM + PVC + Tu + Few + Cus	0.5194
7	A + VSM + Few + Mns + Fes + Cus + Zns	0.5047
8	A + D + VSM + Few + Mns + Fes + Cus + Zns	0.4483
9	A + D + VSM + pH + Tw + Tu + Few + Fes + Cus	0.4007
10	A + D + VSM + pH + Tw + Tu + Few + Fes + Cus + Crs	0.3870
11	A + D + VSM + EC + Tu + Cdw + Few + Mns + Fes + Cus + Zns	0.3667
12	A + D + VSM + Rh + EC + Tu + Cdw + Few + Mns + Fes + Cus + Zns	0.2928
13	A + D + VSM + PVC + pH + Rh + Tu + Cdw + Crw + Few + Mns + Fes + Cus	0.2698
14	A + D + VSM + PVC + pH + EC + Tu + Cdw + Crw + Few + Mns + Fes + Cus + Crs	0.2501
15	A + D + VSM + PVC + pH + Rh + EC + Tu + Cdw + Crw + Few + Mnw + Mns + Fes + Cus	0.2282
16	A + D + VSM + PVC + pH + Rh + EC + Tu + Asw + Cdw + Crw + Few + Mns + Fes + Cus + Crs	0.1949
17	A + D + VSM + pH + Rh + Tw + EC + Tu + Cdw + Crw + Few + Mnw + Mns + Fes + Cus + Crs + Zns	0.1604
18	A + D + VSM + PVC + pH + Rh + EC + Tu + Asw + Cdw + Crw + Few + Mnw + Mns + Fes + Cus + Crs + Zns	0.1182
19	A + D + VSM + PVC + pH + Rh + Tw + EC + Tu + Asw + Cdw + Crw + Few + Mnw + Mns + Fes + Cus + Crs + Zns	0.0772
20	A + D + VSM + PVC + pH + Rh + Tw + EC + Tu + Asw + Cdw + Crw + Few + Mnw + Mns + Fes + Cus + Crs + Cds + Zns	0.0268
21	A + D + MP + VSM + PVC + pH + Rh + Tw + EC + Tu + Asw + Cdw + Cuw + Few + Mnw + Mns + Fes + Cus + Crs + Cds + Zns	−0.0109
22	A + D + MP + VSM + PVC + pH + DO + Rh + Tw + EC + Tu + Asw + Cdw + Cuw + Few + Mnw + Mns + Fes + Cus + Crs + Cds + Zns	−0.0443
23	A + D + MP + VSM + PVC + pH + DO + Rh + Ta + Tw + EC + Tu + Asw + Cdw + Cuw + Few + Mnw + Mns + Fes + Cus + Crs + Cds + Zns	−0.0826
24	A + D + MP + VSM + PVC + pH + DO + Rh + Ta + Tw + EC + Tu + Asw + Cdw + Crw + Cuw + Few + Mnw + Mns + Fes + Cus + Crs + Cds + Zns	−0.1483

A area, *Asw* arsenic concentration in the water, *Cds* cadmium concentration in the sediment, *Cdw* cadmium concentration in the water, *Crs* chromium concentration in the sediment, *Crw* chromium concentration in the water, *Cus* copper concentration in the sediment, *Cuw* copper concentration in the water, *D* minimum distance in meter from the pond to areas of coal tailings deposit, *DO* dissolved oxygen, *EC* electric conductivity, *Fes* iron concentration in the sediment, *Few* iron concentration in the water, *Mns* manganese concentration in the sediment, *Mnw* manganese concentration in the water, *MP* margin profile, *PVC* percentage of vegetation cover on the water surface, *Rh* relative humidity, *Ta* air temperature, *Tu* turbidez, *Tw* temperature of water, *VSM* vegetal strata in the margin, *Zns* zinc concentration in the sediment different factors, since an isolated factor could act distinctly in different communities (Vasconcelos et al. 2009).

The fact that different heavy metals can lead to a variety of effects, in different degree, on the anurans fauna is well documented in some experiments (Loumbourdis et al. 1999, 2007; Brown et al. 2012; Lance et al. 2012; Dal-Medico et al. 2014; Wang et al. 2016) but it seems difficult to prove it in natural environment due to the very complex interactions existing. The composition of species in ponds under the influence of mineral coal mining activity may be a result of historical perturbations, remaining only the most tolerant towards colonization. There is a trend in biological communities to become more homogeneous in response to one or various stress agents (Petsch 2016). From the results obtained in this work it can be suggested that there is a synergistic interaction between factors and the coal mining effects on the anurans fauna of impacted ponds, which will be realized in a near future. Thus, efforts should be directed to elucidate the effects of pollutants exposure levels on the species, the understanding of sub-lethal and lethal effects of each pollutant on each species, as well as how local populations can be maintained through recruiting rates of larvae and anurans of the impacted ponds.

Conclusions

It was observed in this work that there is an influence of the coal mining activity on the physical and chemical characteristics of the ponds, which exert effects on the anuran community structure. In this way, we suggest long-term monitoring, considering individual species characteristics to understand the complexity of interactions and possible synergistic effects on species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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