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Recovery of brown trout populations in streams exposed to atmospheric acidification in the Bohemian Forest

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Abstract. Water chemistry and fish occurrence in outflows from two lakes recovering from acidification were studied during 2005-2010. The two lakes represented the regional extremes: while Lake Laka (LA) was the least acidified among the Bohemian Forest lakes, Lake Čertovo (CT) was the most acidified. Water chemistry improved downstream in either outlet due to mixing with less acidic water from tributaries. While pH of the LA stream was circumneutral and concentrations of ionic aluminium (Al_i) were low (< 50 µg.l^{-1}) except for temporary acidic episodes during snowmelt, the CT stream remained permanently acidified with Al_i concentrations (> 200 µg.l^{-1}) throughout the year and was considered as a reference site. Brown trout (*Salmo trutta* s.l.) survived in the LA stream already at 0.7 km from the lake. A stable trout population, including young-of-the-year fish increasing in numbers, was found in the LA stream between 2005 and 2010 despite the spring short-term acid episodes. Any recovery of the CT stream is unlikely as the outflow continued to be acidic at 1.5 km from the lake and Al_i concentrations were still by one order of magnitude higher than the limit acceptable by trout.

Key words: Czech Republic, streams, longitudinal gradients, *Salmo trutta* s.l., *Cottus gobio*

Introduction

The area of the Bohemian Forest was strongly affected by atmospheric deposition of sulphur and nitrogen compounds in the 20th century. The background data on lake water chemistry clearly documented the peak of atmospheric acidification of the Bohemian Forest lakes in the mid-1980s and certain reversal of their chemistry during the subsequent decades (Veselý et al. 1998a, b, Kopáček et al. 2002, Oulehle et al. 2013). Changes in water chemistry caused significant changes in both plankton and benthos communities that were severely reduced until the mid-1990s (Fott et al. 1994, Vrba et al. 1996, Soldán et al. 1998), exhibiting the lowest biodiversity over the 140-year period (Vrba et al. 2003, Soldán et al. 2012). Acidification caused fish extinction in all the Bohemian Forest lakes, which had been previously inhabited by indigenous brown trout (*Salmo trutta* s.l.) and at least two alien species, rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) and/or brook trout (*Salvelinus fontinalis* (Mitchill, 1814)). Ionic aluminium (Al_i) is recognized a gill toxicant to fish; its toxic action at the gills is ionoregulatory, respiratory, or a mixture of both (for review, see Gensemer & Playle 1999). Malcolm et al. (2014) suggested that aluminium has a critical role in fish recovery. The critical limit, below which the trout gills are disabled, lies between pH 4.4-5.5, depending on Al_i concentrations, i.e. on both total aluminium (Al_t) concentration and its speciation (McDonald 1983, Exley & Philips 1988).

The clear reversal of water chemistry, following the decline in atmospheric deposition with certain hysteresis, has been documented in each Bohemian Forest lake after > 85 %, > 50 %, and ~35 % reductions in emissions and deposition of sulphur (S), oxidized and reduced nitrogen (N) forms, respectively (Kopáček et al. 2011). In theory, such a remarkable drop in atmospheric deposition could release the ecosystem from acid stress. A biotic response of the lake ecosystems, however, has lagged for a decade or

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even longer. The first signs of any plankton recovery appeared in the late 1990s (Vrba et al. 2003), and continue to this day (cf. Nedbalová et al. 2006, Soldán et al. 2012, Vrba et al. 2014, 2016). No natural return of fish has been documented in any Bohemian Forest lake until 2010 (Vrba et al. 2003, 2016). In two German lakes, however, a population of brook trout (*Salvelinus fontinalis*) has established in Kleiner Arbersee, spawning in its main inflow (T. Ring, pers. comm.) and the same species has been observed in Großer Arbersee since 2010 (J. Hoch, pers. comm.; Vrba et al. 2016).

The aims of the present study are to describe the distribution of fish populations in the outflows of two Bohemian Forest lakes (Laka and Čertovo), exhibiting longitudinal gradients in water chemistry but differing in the extent of water acidification, and to evaluate the success of recovery of fish populations in these streams and probability of natural recolonization of the lakes.

Material and Methods

Study sites

Laka and Čertovo lakes are situated in the Bohemian Forest (central Europe, border area between the Czech Republic and Germany) at elevations of 1085 and 1028 m, respectively. Outlets of both Laka and

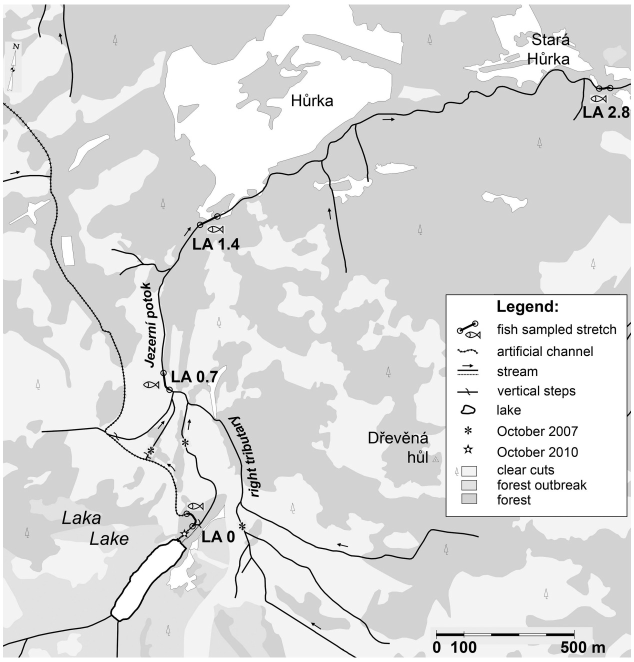

Fig. 1. A local map of the sampled stretch of the outflow of Lake Laka, the stream of Jezerní potok (LA stream in text), and its right tributary (nameless, RT in text). Asterisks (*) and star (✭) indicate the uppermost occurrence of brown trout in particular streams on 12 October 2007 and on 7 October 2010, respectively. Note the extent of forest outbreaks and clear cuts (between 2007 and 2011) in the originally forested area.

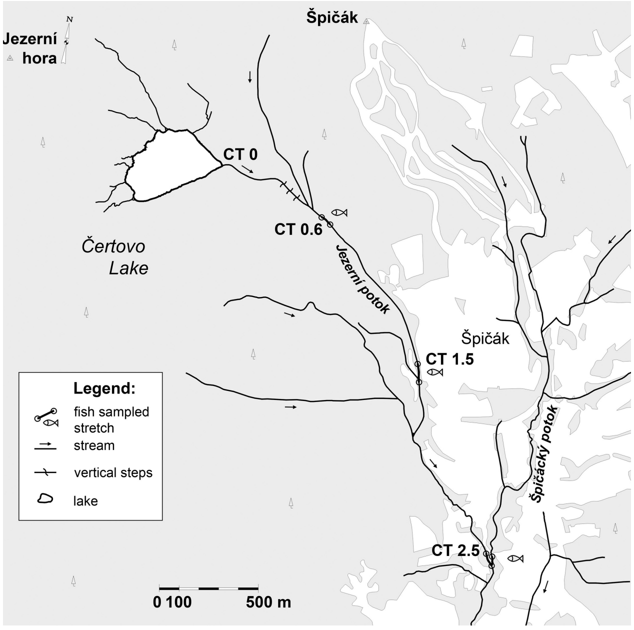

Fig. 2. A local map of the sampled stretch of the outflow of Lake Čertovo, the stream of Jezerní potok (CT stream in text), and its left tributary, Špičácký potok (SP stream in text).

Čertovo lakes are called Jezerní Potok; thus, LA and CT designate the respective stream throughout the text with numbers indicating the downstream distance (in km) from the lake, where studies on water chemistry and fish were performed (i.e. LA-0 = outlet, LA-0.7, LA-1.4, LA-2.8, Fig. 1; and CT-0 = outlet, CT-0.6, CT-1.5 and CT-2.5, Fig. 2).

A century ago, brown trout was regularly stocked into the shallow Lake Laka and its annual crop was approximately 400 specimens (Veselý 1994). Lake Laka was only temporarily acidified in the past and its chemistry has almost recovered during the last two decades (Fig. 3). The lake water has a carbonate buffering system and pH > 5.5 during most of the year (Fig. 4A, B). Clear recovery of macrozoobenthos was observed in the outlet from Lake Laka both along the longitudinal gradient and during the studied period 2005-2007 (Svobodová et al. 2012). While the lake remains fishless until present (Vrba et al. 2016), our preliminary analyses (Svobodová et al. 2012) suggested that the stream water acidity and Al_i concentrations have rapidly reduced below the critical limits for trout (McDonald 1983, Exley & Philips 1988). As the authority of the Šumava National Park supports exclusively natural development and prevents any stocking of fish to lakes, there is an opportunity to study a possible spontaneous return of brown trout (and to determine the necessary threshold conditions) to Lake Laka. The outlet from Lake Čertovo, the most acidic Bohemian Forest lake (Kopáček et al. 2002)

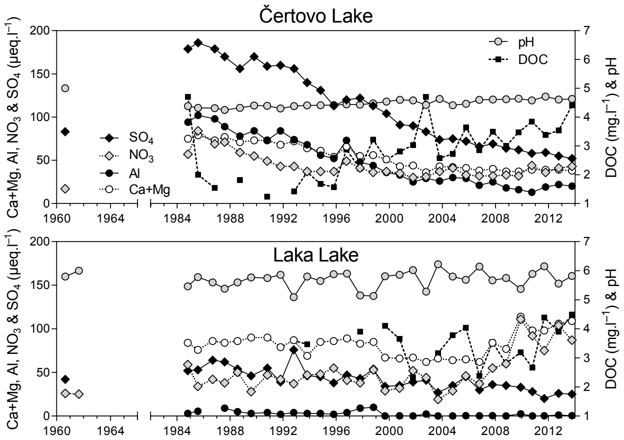

Fig. 3. Long-term trends in water chemistry (surface autumn samples) of Čertovo and Laka lakes. Data from Veselý et al. (1998a, b) and Kopáček (unpubl. data).

may be considered as a control stream, with the water chemistry disabling natural fish return (Figs. 3 and 4). The catchments of both lakes and their outflows were covered almost exclusively by Norway spruce forests for the last century. Forests in the Laka catchment were severely damaged by windthrows in 2007 and 2008 followed by bark beetle outbreak. In consequence, forest dieback affected as much as 95 % of the catchment and limited salvage logging was applied on approximately 50 % of the area (Oulehle et al. 2013). In addition, extensive salvage logging caused large clear cuts on one third of adjacent spruce forests downstream the LA outflow (see forest outbreak and clear cuts in Fig. 1). The fish sampling profiles are located at the altitudes between 906-1083 m a.s.l. and 773-940 m a.s.l. in Laka and Čertovo catchments, respectively. All the studied streams at both localities have been without any fishery management for more than 20 years, so fish populations entirely depend on natural reproduction and/or migration.

All surface water flows from Lake Laka through the artificial canal built in the 19th century for wood transportation and the original natural streambed is only supplied by seepage water below the lake. Lake water comes back to the original LA streambed through three ruptures in the artificial canal successively at distances from 0.1 to 0.7 km below the lake. Downstream, all the water flows through the slightly regulated LA streambed. A right-side tributary (RT) joins the LA stream at about 0.5 km from the lake (Fig. 1). This RT stream flows from a different catchment than tributaries of Lake Laka and has circumneutral water. Both catchments are of similar sizes and specific runoffs (24-48 $l.km^{-2}.s^{-1}$) (Kopáček, unpublished data for 2000-2013). Quantitative

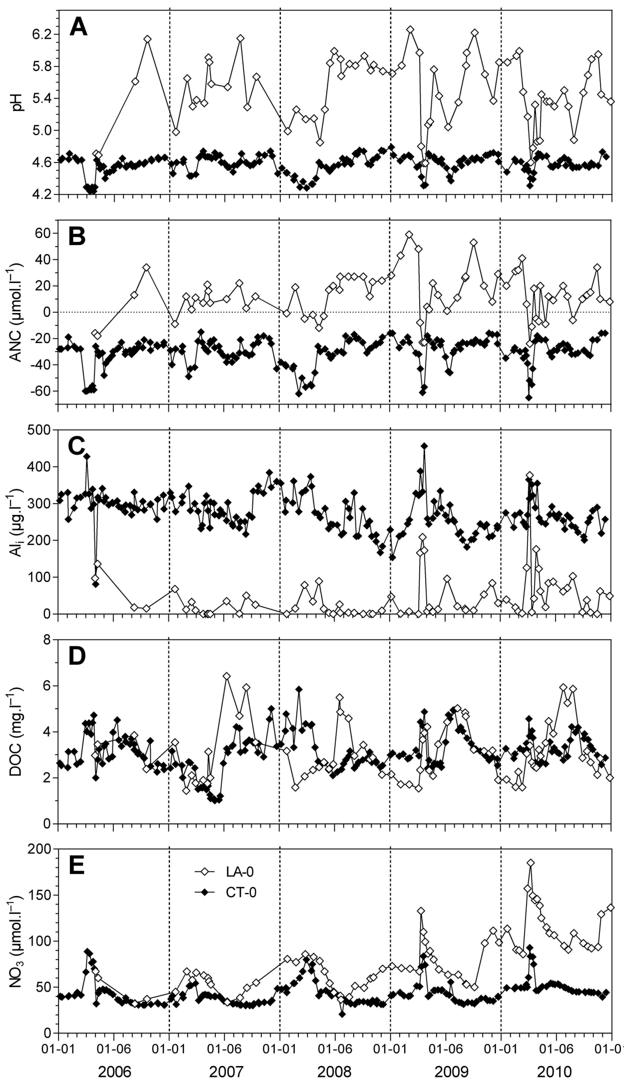

Fig. 4. Basic chemistry of the outlet water from Čertovo and Laka lakes in 2006-2010: pH (A), acid neutralizing capacity (B), and concentrations of ionic aluminium (C), dissolved organic carbon (D) and nitrate (E).

fish sampling was performed within three reaches indicated in Fig. 1, where the stream water was also sampled for chemical analyses in 2007, 2008 and 2010.

The outflow of Lake Čertovo (Fig. 2) flows through steep rocky stretch – the front of the moraine (ca. 0.4-0.5 km downstream from the lake), which forms a natural barrier for fish migration toward the lake. A left-side tributary, Špičácký Potok (SP), with very distinct water chemistry (cf. Table 1), joins the CT stream below its CT-2.5 profile. Stream water was sampled for chemical analyses along the longitudinal profile at three sites in 2007 and 2008 (Table 1); fish sampling was performed within three CT reaches and one SP reach upstream the confluence in July 2007 (Fig. 2).

Table 1. Changes in selected parameters of water chemistry of the lake outlets along their longitudinal profiles – median (minimum, maximum) values from May to October in respective years; n – number of samplings.

Parameters	code[a]	pH	ANC[d]	DOC	Ca^{2+}	Al_t	Al_o	Al_i	n
Units	km		$\mu eq.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$	$\mu g.l^{-1}$	$\mu g.l^{-1}$	$\mu g.l^{-1}$	
LA stream (Laka outlet; 2007, 2008, and 2010)									
lake outlet =	LA-0	5.8 (5.3; 6.2)	13 (8; 22)	4.1 (2.9; 6.4)	0.94 (0.66; 1.09)	120 (76; 228)	76 (50; 134)	26 (< 5; 71)	8
	LA-0.7	6.7 (6.3; 6.8)	92 (33; 104)	2.2 (1.6; 5.5)	2.23 (1.89; 2.29)	48 (30; 124)	33 (18; 107)	6 (< 5; 27)	5
	LA-1.4	6.8 (6.6; 6.9)	110 (84; 120)	3.3 (1.5; 4.9)	2.14 (1.60; 2.56)	43 (28; 115)	27 (16; 78)	5 (< 5; 31)	5
	LA-2.8	6.8 (6.7; 6.9)	111 (105; 144)	3.8 (2.6; 4.4)	2.38 (1.86; 3.02)	92 (34; 94)	14 (10; 48)	36 (5; 55)	3
RT stream[b]	RT	7.1 (6.9; 7.3)	224 (143; 276)	5.5 (3.2; 9.2)	4.65 (3.14; 5.05)	62 (36; 148)	37 (16; 85)	8 (< 5; 31)	5
CT stream (Čertovo outlet; 2007 and 2008)									
lake outlet =	CT-0	4.6 (4.5; 4.7)	−27 (−35; −17)	2.8 (1.6; 3.4)	0.39 (0.30; 0.48)	352 (302; 409)	50 (23; 66)	282 (244; 328)	4
	CT-0.6	4.6 (4.5; 4.8)	−30 (−38; −17)	3.8 (2.9; 4.1)	0.47 (0.31; 0.63)	329 (108; 446)	103 (23; 123)	178 (60; 299)	4
	CT-1.5	5.0 (4.7; 6.1)	−3 (−18; 16)	2.9 (1.3; 3.8)	0.62 (0.36; 0.67)	256 (28; 416)	71 (21; 92)	109 (4; 308)	3
SP stream[c]	SP	(7.1; 7.2)	(225; 235)	(1.56; 2.0)	(7.3; 7.9)	(18; 34)	(7; 9)	(9; 21)	2

[a] code – downstream distance (in km) from the lake outlet.
[b] RT – nameless right tributary of the LA stream at 0.5 km from the lake (see Fig. 1).
[c] SP – Špičácký potok, left tributary of the CT stream at 2.6 km from the lake (see Fig. 2).
[d] ANC – acid neutralising capacity (alkalinity, Gran titration).

Water chemistry

Autumn surface water samples were used to show long-term trends in lake chemistry (1960-2013, Fig. 3). The lake outlets were sampled regularly during 2006-2010 at 1 week to 1 month intervals to reveal seasonal variability in water chemistry and shortened sampling intervals were applied during snowmelt to track possible acid episodes (Fig. 4). Stream water and fish were usually sampled concurrently. All samples were analysed for pH, conductivity, acid neutralising capacity (ANC, Gran titration), dissolved organic carbon (DOC; TOC 5000A analyzer, Shimadzu), total phosphorus (TP; molybdate method after perchloric acid digestion), and aluminium (Al) speciation (Table 1). Total reactive Al (Al_t), total dissolved (< 0.4 µm) Al, and organically bound Al (Al_o) were analysed in unfiltered samples, filtered samples (glass-fibre filter, porosity of 0.4 µm), and cation exchanged samples after their filtration, respectively (Driscoll 1984). The Al_i was calculated as the difference between total dissolved Al and Al_o concentrations. Concentrations of major cations and anions, including nitrate (NO_3^-), sulphate (SO_4^{2-}) and divalent base cations (calcium Ca^{2+}, and magnesium Mg^{2+}) were determined by ion chromatography. The reliability of the analytical results was controlled by means of an ionic balance approach, which is a comparison between measured and calculated conductivities (Kopáček et al. 2000).

Fish

The LA stream was sampled in July 2005, 2007, 2008 and October 2007, 2008, 2010. The CT stream was quantitatively sampled only in July 2007; a qualitative fish sampling was performed at the CT-0.6 site in 2009. Fifty to 100 m long reaches were blocked with 10 mm mesh netting. The total length of fenced reaches was measured as well as the width at least at five transects to calculate the sampled wetted area of the streambed (see Figs. 1 and 2 for their localisation). Samples were taken using multiple pass electroshocking – pulsed DC 150-400/400-750 V, 1.5 kW (FEG-1500 device, Germany) with a prolonged (2.5 m) copper catode due to low conductivity of the water. Each sampling included two passes. Captured fish were measured to the nearest millimeter (fork length – Ls), weighed to the nearest gram (larger fish) or 0.1 g (smaller fish), and allowed to recover before being released at the location of capture. Scale samples were taken for age determination and calculation of growth parameters of trout (Lee 1912).

Fish densities and 95 % confidence limits were calculated using the equations of Seber & Le Cren (1967) as modified by Robson & Regier (1968). Densities were expressed as number of fish per 100 m^2 of the wetted streambed. Where data violated assumptions of these equations (only once, at CT-1.5, more fish were caught in the second run than in the first), the population density was estimated using the total catch and confidence limits were not calculated. These data must be considered as a minimum estimate of the actual stock.

Statistical analyses

Monthly averages of water chemistry data (2006-2010) of both lake outlets were compared by non-parametric Wilcoxon signed-rank test. Kruskal-Wallis test was used to find differences in fish abundance and biomass among three sampling sites along the LA stream (LA-0, LA-0.7 and LA-1.4). Spearman's rank correlation coefficients were used to describe relationships between environmental characteristics (water chemistry), distance off the lake, and fish abundance and biomass using Statistica 13 (Dell Inc. 2015).

Results

Water chemistry

The outlet from Lake Čertovo was significantly more acidic than the outlet from Lake Laka, with more than one order of magnitude higher concentrations of H^+ (on average 25 *vs.* 1.5 μmol.l^{-1}; pH: Z = 5.97, $p < 0.001$) and Al_i (on average 282 *vs.* 26 μg.l^{-1}; Z = 5.97, $p < 0.001$), with the permanently depleted carbonate buffering system in Lake Čertovo versus the positive ANC values in Lake Laka (Z = 5.97, $p < 0.001$; Table 1). While NO_3^- concentrations also significantly differed (Z = 5.97, $p < 0.001$), DOC concentrations did not (Z = 0.39, $p = 0.70$). These results were representative for water compositions of both lakes, as well as for differences between them during the whole study period (cf. Oulehle et al. 2013, Kopáček et al. 2016). Water chemistry exhibited pronounced seasonal trends in both lakes, with the lowest pH and ANC values and highest Al_i concentrations, accompanying NO_3^- leaching from soils during snowmelt periods and after heavy rains (Fig. 4). Increased values of DOC, NO_3^- and Al_i have occurred since forest dieback in the Laka catchment, caused by the windstorm in January 2007 (Fig. 4C-E). A marked increase in pH values was observed in the LA stream downstream the lake, where the right tributary with distinct water chemistry and comparable discharge (not directly measured) apparently neutralized the outflow water at LA-0.7 (Table 1). Distance from the lake was positively correlated with pH, ANC, conductivity, and some ions, and negatively correlated with Al_t concentration (Table 2). Low pH values in the CT stream increased by ~0.5 unit and Al_i concentrations decreased by ~60 % between CT-0 and CT-1.5 due to several small tributaries originating at lower elevations (and less acidified parts) of the catchment (Table 1). Mean concentrations of TP ~5.5 μg.l^{-1} (range: 0.6-10.8 μg.l^{-1}) suggested the oligotrophic character of both studied streams.

Fish

Only two species, brown trout (*Salmo trutta* s.l.) and bullhead (*Cottus gobio* Linnaeus, 1758) were found in the outflows of both lakes, with trout occurring closer to the lakes. An increasing trend in trout densities and biomass was observed in the LA stream during the investigated period 2005-2010 (Table 3, Fig. 5). Five to six age classes of trout were distinguished according to scale readings (Figs. 5 and 6).

The presence of 0+ (young-of-the-year, YOY) fish in July confirms successful natural reproduction of trout in the LA stream. In October 2007, trout were caught in different branches of the LA stream closely to the artificial canal draining most water from the lake and a spent female (releasing residual eggs) was caught in the RT stream at the elevation corresponding to the lake level (see asterisks in Fig. 1). In October 2010, an adult male (Ls = 185 mm) in spawning condition (releasing milt) was caught already in the canal near the lake (see star in Fig. 1). The autumn upstream spawning migration of trout was documented by 1.5-2 times higher densities of fish at sampling reaches LA-0.7 and LA-1.4 in October compared to July values (Table 3). The most important change in trout population was the substantial increase in the share of 0+ fish between 2008 and 2010. The YOY fish accounted for 60-80 % of the trout populations at sampling profiles LA-0.7, LA-1.4, and RT in 2010 (Fig. 5). The occurrence of bullhead was confirmed at LA-2.8 only; numbers of this species were almost equal to trout at this sampling profile.

In the CT stream, only single specimens of trout (Ls = 120 and 186 mm) were caught at the CT-1.5 reach and no fish occurred closer to the lake. Densities comparable with the LA stream were found only at

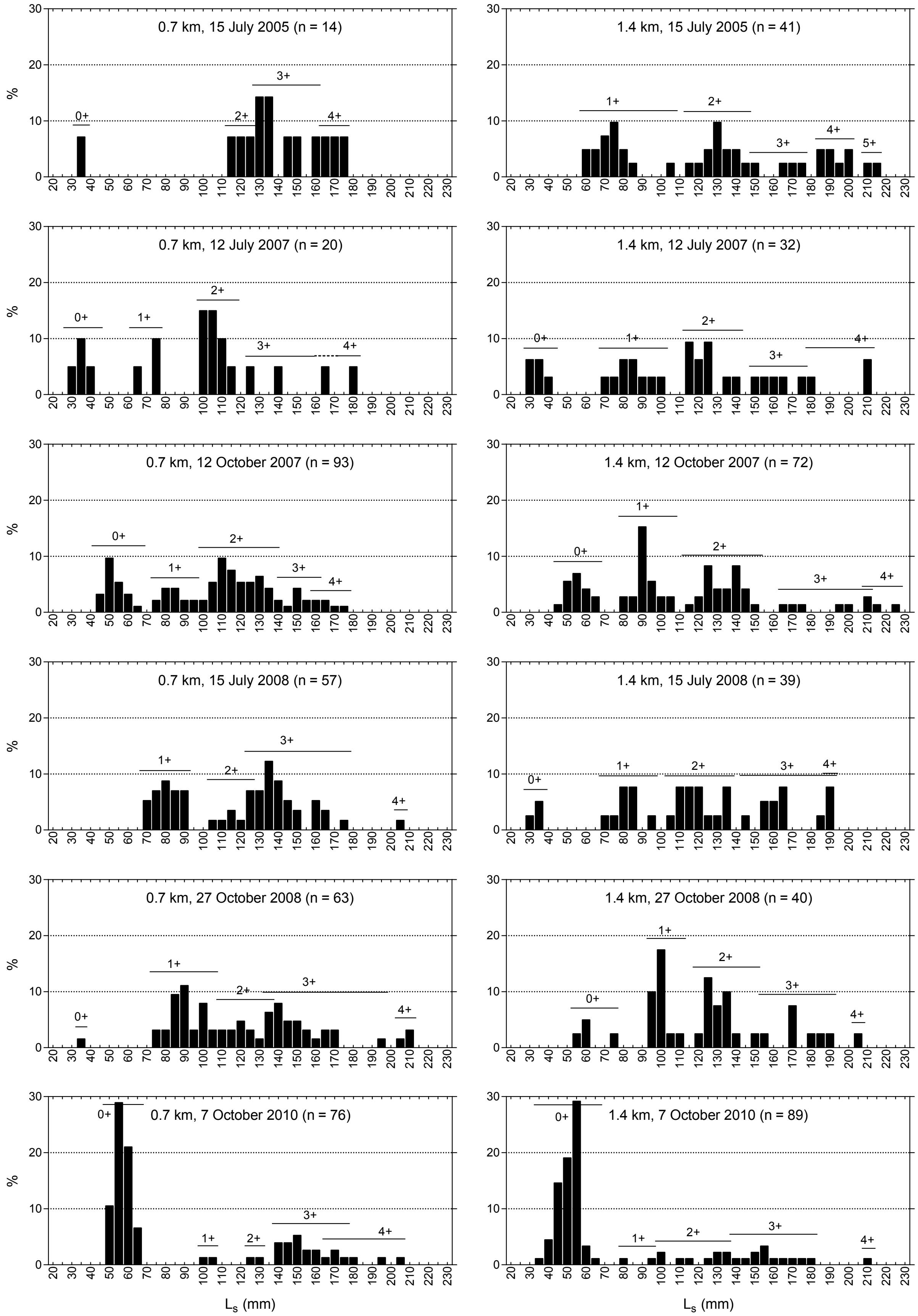

Fig. 5. Length distribution (% of n, n = number of analyzed fish) and age distribution of the trout populations sampled in the LA stream in 2005-2010.

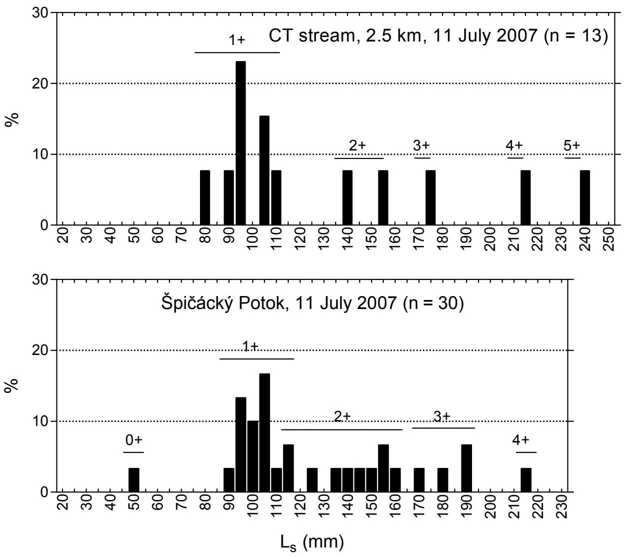

Fig. 6. Length distribution (% of n, n = number of analyzed fish) and age distribution of the trout populations sampled in the CT stream and its tributary of Špičácký potok in 2007.

Table 2. Spearman rank order correlations of brown trout and environmental parametres in the outlet of Lake Laka (LA-0, LA-0.7 and LA-1.4) in 2005-2010. Variables: FA – fish abundance; FB – fish biomass; ANC – acid neutralizing capacity; Al_i, Al_o and Al_t – ionic, organic and total aluminium, respectively; Ca^{2+} – calcium; Cond – conductivity; Dist – distance off the lake; DOC – dissolved organic carbon; Mg^{2+} – magnesium; NO_3^- – nitrate; SO_4^{2-} – sulphate; TP – total phosphorus; bold correlations are significant at $p < 0.05$.

Variables	FA	FB	pH	ANC	Cond	DOC	TP	NO_3^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Al_t	Al_o	Al_i	Dist
FA	1.00														
FB	**0.94**	1.00													
pH	**0.73**	**0.71**	1.00												
ANC	**0.75**	**0.72**	**0.96**	1.00											
Cond	**0.70**	**0.62**	**0.86**	**0.86**	1.00										
DOC	**–0.54**	**–0.65**	–0.41	–0.34	–0.40	1.00									
TP	0.21	0.08	0.36	0.31	0.46	0.08	1.00								
NO_3^-	0.01	–0.13	–0.31	–0.33	0.00	–0.09	0.12	1.00							
SO_4^{2-}	0.21	0.29	0.46	0.46	0.26	0.01	0.39	**–0.75**	1.00						
Ca^{2+}	**0.56**	0.51	**0.90**	**0.89**	**0.90**	–0.30	0.44	–0.29	**0.51**	1.00					
Mg^{2+}	**0.51**	0.36	**0.62**	**0.63**	**0.86**	–0.25	**0.77**	0.24	0.20	**0.75**	1.00				
Al_t	**–0.74**	**–0.73**	**–0.71**	**–0.63**	**–0.64**	**0.73**	–0.14	–0.13	0.00	**–0.51**	–0.45	1.00			
Al_o	**–0.68**	**–0.65**	**–0.56**	**–0.52**	**–0.65**	**0.74**	–0.14	–0.33	0.22	–0.41	**–0.54**	**0.92**	1.00		
Al_i	–0.41	–0.38	**–0.65**	–0.47	–0.42	**0.52**	–0.33	–0.01	–0.12	–0.45	–0.36	**0.76**	**0.61**	1.00	
Dist	**0.72**	**0.71**	**0.82**	**0.83**	**0.68**	–0.28	0.27	–0.40	**0.55**	**0.68**	0.44	**–0.60**	–0.43	–0.33	1.00

CT-2.5 (Table 3). No YOY fish were present in the CT stream (Fig. 6), suggesting unsuccessful natural reproduction of trout in the investigated part of this stream. YOY trout and bullhead were found only in the SP tributary with neutral water, while the 1+ age class of trout was the most numerous in the CT and SP streams (Table 3, Fig. 6). The situation remained unchanged two years later as confirmed by the point sampling at CT-0.6 in 2009.

Trout density and biomass significantly increased with distance from the lake (from LA-0 to LA-1.4; Kruskal-Wallis test, H = 9.55, $p < 0.01$). Both fish parameters were positively correlated with pH, ANC, conductivity, and some ions, but negatively with DOC and Al forms in the LA stream (Table 2).

Discussion

Due to the geologically sensitive catchments, acid deposition caused acidification of the Bohemian Forest lakes and significant changes in their biodiversity (Majer et al. 2003, Vrba et al. 2003). The Al_t concentrations reached their maxima of ~1 $mg.l^{-1}$ in the mid-1980s (Veselý et al. 1998b) and exceeded those in other acidified lake districts of the world (Driscoll et al. 1980, Jansson et al. 1986). Though the Al_t concentrations have declined by > 50 % in the strongly acidified Bohemian Forest lakes during the last two decades (cf. Nedbalová et al. 2006), Al remains the key element controlling their food web structure and plankton dynamics (Vrba et al. 2006, 2014, 2016). Here we first suggest that the current Al concentrations also control biological recovery of the streams and their potential for fish return to the lakes. Bulger et al. (1993) and Malcolm et al. (2014) found that water pH, Al_i concentration, and ANC best predicted the survival of brown trout populations. Lakes with 133 $\mu g.l^{-1}$ of Al_i, pH of 4.8, and ANC of –34 $\mu eq.l^{-1}$

Table 3. Results of fish stock (FS) estimation in the outlets of Laka and Čertovo lakes in 2005-2010. FS per reach means the calculated value from successive catches according to Seber & Le Creen (1967) at the stream profiles sampled (Figs. 1 and 2).

Stream	Streambed reach		Estimated fish stock (FS)			
Date	code	area	FS per reach		density per 100 m^2	
	stream-km	m^2	FS	(95 % conf.)	0+	older fish
LA stream (Laka outlet)						
15 Jul 2005	LA-0.7	260	16	(8-24)	0.5	5.7
	LA-1.4	280	45	(36-54)	5.6	10.5
12 Jul 2007	LA-0.7	220	65	(6-124)	6.0	23.5
	LA-1.4	279	76	(13-139)	4.1	23.1
12 Oct 2007	LA-0.7	314	129	(123-135)	9.0	32.0
	LA-1.4	279	173	(91-255)	5.0	57.0
15 Jul 2008	LA-0.7	220	63	(52-74)	9.1	19.5
	LA-1.4	279	44	(34-54)	1.3	14.5
	RT	97	6	(4-8)		
27 Oct 2008	LA-0.7	220	66	(60-71)	0.6	29.4
	LA-1.4	279	55	(24-87)	1.6	18.1
	RT	82	10	(8-13)		
7 Oct 2010	LA-0.7	315	79	(74-85)	17.6	7.5
	LA-1.4	217	123	(78-169)	43.1	13.6
	RT	120	21	(17-25)		
CT stream (Čertovo outlet)						
11 Jul 2007	CT-1.5	285	2	(n.d.)[a]	0	0.7
	CT-2.5	140	44	(0-104)	0	31.4
SP stream (above the confluence)						
11 Jul 2007	SP	175	34	(24-54)	0.6	18.8

[a] n.d. – no confidence limits were defined for total catch per reach.

had extinct brown trout populations, whereas 11 µg.l^{-1} of Al_i, pH of 6.0, and ANC of 27 µeq.l^{-1} indicated healthy brown trout populations. Lien et al. (1996) have found no damage of trout populations in Norway at ANC ≥ 30 µeq.l^{-1} and suggested ANC 20 µeq.l^{-1} as the critical lower limit based on results from several hundreds of Scandinavian lakes. When applying these thresholds, stream water chemistry in this study (Table 1) predicted the absence of trout population in the CT stream, at least upstream of CT-1.5, and its survival in the LA stream (Table 3). The median water chemistry values of the LA stream were in the range tolerated by adult brown trout, even directly below the lake (Al_i < 50 µg.l^{-1}, Table 1). On the contrary, the high Al_i concentrations (~300 µg.l^{-1}) in the CT stream obviously exceeded the threshold for brown trout (Sadler & Turnpenny 1986, Bulger et al. 1993) by one order of magnitude along the whole stream down to the confluence with the SP stream (Table 1).

The CT stream still has been strongly acidified with a depleted carbonate buffering system. Trout reproduction does not take place above the confluence with the SP stream, where only occasional migrants were caught in the CT stream.

The two lakes in our study remain fishless (Vrba et al. 2003, 2016) and represent the regional extremes: while Lake Laka was the least influenced by atmospheric acidification among the Bohemian Forest lakes, Lake Čertovo was the most acidified (Fig. 3; Veselý et al. 1998a, b, Vrba et al. 2016). The difference between their outlets has become even more pronounced along their longitudinal profiles with contrasting impacts on their community (this study, Svobodová et al. 2012).

On the other hand, the seasonal course of lake water chemistry showed that periods with low pH, negative ANC and high concentrations of NO_3^- and Al_i occurred also in the Laka outlet during spring snowmelt (Fig. 4). Yet these spring peaks of acidity and Al_i differ in their extents and duration from year to year; hence, they differently affect the success of trout reproduction in the LA outflow upstream the RT confluent. This tributary supplies similar amounts of neutral (pH = 6.5-7.0) water that has increased the outflow pH by at least one unit at LA-0.7 even during acid spring episodes in all years studied. Moreover, the RT stream with circumneutral water can serve as a refugium for fish to avoid acid episodes.

The forest dieback in the LA catchment also caused a delay in chemical recovery from acidity and even temporarily reversed the ongoing recovery trends for some constituents (e.g. NO_3^-, Ca^{2+}, and Al_i; Fig. 4 and Oulehle et al. 2013). Experience from similarly affected sites in the Bavarian Forest National Park (Huber 2005, Vrba et al. 2014), shows that high variability in stream water chemistry (Fig. 4) should last only for several (5-8) years, and then it should return to the previous trend before 2007, as it has recently been confirmed (Kopáček, unpubl. data). The stable trout population in the LA stream indeed suggests that fish are able to survive short episodes of harmful conditions. The strong 0+ cohort at LA-0.7 and LA-1.4 in 2010 (Fig. 5) clearly documents that these fish had to survive the acid spring episode in this year (Fig. 4). In contrast, perennial elevated Al_i concentrations in the CT stream clearly form a bottleneck for fish reproduction and recovery (cf. Malcolm et al. 2014).

YOY trout were caught in the LA-0.7 reach in all the years investigated (Fig. 5). Hence, two hypotheses can be formulated: (i) trout spawn successfully in the RT stream, with circumneutral pH (Table 1), and all the offspring originates from this tributary; or (ii) trout spawn successfully in the LA stream. We documented the autumn upstream spawning migration of trout in this stream in October (Fig. 1). Slavík et al. (2012) found the maximum of the annual migrating activity of brown trout in October with a total distance ranging from several dozens of metres to approximately 4 km for individually marked fish in the same river basin as we studied. If the second hypothesis can be proven, then natural re-colonisation of Lake Laka by trout is likely as a single male was caught directly below the lake in the derivation canal in 2010 and no obstacles prevent the further migration to the lake. Until present, however, there is no evidence of fish occurrence in Lake Laka, whereas an abundant *Chaoborus* population suggested that this lake remained fishless during the studied period (Vrba et al. 2016).

The bullhead was found only at the lowest reaches studied. While McCahon & Pascoe (1990) ranked it as rather acidotolerant, our results may indicate that bullhead is more vulnerable to acidification than brown trout. Moreover, the upstream migration ability of bullhead is much lower compared with trout (Utzinger et al. 1998). Hence, its recovery will likely be slower compared to trout.

The present study is the first survey of fish populations in the outflows of two Bohemian Forest lakes and the first attempt to explore a potential of acidified headwater streams for fish recovery in this strongly acidified region. Our results confirm recent occurrence of brown trout and bullhead in the studied streams. Moreover, the LA streambed and its current water chemistry offer a good opportunity to study natural return of fish to Lake Laka that is very likely in the next future. The important result is that the forest dieback and/or clearcut of ~95 % of the area in the LA catchment and the following chemical changes of surface waters could adversely affect fish populations but did not hamper their general recovery from atmospheric acidification.

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