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RESEARCH PAPER

Impact of multiple stressors on the fish community pattern along a highly degraded Central European river – a case study

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Abstract. In this study, we provide a descriptive assessment of how chemical and hydro-morphological stressors have affected the fish community along one of the most impacted rivers in Central Europe. In addition to the toxicity of combined pollutants (expressed in toxic units), a range of hydro-morphological characteristics were measured to assess which stressors have had an impact. No longitudinal spatial trend was observed in fish assemblage characteristics as individual sites were affected by different stressors. Instead, five largely artificial assemblage "zones" were identified corresponding to different combinations of stressors. Water quality (principally dissolved O_2) and hydromorphology were the main drivers affecting fish presence and density, with self-purification processes, restocking from tributaries and geomorphology promoting fish survival and/or recovery, despite increasing toxic pressure downstream. Our results suggest that a) toxic units alone are insufficient to establish causative factors in fish community loss as they do not take account of hydro-morphological stressors, many of which interact with and/or mask each other, and b) that a single WFD monitoring site in such heavily impacted rivers is insufficient to assess ecological status; rather, the ecological status of specific "zones" (identified based on fish assemblage structure, habitat and water quality) should be assessed, with the ultimate aim of merging the zones and returning the river to a single functioning longitudinal ecosystem, accepting that this is unlikely to resemble the natural pre-industrial status of the river.

Key words: channelisation, fish assemblage, oxygen sag, river zonation, toxic pollution

Introduction

Water resources in Europe are subject to strong anthropogenic stressors (i.e. water pollution, habitat degradation, loss of connectivity and flow modification) resulting in poor ecological status and loss of biodiversity. Assessments of stressor impact, however, are hampered by severe gaps in our knowledge of cause-and-effect relationships between single or multiple stressors and biodiversity loss (e.g. Niimi 1990, Rose 2000, van der Geest et al. 2002, Pont et al. 2007, Segner 2007, Couillard et al. 2008).

In assessing the ecological status of a water body, the impact of all stressors should be

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integrated, whether present simultaneously or separated in space and time. Even without new inputs of contamination, for example, changing environmental conditions may affect transport, distribution and bioavailability of contaminants. Conversely, some toxic chemicals may modify exposure of aquatic species to other stressors by affecting distribution, behaviour or habitat use (Couillard et al. 2008). Importantly, the combined impact of multiple stressors is not only a function of magnitude, frequency or duration but also of species character, i.e. the ability of an ecosystem or species community to resist the stressor(s) (Wenger et al. 2010, Jacquin et al. 2020).

Traditionally, toxic chemicals are considered the main stressor in river systems. Ecotoxicological studies, however, have tended to concentrate on the effects of single (or a limited set of) compounds on single species and, even now, most regulatory methods rely on single-substance risk evaluations in combination with basic toxicological models to predict the joint effect of chemical mixtures on single species (de Zwart & Posthuma 2005). Such model-based methods, however, rarely reflect the complexity of natural biological environments. One attempt to address this has been the MODELKEY project (Brack et al. 2005), which has developed models to reflect the impact of multiple toxic inputs on indicator species.

Fish are one of five biological indicators (along with benthic invertebrates, macrophytes, benthic algae and phytoplankton) presently used for ecological status assessment (Simon 1999, European Commission 2006). Such biological indicators are expected to behave in a predictable manner to anthropogenic stressors, allowing classification of ecological quality based on their functional response to a stressor (European Commission 2006, Sandin & Solimini 2009). Due to their complex ecological requirements, fish are sensitive indicators for habitat quality at various spatial scales, each species having specific requirements for breeding, feeding, growth, recruitment and survival (Balon 1975, Schiemer & Waidbacher 1992). However, while higher level responses (i.e. population or community level) attributable to single or multiple stressors have been relatively well-defined for invertebrates, they are less well known for fishes (Elliott 1994, Walker et al. 1996, Elliott & Hemingway 2002, Posthuma & de Zwart 2005).

In this study, we examine the effect of multiple stressors on the fish community along perhaps the most impacted river in Central Europe, the River Bílina (Czech Republic). In doing so, we assessed the effect of multiple toxic pollutants, water temperature, dissolved O_2, pH, conductivity and a range of hydro-morphological characteristics (depth, current velocity, substrate) on a range of fish community measurements (presence, density, species richness and community structure).

Information on the River Bílina fish community is extremely limited, there having been no full fish-stock data published since the 1940s and fishing data from the end of the 20th century being scarce (Svobodová et al. 1993). Further, only one site on the River Bílina is regularly monitored under the EU Water Framework Directive (WFD; between sites 15 and 16; Fig. 1). In addition to providing a deeper understanding of the processes acting on the river's fish population, therefore, this study also provides a first in-depth overview of fish status along the River Bílina, allowing more effective management in the future.

Material and Methods

Study site

Originating from springs in the Krušné hory Mountains (785 m a.s.l.; North Bohemia, Czech Republic), the River Bílina (watershed area 1,070.9 km^2; length 84.2 km), a tributary of the River Elbe, drains the Czech-German border region north-west of Prague (Fig. 1).

Historically a trout river throughout its length, the River Bílina has been heavily impacted by a high concentration of agriculture, heavy industry, brown-coal mining and associated power plants and chemical industries since at least the Second World War (Carter & Turnock 1996). Despite a reduction in pollution since the 1990s, the River Bílina remains the most polluted and impacted river in the Czech Republic, and probably Central Europe (Ministry of Agriculture of the Czech Republic 2007). In addition to inputs of toxic pollutants, the river's geomorphology and hydrology have been heavily altered. Two reservoirs interrupt the main channel, the 16 ha Jirkov drinking-water Reservoir in the upland trout-zone section (river km 72.7) and the 152 ha Újezd Reservoir in the lowland trout-zone (river km 66.8), used for water retention, flood protection

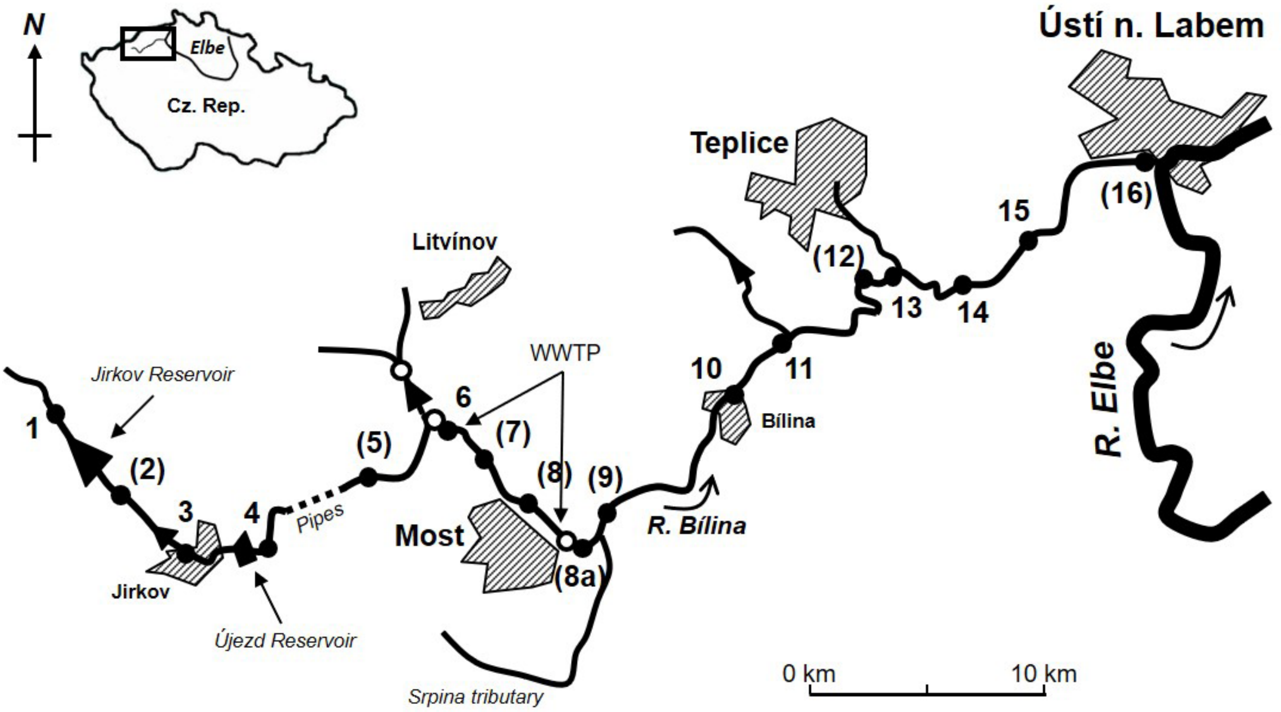

Fig. 1. Schematic map of the River Bílina (Czech Republic). Numbers/black circles = sampling sites (sites in parentheses were used for toxic unit analysis), hatched areas = main urban sites, empty circles = weirs, dotted line = diversion piping with turbine, WWTP = wastewater treatment plant.

and recreation (see Vlček et al. 1984; Fig. 1). The overall length of the impoundments is about 2 km, representing around 2.3% of the river's length. The river channel has been diverted several times to make way for opencast coal mining and a 3 km stretch presently flows through piping (four pipes, each of 2 m diameter) with a turbine at their outlet. The stretch between the Újezd Reservoir and the diversion pipes has been channelised with boulder banks or concrete stabilisation.

Two major wastewater treatment plants (WWTPs) discharge into the river between river km 55.4 and 53.2, the largest being the Litvínov-Záluží WWTP servicing the town of Litvínov (population 27,397) and its associated industrial area (oil refinery and chemical), which discharges from five closely-spaced outlets. Estimates based on discharge data for the River Bílina and the WWTP suggest the proportion of contaminated wastewater downstream of the Litvínov WWTP can reach up to 50% at low discharge. Outflow from the second WWTP outlet, serving the downstream town of Most, maximally increases the proportion of wastewater (mainly municipal) by another 15% (G. Streck, unpublished data) and adds sorption surfaces (bulking sludge was observed in the river during both sampling periods). Approximately 300 m downstream (river km 46.8), a weir hinders fish migration and removes particulate matter through sedimentation. At river km 45.3, the Srpina tributary increases discharge by approximately 50%. Agriculture, particularly around tributaries and the lower reaches of the River Bílina, results in diffuse organic run-off that contributes to eutrophication.

The River Bílina connects with the River Elbe at the town of Ústí nad Labem (132 m a.s.l.), which supports a large chemical industry. In the past, wastewater discharges here contained significant levels of chlorinated compounds and high concentrations of chlorobenzenes (CBs), 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT), hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs), and elevated values are still found in sediments and water at the River Bílina's mouth (Heininger et al. 2004, Stachel et al. 2005). Polycyclic aromatic hydrocarbons (PAHs) are also suspected to play an important role in the River Bílina due to continuing input from brown coal mining, oil refining and power production (Stachel et al. 2005).

Because of the high risk of poisoning, only limited fish stocking is performed by the local Anglers Association (T. Kava & M. Urych, in litt.). Hence, fish assemblages in the main river are expected to originate primarily from natural reproduction.

Table 1. Sample sites surveyed along the River Bílina in 2006 and 2007 with their respective habitat variables. Ds = downstream, Slope = ratio of depth and distance to the bank (1 m), CV = coefficient of variance (in %), velocity = current velocity, veg = vegetation cover (in %), mod = modification type (N = natural-like, F = channelised with fast current, S = channelised with slow current), impact = stressors/mitigators immediately upstream of site.

no.	site	river km	altitude (m a.s.l.)	coordinates	slope	depth (m)	depth CV	velocity ($m.s^{-1}$)	velocity CV	veg	mod	impact
1	Orasín	75.8	542	50°31′59″ N 13°23′58″ E	0.23	0.22	70	0.26	117	0	N	
2	Jirkov – Ds of reservoir	72.0	359	50°30′04″ N 13°25′21″ E	0.23	0.22	48	0.56	66	10	N	Jirkov Reservoir
3	Jirkov – Ds of pond	70.3	332	50°29′43″ N 13°26′06″ E	0.40	0.41	40	1.00	49	11	N	pond, urban
4	Ds of reservoir	65.2	277	50°30′45″ N 13°29′18″ E	0.55	0.66	22	0.89	17	0	F	Újezd Reservoir, urban
5	Komořany	59.0	231	50°31′51″ N 13°33′35″ E	0.51	0.72	30	0.63	45	0	F	pipes with turbine
6	Ds of Jiřetín weir	55.4	231	50°32′52″ N 13°35′34″ E	0.59	0.79	26	0.22	65	0	S	weir, tributary
7	Litvínov-Záluží	53.2	230	50°32′33 ″ N 13°37′00″ E	0.92	1.25	20	0.29	51	26	S	WWTP
8	Most	49.3	229	50°30′55″ N 13°38′59″ E	0.69	0.94	17	0.40	62	96	S	
9	České Zlatníky	45.1	209	50°30′49″ N 13°41′54″ E	0.58	1.11	34	0.28	66	37	S	WWTP, weir, urban, tributary
10	Bílina	35.4	198	50°33′16″ N 13°46′24″ E	0.54	1.11	29	0.31	37	78	S	urban
11	Hostomice	28.6	196	50°34′44″ N 13°48′15″ E	0.53	0.72	22	0.38	53	52	S	
12	Lbín	22.5	178	50°36′23″ N 13°51′29″ E	0.45	0.61	29	0.92	52	47	F	
13	Velvěty	18.5	172	50°36′21″ N 13°53′39″ E	0.68	0.86	19	0.34	49	43	S	tributary
14	Brozánky	12.9	160	50°36′35″ N 13°56′24″ E	0.52	0.83	27	0.86	43	22	F	
15	Stadice	9.7	154	50°37′12″ N 13°58′26″ E	0.53	0.66	26	1.46	31	3	F	
16	Ústí nad Labem	0.2	136	50°39′24″ N 14°02′18″ E	0.38	0.48	28	0.78	44	23	N	contaminated sediment

Water quality and hydro-morphological habitat parameters

Water temperature (°C), dissolved O_2 (mg/l), pH and conductivity (µS/cm) were measured at each of the 16 sites using a WTW multi 350i multimeter (WTW, Weilheim, Germany) at the same time as fish sampling. Habitat characteristics were assessed using the standard Instream Flow Incremental Methodology protocol (Orth & Maughan 1982). Depth was determined using a measuring rod (cm) and current velocity (m/s) using a digital MiniAir20 flow measurement device (Schiltknecht, Switzerland). For each station, scores were calculated for a) relative proportion of silt, sand, fine gravel, course gravel, cobbles, boulders or bedrock, b) percentage vegetation cover, c) mean depth, d) velocity and slope (measured 1 m from the bank; see Fig. 2) and e) within-site variability (coefficient of variance – CV) of depth and velocity (data summarised in Table 1).

Chemical sampling and analysis

More detailed descriptions of the sampling process and analysis of organic pollutants have been published elsewhere (Streck et al. 2008, Wenger et al. 2010). Briefly, the aqueous phase was sampled continuously using semi-permeable membranes deployed for periods of between 21 and 23 days between June and July of 2007. Sampling was restricted to eight sites (2, 5, 7, 8, 8a, 9, 12 and 16; Fig. 1) downstream of WWTP outlets or main tributaries (where changes in pollution extent and pattern were most likely to occur) as the samples also formed part of other studies. All sampling sites corresponded with the respective fish sampling sites, except site 8a (Most-Chanov), which was located downstream of the Most WWTP but upstream of the tributary (Fig. 1). This additional site was chosen to better distinguish pollution originating from the WWTP and to observe any dilution effect from the tributary, a pilot screening study having confirmed that this river section was without fish.

In total, 115 compounds were analysed, 51 of which (including PAHs, PCBs, CBs, DDT and musk compounds) had concentrations above the limit of detection and were subsequently used for assessing toxicity (see Streck et al. 2008). The musk compounds HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran) and AHTN (6-Acetyl-1,1,2,4,4,7-hexamethyltetraline) served as indicators for municipal/domestic wastewater pollution.

Characterisation of risk using toxic units (TU)

A more detailed description of the process for characterising risk has been published elsewhere (Streck et al. 2008, Wenger et al. 2010). Briefly, for a first estimation of risk, toxicity values for a standard test organism (fathead minnow *Pimephales promelas*) were calculated using quantitative structure activity relationships and ecological structure activity relationships (ECOSAR) software (US EPA 2009). No-observed-effect concentrations (NOECs) corresponding to chronic toxicity (30 days, mortality as endpoint) were estimated using ECOSAR, allowing calculation of both baseline toxicity values ($NOEC_{baseline}$) and excess toxicity ($NOEC_{excess}$). Toxic units were calculated for both values as a ratio of the measured concentration (C) and the toxicity value for each compound (subscript "i") using the formula: $TU_i = C_i/NOEC_i$.

Baseline toxicity describes narcosis as a non-specific mode of action exhibited by every chemical. The combined effect of all compounds in a sample, therefore, can be estimated by concentration addition. Consequently, the sum of $TU_{baseline}$ values for each sample was calculated as: $\Sigma TU_{baseline} = \Sigma_i TU_{i\ baseline}$.

In risk assessment, the general method for deriving a predicted-no-effect concentration from a NOEC value for a single compound is through multiplication with an assessment factor. If a long-term NOEC for fish is available, the EU's technical guidance document on risk assessment recommends a factor of 100 (European Commission 2003). In general, as resultant $\Sigma TU_{baseline}$ values increase, total combined toxicity of compounds in the river also increases. Values are displayed as negative $\Sigma TU_{baseline}$ (i.e. decreasing values indicate decreasing water quality). Applying an assessment factor of 100, negative $\Sigma TU_{baseline}$ values would indicate a toxic risk if values are < 2.

Fish population survey and sampling

Sixteen sites were sampled along the river's length in July of 2006 and 2007, with site 1 situated 6.5 km from the source spring and site 16 at the town of Ústí nad Labem, 0.2 km upstream of the confluence with the River Elbe (Fig. 1, Table 1). Site 6, downstream of the Jiřetín weir and upstream of the Litvínov-Záluží WWTP outlets, was added in 2007 in order to further define the source of habitat and pollution stressors identified in 2006.

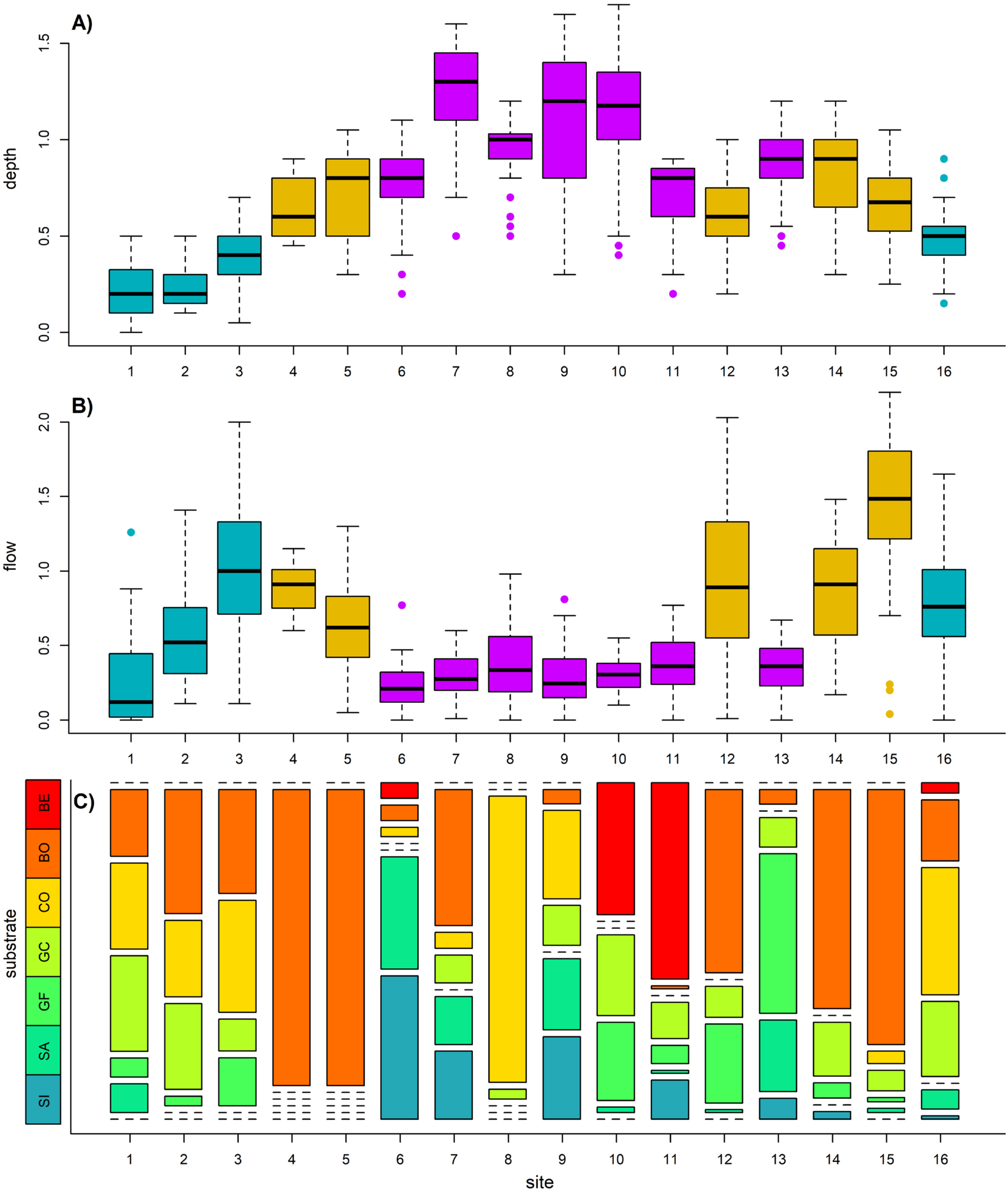

Fig. 2. Values for the most important habitat features at each of the 16 sites sampled along the River Bílina in 2007: A) depth, B) flow, C) substrate. Panels A) and B): vertical bar = median, box = interquartile range, whiskers = non-outlier range (1.5 × interquartile range), points = outliers, box and point colours correspond to *a priori* determined habitat types (green = natural-like, orange = channelised fast flow, purple = channelised slow). Panel C): proportion of each of seven substrate types. BE = bedrock (or concrete), BO = boulders, CO = cobbles, GC = course gravel, GF = fine gravel, SA = sand, SI = silt.

Fish were sampled using single-pass continual electrofishing (backpack type SEN, 220-240 V, 1.5-2 A, 80-90 Hz) as per the requirements of the WFD (CEN 2003). Two anodes were used in stretches wider than 5 m. At each sample site, fish were prevented from escaping upstream by natural (shallow riffles, boulder ramp) or artificial (weir, stop-net) transversal barriers. In general, the

River Bílina was shallow enough (maximum 0.8 m) to allow electrofishing by wading. Only at site 8, downstream of the Jiřetín weir near the town of Most, was sampling undertaken from a small boat due to a thick layer of soft sediment on the river bottom. At all sites, fish were immediately identified on the bank and released back into the water. Data are presented as species richness and population density (ind./ha) and relative community structure (% relative abundance).

Statistical analysis

We identified three groups of predictors *a priori* that could influence fish assemblages: 1) habitat modification type (three-level categorical predictor); 2) pollution (characterised by $TU_{baseline}$ units); and 3) water quality (O_2 and temperature only as a) no pH data were available for 2007, and b) conductivity showed a strong correlation with temperature (Pearson correlation moment, $R = 0.79$, $df = 14$, $P < 0.001$). Where necessary, $TU_{baseline}$ values for each fish sampling site (only calculated for sites 2, 5, 7, 8, 9 and 16) were interpolated as the mean of up- and downstream values. As $TU_{baseline}$ values were strongly correlated with longitudinal gradient (river km; Pearson correlation moment, $R = 0.87$, $df = 13$, $P < 0.001$), river km was not included in the analysis. Replacement of $TU_{baseline}$ units by river km in our models caused no change in the final results of the stepwise regression (results not presented).

The effect of each predictor on the fish assemblage was analysed at four levels: a) presence/absence of fish (disregarding species), b) species richness, c) relative density (ind./ha, disregarding species), and d) community structure.

For levels a-c, the effects of multiple predictors were tested using generalised linear models (GLM), with binomial (Bernoulli) models used for fish presence and Poisson models for species richness and density (using fish count as response variable and area sampled as offset parameter). If detected, the models were modified for overdispersion (quasi-Poisson, quasi-binomial models). For each of the three response variables, the full models consisted of four predictors, i.e. O_2, temperature, $TU_{baseline}$ units and habitat modification type.

The full models were simplified through stepwise removal of redundant variables based on likelihood ratio tests comparing models with and without the variable.

We used cluster analysis (complete linkage, Bray-Curtis distance, cut at 95% of dendrogram height) in order to discriminate groups of sites that differed in fish community structure (response variable d).

All statistical analyses were undertaken using R version 2.7.1 (R Foundation for Statistical Computing, Vienna, Austria), using the "vegan" (Oksanen et al. 2012) and "stats" (R Core Team 2012) packages.

Results

Habitat and water quality parameters

The three *a priori* habitat modification groups (i.e. natural-like, channelised fast flow, channelised slow flow) were distinguishable from each other in the PCA ordination as they each possessed different habitat features (Fig. 3; PC axis 1 explaining 32.1% of data variation and PC axis 2 19.9%). Natural sites were distinguishable from modified sites by low depth and slope and high depth variation, while fast-flowing and slow-flowing channelised sites were distinguishable by current and substrate (Figs. 2, 3).

In June 2006, dissolved O_2 dropped to 2.4 mg/l downstream of the Litvínov WWTP, with subsequent downstream recovery relatively linear (Figs. S1, S2). Dissolved O_2 gradually improved to 3.3 mg/l at site 13, 34.7 km downstream of the WWTP. In 2007, however, concentrations dropped to below the critical level for fish survival (0.08 mg O_2/l), with O_2 remaining well below 8 mg/l until just above site 16 (Figs. S1, S2). In general, patterns for conductivity, an indicator of inorganic mineral load, were opposite to those of O_2 (Fig. S2), with levels peaking dramatically just downstream of the Litvínov-Záluží WWTP, reaching 1,400 μ/cm in 2006 and just under 1,100 μ/cm in 2007. In both years, downstream levels settled at around 1,000 μ/cm. In 2006, water temperatures rose gradually from around 12 °C at site 1 to around 16.5 °C around the Litvínov WWTP, downstream of which temperatures rose rapidly to around 23 °C and remained so up to the River Elba confluence (Fig. S2). In 2007, temperatures were generally a little higher than in 2006, though the pattern was similar (Fig. S1). Water pH values remained relatively neutral between sites with a mean of 7.4 (range 6.3-7.9). Impacts of reservoirs, tributaries and the Most WWTP, while visible in the data, were nevertheless small (Fig. S2).

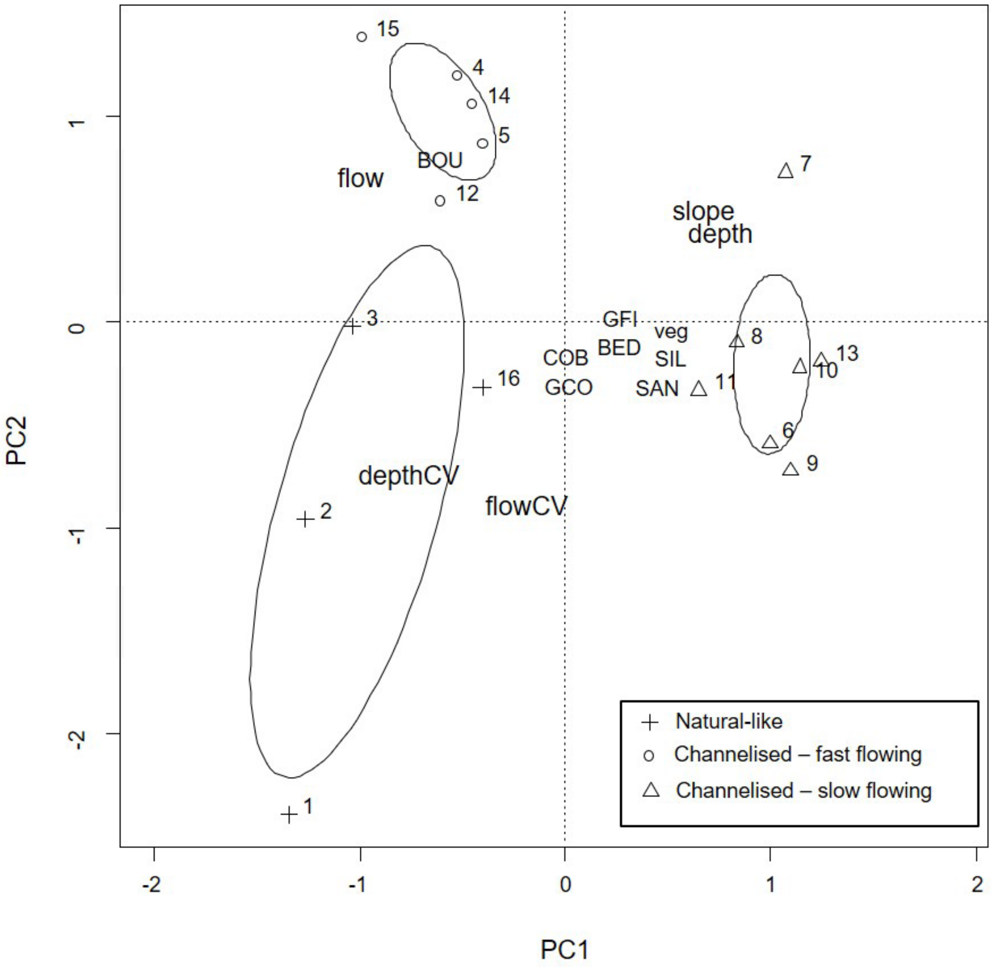

Fig. 3. PCA ordination of sites (numbers) according to stream morphology parameters (abbreviations), plotted in direction of increasing value with relation to the first two PCA axis gradients. PC axis 1 explains 32.1% of variation and PC2 19.9%. Ellipses denote 95% confidence limit of the group centroids. SIL = silt, SAN = sand, GFI = fine gravel, GCO = course gravel, COB = cobbles, BOU = boulders, BED = bedrock, veg = vegetation, depth = mean depth, depth CV = depth variability, flow = mean water velocity, flow CV = velocity variation, slope = mean lope.

Chemical analysis

The main source for PAHs in the River Bílina was the Litvínov-Záluží WWTP (Fig. 4a), along with pollution products from industrial activities such as oil refining. PAH concentrations increased more than five-fold downstream of the WWTP, with concentrations decreasing with increasing distance downstream. Moderate PCB levels were recorded, but these showed a steady increase from the uppermost site to the confluence with the River Elbe (Fig. 4b). CBs showed a pronounced increase at Ústi nad Labem (Fig. 4b), with the bulk of this increase attributable to HCB, though tetra-chlorobenzenes and pentachlorobenzenes also showed enhanced levels. The two musk compounds, HHCB and AHTN, were found at all sampling sites (Fig. 4c), the profile pointing toward the Most WWTP as the main entrance pathway, followed by the Litvínov-Záluží WWTP.

The sum of $TU_{baseline}$ values indicated increasing toxicity along the downstream profile (Fig. 4d). In general, the river could be separated into three distinct zones: the upstream stretch, with a negative log $\Sigma TU_{baseline}$ of approximately 3.2; the middle stretch, dropping to 2.7 following input of wastewater from the Litvínov-Záluží WWTP; and the lower stretch, dropping to 2.4 downstream of the Most WWTP. There was no further change in $\Sigma TU_{baseline}$ downstream to the confluence with the River Elbe, despite changes in chemical composition. At all sites, 2,6-diisopropylnaphthalene contributed most to the $\Sigma TU_{baseline}$. Along the upstream stretch, DDT and sum of benzofluoranthenes were next most important, with HHCB musk compounds, introduced via WWTPs, second most important at all other sites (Fig. 4c). Other compounds, such as PCBs, PAHs and other musk compounds, were also strong contributors to $\Sigma TU_{baseline}$ values. At all

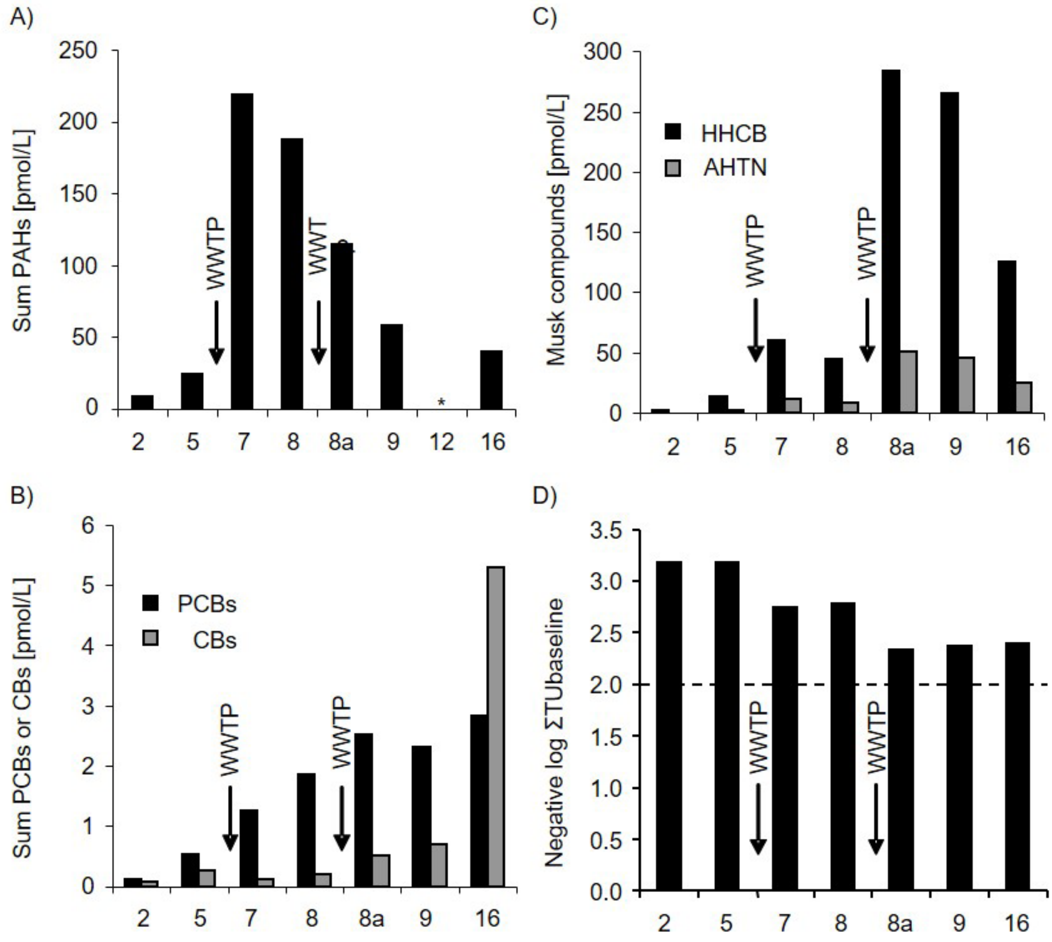

Fig. 4. Distribution of selected compound classes sampled with semi-permeable membrane devices along the River Bílina. A) Dissolved polycyclic aromatic hydrocarbons (PAH); * = no data available. Sum of PAHs includes: acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluroanthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[cd]pyrene, dibenzo[ah] anthracene, benzo[ghi]perylene. B) Sum of polychlorinated biphenyls (PCB) and chlorobenzenes (CB). Sum of PCBs includes: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180, PCB-194. Sum of CBs includes: 1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene. C) The musk compounds HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8 hexamethylcyclopenta-gamma-2-benzopyran) and AHT (6-Acetyl-1,1,2,4,4,7-hexamethyltetraline). D) Negative log ($\Sigma TU_{baseline}$) values along the longitudinal profile of the River Bílina in summer 2007. The dotted line indicates the $\Sigma TU_{baseline}$ value at which a toxicity risk to fish (*Pimephales promelas*) could be expected with an assessment factor of 100. For site names see Table 1.

sites the negative log $\Sigma TU_{baseline}$ did not drop below 2 (Fig. 4d).

Fish assemblage

A total of 786 1+ and older fish (370 in 2006 and 416 in 2007) from 22 species were recorded from the 16 sampling sites (Table 2, Table S1). Cluster analysis identified several distinct groups of fish assemblages along the river (Fig. 5). Cluster 1, including sites 1-3 from the headwater stretch of the river, represented a typical "trout-zone" assemblage. Sites up to the Újezd Reservoir (1-3) had low species diversity but high density (Fig. 6a, b; Fig. S1), with sites 1 and 2 comprising brown trout only, and site 3 showing occasional occurrence of five other species in 2006 (Table S1). Cluster 2 (sites 4-6) comprised typical "lowland cyprinid river" assemblage-types, dominated primarily by perch and roach, with rheophilous species rarely caught, and trout not at all, below site 4 (Table S1). In 2006, the highest fish density (4,494 ind./ha) of any site or year was recorded at site 4 between the Újezd Reservoir and the diversion piping, with roach and perch as the dominant species. A similar but less pronounced peak was observed in 2007 (Fig. 6b, Table S1). Species richness, however, remained similar over the whole site cluster (Fig. 6a). Downstream of the diversion piping, fish density was about 100 times lower than upstream in 2006 (approx. six times lower in 2007; Fig. 6b, Table S1). Cluster 3 (not shown in Fig. 5) comprised sites where no fish were caught (sites 7, 8 and 10, and

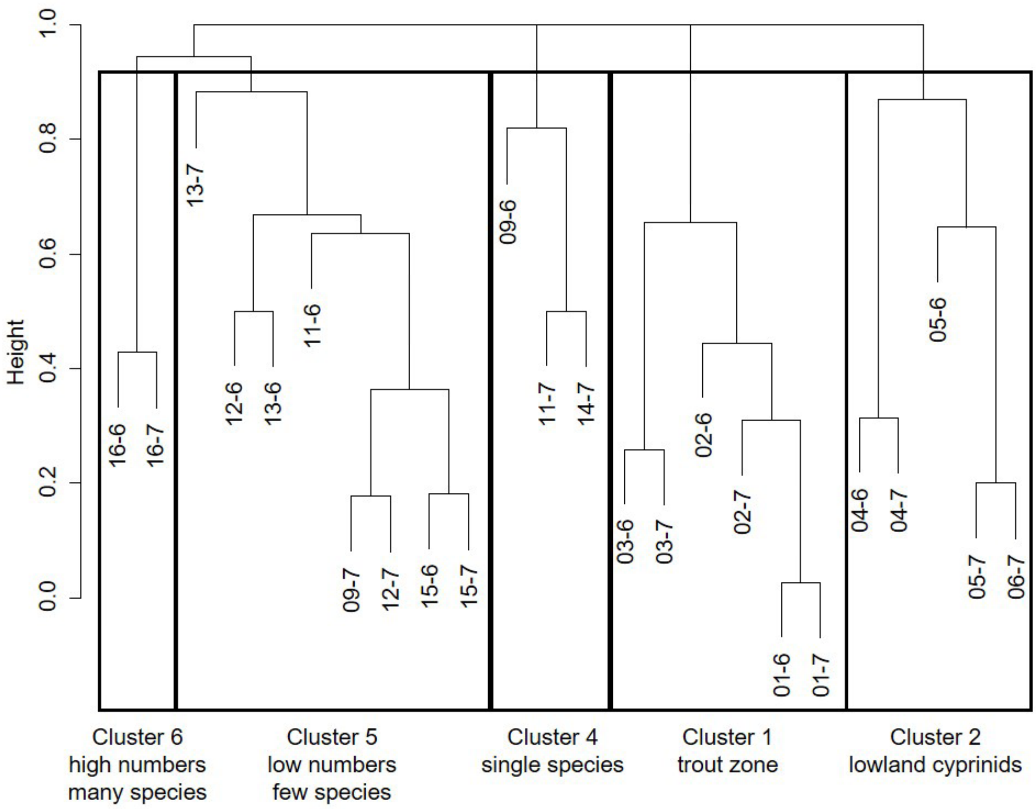

Fig. 5. Results of cluster analysis based on similarity of fish assemblages between samples (Bray-Curtis distance, complete linkage). Rectangles discriminate clusters based on a 95% cut of dendrogram height. Sites are displayed as "site-year". Fish were not sampled at site 6 in 2006. No fish were caught at sites 07-6, 07-7, 08-6, 08-7, 10-6, 10-7 and 14-6, hence they were not considered during cluster analysis (arbitrarily forming cluster 3).

site 14 in 2006). Clusters 4 and 5 were similar, with cluster 4 represented by single-species assemblages (sites 11 and 14 in 2007) and a single outlying site (site 9) dominated by low numbers of eel in 2006, and cluster 5 represented by assemblages with low numbers of few species (site 9 in 2007, and sites 11-15; Fig. 6b). Cluster 6 was represented by a single site (site 16) close to the confluence with the River Elbe, which maintained a rich and dense fish assemblage (12 species, dominated by chub, gudgeon and roach) with varied age classes and increased numbers of rheophilous species (Fig. 6a, b; Table S1).

Effect of multiple stressors

Dissolved O_2 was the only significant predictor explaining fish presence and density, the other predictors being eliminated during the stepwise process (Fig. 7, Table 3, Table S2). None of the predictors had a significant effect on species richness (Fig. 7, Table 3, Table S2).

Discussion

The "coarsest" fish community indicators in this study, fish presence and density, were explained solely by O_2. Dissolved O_2 is a defining and limiting parameter for fish, affecting survival, growth, spawning, swimming performance, larval development and migration behaviour (Doudoroff & Shumway 1970, Chapman 1986). Fish populations on the River Bílina disappeared completely following a major drop in O_2 below the Litvinov WWTP (site 7), recovering only slowly from site 10 onwards. The O_2 sag observed below the Litvínov WWTP is indicative of high levels of organic pollution and/or the release of incompletely treated sewage, the increase in organic matter resulting in an increase in biological oxygen demand (Hynes 1960). The gradual reappearance of fish populations along the River Bílina was supported by "donated" fish and a gradual increase in O_2 levels caused by inflow from the Srpina and other tributaries. Thus, tributaries improve local (and downstream) water quality, act as refuges against declining conditions in the main river and act as sources of new individuals to restock the main channel (Hitt & Angermeier 2008).

Lack of predictor effect on species richness was most likely connected with the different assemblages

Table 2. List of fish species caught along the River Bílina in 2006 and 2007. Ecological guilds after Schiemer & Waidbacher (1992). For fish dominance (in %) at sites along the River Bílina in 2006 and 2007, see Table S1.

code	scientific name	common name	ecological guild
	Salmonidae		
ST	*Salmo trutta m. fario*	brown trout	rheophilic
	Esocidae		
EL	*Esox lucius*	pike	eurytopic
	Cyprinidae		
RR	*Rutilus rutilus*	roach	eurytopic
LL	*Leuciscus leuciscus*	dace	rheophilic
SC	*Squalius cephalus*	chub	rheophilic
LI	*Leuciscus idus*	ide	rheophilic
AU	*Aspius aspius*	asp	rheophilic
TT	*Tinca tinca*	tench	limnophilic
GG	*Gobio gobio*	gudgeon	rheophilic
GL	*Romanogobio belingi*	northern whitefin gudgeon	rheophilic
BB	*Barbus barbus*	barbel	rheophilic
AA	*Alburnus alburnus*	bleak	eurytopic
BJ	*Abramis bjoerkna*	silver bream	eurytopic
AB	*Abramis brama*	common bream	eurytopic
VV	*Vimba vimba*	vimba	rheophilic
CA	*Carassius gibelio*	Prussian carp	eurytopic
CY	*Cyprinus carpio*	carp	eurytopic
	Balitoridae		
NB	*Barbatula barbatula*	stone loach	rheophilic
	Anguillidae		
AN	*Anguilla anguilla*	eel	eurytopic
	Percidae		
PF	*Perca fluviatilis*	perch	eurytopic
GC	*Gymnocephalus cernuus*	ruffe	eurytopic
SL	*Sander lucioperca*	pikeperch	eurytopic

encountered in different parts of the river. Upper trout zone assemblages in unimpacted streams, for example, usually consist of no more than two species, independent of any stressor (Adámek & Jurajda 2001), while lowland cyprinid zones, which naturally harbour high species richness, will still carry more species following anthropogenic impact than unimpacted upper trout zones, as in the case of the artificially created cyprinid zones along the River Bílina. Fish species richness, therefore, is a poor indicator for predicting impact of multiple stressors at a larger scale.

Table 3. Steps of backward stepwise regression for models predicting fish density, richness and presence at 15 sites on the River Bílina in 2007. *P* to remove = *P* value of log-likelihood tests comparing the model with the simpler one (i.e. *P* to remove the term missing in the next model).

response	model	predictors	*P* to remove
density	full	oxygen + habitat + TU + temperature	0.478
	m1	oxygen + habitat + TU	0.079
	m2	oxygen + habitat	0.541
	final	oxygen	0.012
richness	full	oxygen + habitat + TU + temperature	0.136
	m1	oxygen + habitat + TU	0.972
	m2	oxygen + habitat	0.952
	m3	oxygen	0.208
presence	full	oxygen + habitat + TU + temperature	0.692
	m1	oxygen + habitat + TU	0.643
	m2	oxygen + habitat	0.118
	final	oxygen	< 0.001

Similarity in fish community structure, the “finest” community indicator used in our study, was best explained through hydro-morphological zonation of the river due to anthropogenic impact and O_2. Distinct fish assemblage types were distinguished by cluster analysis, resulting in five zones with different community structure (corresponding to four breakpoint sites; Table 4). While the effect of hydro-morphological zonation could not be proved directly, it was clear that changes in hydromorphology and O_2 corresponded directly with changes in fish assemblage (Table 4).

Cluster 1 (sites 1-3), representative of a natural headwater trout zone (Svobodová et al. 1993) with reproducing populations of brown trout, had low to negligible levels of habitat degradation, high O_2 and low temperatures and, as none of the stressors analysed in this study (i.e. habitat degradation, water quality parameters or toxicity) affected this section, the sites can be taken as a reference for past conditions over much of the river. Cluster 2, downstream of the Újezd Reservoir up to the Jiřetín weir (sites 4-6), is indicative of a sudden switch from upland trout zone to a typical lowland cyprinid zone dominated by generalist or eurytopic species (perch and roach; Table 2). The cluster is subject to two strong

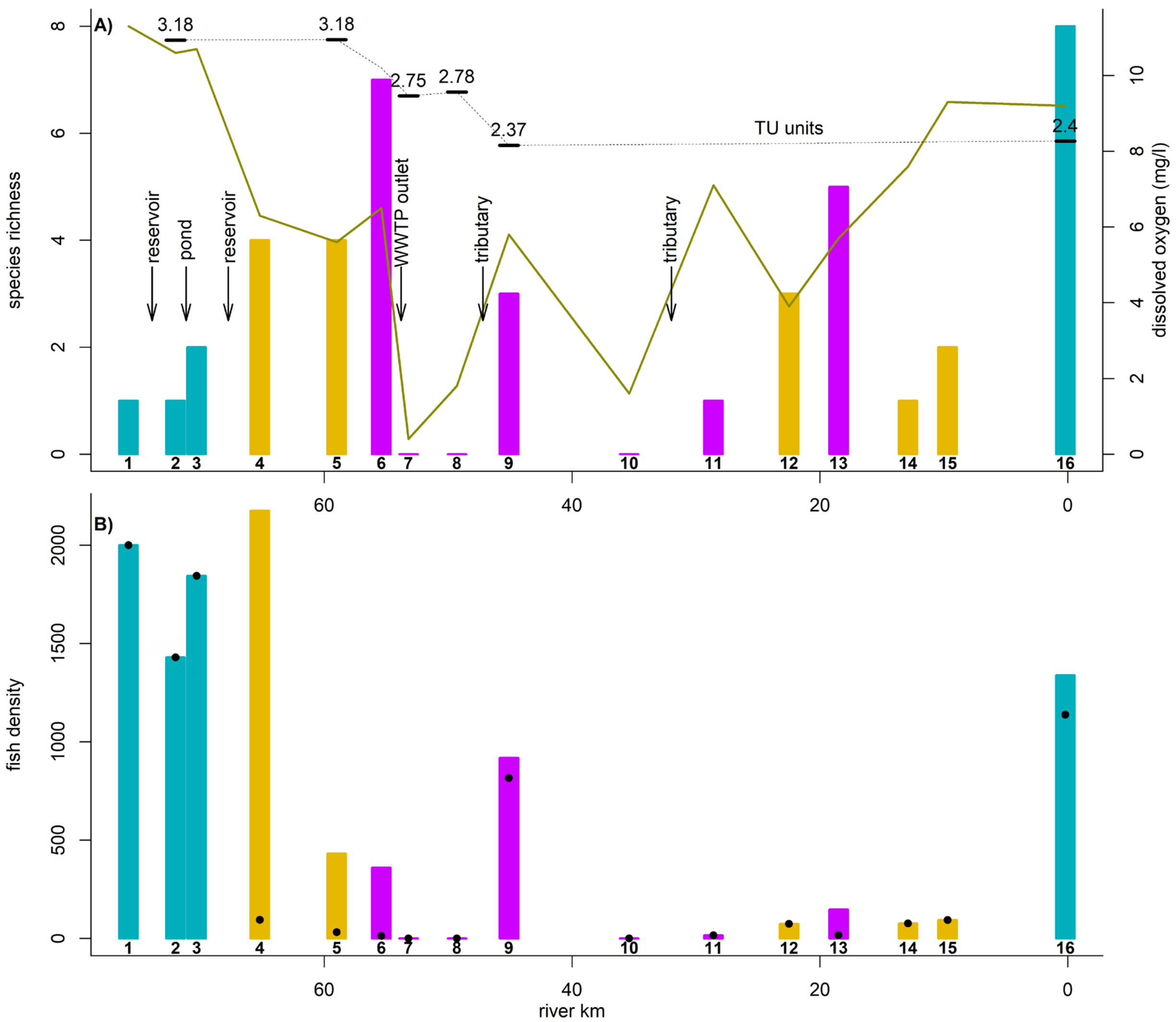

Fig. 6. A) Fish species richness, B) population density (ind./ha) for sites sampled along the River Bílina in 2007. Bar colours correspond to *a priori* determined habitat types (green = natural-like, orange = channelised fast flow, purple = channelised slow). The x-axis shows river km and position of sample sites. Concentration of dissolved oxygen (mg/l) and TU are presented in panel A. The dotted line represents predicted TU values approximated from measured (black lines with numbers) values. The black points in panel B show the population density of rheophilic species (ind./ha). The approximate positions of the main stressors/mitigators are indicated in panel A.

hydro-morphological stressors: 1) channelisation, which has, amongst other things, removed the gravel substrate and variable flow preferred by rheophilic species for reproduction (Jurajda et al. 2001), and 2) the 3 km diversion pipes/turbine below site 4 (Peter 1998), through which fish cannot migrate. As such, the population is most likely maintained through either fish escapes or juvenile drift from the reservoir. As toxicity levels remained low in this section, and water quality levels changed only marginally, the Újezd Reservoir and habitat degradation appear to be the most important stressors affecting fish community structure in this section. Cluster 3 represented sites with no fish, all in the middle zone of the river. These sites were downstream of the WWTP outlets, below which water quality declined dramatically, with an increase in toxicity and a sharp drop in dissolved O_2. Poor habitat availability along this channelised stretch will also have contributed to the decline in fish. As O_2 is a limiting parameter for fish, however, low levels tend to mask the effects of habitat, water quality or toxicity. Cluster 4 (single-species assemblages) and cluster 5 (low density assemblages) were both dominated by chub and gudgeon (Table S1). This lower stretch (sites 9 and 11-15) was characterised by self-purification processes resulting in a steady increase in O_2, contributing to increased macroinvertebrate (Orendt et al. 2012) and fish populations (Table 3, Fig. 6a, Fig. S1). As habitat quality along this stretch did not differ from that at sites 7-10, and $TU_{baseline}$ toxicity actually improved (Figs. 4, 6a), increasing water quality (mainly O_2) appears to be the one

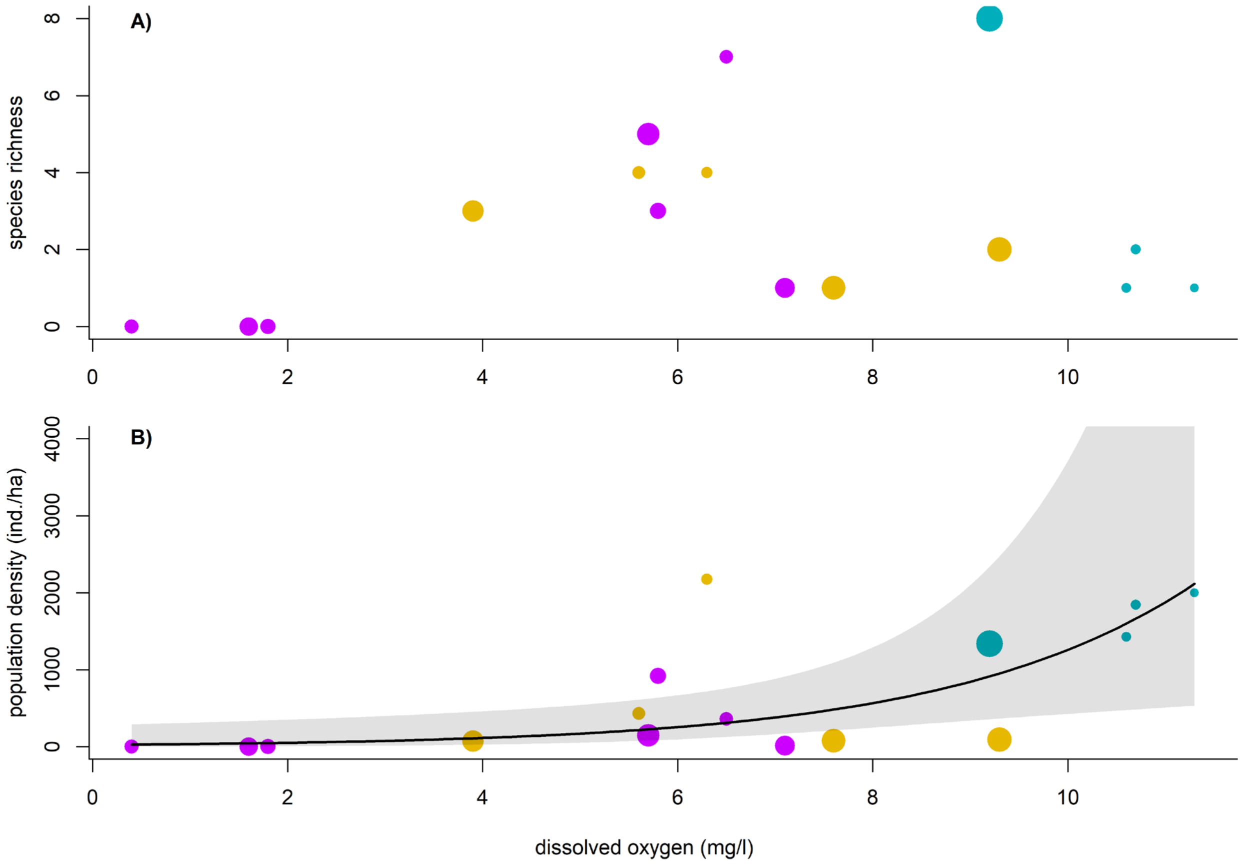

Fig. 7. Effect of dissolved oxygen concentration on species richness (upper panel) and population density (lower panel) on the River Bílina in 2007. Curve predicted by GLM with 95% confidence intervals shown for the density model only, where the effect of dissolved oxygen was significant. Point colours correspond to habitat types (green = natural-like, orange = channelised fast flow, purple = channelised slow), point size corresponds to distance from source (site 1 = smallest point, site 16 = largest point). A binomial model predicting fish presence is not shown due to 0/1 probabilities: no fish occurred at three sites below (sites 7, 8 and 10), and occurred at all sites above, an interval of 1.80-3.90 mg/l.

factor initiating recovery of fish assemblages. Cluster 6 (confluence with the River Elbe) had a fish assemblage typical of a lowland cyprinid zone with high species richness and density dominated by chub, gudgeon and roach of varied age classes (Table S1). As water quality and levels of toxic compounds at the site were as bad, if not worse, than those at upstream sites with almost no fish, improved geomorphological character (e.g. riffles, natural banks) appears to have been the main factor responsible for the change in community structure. High fish species richness at this site is also supported by fish immigration from the River Elbe (as with the tributaries) and an increase in the number and diversity of macroinvertebrates (Jurajda et al. 2010, Orendt et al. 2012), once again connected to improved geomorphological character and O_2 levels. Unfortunately, data on temporal variability of the fish community (individuals) is lacking and it remains unclear whether the fish caught at site 16 are permanently resident in this stretch, temporary residents that move to-and-from the River Elbe or are immigrants that are being prevented from further upstream movement by the deep and fast-flowing waters upstream (site 15). Both our own experience and

Table 4. The four "breakpoint" sites separating five zones with different community structure identified through cluster analysis. Each breakpoint is associated with an assemblage shift corresponding to a unique combination of stressor shifts.

breakpoint	assemblage shift	water quality shift	toxicity shift	hydromorphology shift
site 4	salmonid > cyprinid	no	no	yes
site 7	cyprinid > "none"	yes	yes	no
site 10	"none" > "poor"	yes	no	no
site 16	"poor" > "rich"	no	no	yes

that of our fisheries colleagues, however, suggests that, while all three may be true to some extent, the high species richness and density and the wide range of age/size classes suggests that the fish at this site/zone are predominantly resident. As much of the middle and lower stretch of the river now appears more suitable for cyprinids than trout, it is quite likely that these fish could go on to repopulate upstream sites were it not for the barrier created by the deeper, fast-flowing stretch immediately upstream. This further suggests that hydromorphology and water quality (O_2) are more important than toxic components in dictating fish presence/absence.

The River Bílina has been heavily impacted by a high concentration of urban sewage, heavy industry, brown-coal mining and associated power plants and chemical industries. Toxic pollution, therefore, was expected to be the main stressor dictating fish ecological status along the river. In our study, however, toxic pressure appears not to have been strong enough to adversely affect fish ecological status, suggesting that toxic chemical pollution was unlikely to be the main driver/stressor affecting fish population status along the River Bílina (though it may have been in the past when inputs were higher). It is worth noting here that a number of studies have found that sum of toxicity units tends to underestimate effluent toxicity (Bervoets et al. 1996, Guerra 2001); hence toxicity cannot be ruled out as a contributing factor, particularly at downstream sites. Nevertheless, our data strongly suggests that fish population status was most strongly affected by O_2 concentration and hydro-morphological zonation, rather than pollution. Similarly, Yuan & Norton (2003) noted the importance of substrate and riparian habitat quality on macroinvertebrates and fish, both as refuge habitat and as pollutant "filters". Further, Comte et al. (2010) found that water quality characteristics were more important than exposure to toxicants in predicting macroinvertebrate presence in rivers subject to multiple stressors, while Posthuma & de Zwart (2005) noted that a fish's response to toxicity depended on other stressors, such as habitat quality and O_2 concentration. Finally, Hitt & Angermeier (2008) have previously highlighted how lack of river-stream connectivity can have a negative effect on bioassessment performance.

Our results suggest that fish bioassessment surveys using toxic units, while able to identify increasing "toxic potential" along the river's course, are unable to establish causative factors impacting fish at the population and community level (at least on such highly degraded rivers such as the River Bílina) as they do not take account of other hydro-morphological stressors, many of which interact with and/or mask each other. While still useful for identifying fish unfit for human consumption, they should be used with care when assessing the reasons for species presence/absence. Secondly, the WFD sampling approach presently in use in the Czech Republic (i.e. a single monitoring site per river) is clearly inadequate for assessing the ecological status of rivers as heavily impacted as the River Bílina. Our results clearly show that a range of strong anthropogenic stressors (i.e. water pollution, habitat degradation, loss of connectivity and flow modification) has resulted in a number of largely artificial assemblage "zones" corresponding to different combinations of stressors, each hosting a specific fish assemblage (or lack thereof) unconnected with natural river "zonation". In such cases, it is highly unlikely that the river as a whole can be brought back to a "good ecological status". We suggest that future WFD monitoring efforts in such heavily impacted rivers need first to identify the main anthropogenic stressors, then assess the ecological status of each "zone" identified based on the fish assemblage present, and finally to implement improvement measures within each zone separately (taking account of other confounding factors such as up- to downstream gradient), with the ultimate aim of merging the zones and returning the river to a single functioning longitudinal ecosystem (accepting that this is unlikely to resemble the natural pre-industrial status of the river).

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were undertaken to strict Czech standards of animal welfare. Author contributions: P. Jurajda and G. Streck designed the study and obtained all permissions to fish. P. Jurajda, G. Streck, M. Janáč and Z. Jurajdová participated in the sample collection and habitat characterisation. M. Janáč undertook the statistical analysis. K. Roche wrote the paper, which was reviewed and approved by the co-authors.

Literature

Adámek Z. & Jurajda P. 2001: Stream habitat or water quality – what influences stronger fish and macrozoobenthos biodiversity? *Ecohydrol. Hydrobiol. 1: 305–311.*

Balon E.K. 1975: Reproductive guilds of fishes: a proposal and definition. *J. Fish. Res. Board Can. 32: 821–864.*

Bervoets L., Baillieul M., Blust R. & Verheyen R. 1996: Evaluation of effluent toxicity and ambient toxicity in a polluted lowland river. *Environ. Pollut. 91: 333–341.*

Brack W., Bakker J., de Deckere E. et al. 2005: MODELKEY – models for assessing and forecasting the impact of environmental key pollutants on freshwater and marine ecosystems and biodiversity. *Environ. Sci. Pollut. Res. 12: 252–256.*

Carter F.W. & Turnock D. 1996: Environmental problems in Eastern Europe: new updated edition. *Routledge, London.*

CEN 2003: Water quality – sampling of fish with electricity. *European Committee for Standardization, Brussels.*

Chapman G. 1986: Ambient water quality criteria for dissolved oxygen. *U.S. Environmental Protection Agency, Washington.*

Comte L., Lek S., De Deckere E. et al. 2010: Assessment of stream biological responses under multiple-stress conditions. *Environ. Sci. Pollut. Res. 17: 1469–1478.*

Couillard C.M., McDonald R.W., Courtnay S.C. & Palace V.P. 2008: Chemical-environmental interactions affecting the risk of impacts on aquatic organisms: a review with a Canadian perspective – interactions affecting exposure. *Environ. Rev. 16: 1–17.*

de Zwart D. & Posthuma L. 2005: Complex mixture toxicity for single and multiple species: proposed methodologies. *Environ. Toxicol. Chem. 24: 2665–2675.*

Doudoroff P. & Shumway D.L. 1970: Dissolved oxygen requirements of freshwater fishes. *Food and Agriculture Organization of the United Nations, Rome.*

Elliott M. 1994: The analysis of macrobenthic community data. *Mar. Pollut. Bull. 28: 62–64.*

Elliott M. & Hemingway K.L. 2002: Fishes in Estuaries. *Blackwell, Oxford.*

European Commission 2003: Technical guidance document on risk assessment Part II: environmental risk assessment. *European Commission, Ispra.*

European Commission 2006: Indicators and methods for the ecological status assessment under the water framework directive: linkages between chemical and biological quality of surface waters. *European Commission, Ispra.*

Guerra R. 2001: Ecotoxicological and chemical evaluation of phenolic compounds in industrial effluents. *Chemosphere 44: 1737–1747.*

Heininger P., Pelzer J., Claus E. & Pfitzner S. 2004: Results of long-term sediment quality studies on the river Elbe. *Acta Hydrochim. Hydrobiol. 31: 356–367.*

Hitt N.P. & Angermeier P.L. 2008: River-stream connectivity affects fish bioassessment performance. *Environ. Manag. 42: 132–150.*

Hynes H.B.N. 1960: The biology of polluted waters. *Liverpool University Press, Liverpool.*

Jacquin L., Petitjean Q., Cote J. et al. 2020: Effects of pollution on fish behaviour, personality, and cognition: some research perspectives. *Front. Ecol. Evol. 8: 1–11.*

Jurajda P., Adámek Z., Janáč M. & Valová Z. 2010: Longitudinal patterns in fish and macrozoobenthos assemblages reflect degradation of water quality and physical habitat in the Bílina river basin. *Czech J. Anim. Sci. 55: 123–136.*

Jurajda P., Reichard M., Hohausová E. & Černý J. 2001: Comparison of 0+ fish communities between two differently modified stretches of the River Morava. *Arch. Hydrobiol. Suppl. 135: 187–202.*

Ministry of Agriculture of the Czech Republic 2007: Report on the state of water management in the Czech Republic in the year 2006. *Ministry of Agriculture of the Czech Republic, Praha.*

Niimi A.J. 1990: Review of biochemical methods and other indicators to assess fish health in aquatic ecosystems containing toxic chemicals. *J. Gt. Lakes Res. 16: 529–541.*

Oksanen J., Blanchet F.G., Kindt R. et al. 2012: Community ecology package. R package version 2.0-3. *http://CRAN.R-project.org/package=vegan*

Orendt C., Wolfram G., Adámek Z. et al. 2012: The response of macroinvertebrate community taxa and functional groups to pollution along a heavily impacted river in Central Europe (Bílina River, Czech Republic). *Biologia 67: 180–199.*

Orth D.J. & Maughan O.E. 1982: Evaluation of the incremental methodology for recommending instream flows for fish. *Trans. Am. Fish. Soc. 111: 413–445.*

Peter A. 1998: Interruption of the river continuum by barriers and the consequences for migratory fish. In: Jungwirth M., Schmutz S. & Weiss S. (eds.), Fish migration and fish bypasses. *Fishing News Books, Vienna: 99–112.*

Pont D., Hugueny B. & Rogers C. 2007: Development of a fish-based index for the assessment of river health in Europe: the European fish index. *Fish. Manag. Ecol. 14: 427–439.*

Posthuma L. & de Zwart D. 2005: Predicted effects of toxicant mixtures are confirmed by changes in fish species assemblages in Ohio, USA, rivers. *Environ. Toxicol. Chem. 25: 1094–1105.*

R Core Team 2012: R: a language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria.*

Rose K. 2000: Why are quantitative relationships between environmental quality and fish populations so elusive? *Ecol. Appl. 10: 367–385.*

Sandin L. & Solimini A. 2009: Freshwater ecosystem structure-function relationships: from theory to application. *Freshw. Biol. 54: 2017–2024.*

Schiemer F. & Waidbacher H. 1992: Strategies for conservation of a Danubian fish fauna. In: Boon P.J. (ed.), River conservation and management. *Wiley, New York: 363–382.*

Segner H. 2007: Ecotoxicology – how to assess the impact of toxicants in a multi factorial environment? In: Mothersill C., Mosse I. & Seymour C. (eds.), Multiple stressors: a challenge for the future. NATO science for peace and security series. *Springer, Dordrecht: 39–56.*

Simon T.P. 1999: Assessing the sustainability and biological integrity of water resources using fish communities. *CRC Press, Boca Raton.*

Stachel B., Jantzen E., Knoth W. et al. 2005: The Elbe flood in August 2002 – organic contaminants in sediment samples taken after the flood event. *J. Environ. Sci. Health A 40: 265–287.*

Streck H.G., Schulze T. & Brack W. 2008: Accelerated membrane-assisted clean-up as a tool for the clean-up of extracts from biological tissues. *J. Chromatogr. A 1196–1197: 33–40.*

Svobodová Z., Vykusová B., Máchová J. et al. 1993: Monitoring of foreign substances in fishes from the Bílina River. *Bull. VÚRH Vodňany 4: 123–132.*

US EPA 2009: ECOSARTM Version v1.00a. *United States Environmental Protection Agency, Washington D.C.*

van der Geest H.G., Soppe W.J., Greve G.D. et al. 2002: Combined effects of lowered oxygen and toxicants (copper and diazinon) on the mayfly *Ephoron virgo*. *Environ. Toxicol. Chem. 21: 431–436.*

Vlček V., Kestřánek J., Kříž H. et al. 1984: Water courses and reservoirs. *Academia, Praha.*

Walker C.H., Hopkin S.P., Sibly R.M. & Peakall D.B. 1996: Principals of ecotoxicology. *Taylor & Francis Inc., Bristol.*

Wenger M., Ondračková M., Machala M. et al. 2010: Assessing relationships between chemical exposure, parasite infection, fish health, and fish ecological status: a case study using chub (*Leuciscus cephalus*) in the Bílina River, Czech Republic. *Environ. Toxicol. Chem. 29: 453–466.*

Yuan L.L. & Norton S.B. 2003: Comparing responses of macroinvertebrate metrics to increasing stress. *J. N. Amer. Benthol. Soc. 22: 308–332.*

Supplementary online material

Table S1. Fish dominance (in %) at sites along the River Bílina in 2006 and 2007 (sites 7, 8 and 10 were fishless; site 6 was only sampled in 2007).

Table S2. Models predicting the effect of dissolved oxygen on fish presence, richness and density at 15 sites on the River Bílina in 2007.

Fig. S1. A) Fish species richness, B) population density (ind./ha) for sites sampled along the River Bílina in 2006. Bar colours correspond to *a priori* determined habitat types (green = natural-like, orange = channelised fast flow, purple = channelised slow). The x-axis shows river km and position of sample sites (note no sampling at site 6). Concentration of dissolved oxygen (mg/l) is presented in panel A. The dotted line represents predicted dissolved oxygen approximated from the nearest measured point (marked by points). Black points in panel B mark population density of rheophilic species (ind./ha). The approximate positions of the main stressors/mitigators are indicated in panel A.

Fig. S2. Mean water temperature (°C), dissolved O_2 (mg/l), pH and conductivity (μS/cm) measured during fish sampling along the River Bílina in 2006 (left) and 2007 (right). x-axis = site. Note: no pH data available for 2007. The approximate position of the wastewater treatment plants (WWTP) and the Srpina tributary are indicated.

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