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Mayflies (Ephemeroptera) as indicators of environmental changes in the past five decades: a case study from the Morava and Odra River Basins (Czech Republic)

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ABSTRACT

1. Large-scale and intensive human activities have caused widespread and profound changes in the diversity and structure of biological communities. Historical or long-term data are an important tool for the quantification and understanding of temporal changes. A comparison of Ephemeroptera collected at 60 streams in the Morava and Odra river basins in two periods, 1955–1960 and 2006–2011, enabled an estimate of changes in their assemblages induced by various human pressures to be made.

2. The taxonomic composition of mayfly assemblages was substantially dissimilar between the two periods, although the changes were not associated with a decline in regional or local diversity. Only lowland rivers lost several of their original species, mostly habitat specialists. Species replacement, a leading driver of dissimilarity, caused shifts towards more simplified, less specialized assemblages in large rivers, and towards more pollution- and siltation-tolerant assemblages in small rivers. The increase in cold-water specialists and a stable share of generalists suggested the maintenance of a certain level of specialization in the assemblages of brooks.

3. The most marked change in the assemblage was associated with the impaired or bad water quality of rivers in the 1950s, which persisted or further deteriorated in the ensuing decades. Assemblages that were influenced by a slight deterioration or improvement in water quality were less altered, unless affected by other pressures (such as channel or discharge modifications).

4. The results indicate that the causes of changes in the assemblages are many and complex, although heavy pollution overrides other influences. Direct habitat loss or their degradation by siltation appear to be leading contributors to changes in assemblages. The results imply the need for the application of a catchment-scale perspective for the restoration of streams and the conservation of the remaining well-preserved stretches of lowland rivers.

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KEY WORDS: long-term trends; species turnover; species loss; biodiversity; species traits; water quality; multiple stressors; streams

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INTRODUCTION

Contemporary biological communities are the consequence of more than a hundred years of intensive human impacts. However, knowledge on how and to what extent they have been affected is limited, particularly owing to the lack of reliable historical data (Jackson and Füreder, 2006). Historical or long-term data can provide valuable insights into past constraints that limit different populations, which significantly contribute to an understanding of their resilience, responses to stressors and present distribution (Magurran *et al*., 2010). Large-scale surveys of the distribution of species belonging to different taxonomic groups over the past century have focused on the quantification of biodiversity losses in particular. These surveys have accumulated evidence for the considerable extinction of populations of both rare and widespread species, which is distributed across the areas studied rather than concentrated in a few degraded regions (Thomas *et al*., 2004; Conrad *et al*., 2006; van Dyck *et al*., 2009; Cameron *et al*., 2011). Widespread degradation and habitat fragmentation have been among the leading causes implying a need for additional conservation strategies in the wider landscape, to reduce the rate of biodiversity loss.

Comparably less information on long-term changes in assemblages or populations is available for freshwater rather than for terrestrial biota, particularly for aquatic insects. The only existing large-scale surveys have documented considerable species loss of Plecoptera and the impoverishment of their assemblages during the past century, which has been mainly caused by heavy water pollution (Aubert, 1984; DeWalt *et al*., 2005; Bojková *et al*., 2012, 2014). The concentration of human impacts at low and mid-altitudes and the persistence of relatively intact stream habitats at high altitudes have led to a dramatic restriction of species ranges or to a shift in the altitudinal preference of some species (Bojková *et al*., 2012). However, stoneflies, which include predominantly sensitive species with narrow ecological ranges, might not be objective indicators of environmental changes. Case studies on long-term changes in benthic assemblages of large European rivers (the Loire, Rhône, Rhine and Elbe), which included invertebrates with different ecological requirements, documented truly complex and rather dynamic assemblage changes induced by various human impacts (Usseglio-Polatera and Bournaud, 1989; Zwick, 1999; Marten, 2001; Daufresne *et al*., 2003, 2007; Floury *et al*., 2013). In addition to the unprecedented impact of heavy organic or industrial pollution, rivers have been affected by physical changes in habitats caused by increased siltation, riffle removal and flow conditions altered by channelization, impoundments and hydropeaking. The consequent exchange of fauna driven both by species loss and colonization has led to highly dissimilar communities compared with the original ones, both taxonomically and functionally (Usseglio-Polatera and Bournaud, 1989; Marten, 2001). The recent elimination of heavy pollution and some restoration have enabled re-colonization of some native sensitive species (Marten, 2001; Bojková *et al*., 2012; Floury *et al*., 2013). However, where habitats have been profoundly altered, communities are developing under continuing pressures (such as global warming and its interactions with other pressures) not necessarily towards the 'original' species and functional composition (cf. Marten, 2001). Thus, information on original communities provides a yardstick for quantifying how much a community has shifted to match the change in its environment, rather than the target situation, the attainment of which is illusory.

In contrast, benthic assemblages that inhabited streams not affected by profound and irreversible habitat alterations and long-lasting pollution, show relatively high resistance or resilience (Bradt *et al*., 1999; Woodward *et al*., 2002). A core community with little turnover during a long time period can comprise the majority of the total invertebrate abundance, although the recovery time following disturbances can be variable (Bradt *et al*., 1999; Woodward *et al*., 2002). The resilience of communities is fundamentally influenced, among other factors, by the accessibility of refugia and the spatial connectivity of populations. Resilience can be limited especially in fragmented and disconnected river systems, and also in streams isolated from possible source populations for re-colonization (Sedell *et al*., 1990; Zwick, 1992).

This study assessed the impact of human pressures in the Morava and Odra river basins in the Czech Republic during the past five decades on the diversity and composition of current Ephemeroptera assemblages. A comparison of original data on mayflies of 60 sites collected in 1955–1960 with the present data, enabled the quantification of changes in the assemblages in various types of running waters. The period 1955–1960 represents the beginning of large-scale and radical changes in land-use and the intensification of industry and agriculture in the former Czechoslovakia, which resulted in the severe pollution of streams, acid deposition, and alterations in the morphology and flow conditions of streams (Bičík *et al*., 2001; Horák *et al*., 2001; Kopáček and Veselý, 2005). The study area was affected mainly by intensive agriculture on large fields, the draining of fertile soil for agricultural use and profound water quality alterations, largely due to organic pollution and siltation. Ephemeroptera assemblages were expected to be affected at the majority of sites. However, as mayflies are a group that comprises sensitive habitat specialists as well as eurytopic, very flexible species (cf. Bauernfeind and Soldán, 2012), we hypothesized that local species loss caused by human effects would be counterbalanced by colonization by other species, mostly generalists, and local diversity would remain the same. It was also expected that the extent of assemblage change would be different at sites affected by different human pressures. As human activities affected large rivers the most, we expected that their assemblages would undergo marked changes in taxonomic structure and a shift toward generalized and simplified composition.

METHODS

Study area and sites

The study sites were located in two of the three main river basins in the Czech Republic: the Morava river and Odra river basins (Figure 1), which belong to the Black Sea drainage basin and Baltic Sea drainage basin, respectively. The total area of both river basins covers 27 493 km^2 in the Czech Republic and their altitude ranges from 148 to 1492 m a.s.l. Lowlands below 200 m a.s.l. as well as mountains above 800 m a.s.l. cover only a small part of the area. The western part of the area

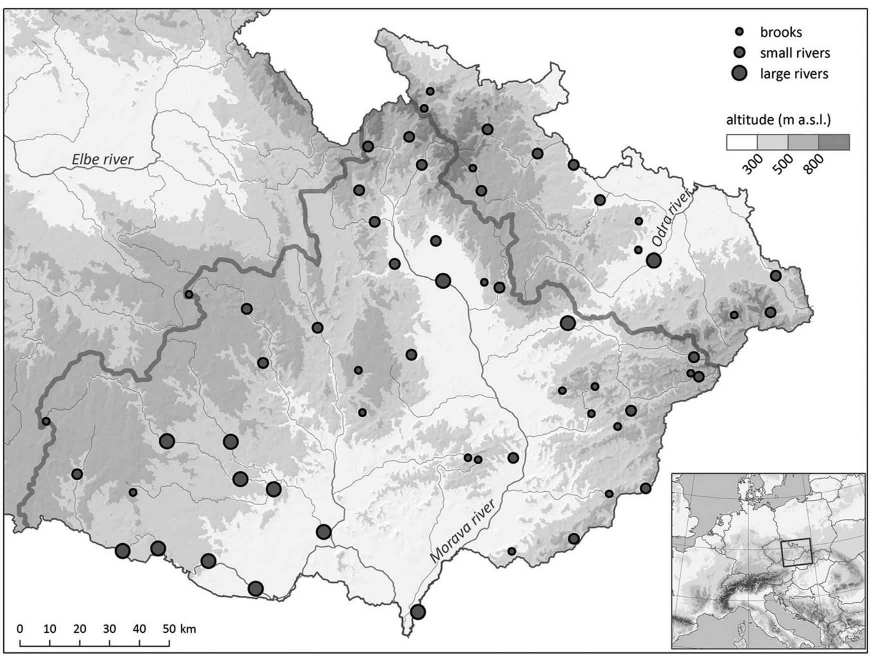

Figure 1. Map of the Morava and Odra river basins with 60 sites studied.

belongs to the Hercynian mountain system and the eastern part of the area belongs to the Carpathians (the Alpine-Himalayan mountain system). The study area is located on the boundary of four ecoregions: the Central Highlands, Carpathians, Hungarian Lowlands, and Central Plains (Nos 9, 10, 11, 14 according to Illies, 1978). There are 37 large dams and 169 small reservoirs (including large fish-ponds) in the Morava and Odra river basins in the Czech Republic.

The study sites were chosen on the basis of the detailed faunistic survey of aquatic insects of the former Czechoslovakia, organized by the Czechoslovak Academy of Science in 1955–1960. During that period, 207 sites were studied, evenly distributed throughout the Morava and Odra river basins. Mayflies were sampled from one to 10 times at these sites. The sites for the present study (2006–2011) were chosen according to the number of samples taken in 1955–1960 and with respect to seasonality and habitat. In total, 60 sites were selected from those with four and more samples over all seasons, to cover a range of altitude and stream sizes as evenly as possible (Figure 1). These sites were located according to old field notes and photographs, so that the contemporary samples were taken at exactly the same locations as in 1955–1960.

Sampling methods and harmonization of species data

Samples were collected from three to five times per year during the period 2006–2011 by the same sampling method and instruments as those used in the 1955–1960 survey. Mayfly larvae were sampled semi-quantitatively by kick-sampling using a metal strainer with a 0.5 mm mesh size over a 50 m stretch for a total of 30 min. All mesohabitats present (riffle, run, pool, woody debris, and aquatic vegetation) were sampled according to their area. Larvae were preserved in 75% alcohol and were identified by B. Zedková. The material is deposited in the collection of the Department of Botany and Zoology, Faculty of Science, Masaryk University in Brno (Ephemeroptera, collection S. Zahrádková).

Unfortunately, the historical material of mayflies collected in 1955–1960 was damaged and revision of previous species identification was difficult. Thus, species data based on previous identification had to be corrected according to valid species status. Several species were not described or reliably identified previously; therefore, taxonomic harmonization of species data collected in 1955–1960 and 2006–2011 was necessary. The list of species and species groups and their full scientific names are given in Table 1.

Classification of sites and species

Sites were classified according to stream size, altitude, and prevailing human pressures. Stream-size classification was based on Strahler's stream order: brooks (stream order 1–3; 21 sites), small rivers (stream order 4–6; 26 sites), and large rivers (stream order 7–8; 13 sites). Classification according to altitude was defined as follows: lowland sites (≤300 m a.s.l.; 20 sites), mid-altitude sites (301–500 m a.s.l.; 25 sites) and submontane sites (501–700 m a.s.l.; 15 sites).

Classification according to prevailing human pressures was based on a detailed assessment of hydromorphological degradation and water quality. Hydromorphological degradation was determined using the hydromorphological assessment protocol for the Slovak Republic (Pedersen *et al*., 2004). The hydromorphological quality score (HQS) is a continuous variable ranging from 1 to 5 and classifies sites into one of five quality classes (1 – high, 2 – good, 3 – moderate, 4 – poor and 5 – bad). Pollution was assessed using four chemical parameters (total P, NH_4-N, NO_3-N and BOD_5) measured in 2006–2008. According to their thresholds, sites are classified into three classes of water quality (1 – very good, 2 – good and 3 – bad; Rosendorf, 2011). Thresholds of the fourth class of water quality (very bad) are not available; thus, the bad water quality class also includes heavily polluted sites. Pollution in 1955–1966 was determined using notes in field protocols and data from yearbooks of water quality (Anonymous, 1959, 1960, 1963, 1964, 1971). More details on the assessment of human pressures and their data sources, and site classifications are available in Bojková *et al*. (2012).

The sites were classified according to prevailing human pressures as follows: (i) 'Pollution' – sites with bad water quality (class 3); (ii) 'Channelization' – sites with poor and bad hydromorphological

Table 1. List of mayflies found at the sites studied in 1955–1960 and 2006–2011, numbers of occurrences based on the original 60 sites and classification according to their habitat specialization and sensitivity (codes of categories available in Table 2). NA = not available

Ephemeroptera species/taxa	Occurrences in 1955–1960	Occurrences in 2006–2011	Body form / locomotion	Altitudinal preference	Stream zone preference	Sensitivity to organic pollution	Sensitivity to siltation
Ameletidae							
Ameletus inopinatus Eaton, 1887	1	1	Swimmer	1	2	1	2
Siphlonuridae							
Siphlonurus aestivalis Eaton, 1903	0	1	Swimmer	2	3	3	3
Siphlonurus armatus Eaton, 1870	0	1	Swimmer	2	2	3	3
Siphlonurus lacustris Eaton, 1870	2	1	Swimmer	3	3	2	3
Baetidae							
Baetis alpinus (Pictet, 1843)	18	11	Swimmer	3	2	2	1
Baetis buceratus Eaton, 1870 / *B. nexus* Navás, 1918	14	14	Swimmer	1	1	3	2
Baetis fuscatus (Linnaeus, 1761) / *B. scambus* Eaton, 1870	57	42	Swimmer	2	2	4	1
Baetis lutheri Müller-Liebenau, 1967 / *B. vardarensis* Ikonomov, 1962	16	23	Swimmer	2	2	2	1
Baetis vernus Curtis, 1834	12	29	Swimmer	3	2	3	2
Baetis muticus (Linnaeus, 1758)	40	26	Swimmer	3	2	2	1
Baetis niger (Linnaeus, 1761)	3	10	Swimmer	2	2	3	1
Baetis rhodani (Pictet, 1843)	57	56	Swimmer	3	3	3	1
Baetopus tenellus (Albarda, 1878)	0	1	Swimmer	1	1	3	1
Centroptilum luteolum (Müller, 1776)	7	24	Swimmer	3	3	3	3
Cloeon dipterum (Linnaeus, 1761) s. lat.	5	5	Swimmer	3	3	4	3
Procloeon bifidum (Bengtsson, 1912)	0	3	Swimmer	1	3	3	3
Procloeon ornatum Tshernova, 1928	0	1	Swimmer	2	1	3	3
Procloeon pennulatum (Eaton, 1870)	2	6	Swimmer	2	2	3	NA
Procloeon pulchrum (Eaton, 1885)	0	1	Swimmer	1	2	3	3
Isonychiidae							
Isonychia ignota (Walker, 1853)	1	0	Streamlined	1	1	2	NA
Oligoneuriidae							
Oligoneuriella rhenana (Imhoff, 1852)	9	1	Streamlined	2	2	2	NA
Heptageniidae							
Ecdyonurus aurantiacus (Burmeister, 1839)	0	3	Clinger	1	1	3	1
Ecdyonurus dispar (Curtis, 1834)	5	7	Clinger	2	2	3	1
Ecdyonurus insignis (Eaton, 1870)	8	1	Clinger	1	2	3	1
Ecdyonurus venosus species group[a]	50	34	Clinger	3	2	3	1
Ecdyonurus subalpinus Klapálek, 1907	3	17	Clinger	2	2	2	1
Electrogena spp.[b]	16	16	Clinger	3	2	3	1
Heptagenia coerulans Rostock, 1878	2	1	Clinger	1	1	3	1
Heptagenia flava Rostock, 1878	10	6	Clinger	1	1	3	1
Heptagenia sulphurea (Müller, 1776)	6	9	Clinger	2	2	3	1
Epeorus assimilis Eaton, 1885	27	19	Clinger	2	2	2	1
Rhithrogena beskidensis Alba-Tercedor and Sowa, 1987	0	2	Clinger	2	1	2	1
Rhithrogena corcontica Sowa and Soldán, 1986	2	3	Clinger	2	1	1	1
Rhithrogena semicolorata species group[c]	44	38	Clinger	3	2	3	1
Rhithrogena hercynia Landa, 1969	0	6	Clinger	1	1	2	1
Leptophlebiidae							
Choroterpes picteti (Eaton, 1871)	2	0	Fissicole	1	3	2	2
Habroleptoides confusa Sartori and Jacob, 1986	41	33	Fissicole	2	2	3	2
Habrophlebia fusca (Curtis, 1834)	8	6	Fissicole	2	2	3	2
Habrophlebia lauta Eaton, 1884	22	20	Fissicole	5	2	3	2
Leptophlebia marginata Linné, 1767	2	2	Sprawler	3	1	3	3
Leptophlebia vespertina (Linnaeus, 1758)	0	1	Sprawler	2	2	3	3
Paraleptophlebia cincta (Retzius, 1783)	0	2	Fissicole	1	1	3	1
Paraleptophlebia submarginata (Stephens, 1836)	19	29	Fissicole	3	2	3	2

(Continues)

Table 1. (Continued)

Ephemeroptera species/taxa	Occurrences in 1955–1960	Occurrences in 2006–2011	Body form / locomotion	Altitudinal preference	Stream zone preference	Sensitivity to organic pollution	Sensitivity to siltation
Ephemeridae							
Ephemera danica Müller, 1764	24	27	Burrower	3	3	3	3
Potamanthidae							
Potamanthus luteus (Linné, 1767)	9	9	Sprawler	2	1	3	2
Ephemerellidae							
Ephemerella ignita (Poda, 1761)	52	38	Sprawler	3	3	4	1
Ephemerella mesoleuca (Brauer, 1857)	1	0	Sprawler	1	2	3	1
Ephemerella mucronata (Bengtsson, 1909)	10	18	Sprawler	2	1	2	1
Ephemerella notata Eaton, 1887	6	0	Sprawler	1	2	3	2
Torleya major (Klapálek, 1905)	21	14	Sprawler	3	2	3	1
Caenidae							
Brachycercus harrisellus Curtis, 1834	1	1	Sprawler	1	1	3	3
Caenis spp.[d]	15	28	Sprawler	2	2	4	3

[a]Comprised of *Ecdyonurus macani* Thomas and Sowa, 1970, *E. starmachi* Sowa, 1971, *E. submontanus* Landa, 1969, *E. torrentis* Kimmins, 1942 and *E. venosus* (Fabricius, 1775)
[b]Comprised of *Electrogena affinis* (Eaton, 1887), *E. lateralis* (Curtis, 1834), *E. quadrilineata* (Landa, 1969) and *E. ujhelyii* (Sowa, 1981)
[c]Comprised of *Rhithrogena carpatoalpina* Kłonowska, Olechowska, Sartori and Weichselbaumer, 1987, *R. iridina* (Kolenati, 1859), *R. picteti* Sowa, 1971, *R. semicolorata* (Curtis, 1834) and *R. puytoraci* Sowa and Degrange, 1987
[d]Comprised of *Caenis horaria* (Linnaeus, 1758), *C. luctuosa* (Burmeister, 1839), *C. macrura* Stephens, 1836, *C. pseudorivulorum* Keffermüller, 1960 and *C. robusta* Eaton, 1884.

quality (HQS > 3.4) and very good and good water quality (classes 1 and 2); (iii) 'Discharge fluctuation' – sites on sandstone or flysh bedrock with naturally fluctuating discharge and seasonal drying of some stream sections, and sites influenced by water diversion; (iv) 'Mixed pressure' – sites affected by more than one human pressure included those influenced by morphological degradation (HQS > 3.4) and simultaneously by pollution (class 3) or a dam up to 20 km upstream; (v) 'Other' – two sites affected by acidification and uranium mining; (vi) 'Near natural'– sites not affected by any of the above-mentioned pressures. No distinct differences in water quality and stream morphology between the two sampling periods were evident and sites have very good and good water quality (classes 1 and 2), high, good, and moderate hydromorphological quality (HQS ≤ 3.4) and no dam upstream.

Species were classified into three categories representing their habitat specialization based on stream zonation, altitudinal preference, and body form/locomotion (Table 2). The eight stream zones (crenal, epi-, meta-, hyporhithral, epi-, meta-, hypopotamal, and littoral), defined according to Illies and Botosaneanu (1963) and Buffagni *et al.* (2009), were used to classify species according to their stream zonation preference. The six zones of altitude (lowland, mid-altitude, submontane, montane, subalpine and alpine), defined according to Buffagni *et al.* (2009), were used for classifying altitude preference. The six categories of body form and locomotion of larvae (see Table 2 for their definitions) were based on Bauernfeind and Soldán (2012).

The classification of species according to their sensitivity to organic pollution was based on saprobic values (Bauernfeind *et al.*, 2002; Zahrádková *et al.*, 2009). Species were classified into four categories based on the assignment of 10 points in five categories of saprobity (xeno-, oligosaprobic, beta-, alpha-mesosaprobic, and polysaprobic) (see Zelinka and Marvan, 1961 and Zahrádková and Soldán, 2008 for details of the 10-point assignment system). The classification of species according to their sensitivity to siltation was based on Fine Sediment Sensitivity Rating (Extence *et al.*, 2013).

Data analysis

Presence–absence data were used in all analyses. The alpha diversity of all sites and categories was calculated as the mean and standard deviation of species richness per site, and gamma diversity was expressed as the total number of species.

Table 2. Summary of the classificationn of species and definition of categories

Categorization	Category	Code	Definition
Altitudinal preference	Altitude_stenotopic	1	Species occurs in one or two zones of altitude.
	Altitude_mesotopic	2	Species occurs in three or four zones of altitude.
	Altitude_eurytopic	3	Species occurs in five or six zones of altitude.
Stream zone preference	Stream size_stenotopic	1	Species occurs in one to three stream zones.
	Stream size_mesotopic	2	Species occurs in four to five zones.
	Stream size_eurytopic	3	Species occurs in six or more zones.
Sensitivity to organic pollution	Pollution_sensitive	1	Species occurs in xeno- and oligosaprobic waters.
	Pollution_slightly sensitive	2	Species occurs also in beta-mesosaprobic waters.
	Pollution_tolerant	3	Species occurs predominantly in beta- and alpha-mesosaprobic waters.
	Pollution_very tolerant	4	Species occurs also in polysaprobic waters.
Sensitivity to siltation	Highly sensitive	1	Fine Sediment Sensitivity Rating A (highly sensitive)
	Moderately sensitive	2	Fine Sediment Sensitivity Rating B (moderately sensitive)
	Insensitive	3	Fine Sediment Sensitivity Rating C, D (moderately insensitive and highly insensitive)
Body form/locomotion	Swimmers	Swimmer	Fish-shaped larvae; their usual way of movement is swimming.
	Clingers	Clinger	Larvae with flattened body shape cling on the substrate and swim only occasionally or never.
	Streamlined body	Streamlined	Streamlined larvae do not actively swim.
	Sprawlers	Sprawler	Cylindrical larvae sprawl on the surface of the sediments or macrophytes.
	Fissicole species	Fissicole	Wriggling larvae preferring stone crevices in flowing water.
	Burrowers	Burrower	Burrowing larvae construct tubes or freely burrow in the sediment.

Differences in alpha diversity between the historical (1955–1960) and contemporary (2006–2011) periods were tested by ANOVA.

Only sites with at least five species in at least one period were included in the following analyses (59 sites). The beta diversity of Ephemeroptera assemblages in the two periods was expressed as pair-wise Jaccard dissimilarities (β_{jac}) and subsequently disentangled into its two components: species turnover (β_{turn}) and differences in species richness (β_{rich}) (Carvalho *et al.*, 2013). Differences in beta diversity and its components between categories of sites were tested by the Kruskal–Wallis test. The difference between the two components of beta diversity in each site category was tested by ANOVA. The relationship between the two components of beta diversity of all sites was displayed using a scatterplot, where the six categories of sites according to prevailing human pressures were highlighted by different colours, and centroids of the categories were shown. Grey dashed lines were used to define the area with a 30–90% change in assemblages between the two time periods.

As several sites were influenced by different pressures and many sites were affected by channelization and pollution to different extents, even in the 1950s, combinations of pressures at each site were explored in detail using the scatterplots, which showed the relationship of the Jaccard dissimilarity of assemblages and pollution in both time periods. Pollution was expressed as arrows when the pollution of a site was different in the periods and by points when it was the same. Sites were further classified according to the HQS and stream size.

The SDR–simplex approach (Podani and Schmera, 2011) was used for displaying changes in Ephemeroptera species occurrence between the two periods. Results were shown in the ternary plot, which allows visualization of the relationship between species loss, gain, and persistence at sites.

Changes in the representation of species categories at sites were expressed as proportional loss (the percentage of sites at which species belonging to each category occurred only in the previous period), gain (the percentage of sites at which the species belonging to each category occurred only recently) and survival (the percentage of sites at which the species belonging to each category occurred in both periods). Only species categories with a survival at more than 50% of sites and with a loss/gain of more than 20% were displayed for each category of stream size.

All analyses were performed in R Statistics (version 2.15.1; R Development Core Team, 2012) using the 'vegan' and 'plotrix' packages (Lemon *et al*., 2006; Oksanen *et al*., 2011).

RESULTS

Changes in species richness and diversity

The total number of taxa (gamma diversity) found in the periods 1955–1960 and 2006–2011 was similar: 42 taxa were found in the previous period and 49 taxa recently (Tables 1 and 3). In total, 53 taxa were recorded in both periods. Eleven species were not found in the previous period, and four species recorded recently were not recorded in 1955–1960 (Table 1). Gamma diversity increased with decreasing altitude in both periods and with increasing stream order in the previous period. In the later period, gamma diversity was the same in different stream orders (Table 3). The number of species found at sites (alpha diversity) was not significantly different between the two periods (Table 3).

Pair-wise Jaccard dissimilarities between the previous and contemporary assemblages increased from montane to lowland streams (Figure 2(B)) and were higher in large rivers than in brooks and small rivers (Figure 2(A), cf. Figure 4(A)). However, the differences were not significant. The partitioning of pair-wise dissimilarities showed that the overall dissimilarity was driven primarily by species turnover (Figure 2). Species turnover was significantly ($P < 0.05$) higher than the difference in richness (i.e. species loss/gain) in all categories of sites. Species turnover and the difference in species richness were, however, similar among the categories of altitudes (Figure 2 (B)) and among the categories of stream size (Figure 2(A)).

Pair-wise dissimilarities were highest at sites affected by multiple pressures (pollution, dam, and channelization) and were relatively low at near-natural sites and some polluted sites (along the diagonal of the plot; Figure 3). Relatively low differences in species richness ($\beta_{rich} \leq 0.2$), which expresses pure loss/gain of species, were observed in the assemblages of near-natural and channelized sites. In contrast, the highest differences in species richness were found in the majority of assemblages affected by pollution and multiple pressures (Figure 3). A high species turnover ($\beta_{turn} \geq 0.5$) was found in assemblages of sites with multiple pressures (Figure 3). However, assemblages affected by pollution, multiple pressures, and discharge fluctuation showed a wide range of turnover and difference of species richness. Fluctuations in the discharge in cold, mostly high-altitude streams mainly led to species turnover (Figure 3, bottom part), whereas fluctuations in warmer streams caused high species loss (Figure 3, upper part). However, the dataset, which comprised only 60 sites, did not enable an analysis of the combined effects of pressures and other environmental descriptors of sites (such as variables connected with temperature and hydrological regimes) in detail. Nevertheless, the distribution of pressures at sites in relation to the dissimilarity of assemblages found between the periods showed the leading role of water quality in both periods that can be enhanced by combination with other pressures. High Jaccard dissimilarity ($\beta_{jac} \geq 0.55$) was clearly associated with bad water quality in at least one period, with the highest

Table 3. Alpha (α, mean ± standard deviation) and gamma (γ) diversity of assemblages in different categories in two time periods

	α diversity		γ diversity	
Categories	**1955–1960**	**2006–2011**	**1955–1960**	**2006–2011**
All streams	10.7 ± 3.6	10.8 ± 3.9	42	49
Brooks	9.9 ± 3.4	9.9 ± 3.8	27	32
Small rivers	10.8 ± 2.2	11.2 ± 4.1	30	32
Large rivers	11.8 ± 5.8	11.5 ± 3.7	36	32
Lowland streams	11.2 ± 4.1	10.2 ± 3.8	38	38
Mid-altitude streams	10.8 ± 3.9	10.7 ± 4.2	34	36
Mountain streams	9.9 ± 2.3	11.8 ± 3.6	25	31

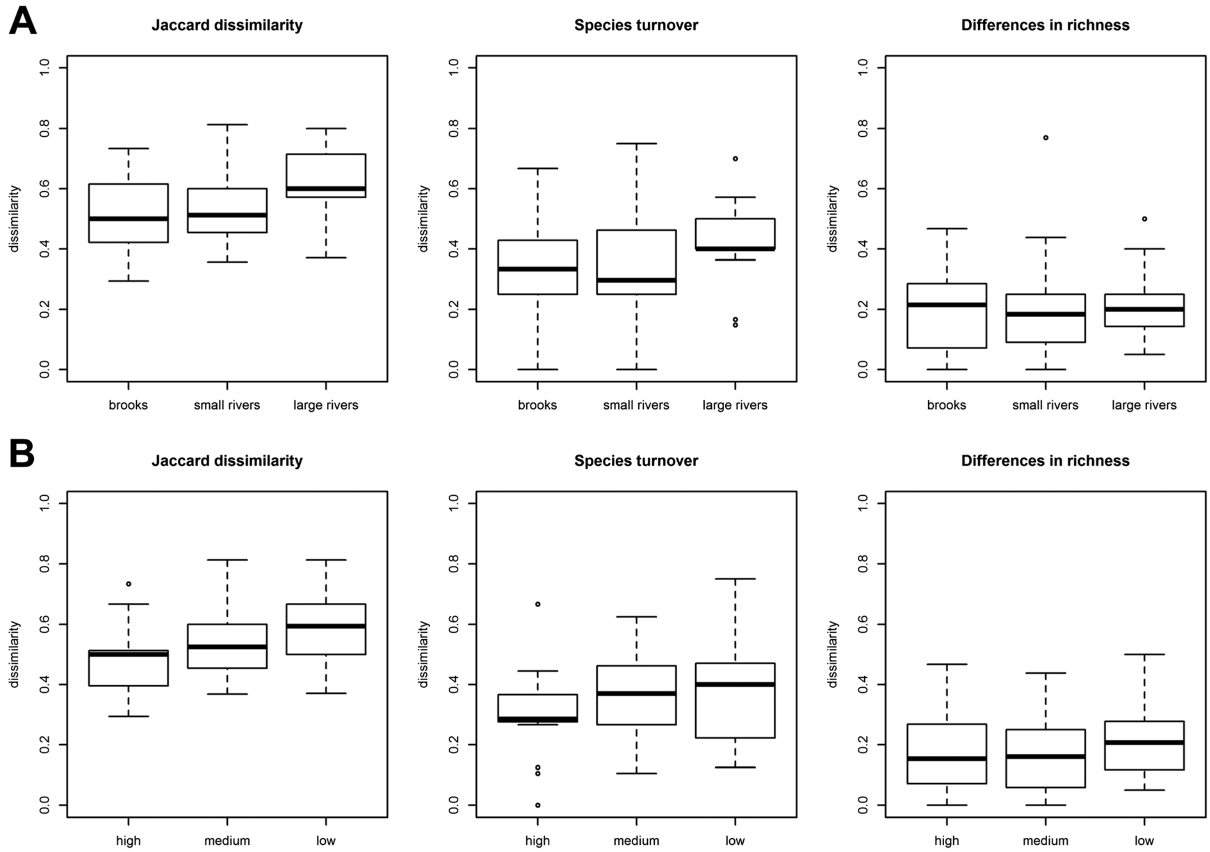

Figure 2. Pair-wise Jaccard dissimilarities of Ephemeroptera assemblages between the two time periods (1955–1960 and 2006–2011) and their partitioning into two components, species turnover and differences in species richness. Sites are classified according to altitude (A) and stream size (B). Only sites with five or more species in at least one of the periods are included (59 sites).

values at sites with degraded water quality (classes 2 or 3) since the 1950s (Figure 4(B)). High dissimilarities also occurred when water quality improved from bad to good, but sites were affected by other pressures, i.e. extreme discharge fluctuation, impoundment, acidification, uranium mining (sites marked by asterisks in Figure 4(B)) or impaired hydromorphological quality (red and black in Figure 4(B)). Low Jaccard dissimilarity ($\beta_{jac} < 0.45$) was predominantly associated with the decrease of water quality from very good to good or, in a few cases, with strongly altered sites, where extremely bad water quality in both periods caused only slight changes in their species-poor assemblages. Contrary to water quality, hydromorphological quality has not changed at most sites since the 1950s, because they were channelized in the first half of the 20th century (mainly in the 1920s and 1930s) or even earlier. HQS increased by more than 0.4 only at 13 sites and such increase was associated with high Jaccard dissimilarity of assemblages only in a few cases (data not shown).

Changes in the occurrence of species and their categories

A ternary plot based on species occurrences at sites in 1955–1960 and 2006–2011 (Figure 5) displayed five groups of species with different changes in occurrences at sites. The first group (group 1 in Figure 5) included pollution-sensitive species and potamal specialists, which became extremely reduced (*Oligoneuriella rhenana*, *Ecdyonurus insignis* and *Ephemerella notata*) or even became regionally extinct (*Isonychia ignota* and *Ephemerella mesoleuca*; Table 1), i.e. there was no new occurrence of these species in the contemporary period and they survived only at a few sites in the study area. These species are

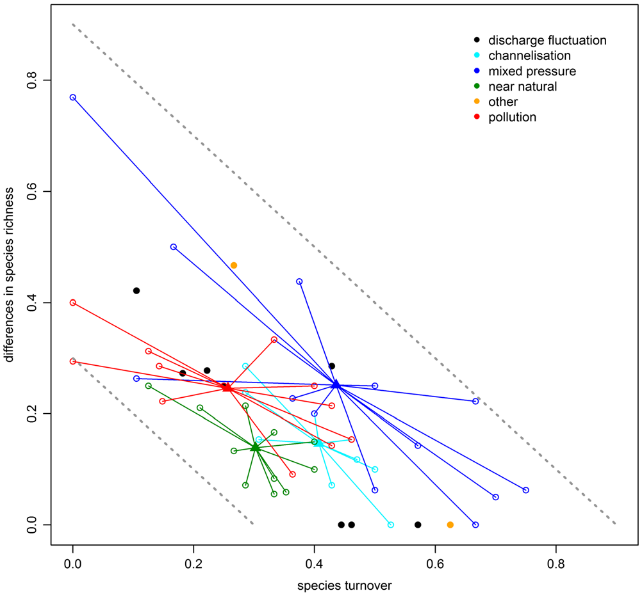

Figure 3. Relationship between the two components of beta diversity, species turnover and differences in species richness (pair-wise dissimilarities between 1955–1960 and 2006–2011), at each site. Sites are displayed as circles and different types of prevailing pressure at sites are shown in different colours. Triangles represent the centroid of the sites with the same pressure. Dashed lines indicate 30% and 90% changes in assemblages between the two periods. Only sites with five or more species in at least one of the periods are included (59 sites).

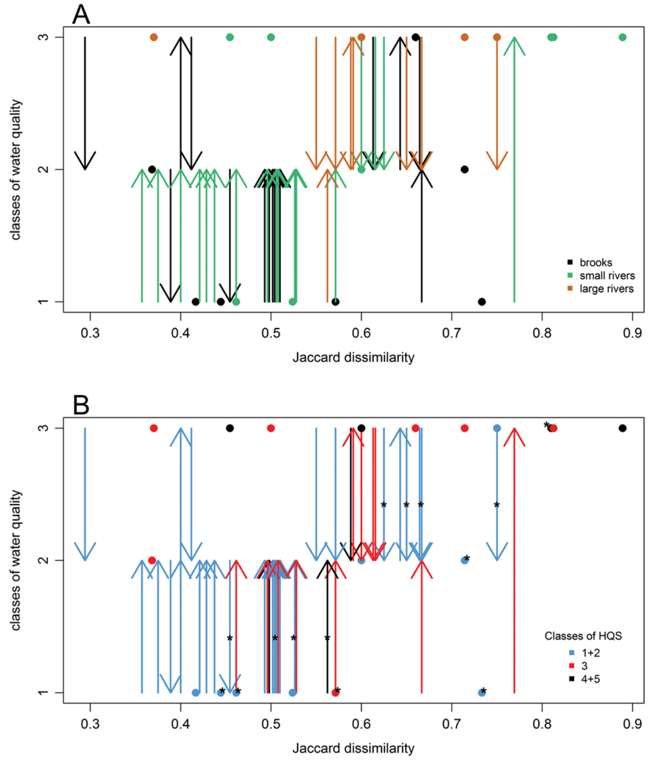

Figure 4. Relationship between pair-wise Jaccard dissimilarities and water quality. For each site, arrows display the direction and extent of change in the class of water quality between the periods 1955–1960 and 2006–2011 and points display no change in water quality. Colours of arrows and points represent: (A) categories of sites according to their stream order; and (B) classes of hydromorphological quality scores (HQS) at present (2006–2011). Presence of other pressures at sites (discharge fluctuation, impoundment, acidification, and uranium mining) are marked with asterisks. Only sites with five or more species in at least one of the periods are included (59 sites).

included in the Red List of the Czech Republic (Soldán, 2005). The second group consisted of species that were more frequent in the contemporary period than in the previous period (Figure 5). A few species (*Centroptilum luteolum* and *Baetis niger*) appeared to expand within the study area. Other species, such as *Rhithrogena hercynia* and *Ecdyonurus subalpinus*, may have been overlooked in the previous period owing to unclear taxonomy of their species groups at that time. The third group comprised eurytopic species, such as *Baetis rhodani*, *Baetis fuscatus* and *Habroleptoides confusa*, which were very common in the study area in both periods and experienced almost no loss or turnover of sites (Figure 5). The fourth and fifth groups included species with a relatively low turnover of sites (<20%) and a high (30–50%) loss or gain of sites, respectively (Figure 5). Mostly common species such as *Baetis alpinus* and *Epeorus assimilis* were found in group 4 (site loss), and *Baetis vernus* and *Caenis* spp. in group 5 (site gain). The sixth group represented species with a high site turnover (>20%), which increased from the top of the dashed-line triangle towards the centre of the base-line, where total site-turnover occurred, i.e. from *Potamanthus luteus* to *Cloeon dipterum* s. lat. (Figure 5).

The occurrence of species with different habitat specializations and sensitivity to pollution and siltation in the two periods also showed a high turnover. Species of a particular specialization or sensitivity that disappeared in some of the sites were usually replaced by other species of the same specialization or sensitivity. This turnover ranged from 15% to 30% of the total number of occurrences in most categories. These species were replaced partly at the same site and partly at other sites of the same category of stream size, independent of the type of human pressure that

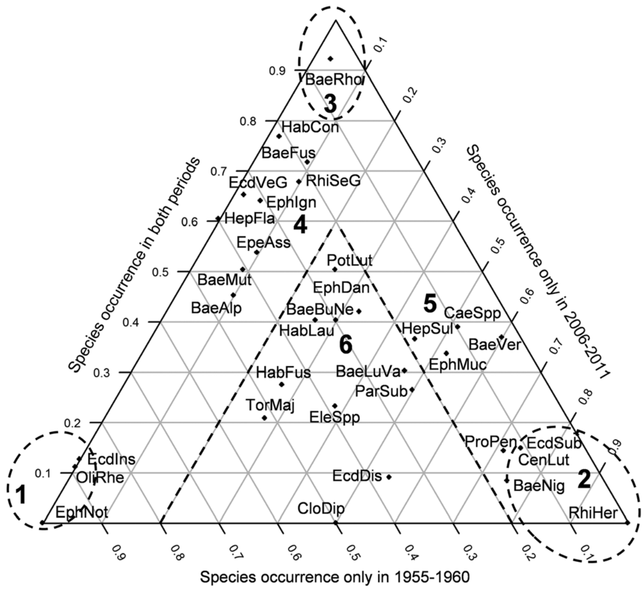

Figure 5. Ternary plot showing the relationship among the proportion of sites where the species occurred in both periods (a), and the proportions of sites where the species occurred in either period (b, c); i.e. a+b+c=100%. Dashed lines represent 20% turnover. Six groups of species differing in the relationship of these three components are indicated by numbers. Species are abbreviated as follows: BaeAlp – *Baetis alpinus*, BaeBuNe – *B. buceratus/nexus*, BaeFus – *B. fuscatus*, BaeLuVa – *B. lutheri/vardarensis*, BaeMut – *B. municus*, BaeNig – *B. niger*, BaeRho – *B. rhodani*, BaeVer – *B. vernus*, CaeSpp – *Caenis* spp., CenLut – *Centroptilum luteolum*, CloDip – *Cloeon dipterum*, EcdDis – *Ecdyonurus dispar*, EcdIns – *E. insignis*, EcdSub – *E. subalpinus*, EcdVeG – *Ecdyonurus* gr. *venosus*, EleSpp – *Electrogena* spp., EpeAsi – *Epeorus assimilis*, EphDan – *Ephemera danica*, EphIgn – *Ephemerella ignita*, EphMuc – *E. mucronata*, EphNot – *E. notata*, HabCon – *Habroleptoides confusa*, HabFus – *Habrophlebia fusca*, HabLau – *H. lauta*, HepFla – *Heptagenia flava*, HepSup – *H. sulphurea*, OliRhe – *Oligoneuriella rhenana*, ParSub – *Paraleptophlebia submarginata*, PotLut – *Potamanthus luteus*, ProPen – *Procloeon pennulatum*, RhiHer – *Rhithrogena hercynia*, RhiSeG – *Rhithrogena* gr. *semicolorata*, TorMaj – *Torleya major*.

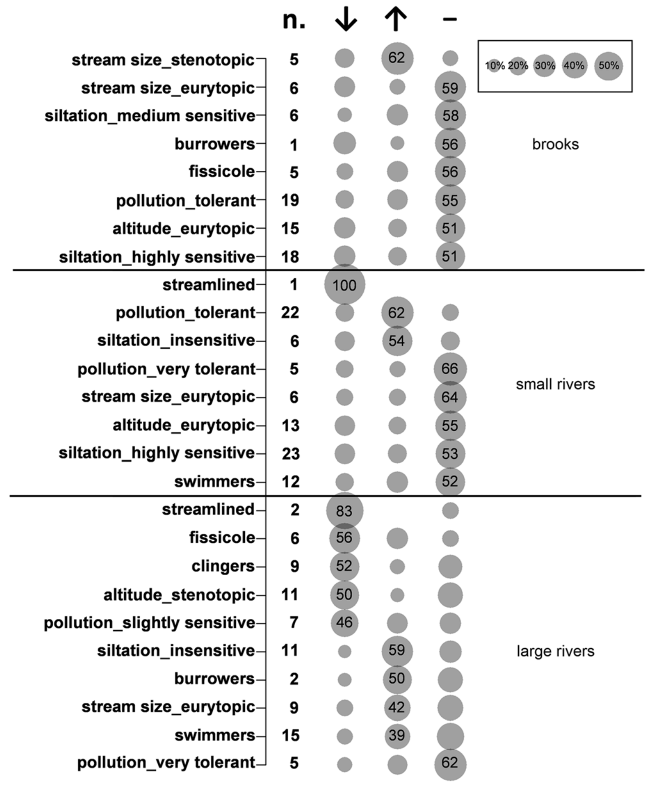

Figure 6. Percentage of colonization of new sites (↑) by the species of a particular species category at the present time, local loss (↓) of the species at the present time and survival (–) of the species in both periods from the total number of occurrences of a particular species category in both periods. All percentages are expressed by the size of circles. The numbers of species included in individual species category (n) are shown.

affected the sites (results not shown). Figure 6 shows only species traits that mainly persisted at sites (>50%), and species traits with a loss or gain greater than 20%, which illustrated the main shifts in the occurrence of generalists/specialists and sensitive/tolerant species in brooks, small rivers, and large rivers. Most mesotopic and moderately sensitive species, and body form and locomotion types showed a substantial turnover and did not increase or decrease in any stream habitat (results not shown). The occurrence of body form and locomotion types changed only in large rivers and that of streamlined and fissicole species and clingers markedly decreased and that of burrowers and swimmers increased (Figure 6).

The assemblages of brooks did not experience any remarkable loss of species categories; on the contrary, the occurrence of some stenotopic species increased (mainly *Rhithrogena hercynia*, *Paraleptophlebia cincta* and *Ephemerella mucronata*). Eurytopic, pollution-tolerant, siltation-sensitive, fissicole, and burrowing species predominantly survived (Figure 6). Small rivers gained some generalists (siltation-insensitive and pollution-tolerant species) and other generalists together with swimmers predominantly survived. Only streamlined species *Oligoneuriella rhenana* disappeared (Figure 6). In addition to the remarkable changes in the occurrence of body form and locomotion types mentioned above, large rivers lost some stenotopic species (lowland specialists such as *Ephoron virgo*, *Isonychia ignota*, *Choroterpes picteti*, *Ephemerella notata* and *Ephemerella mesoleuca*) and species slightly sensitive to pollution (no truly sensitive species, i.e. those preferring xeno- and oligo-saprobity, were found in large rivers in either period), and gained widespread species together with siltation-insensitive species (Figure 6).

DISCUSSION

The majority of case studies that have focused on long-term trends in the diversity of aquatic insects have stressed the role of species loss that occurred around the mid-20th century in the structuring of contemporary assemblages (Aubert, 1984; Landa and Soldán, 1989; Zwick, 1992; Küry, 1997; DeWalt *et al.*, 2005; Bojková *et al.*, 2012). A comparison of contemporary and historical mayfly assemblages of the Morava and Odra river basins in the Czech Republic did not show any anticipated decline in the local or regional diversity of mayflies. On the contrary, slight increases in species richness in all stream types, except lowland streams and large rivers, and in the total number of species recorded were documented (Table 3). Nevertheless, contemporary assemblages were highly dissimilar to historical ones (33 out of 60 sites had a dissimilarity higher than 50%; Figure 4), particularly because of a high species turnover (Figure 2). This indicates a possible complex change in mayfly assemblages under the long-term pressure of human impacts.

Some of the highly specialized and sensitive species have disappeared. Such a decline, which is particularly evident in potamal sections of streams, has also been noted in many European rivers (Landa and Soldán, 1989; Usseglio-Polatera and Bournaud, 1989; Zwick, 1992; Marten, 2001; Floury *et al.*, 2013). However, the main changes in assemblages have been caused by the loss or turnover of common and rather widespread species (cf. Figure 5), which is generally less apparent from the present-day perspective without using historical data for comparison. Several long-term and large-scale studies have recently provided evidence that common, widespread species have experienced profound changes in their distribution (Conrad *et al.*, 2006; van Dyck *et al.*, 2009; Bojková *et al.*, 2012; Brooks *et al.*, 2012), although there is a relative paucity of quantitative information on these changes (Gaston and Fuller, 2007). This might be attributable to the rapid ecological changes in the wider landscape, resulting from the intensification of land use, land or river management, and pollution that led to the wide-scale habitat degradation or changes in conditions important for particular species (van Dyck *et al.*, 2009; Brooks *et al.*, 2012; Fox, 2013; Bojková *et al.*, 2014). On the other hand, common, widespread species are consequently habitat generalists with less-demanding ecological requirements that are able to counterbalance their losses by colonizing new habitats (or re-colonizing restored habitats), exhibiting a certain level of resilience (Bojková *et al.*, 2014). Therefore, changes in species occurrence induced by various environmental alterations at the regional scale seem to be rather dynamic.

The interpretation of relatively heterogeneous assemblage changes between such distant time periods is not simple. Although pairwise dissimilarities in assemblages have generally increased from near-natural sites to sites affected by multiple pressures, the extent of species turnover and differences in species richness was wide in all pressure categories (Figure 3). This highlights a crucial role of local conditions and local interaction of pressures, which were also related to the conditions in the 1950s, as many sites were affected by some pressures even in that period. The greatest change in assemblages was associated with impaired or bad water quality in rivers in the 1950s, which has continued to become worse until now (Figure 4(B)). Assemblages influenced by a slight deterioration or improvement of water quality were less dissimilar, unless another pressure (such as channel or discharge modifications) occurred. The influence of channelization could not be distinguished because most streams were already channelized in the 1950s, and the effect co-occurred with the influence of pollution. Quantifiable hydromorphological changes have taken place in few streams since then. The co-occurrence of pressures and their different intensities that have affected streams in both periods prevents an assessment of species or assemblage responses to particular pressures to be made in more detail, which is one of the main challenges in the evaluation of long-term data. Larger datasets that cover different combinations of pressures in both periods are necessary to disentangle their effects. In fact, the majority of studies that have attempted to explain temporal changes in fauna have faced similar limitations, because they all had such multiple-stressor contexts. For example, continuous changes in water quality or in hydrology might confound the possible effects of increasing water temperature, and habitat degradation might be synergistic with pollutants (Daufresne *et al*., 2003, 2007; Durance and Ormerod, 2009; Bojková *et al*., 2012; Rasmussen *et al*., 2012; Floury *et al*., 2013).

In these situations, the detection and interpretation of impacts based on species traits, which can respond quite specifically to certain sources of human pressure (Townsend *et al*., 2008; Statzner and Bêche, 2010), is an appropriate and promising approach. The present study attempted to infer the direction of the assemblage change from loss, gain, and survival of species with different habitat specializations, sensitivity to pollution and siltation, and body type (Figure 6) which is a surrogate for other life strategies, such as locomotion, microhabitat preference, and feeding habits (cf. Buffagni *et al*., 2009; Bauernfeind and Soldán, 2012). The results suggest that assemblages of brooks, small rivers, and large rivers exhibited quite different changes.

The reappearance of cold-water specialists and a stable share of generalists in brooks, and the tendency for a shift towards a pollution- or siltation-tolerant assemblage that is not accompanied by loss of specialists in small rivers, suggest the maintenance of a certain level of specialization in the assemblages. In contrast, large-river assemblages underwent profound changes that led to evident simplification in their taxonomic and ecological diversity. Potamal specialists were replaced by habitat generalists, although the original potamal specialists were recorded rather marginally in the data from the mid-20th century, owing to their rapid large-scale extirpation during the first half of that century (cf. Landa and Soldán, 1985, 1989; Landa *et al*., 1997). There was also loss of larvae with a specialized way of life: wriggling larvae preferring stone crevices, streamlined larvae living in swift flow, and flattened larvae clinging to stones. Thus, persisting larvae were mostly fish-like, swimming or sprawling on substrates, or were burrowers. Such a shift towards less specialized assemblages might be attributable partly to habitat degradation caused by channel modifications and increased sedimentation, and partly to simultaneous organic pollution, as indicated by the decline in species slightly sensitive to pollution and an increase in siltation-insensitive larvae and burrowers. Analogous shifts in insect assemblages were observed in regulated large lowland rivers that carried the flood water and waste from densely populated and intensively farmed land during the 20th century (Usseglio-Polatera and Bournaud, 1989; Zwick, 1992). Nevertheless, reversal from severe pollution at the turn of the 20th century resulted in the colonization of several species with higher demands for water quality and oxygenation (cf. Marten, 2001; Floury *et al*., 2013). Similar positive trends might also have influenced the data from large rivers in the contemporary period as water pollution was substantially reduced after 1989 in the Czech Republic (Bojková *et al*., 2014 and references herein). However, the present water quality of rivers is still far from natural conditions. Furthermore, new data document the continuing gradual deterioration caused mainly by non-point pollution (IzaR, 2010; Esteban and Albiac, 2012).

Implications for conservation and assessment of ecological status

The outcomes of the study stress the importance of well-developed and complex methodology for assessing the ecological status of streams to show changes caused by species turnover and restructuring of assemblages without substantial increase or decrease of species richness. In such cases, most frequently used indices based on species richness or basic compositional characteristics of assemblages (Birk *et al*., 2010, 2012) can fail. One of the most suitable approaches is comparison with a reference near-natural assemblage predicted using environmental data from existing near-natural conditions using an approach based on RIVPACS (Wright *et al.*, 1993). This enables the extent of shifts in both taxonomic and functional composition of an assemblage to be quantified and the species that probably disappeared and colonized an assemblage to be estimated. This assessment has also been developed in the Czech Republic (Kokeš *et al.*, 2006) and included in the multimetric indices used for the ecological status assessment of brooks and small rivers under the EC Water Framework Directive (Directive 2000/60/EC; Council of the European Communities, 2000). However, the method has not been developed for large rivers in the Czech Republic owing to the lack of species data from near-natural conditions. A possible solution can be the reconstruction of original (or at least characteristic) assemblages based on either historical faunistic records or available recent analogies (cf. Němejcová *et al.*, 2013). The reference assemblages would also serve to establish conservation and management planning targets (cf. Nel *et al.*, 2009).

The loss of original diversification and specialization particularly endangers aquatic assemblages of large lowland rivers draining intensively exploited landscapes. Therefore, a strategy is urgently needed to protect their remaining well-preserved stretches. Targeting conservation management towards this specific habitat is difficult when based on those defined in the EC Habitats Directive (Council of the European Communities, 1992). Of the five natural habitat types connected with running waters, only the type 3270 – 'Rivers with muddy banks with *Chenopodion rubri* p.p. and *Bidention* p.p. vegetation', and the type 3260 – 'Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation', cover some lowland rivers. However, these types include fairly heterogeneous stream habitats, which are currently protected in 28 Special Areas of Conservation (SACs) in the Czech Republic and only weakly correspond with the river stretches inhabited by endangered aquatic assemblages. The current list of animal species of Community importance whose conservation requires the designation of SACs (Annex II of the Habitats Directive) is insufficient too. It includes only five lotic invertebrates (*Margaritifera margaritifera* (Linnaeus, 1758), *Unio crassus* Philipsson, 1788, *Austropotamobius torrentium* (Schrank, 1803), *Ophiogomphus cecilia* (Fourcroy, 1785) and *Coenagrion ornatum* Sélys, 1850), which inhabit 35 stretches of streams currently protected under the Habitats Directive in the Czech Republic. There are also 14 fish species listed in Annex II; however, most of these species occur in small areas in the borderland of the Czech Republic (the Elbe River, Morava River). Many characteristic invertebrates of lowland rivers are included in the Red List in the Czech Republic (Farkač *et al.*, 2005), but only a few of them are legislatively protected. The extension of the list of specially protected species to include the appropriate representatives of the lowland river assemblages (such as mayflies *Ephoron virgo* and *Choroterpes picteti*) would enable effective conservation of some stretches of lowland rivers.

The main challenge for freshwater conservation planning is not only the rehabilitation of the physical heterogeneity of river channels and riparian zones, but an appropriate management of entire catchments (Harper *et al.*, 1999; Jähnig *et al.*, 2009; Nel *et al.*, 2009). In practice, real progress in catchment management could be achieved through the river basin management plans (RBMPs) that should integrate water management and relevant measures under other EU legislation. The measures needed under the Habitats Directive should be included in the second RBMPs (effective from 2016). Precisely prepared plans and their consistent implementation would play a crucial role in maintaining biodiversity and habitat heterogeneity in fresh waters.

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